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Selection of aluminum tolerant cereal genotypes strongly influences the arbuscular mycorrhizal fungal communities in an acidic Andosol

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ABSTRACT

In Chile, cereals cultivation is mainly in volcanic soils with pH values typically between 4.5–5.5 and high levels of exchangeable aluminum (Al) and low P availability. In this context, arbuscular mycorrhizal fungi (AMF) provide or enhance protection against this environmental stress. The aim of this study was to investigate the impact of the breeding process of Al-tolerant cereal plants on AMF community structure and diversity associated to cereals species. This breeding program has been developed since 1980 in our country and consists of obtaining cereal plants that can tolerate stress by Al. For this, we contrast cereals species and genotypes in which Al-stress has been included or not in this breeding program. Rhizosphere soils from Al-tolerant cereals recently developed (*Avena sativa, Hordeum vulgare, Triticum durum*, x. *Triticosecale Wittmack*, *Secale cereale* and *T. aestivum*) were collected from field plots in South-Central Chile. In addition, two cereals with recognized Al-tolerance (Crac wheat cultivar and rye) were also analyzed. AMF identification and taxonomy was performed based on spore morphological analyses. Colonization and glomalin related soil protein (GRSP) was also evaluated. In general, up to 80% of root colonization in all cereal was found. Extraradical mycelium reached levels close to 3 m g^{-1} of soil in the rhizosphere of *S. cereale*, *A. sativa* and *H. vulgare* selected under Al stress. While, GRSP values were statistically similar among selected or not selected genotypes under Al stress, this trend was not observed in *H. vulgare*, where a difference of 20 μg GRSP g^{-1} of soil was found. Moreover, large differences in AMF spore densities were observed, being 340 spores in 100 g soil the lowest and 1900 the highest one, in non Al tolerant *H. vulgare* and Al tolerant x. *Triticosecale Wittmack*, respectively. From a total of 10,000 AM fungal spores, 21 AMF species were identified, belonging to three classes, six orders, and eight families. The alpha diversity was higher in Al tolerant *T. durum* and almost similar to *T. aestivum*. Evenness index was significantly higher in Al tolerant *H. vulgare*. As conclusion, the use of target AMF species and cereals obtained under Al stress could be determinant factors for the appropriate AMF community establishment, potential inoculation assays and agricultural practices, especially oriented to soils with high Al levels.

1. Introduction

Worldwide, acid soils restrict agricultural production (von Uexküll and Mutert, 1995). In Chile, cereals cultivation is mainly carried out in Andosols characterized by pH values typically between 4.5 and 5.5 and low P availability. In general, these soils have undesirable properties, such as high P-adsorption and high levels of exchangeable aluminum (Al^{3+}), Mn^{2+} and H^{+} ions. These soil conditions create a significant decline in plant growth by a reduction of root length, which are limiting their capacity for absorbing water and nutrients (Seguel et al., 2013; Aguilera et al., 2015). It is recognized that phytotoxicity caused by the high levels of Al damages the cell membrane resulting in slower growth and root cell elongation (Magalhaes et al., 2007; Seguel et al., 2013; Aguilera et al., 2015).

The strategies that have allowed the cultivation of cereals in Chile, specifically on Andosols, are principally focused in the developing of genotypes differing in their capacity to tolerate Al phytotoxicity. The obtained cereal cultivars have been generated through breeding programs based on the introduction of cultivars which have been subjected to landraces adapted to local conditions from Southern-Central Chile. As a whole, this has caused an improvement on the germplasm characteristics, such as the tolerance to high Al levels (von Baer, 2007;

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Seguel et al., 2016a, 2016b). Additionally, these traits have been enhanced by associated soil indigenous microorganisms, which improve the plant protection against environmental stress (Meier et al., 2012; Seguel et al., 2013; Aguilera et al., 2011, 2015).

In the context of Al environmental stress, several studies have shown that arbuscular mycorrhizal fungi (AMF) favor biological adaptation of cereals living under abiotic stress conditions, such as low P bioavailability (Castillo et al., 2012) and Al phytotoxic levels (Cumming and Ning, 2003; Seguel et al., 2013, 2015, 2016b). Based on the above, among agricultural management alternatives of cereal growing under these conditions, it is considering the use of AMF in programs oriented to inoculants generation able to attenuate Al phytotoxicity and increasing P acquisition. In this way, it could increase plant productivity and grain production (Borie et al., 2010; Aguilera et al., 2015).

This work is part of a research line focused on elucidating the main mechanisms by which AMF help to reduce negative effects of high Al levels and to find efficient AMF strains able to be used as inoculants in crop production in acidic soils. According to previous studies (Castillo et al., 2006; Aguilera et al., 2014; Seguel et al., 2015, 2016a, 2016b; Marín et al., 2016) we hypothesized that genotypes developed under Al stress will positively influence on AMF diversity represented by the dominance of generalist AMF species for this soil conditions. Thus, few AMF species will be more functionally compatible with these cereals.

Generally, AMF propagules and glomalin related soil protein (GRSP) have been used as a tool for determining host-AMF interaction. Then, we also hypothesized that there will be higher AMF propagules volumes associated with cereals obtained under Al stress differentiate mass selection, because cereals could be depend in great magnitude of AMF symbiosis for overcoming this environmental stress condition. Consequently, the aims in this study were i) to determine the effect of Al-tolerance on AMF community structure and diversity in six cereals species, and ii) to compare AMF propagules interaction with these cereals in a long-term field assay under conventional farming.

2. Materials and methods

2.1. Study site and cereal species

For this study, 6 plots located in the South-Central Chile that belong to an Experimental Station dedicated to cereals breeding oriented to develop Al-tolerant cereal genotypes, were selected (39°06′14″S and 72°41′16″W). Genotypes of four cereal species (*Avena sativa* L., *Hordeum vulgare L*, *Triticum durum*, x. *T. Wittmack*) have been developed recently by means of breeding process based on mass selection including Al-stress as principal factor (see Table 1). Moreover, two genotypes of cereal species with a recognized Al-tolerance (*Secale cereale* L. and *Triticum aestivum* L. cv. Crac) were also included in our study.

Table 1
Cereal species and genotypes selected in a long-term assay in field conditions in an Andosol from south-central Chile in a breeding process under Al-stress and the respective cereal code.

Cereal species	Cultivar selection under Al-stress[a]	Cereal code
Avena sativa	−	AS1
Avena sativa	+	AS2
Hordeum vulgare	−	HV1
Hordeum vulgare	+	HV2
Triticum durum	−	TD1
Triticum durum	+	TD2
x. *Triticosecale Wittmack*	−	TW1
x. *Triticosecale Wittmack*	+	TW2
Secale cereale	+	SC
Triticum aestivum	+	TA

[a] (+) indicates genotypes selected under Al-stress and (−) indicates the genotypes without Al-stress. *A. sativa*, *H. vulgare*, *T. durum*, x *Triticosecale Wittm.* have been recently developed; whereas, *S. cereale* and *T. aestivum* correspond to proved Al-tolerant genotypes widely used in acidic soils from southern Chile, also included in this study.

