Drivers of Arbuscular Mycorrhizal Fungal Diversity Across 1,000 km of Chilean Vineyards

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Abstract

Arbuscular mycorrhizal fungi (AMF) communities associated to several grapevine cultivars were analyzed from 1,000 km long of crop area in Chile. The effect that different cultivars of *Vitis vinifera* L. may have on AMF communities has been scarcely studied, especially in non-organic managements. Our objective was to describe the AMF communities and quantify the arbuscular mycorrhizal root colonization associated to several grapevine cultivars in different grapevine ages along a 1,000 km long cropping surface and to test whether factors such as grapevine cultivar and grapevine age, as well as soil chemical factors shapes AMF communities. The vineyards were distributed along 1,000 km across Chile, passing through several administrative regions of the country. The different grapevines ranged from 1 to 41 years old. AMF identification and taxonomy was performed based on spore morphological analyses. AMF spores abundance, root colonization and extraradical mycelium (ERC) were also evaluated. More than 94,000 AMF spores were identified in the 34 vineyards investigated. In total, 15 AMF species were identified in this study. The AMF community was mainly represented by species belonging to the families *Acaulosporaceae*, *Entrophosporaceae* and *Glomeraceae*, dominated by the genera *Acaulospora*, *Claroideoglomus*, *Septoglomus* and *Simiglomus*. Regardless of grapevine age, soil chemistry and geographic location, the AMF community structure was influenced by grapevine cultivar. Grapevine age, soil chemistry and geographic location no affect AMF richness, AMF spores abundance, root colonization and extraradical mycelium (ERC). The AMF species identified in our work could be indicators of agricultural systems exposed to biotic and abiotic stresses in different grapevine age, soil chemistry and geographic location, according to the transect investigated. A role of vineyard cultivar in determining the structure of the AMF community was revealed. The use of AMF species target in regenerative vineyards management could be determining factors for the AMF community establishment.

Keywords Grapevine · Terroir · Chile · Mycorrhizal field inoculation

1 Introduction

Grapevine (*Vitis vinifera* L.) was one of the first domesticated fruit species (Keller 2010) and nowadays are one the world's most economically important fruit crops (FAO 2023). In 2022, global grapevine production surface reached 7.3 million ha, while world wine production and consumption were estimated at 258 mhl and 232 mhl, respectively (OIV 2022).

However, this industry is on alert due to the current climate crisis (Calvin et al. 2023). Also, production areas face an increase in average temperatures and lowering water for irrigation, especially in regions with warm and dry climates (Keller 2023). Under climate change scenario, it is also predicted an increase of the incidence, severity, and outbreak time of *V. vinifera*-related pathogens (Bove et al. 2020; Salinari et al. 2006). Thus, wine producers need to adopt strategies of adaptation and new sustainable cultivation practices to face growing constrains (OIV 2016). As such, it has been proposed to move grapevine crops to colder climate zones where water for irrigation is not limiting, for example.

Research Highlights
- We detected 15 AMF species from 1,000 km of vineyards in Chile.
- Location, variety and age no affect AMF species richness or AM fungal propagules.
- AMF community structure was influenced by grapevine variety.
- We suggest a key role of AMF species target in sustainable vineyards.

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Pointing to sustainable agrotechnical practices, the use of biostimulants has been also proposed to increase the water use efficiency and to reduce agrochemicals and pesticides by crops (Aguilera et al. 2022). In this context, the use of arbuscular mycorrhizal fungi-based biostimulants is of particular interest. The arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycota phylum (Wijayawardene et al. 2018) are obligate symbiotic organisms which are associated to an estimated of 78% of land plants (Brundrett and Tedersoo 2018). When AMF hyphae enter the root, specific structures such as arbuscules and vesicles are formed; this symbiosis is called arbuscular mycorrhiza (AM). The AMF symbiosis is based on the bidirectional exchange of nutrients (Smith and Read 2008). Moreover, several studies have also shown that AMF favor the tolerance of host plants exposed to abiotic and biotic stressors (Delavaux et al. 2017; Marro et al. 2022; Rasmann et al. 2017). Specifically, Marro et al. 2022 reported that *Acaulospora*, *Claroideoglomus*, *Septoglomus* and *Simiglomus* genera have presented greater functions related to plant performance (biomass, P and N nutrition) and abiotic and biotic stressors (drought, heavy metals, salinity, pathogens). Finally, AMF increases soil structure, stability, and water retention (Rillig et al. 2002; Rillig and Mummey 2006).

The *V. vinifera* is a species that forms AM and it plays an important role for enhancing nutrient absorption from soils (Khalil 2013; Schreiner 2005; Trouvelot et al. 2015; Kuyper and Jansa 2023), resulting in an increase of grapevine plants shoot dry weight and number of leaves (Karagiannidis et al. 2007). In this line, it has been observed that mycorrhized grapevines have a more efficient water use (Valentine et al. 2006) and increased tolerance against pathogens such as *Botrytis cinerea* (Bruisson et al. 2016), *Fusarium oxysporum f. sp. herbemontis* (Vilvert et al. 2017), *Dactylonectria macrodidymum* (Moukarzel et al. 2022; Petit and Gubler 2006), *Armillaria mellea* (Nogales et al. 2009, 2010), *Plasmopara viticola* (Bruisson et al. 2016; Cruz-Silva et al. 2021), and grapevine fanleaf virus (Hao et al. 2018). Thus, the use of AMF as biostimulants seems a suitable biotechnological tool, and there are many commercial AMF-based products validating it (Hart et al. 2018). However, there are also evidence indicating that some of these products are not efficient to produce a positive effect (Hart et al. 2018; Salomon et al. 2022). A possible explanation for that is the lack of adaptation of the AMF inoculated to conditions different that they were obtained (Rúa et al. 2016). It has been demonstrated the importance of the sympatric combinations of plants, AM fungi, and soil to reach enhanced plant biomass (Rúa et al. 2016).

