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RESEARCH ARTICLE

Arbuscular mycorrhizal fungal diversity in wheat agro-ecosystems in Southern Chile and effects of seed treatment with natural products

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Abstract

Arbuscular mycorrhizal (AM) fungi are important for P uptake in Andisols cultivated with wheat. We assessed AM fungal diversity in field experiments established with wheat cultivated after AM host plants and non-host plants at three locations of the Araucanía Region. Wheat seed was treated with two natural products: Fosfobio (FOS), mixture of P-solubilizing bacteria and N_2-fixing bacteria; and Myconate (MYC), product containing formononetin. For investigations of AM fungal diversity, soil samples were taken before planting and after harvest of wheat. The morphological spore analyses resulted in 26 species, belonging to 10 families and 16 genera; 5 species belonged to *Acaulospora* (31.2% of total), and 3 to *Claroideoglomus* (18.8%). *Claroideoglomus claroideum* was the prevalent while *Ambispora leptoticha*, *Dominikia aurea*, and *Glomus badium* presented the lowest frequency. The AM fungal species distribution was strongly dependent on the location, and richness at planting of wheat was higher when a non-host for AM fungi had been grown before. There appeared to be a tendency that through wheat cultivation, the richness of AM fungal species decreased from time of planting to harvest when the pre-crop was a non-host; when the pre-crop was AM host there was no apparent decrease in AM species richness through wheat cultivation. Natural products did not significantly influence grain yields. However, there was a tendency that MYC increased average grain yields by 7%. It is discussed that increased AM root colonization, as by MYC and improved P-uptake by AM fungi is more important than inoculation of seed with P-solubilizing microbes.

Keywords: Crop rotation, host plants, natural products, species richness

1. Introduction

Southern Chile has the country's main cereals producing area. Wheat is planted on around 106,790 ha, contributing some 41.6% of Chile's total wheat production (Larraín and Olfos, 2013). The Andisols in the area are low in available nutrients and phosphorus (P) deficiency can limit wheat production (Pino *et al*., 2002). Several studies from the area have shown that most crops depend on arbuscular mycorrhizal (AM) fungi for growth in these Andisols (Borie *et al*., 2010). Wheat roots, too, are symbiotically associated with AM fungi, as is known since long time. It has been postulated that management of indigenous AM fungi may be a valuable agronomic tool for optimising P acquisition in cereals by biological means, thus increasing soil productivity and grain yields (Sieverding, 1991). Of all the factors influencing AM fungal community dynamics and associations with plants, agricultural practices can be considered to be the most important (Barea, 2015). Soil management, such as the intensity of cultivation, the quality and quantity of herbicides applied and the plant protection strategies used can have severe impacts on AM fungal activity and community structure (Jansa *et al*., 2003). Tillage and fallow periods, crop rotation sequences – including alternating host with non-host plants – have been reported as other critical factors affecting the diversity of AM fungi (Harinikumar and Bagyaraj, 1988). Oehl *et al*. (2003; 2005) reported that increased land use intensity was correlated with a decrease in AM fungal species richness and with preferential occurrence and selections of species in agro-ecosystems in Central Europe. Also, Miranda *et al*. (2005) confirmed that the species richness of AM fungi is influenced by the associated crop and the type of soil management.

Information about the species composition of the AM fungal community appears important for understanding mycorrhizal function in ecosystems. In agro-ecosystems of the La Araucanía Region of Chile, most research into AM fungi has been focused on determining plant responses to symbiosis, regardless of the origin or identity of the AM fungi. In general terms, there is little information on the identity of AM fungi associated with wheat in the region. Only two studies are known: Castillo *et al*. (2006) reported the diversity of fungal species in a crop rotation of oats with wheat, cropped under conventional tillage and non-tillage, and Aguilera *et al*. (2014) reported on the diversity of AM fungi in different wheat varieties tolerant to higher aluminium concentrations in soils.