2.2. Soil sampling

The soil present in the plots was a Dystric Andosol (pH 4.5, -SOM-12.2%, Al-Sat. 25%). Rhizosphere soil was considered the soil adhered to the roots of cereal plants obtained at 0–20 cm depth. Soil sampling was performed in three replicates plots, six rhizosphere soil sub-samples were obtained from each plot and then combined, air dried and sieved through a 2 mm mesh and analyzed as one individual sample per plot. Soil samples were taken at two weeks post-harvest (March, 2015), because previous studies by Cornejo et al. (2007, 2008b, 2008c) have demonstrated that in cereals as wheat growing in acidic Andosols the time between harvest and three months post-harvest represents the maximum densities of AMF spores. In November, 2014, trap cultures were established using field samples (0–20 cm soil depth) according to the methodology proposed by Oehl et al. (2003) in order to improve detection of the whole AMF diversity. Trap cultures of AMF were maintained for 1 year prior to analysis.

2.3. Morphological identification

Spores were extracted from soils using wet sieving and sucrose density gradient centrifugation (Sieverding, 1991). Briefly, 25 g of air-dried field soil were passed through sieves of 500, 125 and 32 μm and thoroughly washed with distilled water. The last soil portion collected in 32 μm mesh and the fraction between 500 and 125 μm were distributed into plastic tubes of 50 mL. To each fraction is added distilled water up to 25 mL. Then, 25 mL of a 70% sucrose solution were inserted at the bottom of the tubes and centrifuged at 2000 rpm for 2 min. Samples were decanted after centrifugation, washed and transferred to *Petri* dishes. Spores were carefully counted under the compound microscope (CX31, Olympus) at up to 400-fold magnification. The number of AMF spores was expressed as spores in 100 g of dry soil. Finally, all spores found in each sample were mounted on microscope slides in polyvinyl alcohol-lactic acid glycerol (PVLG) medium or PVLG mixed 1:1 (v/v) with Melzer's reagent (Sieverding, 1991; Oehl et al., 2003) for their taxonomic identification. The spores were classified based on Glomeromycota system of Oehl et al. (2011a, 2011b). Identification reports (Błaszkowski, 2012; Oehl et al., 2011a, 2011b) and the homepage of the Swiss collection for AMF (SAF; http://www.agroscope.ch/saf) were also used.

2.4. Mycorrhization analyses

Root colonization levels were determined by the gridline intersect method (Giovannetti and Mosse, 1980) after clearing the roots with 2.5% KOH solution (w/v) and staining with a solution of 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970). The extraradical mycelium (ERM) was determined as hyphal length by an adaptation of the filtration-gridline method described by Rubio et al. (2003). Briefly, substrate samples (1 g) were mixed with 4 mL of a solution containing glycerol/12 M HCl/distilled H_2O (12:1:7) and 0.05% trypan blue. Then, the samples were shaken overnight. This suspension was washed thoroughly in 32 μm mesh, suspended in 20 mL distilled H_2O and filtered. An aliquot (1 mL) was taken from suspension that was transferred to a membrane filter of 0.45 μm pore size. To quantify the total hyphal density expressed as ERM the Newman (1966) intersect gridline method was used.

Glomalin-related soil protein was extracted according to Wright et al. (1996), with some minor modifications (Cornejo et al., 2017). Briefly, 1 g substrate in 8 mL of 50 mM citrate buffer pH 8.0 was autoclaved for 1 h at 121 °C. This procedure was repeated until no dark color was obtained in the supernatant. Then, the supernatant was

filtered through Whatman No. 1 paper, and the extracted protein was analyzed using the Bradford assay with bovine serum albumin as standard. Colonization, ERM, AMF spore densities and GRSP were subjected to one way analyses of variance and means were compared using the Tukey's multiple range test. Statistical significance was determined at $p < 0.05$.

2.5. Diversity indices and statistical analyses

In order to test the effectiveness of AMF sampling, a species accumulation curve across pooled individuals (spore counting of AMF) was calculated using the function *accumresult* of the *BiodiversityR* package (Kindt and Coe, 2005) in R 3.2.2 (R Development Core Team, 2015). Moreover, in order to describe AMF diversity patterns associated to cereals, several diversity indices accounting for alpha diversity, dominance, and evenness (Richness, S; Shannon, Ĥ; Simpson, 1-D1; Inverse Simpson, D2; Evenness, Ĵ; Berger, BP) were calculated using the function *diversityresult* of the *BiodiversityR* package (Kindt and Coe, 2005). The differences between diversity indices were analyzed using a one-way ANOVA in which the plot (three samples per plot) was the input variable and diversity indices were the output variable (index ~ plot).

In order to test the effects of host cereal and Al stress on the AMF colonization, hyphal density, GRSP and spores number of AMF, linear mixed effects models were run using the function *lme* of the R package *nlme* (Pinheiro et al., 2016). The model (~Cereal*Al stress, random = ~1|Replicates) with the above-mentioned measurements as response variables, used the three replicates by treatment as a random variable. As *Secale cereale* and *T. aestivum* (cv. Crac) were only tested under Al stress (Table 1), these two cereals were excluded from the models.

Rényi diversity profiles of each site were calculated using the function *renyiresult* of the *BiodiversityR* package (Kindt and Coe, 2005). Rényi diversity profile values (H-alpha) were calculated on the basis of the relative abundance of each AMF species and a scale parameter (alpha), ranging from zero to infinity (Kindt et al., 2006). Rényi profiles are directly related to richness (S) and to the Shannon (Ĥ), Simpson (D1) and Berger (BP) indices. Thus, in a Rényi profile, the H-alpha values reflect diversity (i.e., community A is more diverse than community B if A is plotted above B; Kindt et al., 2006). In the profile, community A is more diverse or had more evenness than community B, if the former is above and never intersects with the latter.

To calculate the alpha, beta, and gamma diversity (measured as contribution to 1-D1) of the plots, the function *contribdiv* in the R package *vegan* (Oksanen et al., 2016) was used. The function *vegdist* of the R package *vegan* (Oksanen et al., 2016) was used to calculate Bray-Curtis dissimilarity, an ecological distance used to generate heat maps of the samples and species, and it was also used in a distance-based Redundancy Analysis (db-RDA). The db-RDA modeled the analyzed mycorrhizal variables (colonization, GRSP content, spores density, no significant for hyphal length) predicting the AMF community structure. The variables order of the db-RDA was given by adding variables according to the Variance Inflation Factor–VIF (VIF < 10); 1000 permutations were carried out. The db-RDA was calculated using the function *capscale* of the R package *vegan* (Oksanen et al., 2016).