Soil chemical factors and grapevine age are important factors that modulate AMF communities (Betancur-Agudelo et al. 2021), consequently, grapevine plants should be studied in interaction with AMF diversity to identify which of the AMF species can be related to the

soil-climatic stress condition and potentially be used as inoculant to face the climate crisis. Given the importance of the use of AMF sympatric to the plant and soil, it is key to first investigate AMF communities associated to the region where they will be used, their environmental drivers (mainly soil conditions), and actually quantify AM root colonization. Then, it can be further determined the key factors involved in the establishment, persistence and positive effects of AMF on the plants of interest (Kokkoris and Hart 2019). However, the effect that different grapevine cultivar of *V. vinifera* may have on AMF communities has been scarcely studied. In contrast, the variability of AMF species richness has been associated with soil chemical factors and vineyard maturity (Betancur-Agudelo et al. 2021).

Chile is an important wine producer with a vineyard area reaching 196,000 ha (OIV 2022). In 2022, Chile had the 8th highest planted area and it was the 6th largest wine producer country, with 12.4 million hl (OIV 2022). Over the last two decades, Chilean viticulture is facing serious challenges, such as an extreme mega-drought with 30% to 45% decrease in precipitation (González et al. 2020). For this reason, the wine production areas have been moves towards the southern regions where water for irrigation is not very limiting yet, but the progressive rise in temperatures has already begun. As a result, in Southern Chile has experienced an increase in planted areas, mostly using cold-climate cultivars, mainly Chardonnay followed by Pinot Noir. Even though plantations have been slowly increasing in the Southern region, viticulture there is facing new constraints, such as its production in Andisol, soils which are very acidic, phosphorous fixing, and prone to induced Al toxicity. The AMF communities and mycorrhizal colonization associated to Chilean vineyards have not been extensively studied, nor the factors than modulate their structure. Up to now, 59 AMF species have been reported in Chile (17% of global biodiversity of the Glomeromycota Phylum) (Marín et al. 2017), documented mainly in agroecosystems (Aguilera et al. 2014, 2015, 2017). Interestingly, a third of those species are shared between agroecosystems and native forests (Marín et al. 2017).

Thus, to properly select AMF species for Chilean viticulture, it is key describe their communities and factors modulating them in vineyards. Thus, the objectives of this study were: 1) to describe the AMF communities and quantify the arbuscular mycorrhizal root colonization associated to different grapevine cultivar and grapevine ages along to 1,000 km long cropping area, including calcareous, neutral and acidic soils and, 2) to test whether factors such as grapevine cultivar and age, as well as soil chemical factors modifies AMF communities. Our hypothesis is that AMF communities and root colonization will be different in grapevines of different areas, grapevines

cultivars and age, and the differences will be explained in relation with the main chemical characteristics of the soil.

2 Materials and Methods

2.1 Study Sites

In order to describe AMF communities, to quantify percentage of root mycorrhization and extraradical mycelium, and to test whether *Vitis vinifera* L. cultivar and age shape AMF communities, and their relation with soil chemical properties, 34 vineyards, hereafter designated as 1 to 34, were surveyed during 2021; being 1 the northernmost and 34 the southernmost sites, respectively, from the main wine grapevine production valleys of Chile, The vineyards were distributed along 1,000 km across Chile, passing through several administrative regions of the country, including Coquimbo (29° 54′ S), Valparaíso (33° 02′ S), O´Higgins (34° 10′ S), Maule (35° 25′ S), Biobío (36° 49′ S), and La Araucanía (38° 44′ S). Those regions have a mean annual rainfall ranging from (125 to 1,246 mm), mostly during winter months. Ten grapevine cultivars were considered: "Cabernet Sauvignon" (CS), "Carménère" (Ca), "Chardonnay" (Ch), "Malbec" (Ma), "Merlot" (Me), "Pedro Jimenez" (PJ), "Pinot noir" (PN), "Riesling" (Ri), "Sauvignon blanc" (SB), and "Pinot" (Pi). "Pinot" is defined as such by the communities and farmers of the La Araucanía Region, referring to the landrace cultivars that have been mass-selected over time. The different vineyards ranged from 1 to 41 years old.

2.2 Soil and Root Sampling

To describe the AMF communities, quantify the percentage of AMF root colonization, and determine soil chemistry, bulk soil samples were taken from 30×30 m plots on each of the 34 vineyards. Each vineyard was considerably larger (5 – 10 ha) than the plot size so no edge effect was generated. Three subsamples were taken from each plot in order to generate a composite sample representative of the vineyard. The samples were taken diagonally across the plot, thus considering two edges and a central point. For each subsample, leaf litter and debris were removed and approximately 1 kg of soil was taken at a depth of 20 cm with a shovel, previously washed with water and disinfected with 70% alcohol. The three subsamples were homogenized in a plastic bag. From this homogenized sample, roots were taken to quantify the percentage of AMF colonization, to extract AMF spores, and for soil chemical analyses.

2.3 AMF Identification

Spores were extracted from soils using wet sieving and sucrose density gradient centrifugation (Oehl et al. 2003). From each vineyard soil sample, a 25 g aliquot was strained though 500, 125, and 32 μm sieves, and subsequently washed with distilled water. The fractions of the 32 and 125 μm sieves were poured in plastic tubes. 25 mL of the spore suspensions were transferred to 50 mL centrifugation tubes. 25 mL of a 70% sugar solution were inserted at the bottom of the tubes and centrifuged at 2000 rpm for 2 min. Samples were sieved after centrifugation, washed with distilled water, and transferred to Petri dishes for sorting and quantification under a dissection microscope (CX31, Olympus) at up to $400 \times$ magnification. The number of AM fungal spores was expressed as spores in 100 g dry soil. Finally, spores were mounted on microscope slides in polyvinyl alcohol-lactic acid glycerol (PVLG) medium for identification (Oehl et al. 2003; Sieverding 1991). The AMF species were identified under a compound microscope based on morphological characteristics of the spores considering the Glomeromycota taxonomy sensu Błaszkowski et al. (2015), and Oehl et al. (2011a, b), which includes spore wall structures, subtending hyphae, and germination structures.