The addition of natural products (NP) to the soil is an alternative agronomic practice that can stimulate root development (Siqueira *et al*., 2002), causing increased susceptibility to colonization by AM fungi and improving recognition between the root and AM fungi (Sarabia-Ochoa *et al*., 2010). Natural products are complex mixtures of ingredients that may include organic acids, plant extracts, hormones or microorganisms. One NP, formononetin is an isoflavon that is extracted from clover roots. Some 20 years ago it was found to improve early AM infection of plants (Baroja *et al*., 2010) and recently also of wheat (Castillo *et al*., 2016a). This active principle showed quite consistent improvements in root mycorrhization and improvement of yields (Westphal *et al*., 2008), and has been reported to enhance AM fungal sporulation (Siqueira *et al*., 1991; Davies *et al*., 2005). Other NP containing free-living soil bacteria can release phosphate ions that are then captured by AM fungal hyphae for the host plant (Bonfante and Anca, 2009; Azcón-Aguilar and Barea, 2015). One of these is Fosfobio (FOS), a NP formed by a mixture of three phosphorus-solubilizing bacteria.

Given the importance associated to mycorrhizal fungal diversity for maintaining the functioning of

ecosystems, a better understanding of the influence of land-use and agricultural practices on AM fungal diversity is needed (Soka and Ritchie, 2015). Therefore, the objective of the research presented here was to investigate whether selected natural products affect AM fungal richness and diversity in the rhizosphere of wheat that grew after host plants (oats) and non-host plants (lupine and oil seed rape) in three locations of the Araucanía Region, and to determine the species richness of AM fungal in these agro-ecosystems. Our hypothesis was that non-host pre-crops will decrease AM fungal diversity in the field and that only by means of NP a higher AM fungal diversity can be obtained in wheat mono-culturing.

2. Materials and Methods

One year experiments with wheat (*Triticum aestivum* L.) were carried out in three different localities in the Araucanía Region: a) El Carmen (Car), where AM non-host lupine (*Lupinus albus* L.) had been grown, (Car_{LW}); b) Huichahue (Hui), where the AM-host oats (*Avena sativa* L.) had been grown (Hui_{OW}) and c) Vilcún (Vil), where in one site the AM non-host oilseed rape (*Brassica napus* L.) (Vil_{RW}), had been grown, and at other site the AM-host oats (Vil_{OW}).

At all locations, the fields were in fallow and covered with mixed pasture grasses and forbs before planting wheat in the experiments. When the study commenced, these fallow plants were ploughed under. The Huichahue soil differed from the others through higher organic matter and available soil phosphate content (Table 1).

Table 1. Geographical location and chemical properties of soils of wheat agro-ecosystems used in this study.

Locality	Location	Soil Order	Crop rotation	pH[A]	OM (%)[B]	Available P (mg kg^{-1})[C]
El Carmen	38°43'S; 72°43'W	Ultisol	Lupine-wheat	5.3	6	4
Huichahue	38°50'S; 72°31'W	Andisol	Oats-wheat	5.4	16	14
Vilcún	38°15'S; 72°50'W	Andisol	Oilseed rape-wheat	5.5	7	5
Vilcún	38°39'S; 72°03'W	Andisol	Oats-wheat	6.1	8	6

[A]Measured in H_2O; [B]Walkley and Black method; [C]Extractable by Olsen method (Zagal and Sadzawka, 2007).

In El Carmen and Huichahue, experiments were established in October 2011 and the wheat was harvested in February 2012. Wheat var. "Otto-Baer" was planted at seed dose equivalent to 200 kg ha^{-1}. Seed had been treated with the fungicide DIVIDEND 030 FS (30 g L^{-1} difenoconazole) at 150 mL 100 kg $seed^{-1}$. The wheat was fertilized with 333 kg ha^{-1} of a mixture of 7-27-8-6-14 N-P_2O_5-K_2O-S-Ca at sowing. All weeds were hand pulled throughout the study; the cultivated wheat was the only host plant for the AM fungi in these field plots. There was no foliar fungicide or insecticide application in these trials. At Vilcún, field trials were established in July 2012, and the wheat was harvested in February 2013. Wheat var. "Crac-Baer" was planted at 200 kg ha^{-1}. The seed had been treated with 150 mL DIVIDEND and 125 mL FORCE 3G (30 g L^{-1} tefluthrin). The wheat was fertilized with 333 kg ha^{-1} of a complete fertilizer mixture of 7-27-8-6-14 N-P_2O_5-K_2O-S-Ca at sowing; two applications every two months followed with potassium nitrate (at 50 kg KNO_3 ha^{-1}). The herbicide TRAXOS (25 g L^{-1} clodinafop + 25 g L^{-1} pinoxaden) was applied at 2.4 L ha^{-1}, to control weeds. The fungicide PRIORI (200 g L^{-1} azoxystrobin + 80 g L^{-1} cyproconazole) was applied at 1 L ha^{-1} at growth stage (GS) 41 to control foliar diseases, and the insecticide ENGEO (141 g L^{-1} of thiamethoxame + 106 g L^{-1} lambda-cyhalothrine) was used at 100 mL ha^{-1} to control insects at GS 41.