3. Results

3.1. Mycorrhization status and GRSP

In general, up 80% of root colonization, by AMF, in all cereals were found (Fig. 1A). Cereal cultivars that showed the lowest percentages of colonization were not Al tolerant *H. vulgare*, *T. durum* and *S. cereale*. Hyphal length reached levels close to 3 m g^{-1} in the rhizosphere of *S. cereale*, *A. sativa* and *H. vulgare* selected under Al stress (Fig. 1B). In *A. sativa*, *H. vulgare* and *T. durum* significant differences among hyphal length levels associated with plants growing under this stress condition were observed.

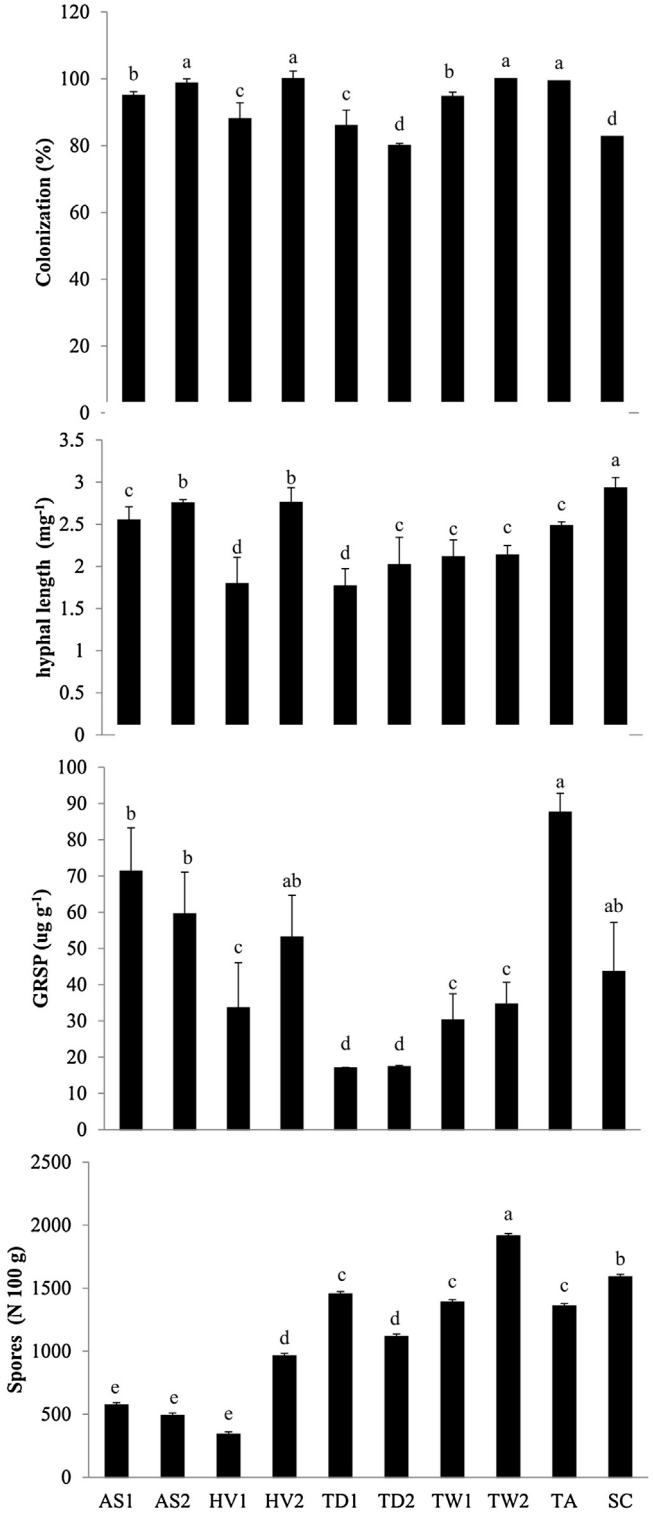

Fig. 1. Arbuscular mycorrhizal fungal propagules and glomalin-related soil protein (GRSP) in the rhizosphere of cereals (AS: *Avena sativa*, HV: *Hordeum vulgare*, TD: *Triticum durum*, TW: x.*Triticosecale Wittmack*, TA: *Triticum aestivum*, SC: *Secale cereale*). Numbers 1 and 2 correspond to mass selection with and without Al stress, respectively, using an Andosol with high Al saturation. *T. aestivum* and *S. cereale* were used as control of cereal with Al-tolerance. Means (S.E.) with different letters are significantly different according to Tukey's multiple range test ($P < 0.05$).

In this study, high differences in GRSP levels in *T. aestivum* and *T. durum* were observed (Fig. 1C). Particularly, in *T. aestivum* rhizosphere GRSP levels close to 90 mg g^{-1} soil were determined, while in *T. durum*

Table 2
Mixed linear effects model showing the main effects (F-value) of cereal host, Al-stress and interactions on the AMF propagules and GRSP in a long-term field assay in an Andosol.

Effect	Colonization	Hyphal length	GRSP*	Spores number
Cereal	21.414^{***}	5.828^{**}	5.825^{**}	28.921^{***}
Al-stress	3.904^{ns}	7.435^{*}	0.136^{ns}	1.566^{ns}
Cereal x Al-stress	6.249^{**}	2.477^{ns}	0.590^{ns}	0.414^{ns}

P-value: *** < 0.001, ** < 0.01, * < 0.1, ^{ns} < non-significant. *Glomalin related soil protein

levels were close to 20 mg g^{-1}soil. While, GRSP values are statistically similar among selected or not under Al stress, this trend was not observed in *H. vulgare,* where a difference of 20 mg g^{-1}soil was found. Moreover, large amplitude in AMF spore density was found being 340 spores in 100 g^{-1} soil the lowest and 1.900 the highest, in non Al-tolerant *H. vulgare* and in Al-tolerant x. *Triticosecale Wittmack*, respectively (Fig. 1D).

The host cereal significantly affected AMF propagules present in the rhizosphere (root colonization, hyphal length and spores number) and GRSP (Table 2). It is noticeable that Al stress condition just significantly affected hyphal density and AMF colonization when interacting with host cereal (Table 2).

3.2. AMF diversity

In this study, from 10,000 AMF spores for each simple sample (Fig. 2), 21 AMF species were identified (Table 3). These species belong to three classes, six orders, and eight families: five Acaulosporaceae species, one *Pacispora* (Pacisporaceae), two *Claroideoglomus* (Entrophosporaceae), one *Dominikia*, three *Glomus*, one *Rhizoglomus*, one *Funneliformis*, one *Septoglomus*, one *Simiglomus* (Glomeraceae), one *Scutellospora* (Scutellosporaceae), one *Cetraspora* (Racocetraceae), two *Ambispora* (Ambisporaceae) and one *Paraglomus* (Paraglomeraceae) were identified. Within which, six species could be potentially undescribed AMF at the date. Subsequently, thirteen genera of the Glomeromycota (Table 3) were identified according to methods by Oehl et al. (2011a, 2011b).