2.4 Quantification of AMF Root Colonization and Extraradical Mycelium

Root colonization was determined by the gridline intersect method (Giovannetti and Mosse 1980) after clearing the roots with a 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 (ERC) was determined as hyphal length per soil gram 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$_2$O (12:1:7) and 0.05% trypan blue. Then, the samples were shaken overnight. This suspension was washed thorough a 32 μm mesh and suspended in 20 mL distilled water. An aliquot (1 mL) was taken from the suspension, and transferred to a membrane filter of 0.45 μm pore size. To quantify the total hyphal density expressed as extraradical mycelium (ERC) the Newman (1966) intersect gridline method was used.

2.5 Soil Chemical Analyses

Soil chemical analyses were done according to Marín et al. (2023). Briefly, soil pH was determined in a 1/2.5 0.01 M CaCl$_2$ solution, while electrical conductivity (EC) and redox potential (Eh) were determined in a water solution (1/2.5; at 20 °C). Total Carbon (C) and Nitrogen (N) were determined in a CN Elemental Analyzer. Olsen P was determined by

Table 1 Arbuscular mycorrhizal fungal species associated to vineyards in Chile

	Glomeromycetes													Archaeosporomycetes	
	Diversisporales					Glomerales							Gigasporales	Archaeosporales	
	Acaulosporaceae				Diversisporaceae	Entrophosporaceae		Glomeraceae					Gigasporaceae	Ambisporaceae	
Vineyards	Acaulospora alpina	Acaulospora laevis	Acaulospora paulinae	Acaulospora sp	Diversispora eburnea	Claroideoglomus claroideum	Claroideoglomus etunicatum	Funneliformis badium	Funneliformis mosseae	Dominikia aurea	Septoglomus constrictum	Simiglomus hoi	Gigaspora margarita	Ambispora sp	Ambispora gerdemannii
1(Ch;13)	0	35,12(440)	0	0	2,55(32)	14,9(187)	14,26(179)	0	0	7,87(99)	5,11(64)	13,19(165)	7,02(88)	0	0
2(PN;10)	0	15,74(280)	0	0	3,6(64)	23,98(427)	10,34(184)	0	0	16,49(293)	13,04(232)	2,25(40)	8,39(150)	6,15(109)	0
3(Ch;10)	0	9,24(146)	0	0	5,21(83)	12,6(200)	9,41(149)	0	4,87(77)	18,48(43)	20,16(320)	17,31(275)	2,69(77)	0	0
4(Pi;41)	0	0	0	15,83(136)	**0**	21,73(187)	11,49(99)	0	0	0	0	46,88(403)	4,04(35)	0	0
5(Pi;7)	0	0	0	31,47(107)	0	0	0	0	0	0	0	59(200)	9,44(32)	0	0
6(SB;28)	0	10,63(179)	0	0	5,24(88)	11,43(192)	7,94(133)	42,86(720)	0	0	0	3,02(51)	18,89(317)	0	0
7(Ch;8)	0	11,78(104)	0	0	0	0	0	78,52(693)	0	0	7,85(69)	0	1,81(16)	0	0
8(SB;6)	0	26,47(181)	0	43,99(301)	0	0	19,46(133)	0	10,12(69)	0	0	0	0	0	0
9(SB;22)	0	60,34(280)	0	39,66(184)	0	0	0	0	0	0	0	0	0	0	0
10(CS;18)	0	0	0	0	0	32,24(187)	0	0	0	36,85(213)	10,13(59)	6,91(40)	13,82(80)	0	0
11(CS;35)	0	4,11(67)	0	0	0	29,23(475)	54,19(880)	0	0	0	11(179)	0	1,48(24)	0	0
12(CS;1)	0	0	0	0	0	44,69(720)	7,78(125)	22,84(368)	0	0	24,66(397)	0	0	0	0
13(CS;35)	0	0	0	0	0	19,61(219)	24,63(275)	0	0	0	31,09(345)	15,78(176)	0	0	8,85(99)
14(SB;16)	1,66(24)	43,55(629)	0	40,6(587)	0	0	9,6(138)	0	0	0	0	4,61(67)	0	0	0
15(Ch;7)	0	0	9,85(125)	0	0	0	33,02(280)	58,18(493)	0	0	0	5,97(51)	2,83(24)	0	0
16(Ca;38)	0	0	0	0	0	49,49(130)	35,35(93)	0	0	0	0	15,15(40)	0	0	0
17(Ca;5)	0	34,1(192)	0	0	0	31,73(179)	34,1(192)	0	0	0	0	0	0	0	0
18(Cb;4)	0	45,32(901)	0	0	0	23,73(472)	0	16,36(325)	0	0	13,27(261)	0	1,34(27)	0	0
19(Ch;16)	0	0	0	0	0	48,31(261)	12,32(67)	15,51(197)	0	0	39,43(213)	0	0	0	0
20(Ma;1)	12,58(160)	2,1(27)	0	0	0	16,56(211)	24,74(315)	0	0	0	0	18,66(237)	0	0	0
21(Ca;29)	0	21,1(133)	0	0	0	27,85(176)	51,05(322)	0	0	0	0	0	0	0	0
22(CS;4)	0	0	0	0	0	18,04(157)	34,56(301)	0	0	25,38(221)	0	9,17(80)	12,84(112)	0	0
23(CS;7)	0	29,44(283)	0	0	0	0	14,44(139)	0	0	27,22(261)	14,44(139)	14,44(139)	0	0	0
24(Me;21)	0	18,48(139)	0	0	0	0	27,54(221)	25(184)	0	0	28,99(213)	0	0	0	0
25(Ri;3)	0	15,53(80)	0	0	0	0	8,28(43)	0	0	52,82(272)	17,09(88)	6,21(32)	0	0	0
26(PN;1)	0	7,38(59)	0	0	0	0	39,58(315)	26,5(211)	0	0	26,5(211)	0	0	0	0
27(SB;15)	0	16,83(139)	0	0	0	0	26,86(221)	22,33(180)	0	0	33,98(280)	0	0	0	0
28(Ch;15)	0	25,81(136)	0	0	0	0	25,81(136)	0	0	0	40,48(213)	13,66(72)	0	0	0
29(PN;15)	0	26,2(133)	0	0	0	27,24(139)	31,43(160)	0	0	0	0	0	15,19(77)	0	0
30(Ch;22)	0	32,41(245)	0	0	0	22,19(168)	28,53(216)	0	0	0	0	0	16,91(128)	0	0
31(Pi;22)	0	0	0	0	0	62,07(459)	21,65(160)	0	0	0	2,89(21)	13,35(99)	0	0	0
32(Ch;17)	0	14,39(51)	0	0	0	0	0	0	0	0	0	53,03(187)	32,58(115)	0	0