For the experiments, wheat seeds were treated, either with above fungicides and/or insecticides alone (Control, CON), or in addition with the two natural products:

a) Fosfobio (FOS), which is a commercial product containing a mixture of the phosphorus-solubilizing bacteria: *Bacillus megaterium* and *Bacillus polymyxa,* and *Azotobacter*, a genus of free-living nitrogen fixing bacteria. 100 mL FOS was diluted in 1 L water and was carefully sprayed and mixed on the equivalent of 100 kg seed, after the chemical seed treatment;

b) Myconate® (MYC) is a product of a water-soluble potassium salt of formononetin (7-hidroxy, 4'-metoxy isoflavone). This product is not commercially available in Chile but is known to consistently improve the mycorrhizal colonization of plants. 100 g were dissolved in 1 L of water and sprayed/mixed with the equivalent of 100 kg seed, in an extra step after chemical seed treatment.

Plots were established at 12 m^2 (4 m x 3 m), with four replicates per treatment with a total of 12 experimental units in a complete randomized block design. Seed was planted by hand in furrows at 3-5 cm depth at about 500 seeds m^{-2}, in distance of 20 cm between rows.

For the investigation of the diversity of AM fungal species, representative soil samples were collected from the areas: a) before planting wheat (this means after ploughing the fallow, FAL) and b) after wheat harvest (CON, FOS, MYC). Each soil sample was a composite of eight cores (6 cm diameter × 10 cm deep) and was taken from each of the four field replications. Samples were homogenized and were stored in a refrigerator before AM fungal spores extraction.

AM fungal spores were extracted from 20 g soil by the method of wet sieving and decanting, established by Gerdemann and Nicolson (1963). Briefly, soil was dispersed in water and the suspension was passed through sieves of 425, 53 and 32 µm mash opening while thoroughly washing with water. The last soil portions collected in 32 µm mesh and the soil fraction of 53 µm were mixed and distributed into plastic tubes; 20 mL of a 70% sucrose solution were inserted at the bottom of the tubes and the mixture was centrifuged at 2500 rpm for 7 min. The spores were decanted after centrifugation, washed and transferred to Petri dishes. AM fungal spores were isolated and permanent preparations were made with polyvinyl alcohol in lactoglycerol

(PVLG) and a mixture of PVLG and Melzer's reagent for species identification. The morphological properties and their subcellular structures were observed under high-power light microscopy at 100 × and 400 × magnification. Subsequently, species were identified by morphological analyses of the spores, using the descriptions of AM fungal found in Schenck and Pérez (1990) and Błaszkowski (2012), http://invam.caf.wvu.edu, and latest AM fungal species descriptions.

Species richness is the easiest way to assess the diversity of species within communities. It is based on the number of species present, without considering their relative importance (Moreno, 2001), and is defined as the total number of species observed per sampling site. In this study the richness was expressed as the number of species of AM fungi in a 20 g soil sample. Table 2 presents the occurrence of a fungal species when found in any of the 4 replicates at a trial site.

Grain yields were taken in the experiments and yields were calculated in kg per ha after readjustment of grain humidity to 14%. Average yields are presented.