3.3. Diversity indices

Al-tolerant *Triticum aestivum* genotype showed significantly higher values for the principal diversity indices (richness, Shannon, Simpson and Inverse Simpson, Table 4). Additionally, considering the species richness in the other cereal species, only differences between the Al-tolerant and Al-sensitive genotype in *H. vulgare* and *T. durum* were found. On the other hand, TD2 showed a higher Shannon, Simpson, and Inverse Simpson diversity indices. Dominance measured by the Evenness ($\acute{J}$) index (Table 4) was significantly higher in both HV2 and TW2. The alpha diversity (Fig. 3) was significantly higher in Al-tolerant *T. durum*, almost similar to *T. aestivum*.

The cereal species within each plot clustered as expected with AMF species within AMF propagules (Fig. 4). Here, we found that key community attributes as diversity (Table 3, Fig. 3) and structure (Fig. 4; Fig. 5) of AMF were highly affected by the host cereal species and in a minor degree by the Al-tolerance into each plant species. For example, AMF alpha diversity was higher in *Triticum* rhizosphere (independent of Al stress), and lower on *Avena sativa* rhizosphere, also independent of Al stress (Fig. 3). In general, host-cereal has higher effect determining AMF richness than Al-tolerance (Table 3; Fig. 3), but within each variety, there are clear effects of Al-tolerance (Table 3; Fig. 3).

Arbuscular mycorrhizal fungal community structure also seems to be more related to host-cereal than with Al-tolerance (Fig. 4). At least half of the AMF species here described had high abundances on cereal soils with high Al levels (Fig. 4). The AMF community structure associated with cereals is forming different groups according to cluster, showing certain AMF species in cereals breeding with or without Al-tolerance like as *Glomus*, *Septoglomus* and *Acaulospora* genera and others in specific conditions such as *Scutellopora* in oat (AS1) and barley (HV2), which accounts for a high specificity and compatibility even at the genus level (Fig. 4).

4. Discussion

According to our last findings, AMF are able to induce tolerance to phytotoxic Al levels, which emerges as a significant plant adaptation in acidic soils (Borie et al., 2010; Aguilera et al., 2011; Seguel et al., 2013, 2015, 2016b). Specifically, community level adaptations in AMF associated with cereals roots under Al-stress conditions could result on inoculation recommendations regarding cereal production (von Baer, 2007; Aguilera et al., 2014). High level of AMF propagules (root colonization, hyphal length and AMF spores) and GRSP associated to cereals at post-harvest stage were found, being these cereal genotypes obtained under breeding program in which Al level in soil was the principal factor taken into account for the mass selection program. These high values of AMF characteristics suggest a high cereal host-AMF affinity even at cultivar level, as well as a dependency of these cereals to cope agricultural acidic conditions, where AMF symbiosis could be a key component of Al-tolerance. Moreover, in this study we found that the AMF community depend both on the host cereal and the Al-stress level used for breeding.

According to studies performed by Borie and Rubio (1999) and Seguel et al. (2015), *H. vulgare* is the most sensitive cereal species to Al toxicity; however, here this species showed a difference determined by breeding program, reaching up to 3-fold spores (300–900) in *H. vulgare* rhizosphere which plants growing at high Al levels. Likewise, a big

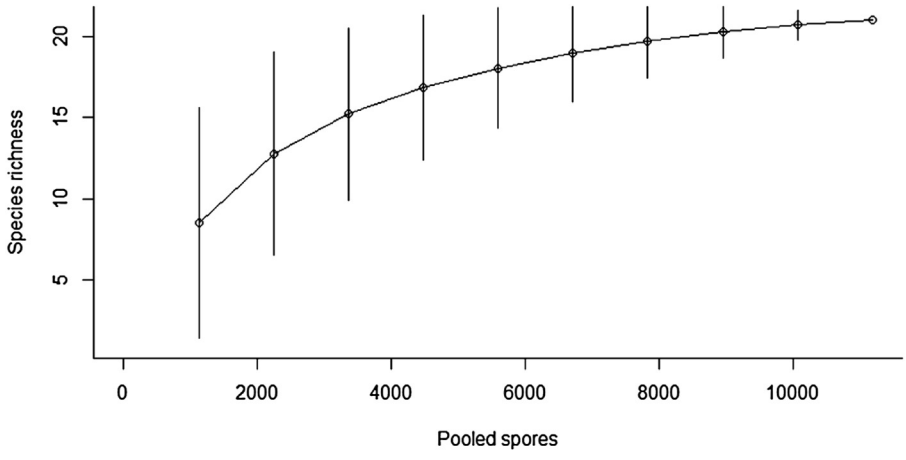

Fig. 2. Species richness accumulation curve with pooled individuals (AMF spore counting). 10,000 AMF spores for each sample were analyzed (n = 3).

Table 3
Arbuscular mycorrhizal species associated with different cereal species and their relative spore abundances (%).

AMF Class	AMF Family	AMF Species	Cereal species									
Order			AS1	AS2	HV1	HV2	SC	TD1	TD2	TA	TW1	TW2
Glomeromycetes												
Diversisporales	*Acaulosporaceae*	*Acaulospora laevis* Gerd. & Trappe	–	25.82	–	6.93	7.16	18.28	4.61	–	–	–
		Acaulospora sp. CL1	–	–	–	–	–	11.95	–	–	–	–
		Acaulospora sp. CL2	–	–	–	–	–	–	2.50	2.58	–	–
		Acaulospora sieverdingii Oehl et al.	–	–	23.53	9.14	9.72	–	–	8.64	6.08	12.58
		Acaulospora paulinae Błaszk.	–	–	7.45	–	–	–	–	–	–	–
	Pacisporaceae	*Pacispora dominikii* (Błaszk.) Sieverd. & Oehl	–	–	–	–	–	–	–	0.84	2.16	–
Glomerales	*Entrophosporaceae*	*Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler	–	–	–	–	–	6.21	5.76	5.71	13.74	9.48
		Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüsser	16.78	–	–	11.22	12.02	7.65	7.58	6.13	10.99	10.40
	Glomeraceae	*Dominikia aurea* (Oehl & Sieverd.) Oehl et al.	–	–	–	18.28	19.82	17.32	13.72	12.61	15.31	15.18
		Glomus sp CL1	18.65	37.77	–	–	–	–	–	–	–	–
		Glomus sp CL2	–	–	30.20	17.59	21.65	–	8.45	–	18.06	–
		Glomus diaphanum J.B. Morton & C. Walker	–	–	–	–	–	7.05	10.08	14.35	8.05	14.68
		Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler	–	22.55	38.82	–	–	–	15.83	10.80	–	–
		Rhizoglomus intraradices N. C. Schenck and G.S. Sm.	–	–	–	–	–	–	–	3.62	–	11.74
		Septoglomus constrictum (Trappe) Sieverd. et al.	20.75	13.86	–	19.81	20.37	14.70	11.80	10.87	14.23	9.73
		Simiglomus hoi (S.M. Berch & Trappe) G.A. Silva et al.	–	–	–	–	–	–	7.29	4.39	–	–
Gigasporales	*Scutellosporaceae*	*Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	33.57	–	–	17.04	9.27	6.57	6.24	9.41	–	7.30
	Racocetraceae	*Cetraspora gilmorei* (Trappe & Gerd.) Oehl et al.	–	–	–	–	–	0.96	0.38	0.56	–	–
Archaeosporomycetes												
Archaeosporales	*Ambisporaceae*	*Ambispora* sp CL1	10.26	–	–	–	–	9.32	5.76	5.71	5.30	3.94
		Ambispora sp CL2	–	–	–	–	–	–	–	–	6.08	4.95
Paraglomeromycetes												
Paraglomerales	*Paraglomeraceae*	*Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker	–	–	–	–	–	–	–	3.76	–	–