Table 1 (continued)

	Glomeromycetes													Archaeosporomycetes	
	Diversisporales					Glomerales							Gigasporales	Archaeosporales	
	Acaulosporaceae				Diversisporaceae	Entrophosporaceae		Glomeraceae					Gigasporaceae	Ambisporaceae	
Vineyards	Acaulospora alpina	Acaulospora laevis	Acaulospora paulinae	Acaulospora sp	Diversispora eburnea	Claroideoglomus claroideum	Claroideoglomus etunicatum	Funneliformis badium	Funneliformis mosseae	Dominikia aurea	Septoglomus constrictum	Simiglomus hoi	Gigaspora margarita	Ambispora sp	Ambispora gerdemannii
33(Pi;13)	0	5,64(59)	0	0	0	44,1(459)	9,49(99)	0	0	0	0	19,74(205)	21,03(219)	0	0
34(Pi;8)	0	10,18(93)	0	15,41(141)	0	38,68(354)	9,01(83)	0	0	0	10,76(99)	15,99(147)	0	0	0

Transect of 1,000 km across Chile. Relative spore abundances (%) and spore average (in brackets)

extraction with 0.5 M $NaHCO_3$ (at pH 8.5), with the extraction diluted (1/2.5) in HNO_3 at 10%, and determined using inductively coupled plasma–optical emission spectrometry (ICP-OES, VARIAN, Palo Alto, USA). The cations (Al, Ca, K, Mg, and Na) were extracted using 1 M NH_4OAc (multi-standards in a matrix of NH_4OAc 1 M, HNO_3 10%, and ultra-pure water), and then determined through ICP-OES.

2.6 Statistical Analyses

The homogeneity of variances and normality of the residuals were checked using the Bartlett test and graphical checks, respectively, before ANOVAs. ANOVAs were performed with the R base function 'aov' in R Studio v.2022.07.1 + 554 (RStudio Team 2022), in order to test the effects of grapevine cultivar, maturity (in years), and their interaction on AMF species richness, number of spores per 100 g of dried soil, root colonization, ERC, and three diversity indices (Simpson, Shannon, inverse Simpson). Diversity indices were calculated with the 'diversity' function of the *vegan* v.2.6–4 R package (Oksanen et al. 2022). The base R function 'TukeyHSD' was used to calculate Tukey tests to check for differences among cultivars regarding AMF species richness, number of spores per 100 g of dried soil, root colonization, and ERC.

To test if grapevine cultivar, maturity, and their interaction had any effect on AMF community structure across the 34 vineyards, a permutational analysis of variance (PERMANOVA) using the Bray–Curtis dissimilarities was run using the function adonis in the R package vegan v.2.6–4 (Oksanen et al. 2022). Such Bray–Curtis distances (among sites and AMF species) were graph as a heatmap. Similarly, a non-metric multidimensional scaling (NMDS, Supplementary Fig. 1) analyses was done across the 34 soil samples, with the 'metaMDS' function in vegan. Finally, to identify which of the edaphic variables best predicted AMF community structure, the 'ordistep' function in *vegan* was used (forward and backward regression at the same time), with the community matrix as the response variable.

Because geographic distance has been shown to influence AMF communities (Marín et al. 2017), two different Mantel tests were run: one to test the effects of environmental distance (calculated based on the 11 soil chemical variables measured; Euclidian distance) on the Bray–Curtis distance among the 34 AMF communities, and other to test the effects of geographical distance on the same response variable.

3 Results

3.1 AMF Species and Genera Richness

A total of 94,752 AMF spores were identified (Table 1) in the 34 vineyards investigated across Chile. In total, 15

AMF species were identified in this study, belonging to 2 classes, 4 orders, 6 families, and 9 genera of the Glomeromycota phylum (Table 1; Fig. 1). 13 AMF species could be unequivocally identified, whereas two others might correspond to undescribed AMF species.

3.2 Effects of Geographic Location, Grapevine Cultivar and Grapevine Age on AMF

When analyzing the Bray–Curtis distances among the AMF communities of the 34 vineyards, it is clear that these did not aggregate by geographic location (Fig. 2). No effects of grapevine cultivar, grapevine age, and their interaction was observed on spore abundance, AMF richness, root colonization, and ERC (Fig. 3a, b, c, d, Supplementary Table 2); these factors also had no effect on the

3.3 Effects of Soil Chemical Parameters on Structure of AMF Communities

To facilitate the understanding of the investigated transect, zones of Chile will be divided into 3, being N: north, C: center and S: south. North zone (NZ) from samples 1 to 9. Central zone (CZ) from samples 10 to 24. South zone (SZ) from 25 to 34. In general, although a range between 2 to 9 AMF species was found in all studied vineyards (Fig. 1), the northern and central zones showed a higher volume of spores expressed in N of spores per 100 g of soil. The main difference between the areas under study was the soil chemistry, where it was found a pH range between 6.4–7.8 in the North zone, 6.0–7.7 in the Central zone, and 5.6–7.2 in the South zone in Andisols characterized by high Al phytotoxic levels and low P availability. Despite this, an ordistep multiple regression (in both directions) indicated