For statistical analyses of the studied variables, one–way ANOVA was performed. Means were compared by Tukey's multiple range test. Statistical significance was determined at $P<0.05$. Analysis of variance was carried out with SPSS software package (version 13.0).

3. Results

In the research area, we recorded 26 AM fungal species in total (Table 2) that belonged to 10 families (Figure 1) and 16 genera.

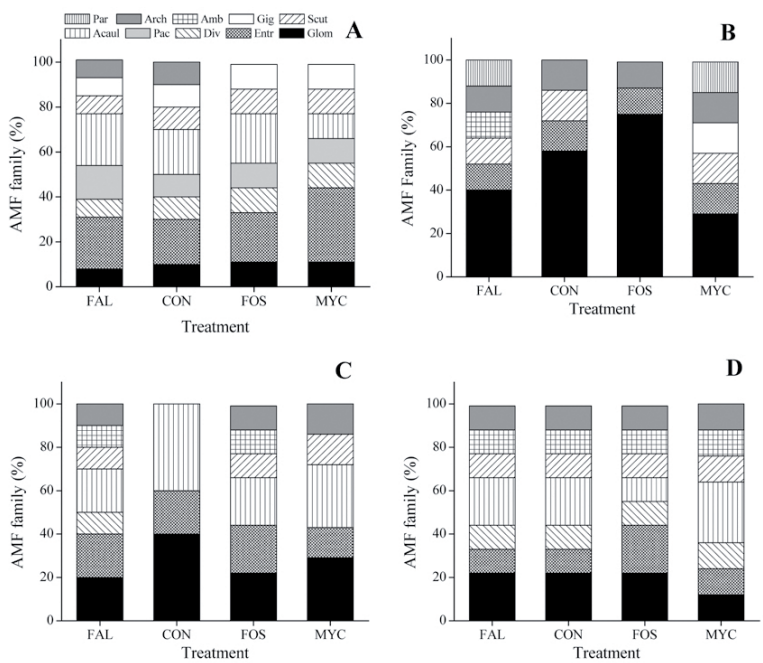

Figure 1. Distribution of arbuscular mycorrhizal fungal families in wheat agro-ecosystems of the Araucanía Region: Car_{LW} (A), Hui_{OW} (B), Vil_{RW} (C) and Vil_{OW} (D) treated with natural products. FAL: fallow; CON: control; FOS: Fosfobio; MYC: Myconate; Glom:Glomeraceae; Entr:Entrophosporaceae; Div:Diversisporaceae; Pac:Pacisporaceae; Acaul:Acaulosporaceae; Scut:Scutellosporaceae; Gig:Gigasporaceae; Amb:Ambisporaceae; Arch:Archaeosporaceae; Par:Paraglomeraceae

It was clearly visible that one location differed strongly in the presence or absence of AM fungi genera: the genera *Dominikia*, *Paraglomus*, *Sclerocystis*, *Septoglomus* and *Simiglomus* were only found at Huichahue while species of Acaulosporaceae and Pacisporaceae were totally lacking at this site (Figure 1, Table 2). Of the other three locations the two fields at Vilcún (Vil_{RW} and Vil_{OW}) were most similar in presence of AM fungal species (Table 2) while differing from El Carmen (Car_{LW}) through absence of *Gigaspora*, a *Glomus* sp., and presence of e.g. *Claroideoglomus lamellosum*.

At locations with pre-crop lupines (Car_{LW}) and rape (Vil_{RW}) the total number of AMF species tended to be higher before planting of wheat than at harvest of wheat at these sites (Table 2). In these plots, wheat cultivation tended to decrease the AMF species richness, whether without or with seed treatment (from 13 and 10 spp. to 9 to 10 and 5 to 9 spp. at Car_{LW} and Vil_{RW}, respectively); the effect was significant at Vil_{RW} in the control without NP seed treatment, only. At locations with pre-crop oats (Hui_{OW} and Vil_{OW}) no effect of wheat cultivation and of seed treatment was observed from planting to harvest (7 to 8 and 8 to 9 species found in all plots).

Grain yields of wheat at the experimental sites (Table 3) were higher in Vilcún than in the others two locations. While FOS had no or even slight negative effect on average yields of the four sites, MYC tended to increase yields. However, the effect of seed treatment with a natural product was statistically not significant at none of the locations.