In total 33,556 spores were identified.
For the code of cereal species, please see Table 1.

difference between both x. *Triticosecale Wittmack* was observed. Root colonization and hyphal density were indeed affected by Al-stress, besides cereal host. Meanwhile, GRSP and spore production were unaffected by Al-stress. Thus, traits related with soil exploration and symbiotic exchanges are indeed affected by Al-stress, while reproductive traits, as AMF spore production and GRSP, are just affected by host cereal. The remarkable effects at AMF community-level here shown, where even at high Al levels reproductive functions are not affected, are really promising for acidic soils.

While, all cereals had a high mycorrhization, significant differences among cereal species and genotypes determined by the selection process under high Al levels were found, especially for *H. vulgare*. On the other hand, *S. cereale* was associated to a high AMF spores density and hyphal length, whereby this species could be included in a crop rotation system considering an AMF species proliferation either hyphal length or fungal spores. These AMF propagules will grant ecosystem benefits to the next crop growing in this kind of acidic Andosols. At the same time, strategies should be implemented for conditioning the inclusion of agronomical practices that give greater sustainability in these agricultural systems favoring the maintenance and survival of indigenous AMF ecotypes. Such practices could be the incorporation of cereals with high affinity for AMF or minimize impacts on soil structure as reduced tillage, which allows the maintenance of a network of AMF structures, especially hyphae (Curaqueo et al., 2011; Säle et al., 2015).

While, *T. aestivum* included in this study corresponds to a cultivar commonly grown in these acidic Andosol, which has been selected under Al-stress, also related to high level of colonization and GRSP accumulation. This cultivar has been characterized as highly dependent of AMF root colonization in this Andosol (Seguel et al., 2015, 2016b, 2017). These GRSP levels will impact mainly on two aspects, firstly as

Table 4
Diversity indices, statistical analysis of the indices and contribution to diversity (alpha, beta and gamma diversity, Simpsońs index) of AMFcommunities in cereals, of a long term assay in field conditions in Andosol of south-central Chile.

Cereal[a]	Richness (S)	Shannon (Ḧ)	Simpson (1-D1)	Inverse Simpson (D2)	Evenness (J́)	Berger (BP)	Contribution to 1-D1		
ALL	21	2.653	0.916	11.950	0.871	0.139	Alpha	Beta	Gamma
AS1	5a	1.539a	0.771a	4.364a	0.956c	0.336d	0.771a	0.142d	0.913a
AS2	4a	1.327a	0.721a	3.579a	0.957c	0.378d	0.721a	0.203d	0.924c
HV1	4a	1.262a	0.697a	3.302a	0.911a	0.388d	0.697a	0.223d	0.920b
HV2	7b	1.887b	0.842b	6.315b	0.970d	0.198c	0.842b	0.059c	0.900a
TD1	10c	2.150c	0.873c	7.870c	0.934a	0.183b	0.873c	0.052b	0.925d
TD2	13d	2.400d	0.901d	10.052d	0.936b	0.158a	0.901d	0.020a	0.921c
TW1	10c	2.171c	0.876c	8.039c	0.943b	0.181b	0.876c	0.044b	0.920b
TW2	10c	2.233d	0.887c	8.861d	0.970d	0.152a	0.887d	0.043a	0.930d
SC	7b	1.866b	0.835b	6.052b	0.959d	0.217c	0.835b	0.065c	0.900a
TA	15d	2.497d	0.908d	10.866d	0.922a	0.144a	0.908d	0.020a	0.928d

Letters indicate quartiles. Model = index ~ plot.
[a] For the code of cereal species, please see Table 1.

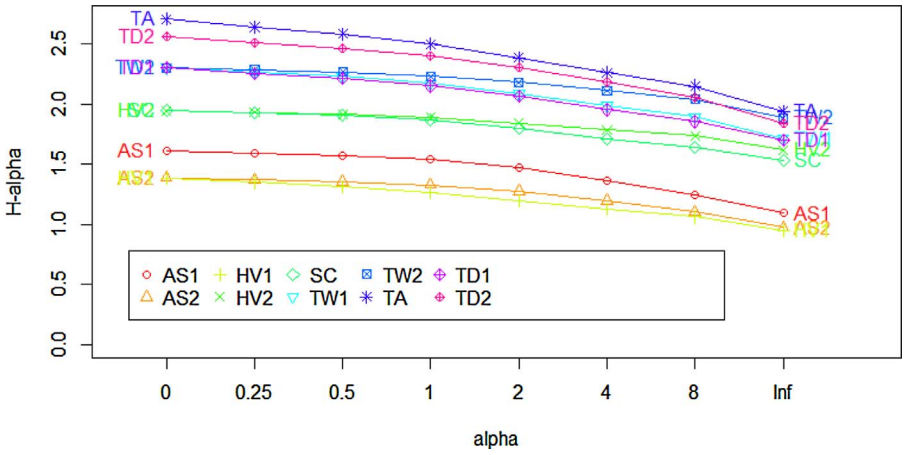

Fig. 3. Rényi diversity profile of arbuscular mycorrhizal fungi per treatment. In a Rényi profile, community A is more diverse or has more evenness than community B, if the former is above and never intersecting with the latter.