Fig. 1 Number of spores per 100 g of dry soil of different species of arbuscular mycorrhizal fungi (AMF) across 34 commercial vineyards in Chile. Grapevine cultivars indicated as: "Cabernet Sauvignon" (CS), "Carménère" (Ca), "Chardonnay" (Ch), "Malbec" (Ma), "Merlot" (Me), "Pedro Jimenez" (PJ), "Pinot noir" (PN), "Riesling" (Ri), "Sauvignon blanc" (SB), and "Pinot" (Pi). In parenthesis, the age (years) of each vineyard is indicated next to the abbreviation of each grapevine cultivar. Plots are represented from the northernmost (1) to the southernmost (34)
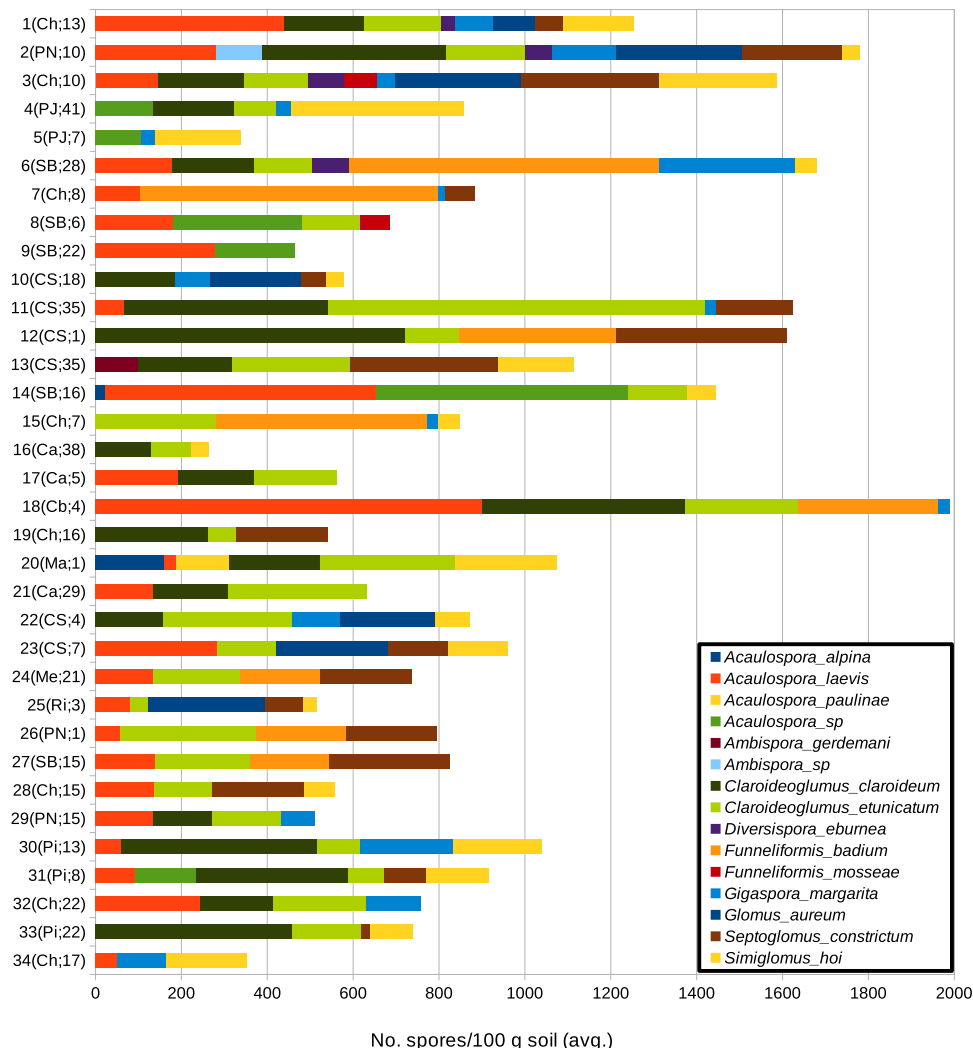
three diversity indices calculated (Table 2).

that none of the soil chemical parameters affected the structure of AMF communities.

The AMF species that showed a higher frequency index (between 50 and 80%) were *Acaulospora laevis*, *Claroideoglomus etunicatum*, *Claroideoglomus claroideum*, *Septoglomus constrictum*, and *Simiglomus hoi* (Fig. 2). Likewise, the species that showed a lower frequency rate (between 2 to 5%) were *Acaulospora alpina*, *Acaulospora paulinae*, both species of the genus *Ambispora*, *Funneliformis mosseae* and *Diversispora eburnea* (Fig. 2).

A Mantel test showed that soil chemistry (Euclidian distance based on the 11 soil chemistry variables measured) did not influence Bray–Curtis distance among the AMF communities (Mantel statistic r: −0.08879, significance: 0.8248, based on 9999 permutations). Similarly, a Mantel test showed that the geographic distance among the 34 vineyards did not affect the Bray distance among the communities (Mantel statistic r: −0.04053, significance: 0.764, based on 9999 permutations). However, community structure was influenced by

grapevine cultivar (but not maturity neither their interaction), as shown by the PERMANOVA analysis (Table 3).

3.4 AMF Colonization and Extraradical Mycelium

A wide range of colonization percentage was visualized inside the roots of the grapevine (Fig. 3c); such colonization was determined by the presence of fungal propagules at a global level inside the observed roots, including arbuscules, vesicles, spores, and hyphae. In general, a range between 2 and 79% of AMF root colonization was found in all grapevine cultivars. The grapevine cultivars with the lowest colonization values were Carménère and Pedro Jiménez. Although other grapevine cultivar showed levels above 70% of root colonization (Fig. 3c). The extraradical mycelium did not show significant differences among grapevine cultivars. The hyphal length had very thin thickness characteristics at microscopic observation and hyphae showed a length between 0,10 and 0,53 mg^{-1} of soil (Fig. 3d).