Table 2. Arbuscular mycorrhizal fungal species in wheat agro-ecosystems treated with natural products in the Araucanía Region.

AM fungal species*	Wheat ecosystem															
	Car_{LW}				Hui_{OW}				Vil_{RW}				Vil_{OW}			
	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC
Acaulospora																
Acaulospora laevis										x	x	x		x	x	x
Ac. mellea	x	x	x	x												
Ac. paulinae	x								x	x	x	x	x	x		x
Ac. punctata									x				x			
Acaulospora sp.	x	x	x													
Ambispora																
Ambispora leptoticha					x											
Ambispora sp.									x		x		x	x	x	x
Archaeospora																
Archaeospora trappei	x	x			x	x	x	x	x		x	x	x	x	x	x
Claroideoglomus																
Claroideoglomus claroideum	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Cl. etunicatum	x			x					x		x				x	
Cl. lamellosum	x	x	x	x												
Diversispora																
Diversispora spurca	x	x	x	x					x				x	x	x	x
Dominikia																
Dominikia aurea							x									
Funneliformis																
Funneliformis mosseae	x					x				x		x				

AM fungal species*	Wheat ecosystem															
	Car_{LW}				Hui_{OW}				Vil_{RW}				Vil_{OW}			
	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC	FAL	CON	FOS	MYC
Gigaspora																
Gigaspora sp.	x	x	x	x				x								
Glomus																
Glomus ambisporum					x				x	X	x	x	x	x	x	x
Gl. badium							x									
Glomus sp.		x	x	x	x	x	x	x								
Pacispora																
Pacispora dominikii	x	x	x	x												
Pacispora sp.	x															
Paraglomus																
Paraglomus occultum					x			x								
Rhizoglomus																
Rhizoglomus fasciculatum					x		x	x	x				x	x	x	
Sclerocystis																
Sclerocystis sp.						x										
Scutellospora																
Scutellospora sp.	x	x	x	x	x	x		x	x		x	x	x	x	x	x
Septoglomus																
Septoglomus sp.							x									
Simiglomus																
Simiglomus hoi						x	x				x					
Richnness (R)**	13	10a	9a	9a	8	7a	8a	7a	10	5b	9a	7ab	9	9a	9a	8a

Vil_{OW}:Vilcún (oats/wheat); Vil_{RW}: Vilcún (oilseed rape/wheat); Hui_{OW}: Huichahue (oats/wheat); Car_{LC}: El Carmen (lupine/wheat); FAL:fallow; CON: control; FOS: Fosfobio; MYC: Myconate. * Presence of species in any four replicates at a trial site indicated by x; **: at each location values followed by different letters indicate a significant ($P<0.05$) difference

Table 3. Grain yields of wheat experiments which were established after non-mycorrhizal pre-crop lupines (Car_{LW}) and rape (Vil_{RW}), and after mycorrhizal pre-crop oates (Hui_{OW}, Vil_{OW}). Wheat seed was either treated with fungicides/insecticides (F/I) (CON) or treated with F/I + Fosfobio (FOS) or F/I + Myconate (MYC).

Location	Grain yield (kg ha^{-1})			
	CON	FOS	MYC	Mean
Car_{LW}	1600a	1800a	1800a	1733
Hui_{OW}	2500a	2400a	3000a	2633
Vil_{RW}	6300a	5900a	6800a	6333
Vil_{OW}	6100a	5700a	6100a	5967
Mean	4125	3950	4425	

Mean values within a row followed by same letters indicate non significant ($P<0.05$) difference.

4. Discussion

The three trials locations differed strongly in the presence or absence of AMF genera and species. While Huichahue had higher organic matter, which could explain the differences in AM fungal genera, the other three locations were quite similar in soil characteristics. However, any relation of occurrence of specific

AM fungal genera or species to soil characteristics is unlikely. For example, *Acaulospora* spp. appear to occur regularly in lower pH soils, as do *Scutellospora* spp. (Castillo *et al*., 2006; Oehl *et al*., 2006). Here however, in locations with soils of similar pH, the presence or absence depended on the location itself. While in areas like Vilcún, where trials were next to each other, the AM fungal community was similar, other locations with different soils and crop cultivation histories had other AM fungal species compositions even though all sites were grown with wheat. This confirms findings of other researchers about site specific AM fungal communities (Oehl *et al*., 2005; Posada *et al*., 2016).