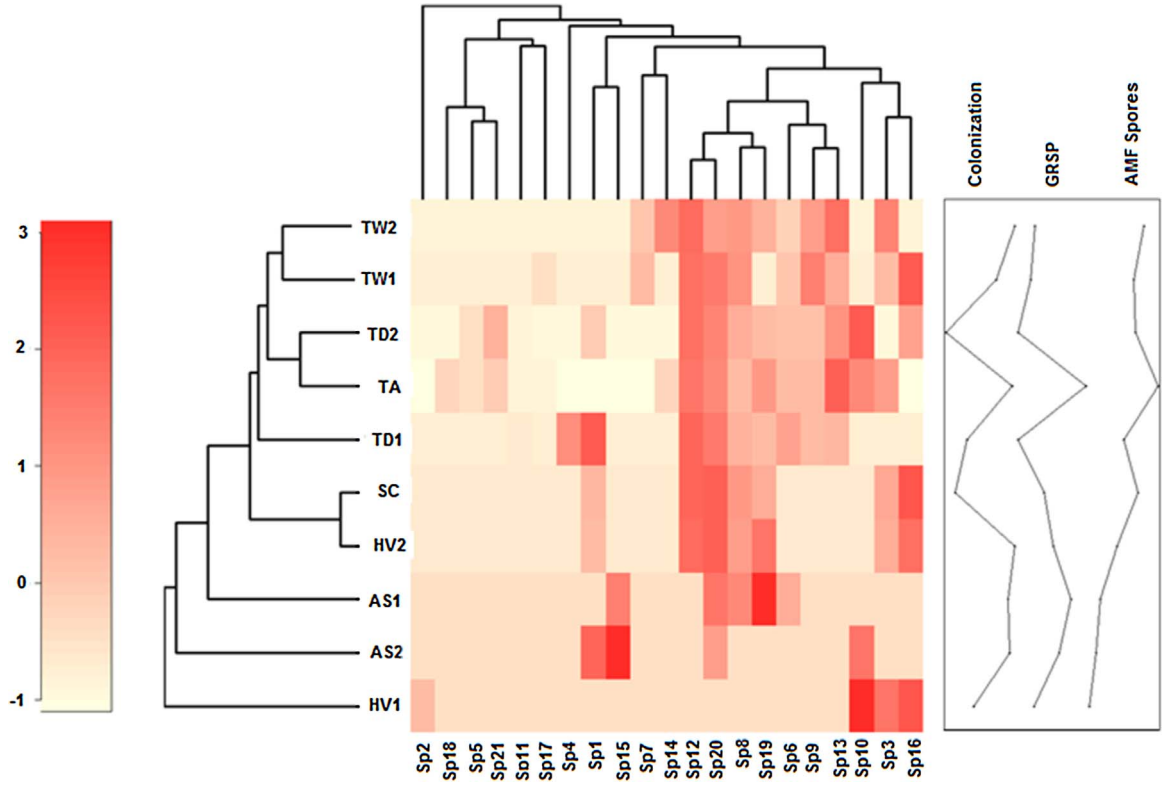

Fig. 4. Heat map and clustering (Bray-Curtis dissimilarity) for the 21 arbuscular mycorrhizal fungi species described in the ten treatments, showing the variables which significantly structure the AM fungi communities. AMF Species legend: Sp1: *Acaulospora laevis*, Sp2: *Acaulospora paulinae*, Sp3: *Acaulospora sieverdingii*, Sp4: *Acaulospora* sp. *CL1*, Sp5: *Acaulospora* sp. *CL2*, Sp6: *Ambispora* sp. *CL1*, Sp7: *Ambispora* sp. *CL2*, Sp8: *Claroideoglumus claroideum*, Sp9: *Claroideoglumus etunicatum*, Sp10: *Funneliformis mosseae*, Sp11: *Gigaspora margarita*, Sp12: *Glomus aureum*, Sp13: *Glomus diaphanum*, Sp14: *Rhizoglomus intraradices*, Sp15: *Glomus* sp. *CL1*, Sp16: *Glomus* sp. *CL2*, Sp17: *Pacispora dominikii*, Sp18: *Paraglomus ocultum*, Sp19: *Scutellospora calospora*, Sp20: *Septoglomus constrictum*, Sp21: *Simiglomus hoi*.

has been previously reported, GRSP has a role in stabilizing soil Al (Aguilera et al., 2011) and it favors soil structure (Morales et al., 2005; Cornejo et al., 2008a; Curaqueo et al., 2011).

The formation and stabilization of aggregates in soil depend of the abundancy and functioning of soil fungi; especially considering the tillage management, where the AM symbiosis contributes with their extensive hyphae network of extraradical mycelium to the formation and stabilization of soil aggregates, with the important contribution of GRSP production as an adhesive. Particularly, in volcanic soils the stabilization of organic matter (OM) by occlusion within soil microaggregates can be chemically stabilized for long time, in addition to the well-known associations with mineral surface, acting as important mechanism of carbon (C) sequestration (Garrido and Matus, 2012).

Worldwide, the agricultural activities as fertilization and tillage significantly affect AMF community composition and are important factors for the variability of the composition and abundance of AMF species, some of them very sensitive to land-use changes (Williams et al., 2016). Spore identification of AMF has been successfully implemented in natural (Castillo et al., 2006) and agricultural settings (Castillo et al., 2010; Aguilera et al., 2014) of volcanic Chilean Andosols. Several studies have been focused in to analyze AMF communities structure obtaining species that occur in most of the locations analyzed, which are known as “generalist”, unlike those whose presence is limited in restricted places, called “specialist” (Castillo et al., 2006; Oehl et al., 2004, 2010; Aguilera et al., 2014). In our study, the AMF species with greater presence in the rhizosphere of cereals growing in this Andosol belong to the genera *Acaulospora, Glomus*, *Septoglomus* and *Scutellospora*, which could be classified as generalist.