Fig. 2 Heatmap showing Bray–Curtis distances among sampling plots and arbuscular mycorrhizal fungal (AMF) species. Cultivars indicated as: "Cabernet Sauvignon" (CS), "Carménère" (Ca), "Chardonnay" (Ch), "Malbec" (Ma), "Merlot" (Me), "Pedro Jimenez" (PJ), "Pinot noir" (PN), "Riesling" (Ri), "Sauvignon blanc" (SB), and "Pinot" (Pi). In parenthesis, the age (years) of each vineyard is indicated next to the abbreviation of each cultivar. Genera of AMF indicated as: Cl: *Claroideoglomus*, Fu: *Funneliformis*, Ac: *Acaulospora*, Se: *Septoglomus*, Si: *Simiglomus*, Gl: *Glomus*, Am: *Ambispora*, Di: *Diversispora*, Gi: *Gigaspora*. The first number is referred to the geographical location of vineyards designated as 1 to 34; being 1 the northernmost and 34 the southernmost sites
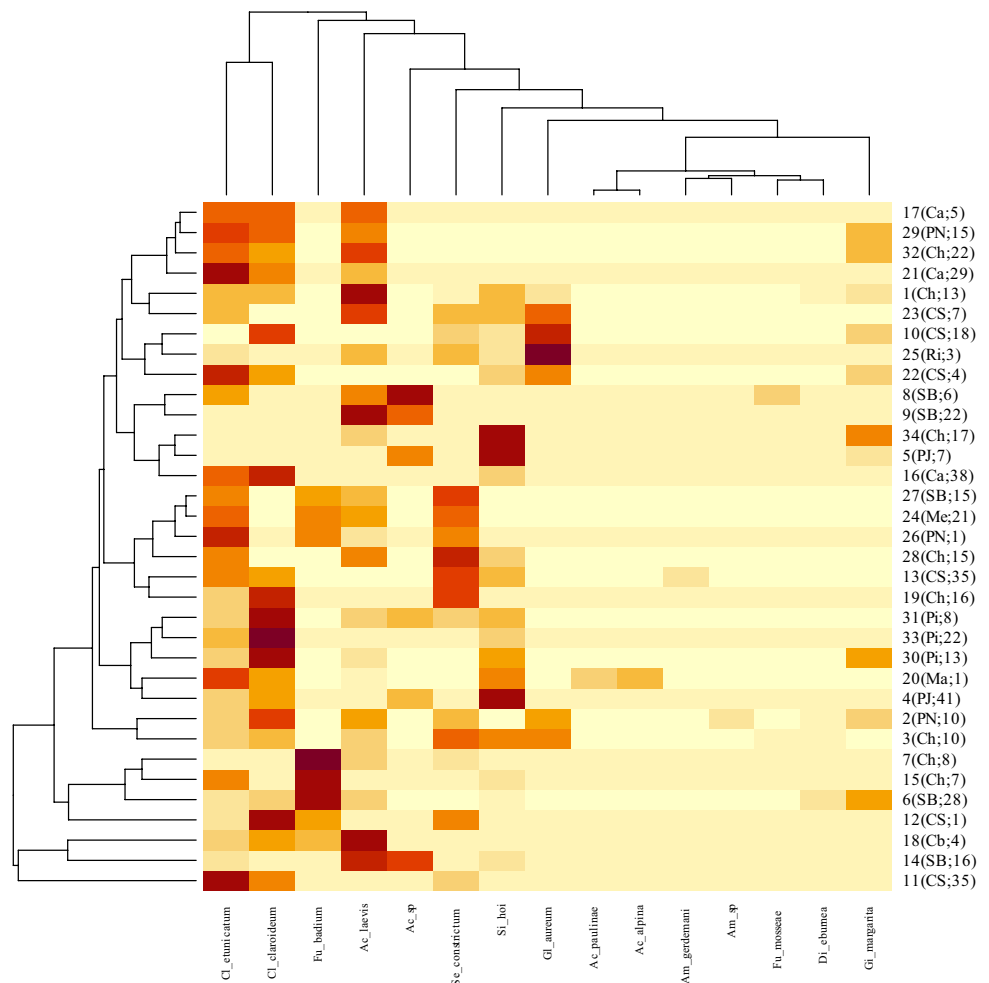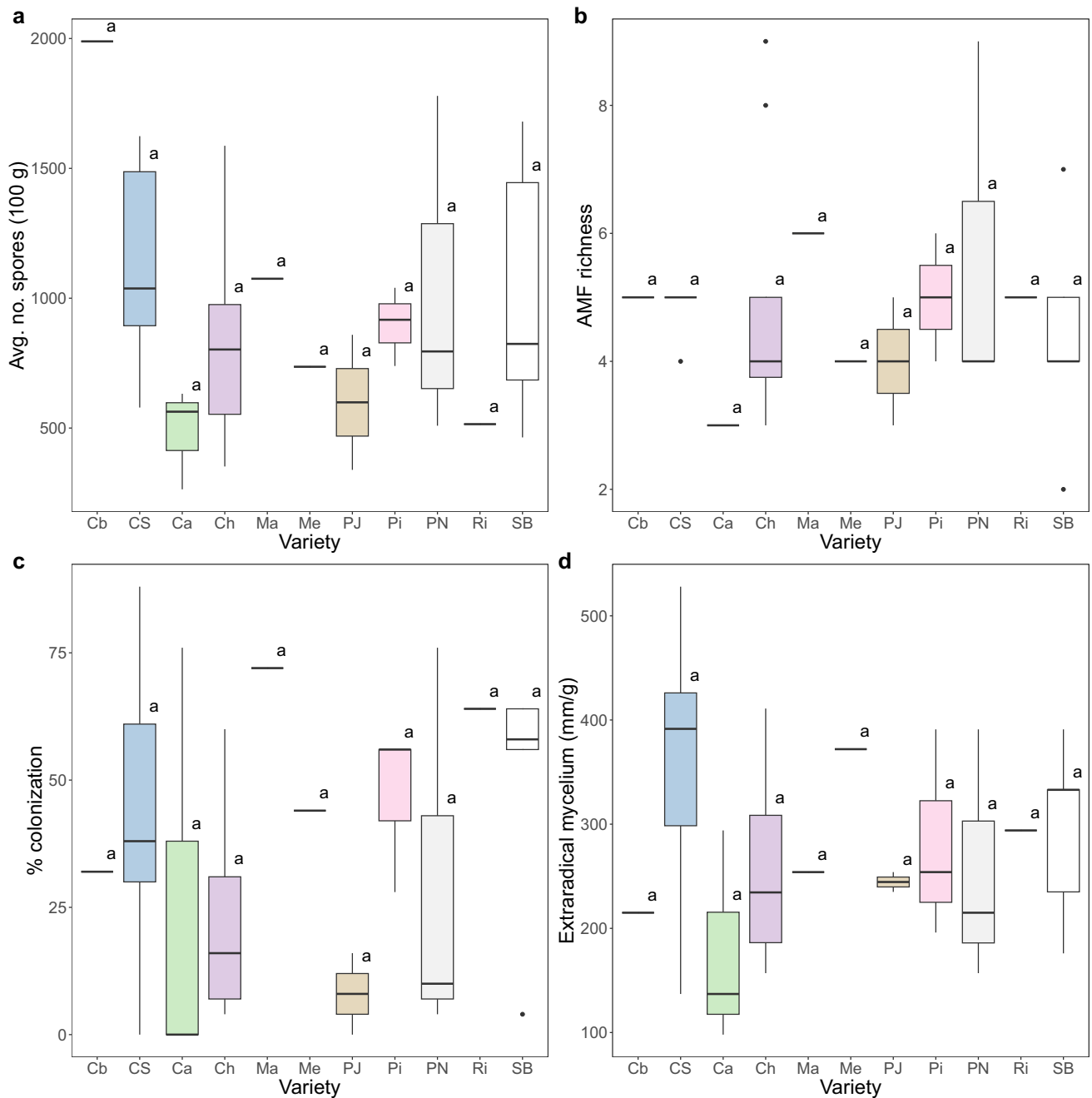
Fig. 3 Boxplot and Tukey tests for different vine cultivars for (**a**) number of arbuscular mycorrhizal fungal (AMF) spores in 100 g of dry soil, **b** AMF species richness, **c** AMF root colonization percentage, and (**d**) Extraradical mycelium of AMF