From our study of wheat agroecosystems in Southern Chile we can confirm also the presence of so-called AM fungal generalists (Oehl *et al*., 2003; Börstler *et al*., 2006), species which occur all over Southern Chile, and independent on soils and crops: such were *Archaeospora trappei*, *Claroideoglomus claroideum* and a *Scutellospora* sp., which was morphologically similar to *S. calospora* (Aguilera *et al*., 2014; Castillo *et al*., 2016b). Other species were rarely found (Table 2): *Ambispora leptoticha* (former *A. appendicula*), *Dominikia aurea*, *Glomus badium*, *Paraglomus occultum*, *Sclerocystis* sp., and *Septoglomus* sp. These species are known to occur in other agroecosystems of South America, and some of them are frequent like *Paraglomus occultum* (Sieverding, 1991) while others are not (Sieverding, 1991; Posada and Sieverding, 2014). So, its infrequent occurrence may indicate that their concentration in soils is low and not found, due do the relative small soil sample investigated, or that the relative short cropping systems did not allow completing the formation of spores in higher concentrations, like may have been the case with *G. badium* and *Sclerocystis* sp., which both form spores in sporocarps. Oehl *et al*. (2009) found that such sporocarpic AM fungal species need at least 9 months to complete the sporulation cycle; so, likely too short cycle for sporocarp formation under the investigated cereals cultivation in Southern Chile.

The expectation of the experiments was that the pre-crop would have had a significant negative effect on the diversity of AM fungi. Lupine and oilseed rape are both known as non-mycorrhizal plants, and several years continuous cropping of a non-mycorrhizal plant like rape decreased the abundance of AM fungal species although it did not eliminate them in soils of Germany (Sieverding and Oehl, 2005). However, in our experiments the sites with non-mycorrhizal pre-crops had even slightly higher number of AM fungi in the fallow before planting wheat (Table 2) than with the pre-crop oats, which is a mycorrhizal plant. There may have been several reasons for this: a) with non-mycorrhizal and mycorrhizal pre-crop there was a period of several months of fallow before planting wheat, during which the indigenous AM fungal population could have recovered and multiplied, b) lupine and rape are generally planted at 50-75 cm distance between the rows and at planting a pre-emergence herbicide is applied for weed control, which have often not long lasting efficacy. In contrast oats are planted denser and more effective post-emergence herbicides are used, which eliminate all weeds until harvest. Planting in more distance between rows and pre-emergence herbicides allow a re-establishment of weeds between rows – weed species that may have reproduced and maintained a divers plant species and divers AM fungal communities in the non-mycorrhizal crops. Oats, on the other hand had almost no weeds and only after harvest new weeds could develop. It is further well known that cereal mono-culture decrease the diversity of AM fungi (Oehl *et al*., 2003; Sharmah and Jha, 2014). Cultivation of wheat after non-mycorrhizal crops consequently decrease the total number of AM fungal species (Table 2), from 13 to 10 in El Carmen, and significantly from 10 to 5 species in Vil-

cún (compare Fallow with Control); while after oats the AM fungal diversity was not strongly modified through the following cereal wheat, likely also because the AM fungal species number was lower in the pre-crop. It also may have been that the AM fungal community did not react to cultivation of wheat after oats because the AM fungal species population was adapted to cereals mono-cultivation systems, already. Such non-reaction of AM fungal population to cereals after cereals culturing is new finding and has not been reported before.