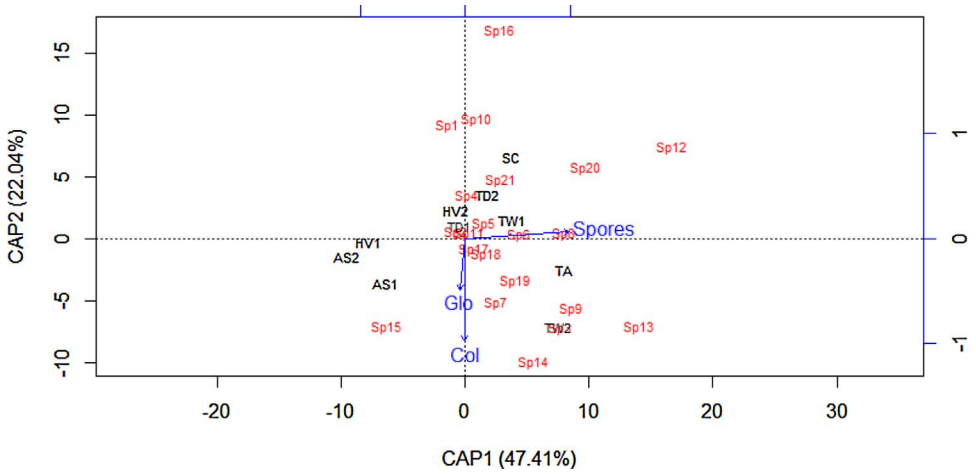

Fig. 5. Distance-based Redundancy Analysis (db-RDA; Bray-Curtis dissimilarity; variance explained in brackets), showing the effect of the variables measured (Col = Colonization, Glo = Glomalin-related soil protein and number of AMF Spores) in clustering the ten treatments and the 21 described AMF species. Permutation test for db-RDA (1000 permutations; model: ~Spores + Glo + Col; pseudo-F: 2.085, significance: 0.014, variables selected due to VIF regression, threshold = 10). AMF Species legend: Sp1: *Acaulospora laevis*, Sp2: *Acaulospora paulinae*, Sp3: *Acaulospora sieverdingii*, Sp4: *Acaulospora* sp. *CL1*, Sp5: *Acaulospora* sp. *CL2*, Sp6: *Ambispora* sp. *CL1*, Sp7: *Ambispora* sp. *CL2*, Sp8: *Claroideoglumus claroideum*, Sp9: *Claroideoglumus etunicatum*, Sp10: *Funneliformis mosseae*, Sp11: *Gigaspora margarita*, Sp12: *Glomus aureum*, Sp13: *Glomus diaphanum*, Sp14: *Rhizoglomus intraradices*, Sp15: *Glomus* sp. *CL1*, Sp16: *Glomus* sp. *CL2*, Sp17: *Pacispora dominikii*, Sp18: *Paraglomus ocultum*, Sp19: *Scutellospora calospora*, Sp20: *Septoglomus constrictum*, Sp21: *Simiglomus hoi*.

Arbuscular mycorrhizal fungi taxonomic and functional diversity studies on agroecosystems are essential in order to predict the response of organisms to environmental stresses. This is based on different compatibility capacities of AMF species have regarding their host and their functionality under certain conditions (van der Heijden et al., 1998). AMF functional variability is even at the intra-specific level (Börstler et al., 2010). Intra- and interspecific functional variability has been reported in AMF species in regard to fungal diversity and their effects on plants exposed to high Al levels in soils (Börstler et al., 2010). Intra- and interspecific AMF differences are functionally and morphologically expressed on traits such as spore germination capacity, hyphae elongation and root colonization; all these traits commonly present a high variation. AMF inoculated plants in soils with high Al content have shown higher growth than non-inoculated plants (Seguel et al., 2012). Therefore, a fundamental aspect is to identify indigenous AM fungal species or ecotypes diversity associated with cereals growing under conditions which has been approached tangentially in *A. sativa* and *T. aestivum* at soil with pH 5.5 and low Al levels (Castillo et al., 2006) and *T. aestivum* growing in soils at pH 4.7 and high Al levels (Aguilera et al., 2014). While measuring community-level physiological adaptations of AMF to high Al stress is a good starting point, the AMF community itself must be measured to make precise inoculation recommendations (Barea et al., 2013; Barea, 2015), not only for the management of indigenous AMF populations but also for the isolation, reproduction and design of AMF based inoculants oriented to cope with specific stressors, as high Al levels in this case.

5. Conclusions

Overall, the breeding process which leads to the cereals selection conditioned by the presence of high Al phytotoxic levels allowed to observe that, in contrasting cultivars of cereals as *H. vulgare* and x. *Triticosecale Wittmack* in a lesser extent, selection under Al-stress condition had a strong impact on the density of AMF propagules present on the rhizosphere of these cereal plants and GRSP. At the same time, the diversity indices determined, as equity, dominance presence, and the study of the affinity between AMF communities with contrasting cereal genotypes expressed through the existence of different AMF propagules densities associated to cereal plants and diversity give support to the existence of a functional compatibility even into a same plant species. The results here reported are key factors that must be taken into account in the implementation of programs aimed to generate AMF-based inoculants and its subsequent inclusion in agro-ecosystems in a framework of sustainable agriculture.

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References

Aguilera, P., Borie, F., Seguel, A., Cornejo, P., 2011. Fluorescence detection of aluminum in arbuscular mycorrhizal fungal structures and glomalin by using confocal laser scanning microscopy. Soil Biol. Biochem. 43, 2417–2431.
Aguilera, P., Cornejo, P., Borie, F., Barea, J.M., von Baer, E., Oehl, F., 2014. Diversity of arbuscular mycorrhizal fungi associated with *Triticum aestivum* L. plants growing in an Andosol with high aluminum level. Agric. Ecosyst. Environ. 186, 178–184.
Aguilera, P., Cumming, J., Oehl, F., Cornejo, P., Borie, F., 2015. Diversity of arbuscular mycorrhizal fungi in acidic soils and their contribution to aluminum phytotoxicity alleviation. In: Panda, S., Baluška, F. (Eds.), Aluminum Stress Adaptation in Plants. Springer International Publishing AG, Cham, pp. 203–228.
Börstler, B., Thiery, O., Sykorova, Z., Berner, A., Redecker, D., 2010. Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. Mol. Ecol. 19, 1497–1511.
Błaszkowski, J., 2012. Glomeromycota. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
Barea, J.M., Pozo, M., López-Ráez, J.M., Aroca, R., Ruíz-Lozano, J.M., Ferrol, N., Azcón, R., Azcón-Aguilar, C., 2013. Arbuscular Mycorrhizas and their significance in promoting soil-plant systems sustainability against environmental stresses. In: Rodelas, B., González-López, J. (Eds.), Beneficial Plant-Microbial Interactions: Ecology and Applications. CRC Press, Florida, pp. 353–387.
Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. J. Soil Sci. Plant Nutr. 15, 261–282.
Borie, F., Rubio, R., 1999. Effects of mycorrhizae and liming on growth and mineral acquisition of Al-tolerant barley cultivars. J. Plant Nutr. 22, 121–137.
Borie, F., Rubio, R., Morales, A., Curaqueo, G., Cornejo, P., 2010. Arbuscular mycorrhizae in agricultural and forest ecosystem in Chile. J. Soil Sci. Plant Nutr. 10, 185–206.
Castillo, C., Borie, F., Godoy, R., Rubio, R., Sieverding, E., 2006. Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile. J. Appl. Bot. Food Qual. 80, 40–47.
Castillo, C., Rubio, R., Borie, F., Sieverding, E., 2010. Diversity of arbuscular mycorrhizal fungi in horticultural production system of Southern Chile. J. Soil Sci. Plant Nutr. 10, 407–413.
Castillo, C., Puccio, F., Morales, D., Borie, F., Sieverding, E., 2012. Early arbuscular mycorrhiza colonization of wheat, barley and oats in Andosols of southern Chile. J. Soil Sci. Plant Nutr. 12, 511–524.
Cornejo, P., Borie, F., Rubio, R., Azcón, R., 2007. Influence of nitrogen source on the viability, functionality and persistence of *Glomus etunicatum* fungal propagules in an Andisol. Appl. Soil Ecol. 35, 423–431.
Cornejo, P., Meier, S., Borie, G., Rillig, M., Borie, F., 2008a. Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci. Total Environ. 406, 154–160 38.
Cornejo, P., Rubio, R., Borie, F., 2008b. Effect of nitrogen source on some rhizospheric properties and persistence of mycorrhizal fungal propagules in an andisol. Chil. J. Agric. Res. 68, 119–127.
Cornejo, P., Rubio, R., Castillo, C., Azcón, R., Borie, F., 2008c. Mycorrhizal effectiveness on wheat nutrient acquisition in an acidic soil from southern Chile as affected by