4 Discussion

This study represents the first investigation into the diversity of AMF species associated with vineyards in Chile, spanning a total distance of 1,000 km. Previous research has explored AMF diversity in the soils of southern Chile identifying a range of 5–19 species associated with horticulture (Castillo et al. 2016). According to our last findings, out of a total of 59 species found

in Chile (Marín et al. 2017), only 20 have been reported in anthropogenically intervened agro-ecosystems and native forest. This encompasses studies conducted in cereals, grasslands and horticulture in Southern zone (Castillo et al. 2016). This finding underscores a significant adaptation of AMF to horticultural practices and, consequently, highlights the potential for viticulture in the southern region of the country, particularly under volcanic soil conditions. However, in this study, between 3 and

Table 2 Diversity indices of arbuscular mycorrrhizal fungal communities associated to vineyard in Chile

Plot	Shannon	Simpson	Inverse Simpson
1(Ch;13)	1.828	0.803	5.064
2(PN;10)	2.015	0.85	6.669
3(Ch;10)	2.041	0.856	6.951
4(PJ;41)	1.358	0.693	3.26
5(PJ;7)	0.898	0.543	2.19
6(SB;28)	1.626	0.746	3.943
7(Ch;8)	0.714	0.362	1.568
8(SB;6)	1.263	0.688	3.209
9(SB;22)	0.672	0.479	1.918
10(CS;18)	1.424	0.726	3.651
11(CS;35)	1.128	0.607	2.546
12(CS;1)	1.241	0.681	3.134
13(CS;35)	1.534	0.771	4.374
14(SB;16)	1.164	0.634	2.735
15(Ch;7)	0.951	0.549	2.215
16(Ca;38)	1.001	0.607	2.543
17(Ca;5)	1.098	0.666	2.997
18(Cb;4)	1.322	0.694	3.268
19(Ch;16)	0.977	0.597	2.481
20(Ma;1)	1.639	0.791	4.778
21(Ca;29)	1.027	0.617	2.611
22(CS;4)	1.507	0.759	4.145
23(CS;7)	1.553	0.777	4.479
24(Me;21)	1.373	0.744	3.899
25(Ri;3)	1.308	0.657	2.915
26(PN;1)	1.264	0.697	3.304
27(SB;15)	1.355	0.734	3.763
28(Ch;15)	1.321	0.718	3.544
29(PN;15)	1.355	0.735	3.78
30(Pi;13)	1.395	0.71	3.452
31(Pi;8)	1.638	0.771	4.37
32(Ch;22)	1.358	0.736	3.788
33(Pi;22)	0.998	0.549	2.215
34(Ch;17)	0.981	0.592	2.453

Transect of 1,000 km across Chile

Varities indicated as: "Cabernet Sauvignon" (CS), "Carménère" (Ca), "Chardonnay" (Ch), "Malbec" (Ma), "Merlot" (Me), "Pedro Jimenez" (PJ), "Pinot noir" (PN), "Riesling" (Ri), "Sauvignon blanc" (SB), and "Pinot" (Pi). In parenthesis, the age (years) of each vineyard is indicated next to the abbreviation of each variety. Plots are represented from the northernmost (1) to the southernmost (34)

Table 3 Permutational analysis of variance (PERMANOVA) of the Bray–Curtis dissimilarities of the arbuscular mycorrhizal fungal community structure in relation to grape variety, crop maturity (age), and their interaction

Variety	1.543 (0.415)*
Maturity	1.083 (0.029)^{ns}
Variety X Maturity	0.738 (0.141)^{ns}
Residuals (R^2)	(0.409)

F value is given, R^2 in parenthesis, and p values as asterisks: F $(R^2)^p$, p: * < 0.05; *NS* Non-significant

was found. Specifically, community level adaptations in AMF associated with *Vitis vinifera* L. roots under several edaphoclimatic conditions could result in inoculation recommendation regarding horticultural production (Aguilera et al. 2022).