The effect of seed treatment with two natural products (FOS and MYC) did not lead to general significant changes in AM fungal species richness (Table 2), as compared to the control. The exception was Vilcún where after rape the wheat cultivation caused irregular effects on AM fungal species richness, depending on the seed treatment. However, because this occurred only at one site, we cannot make general conclusion. It appears that AM species that appeared in the Control, also regularly were found in the FOS and MYC treatments, only varying by the occurrence of some of the non-frequent AM fungal species named above. Our expectation had been that the seed treatments with natural products would increase the concentration of spores in the soil and thus consequently result in a higher AM fungal species diversity. It appears, however, that an increased initial mycorrhizal root colonization rate through seed treatment with natural products in wheat, as reported by Castillo *et al*. (2014), will not result in higher AM fungal species diversity. Natural products applications to cereal seeds, thus, are influencing the mycorrhizal colonization, root growth and initial growth (Castillo *et al*., 2014), but not the AM fungal species richness.

Grain yields of wheat at the experimental sites (Table 3) were higher in Vilcún than in the others two locations. The main reason for this may have been some differences in soil fertility as well in weather conditions and disease pressure. At El Carmen and Huichahue, no foliar fungicides had been applied and foliar diseases like those caused by *Septoria* spp. were not controlled, which may have dramatically affected the yields. In Vilcún, fungicides were applied to control foliar diseases. Whether the pre-crop was a non-mycorrhizal crop (lupine or rape) or a mycorrhizal crop (oats), had no consistent effect on grain yields. The effects of seed treatments by FOS and MYC on grain yields were not significant at none of the locations (Table 3). On average of four trials however, there was a tendency that MYC increased grain yields by an average of about 7% (300 kg ha^{-1}). FOS had no effect. MYC was reported to increase yields of crops in the range of 5-15%, in the past (Westphal *et al*., 2008), and such increases may also be possible in wheat cultivation systems of Southern Chile. Main effect of MYC is improved root colonization by AM fungi (Baroja *et al*., 2010), which as consequence can lead to improved phosphate uptake (Bonfante and Anca, 2009). We had expected that the treatment with FOS would give increased yields but it may be that the inoculation of the wheat seed with P solubilizing bacteria is not the right technology for use of P-solubilizing bacteria. These bacteria live in the rhizosphere of roots and may even be distributed in the soil with the roots. However, P-solubilization in rhizosphere is a natural process also induced by the roots themselves, so that additional P-solubilization by bacteria is unlikely to happen in the same rhizosphere area. It is obvious that additional P, which is not normally available to plant roots can only be taken up and transported to the roots by external AM fungal mycelium, which explores regions not accessible to the roots. Even when P-solubilizing bacteria would have dissolved more P in distance of a few mm from roots, this P would likely not be available to plants without higher AM fungal root colonization rates, and AM fungi external mycelium. Thus, this may explain

why, by assumed higher AM root colonization rates, MYC had tendentially positive effects on grain yield of wheat but FOS not. It can further be assumed from the results of our experiments that AM fungal species richness has little if nothing to do with yield performance of wheat. More important appears to be the actual AM root colonization which results from the indigenous AM fungal population. On the other hand, it is known that some of the members of the AM fungal community are generally quickly and extensively colonizing roots, like *Cl. claroideum*, *Glomus* spp. and *Rhizoglomus* spp. It should thus be the objective of farming practices to maintain such AM fungal species in soils, which can extensively colonize roots of wheat crops in the soils of Southern Chile, which have low available P.

5. Conclusions

In total 26 AMF species were found in wheat agroecosystems of Southern Chile, which corresponds to earlier findings in the area. However, the species richness and occurrence of specific AMF species was site specific, and was unlikely related to soil characteristics. After non-mycorrhizal crops like lupine or rape the AMF species richness is not low, as had been hypothesized, and while wheat cropping after non-mycorrhizal pre-crops decreased AMF richness, wheat after oats did not change the number of AMF species. Seed treatments with the natural products investigated did not increase regularly the richness of AM fungal species in these experiments.

While wheat grain yields were not affected by seed treatments with P-solubilizing and N-fixing bacteria, the formononetin containing tentatively increased grain yields on average of the four trials. Because no relationship was found between wheat grain yields and AMF diversity and species richness, other factors, like increased early mycorrhizal root colonization through formononetin can have been the reason for wheat grain yield increase.

Acknowledgments

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