nitrogen sources. J. Plant Nutr. 31, 1555–1569.
Cornejo, P., Meier, S., Seguel, A., Durán, P., García, S., Ferrol, N., Borie, F., 2017. Contribution of Bradford-reactive soil protein to the copper sequestration in a Cu-polluted soil using *Oenothera picensis*. J. Soil Sci. Plant Nutr. 17, 1–8.
Cumming, J., Ning, J., 2003. Arbuscular mycorrhizal fungi enhance aluminum resistance of broomsedge (*Andropogon virginicus* L.). J. Exp. Bot. 54, 1447–1459.
Curaqueo, G., Barea, J.M., Acevedo, E., Rubio, R., Cornejo, P., Borie, F., 2011. Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agroecosystem in central Chile. Soil Till. Res. 113, 11–18.
Garrido, E., Matus, F., 2012. Are organo-mineral complexes and allophane content determinant factors for the carbon level in Chilean volcanic soils? Catena 92, 106–112.
Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infections in roots. New Phytol. 84, 489–500.
Kindt, R., Coe, R., 2005. Tree Diversity Analysis: A Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies. World Agroforestry Centre, Nairobi PMC id: PMC1156951.
Kindt, R., Van Damme, P., Simons, A.J., 2006. Tree diversity in western Kenya: using profiles to characterise richness and evenness. Biodivers. Conserv. 15, 1253–1270.
Magalhaes, J.V., Liu, J., Guiamaraes, C.T., Lana, G.P., Alves, M.C., Wang, Y.H., Shaffert, R.E., Hoekenga, O.A., Piñeros, M.A., Shaff, J.E., Klein, P.A., Carneiro, N.P., Coelho, C.M., Trick, H.N., Kochian, L.V., 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat. Genet. 9, 1156–1161 37.
Marín, C., Aguilera, P., Cornejo, P., Godoy, R., Oehl, F., Palfner, G., Boy, J., 2016. Arbuscular mycorrhizal assemblages along contrasting andean forest of southern Chile. J. Soil Sci. Plant Nutr. 16, 916–929.
Meier, S., Borie, F., Bolan, N., Cornejo, P., 2012. Phytoremediation of metal polluted soils by arbuscular mycorrhizal fungi. Crit. Rev. Environ. Sci. Technol. 42, 741–775.
Morales, A., Castillo, C., Rubio, R., Godoy, R., Rouanet, J.L., Borie, F., 2005. Glomalin levels in soils from two ecosystems of southern Chile. J. Soil Sci. Plant Nutr. 5, 37–45.
Newman, E., 1966. A method of estimating the total length of root sample. J Appl. Ecol. 3, 139–145.
Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl. Environ. Microbiol. 69, 2816–2824.
Oehl, F., Sieverding, E., Mader, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574–583.
Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M., Sieverding, E., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol. Biochem. 42, 724–738.
Oehl, F., Da Silva, G.A., Goto, B.T., Sieverding, E., 2011a. Glomeromycota: three new genera and glomoid species reorganized. Mycotaxon 116, 75–120.
Oehl, F., Sieverding, E., Palenzuela, J., Ineichen, K., Da Silva, G.A., 2011b. Advances in Glomeromycota taxonomy and classification. IMA Fungus 2, 191–199.
Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Wagner, H., 2016. vegan: Community Ecology Package. R package version 2. 4-0. https://CRAN.R-project.org/package=vegan.
Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. _nlme: Linear andNonlinear Mixed Effects Models_. R packageversion 3. 1–125, < URL: http://CRAN.R-project.org/package=nlme >
R Development Core Team, 2015. R: A Language and Environment for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria. Available online at http://www.R-project.org/.
Rubio, R., Borie, F., Schalchli, C., Castillo, C., Azcón, R., 2003. Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by the source and amount of phosphorus fertilizer and fungal inoculation. Appl. Soil Ecol. 23, 245–255.
Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 84, 38–52.
Seguel, A., Medina, J., Rubio, R., Cornejo, P., Borie, F., 2012. Effects of soil aluminum on early arbuscular mycorrhizal colonization of wheat and barley cultivars growing in an Andisol. Chil. J. Agric. Res. 72, 449–455.
Seguel, A., Cumming, J., Klugh-Stewart, K., Cornejo, P., Borie, F., 2013. The role of arbuscular mycorrhizas in decreasing aluminium phytotoxicity in acidic soils: a review. Mycorrhiza 23, 167–183.
Seguel, A., Barea, J.M., Cornejo, P., Borie, F., 2015. Role of arbuscular mycorrhizal symbiosis in phosphorus-uptake efficiency and aluminum tolerance in barley growing in acid soils. Crop Pasture Sci. 66, 696–705.
Seguel, A., Castillo, C.G., Morales, A., Campos, P., Cornejo, P., Borie, F., 2016a. Arbuscular Mycorrhizal symbiosis in four Al-tolerant wheat genotypes grown in an acidic Andisol. J. Soil Sci. Plant Nutr. 16, 164–173.
Seguel, A., Cumming, J., Cornejo, P., Borie, F., 2016b. Aluminum tolerance of wheat cultivars and relation to arbuscular mycorrhizal colonization in a non-limed and limed andisol. Appl. Soil Ecol. 108, 228–237.
Seguel, A., Cornejo, P., Ramos, A., Von Baer, E., Cumming, J., Borie, F., 2017. Phosphorus acquisition by three wheat cultivars contrasting in aluminum tolerance growing in an aluminum-rich Andisol. Crop Pasture Sci.In press. doi.org/10.1071/cp16224.
Sieverding, E., 1991. Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems. GTZ, Eschborn, Germany.
van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396, 69–72.
von Baer, E., 2007. Wheat breeding for soil acidity and aluminum toxicity. In: In: Buck, H., Nissi, J., Salomon, N. (Eds.), Wheat Production in Stressed Environments, vol. 12. Springer, Nerherlands, pp. 387–394.
von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic impact of acid soils. Plant Soil 171, 1–15.
Williams, A., Manoharan, L., Rosenstock, P R., Olsson, A, Hedlund, K., 2016. Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (*Hordeum vulgare*) mycorrhizal carbon and phosphorus exchange. New Phytol. 213, 874-885.
Wright, S.F., Franke-Snyder, M., Morton, J.B., Upadhyaya, A., 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181, 193–203.