Although no significant differences were found when analyzing the grapevine cultivars or between the 3 zones, we can consider a general average of 30% root colonization for each zone. However, finding percentages over 70% in central zone suggests a high affinity between the fungi and the grapevine, their host, even across different grapevine cultivar. Additionally, it indicated a dependency on this crop to cope with the productive needs where mycorrhizal fungi could even attenuate stress conditions due to salts, presence of metals, nutritional deficiencies and even presence of diseases, where the AMF symbiosis could be a fundamental component for dealing with these adverse conditions.

Although in this study we did not find differences between the quantification of the propagules at intraradical level or between the extraradical mycelium, it was found that the symbiosis dependent on the grapevine cultivar. Other authors have investigated the levels of extraradical mycelium in cereal crops with conventional tillage management in southern Chile, reporting values of hyphal lengths of approximately 3 m per g soil (Aguilera et al. 2017). In this case, in vineyards the values were generally less than 0.53 m (Fig. 3d). Diverse studies conducted on grapevine crop have reported low diversity of these AMF associated with the rhizosphere, typically ranging from one to five AMF species. This has been correlated with soil chemistry, specifically at slightly acidic pH, of around 6.0 (Sas-Paszt et al. 2020).

In this context, a few studies from Iran, Brazil, Italy, Germany and United States, have explored the AMF communities, based on the spore bank of AMF associated to *V. vinifera* L. (Balestrini et al. 2010; Baumgartner et al. 2005; Betancur-Agudelo et al. 2021; Chen and Baumgartner 2004; Danesh et al. 2022; Oehl et al. 2005; Oehl and Koch 2018). In general, in these studies, the species richness in the AMF communities has been quite variable, ranging from 2 to 34 species. The species genera found on those studies are quite diverse, such as, *Acaulospora,*

6 species were found in these acidic soils from Southern Chile. This raises concerns about the necessary conditions that should be considered to promote the proper conservation of the diversity of these fungi in productive ecosystems. A similar situation was found in the central zone with a range between 3 to 6 AMF species. In the northern zone wider between 2 to 9 AMF species

Rhizophagus, Funneliformis, Glomus, Septoglomus, Claroideoglomus, Rhizoglomus, Scutellospora, Gigaspora, Paraglomus, Archaeospora, Ambispora, Entrophospora, Diversispora, Dominikia, Sclerocystis, Palaeospora. Studies that describe AMF communities based on the spore bank of the soil have shown the presence of variable AMF genera. This variability could be associated with the fact that different wine-growing regions grow different grapevine cultivars, and in the present study it was found the importance of grapevine cultivar in the structure of the community.

In contrast to the present study, Betancur-Agudelo et al. (2021) found that a young vineyard (10 years old) had a higher AMF species richness and spore abundance compared with an older vineyard (65 years old) of the same grapevine cultivar, while the opposite pattern was found for root colonization. Besides, here it was not possible to observe this pattern.

In addition, same authors indicate that the most common species in these Polish soils corresponded to *Claroideoglomus claroideum*. They also reported the presence of the genera, *Funneliformis, Gigaspora* and several *Rhizoglomus*.

Furthermore, Balestrini et al. (2010) focused their study on the AMF cohort in two Mediterranean vineyards in Piedmont-Italy, reporting the genera *Glomus, Acaulospora* and *Diversispora*. They concluded that the diversity of AMF species depends on soil conditions, more than the vegetative state or management practices. The most frequently found AMF species in this study, *Claroideoglomus claroideum*, has also been found in the rhizosphere of soils cultivated with "Solaris" and "Regent" grapevines in Polish soils (Sas-Paszt et al. 2020) as it was found here as well, with other species. They also reported the presence of the genera, *Funneliformis*, *Gigaspora* and several *Rhizoglomus*.

5 Conclusions

This study shows that there is an effect of grapevine cultivar on arbuscular mycorrhizal fungi (AMF) community structure. Nevertheless, geographic location, grapevine cultivar and grapevine age no affect AMF richness, AMF spores abundance, root colonization and Extraradical mycelium (ERC). In addition, soil chemistry had no effect on AMF community structure. On the other hand, our results suggest usefulness of exploring the current state of AMF diversity based on the identification of species from the visualization of spores as resistance propagules. To enhance the sustainability of viticulture management practices, it is recommended to carefully select AMF species for exogenous inoculation, prioritizing those that are most compatible with the existing community structure in relation with grapevine cultivar. However, more background will be needed to understand the role of specific AMF species that make up the core microbiota of grapevines grown with conventional management that should incorporate environmentally friendly management.

Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Supplementary Table 1. Analyses of variance (ANOVAs) showing the effects vine variety, grapevine age, and their interaction on arbuscular mycorrhizal fungi richness, spore abundance, root colonization, and extra-radical mycelium (ERC).

	Richness	Abundance	Colonization	ERC
Variety	0.337ns	0.702ns	1.681ns	0.871ns
Age	0.013ns	0.089ns	1.736ns	0.302ns
Variety X Age	0.412ns	1.001ns	1.141ns	1.083ns
	R^2: 0.295	R^2: 0.485	R^2: 0.639	R^2: 0.525

F value is given, with *p* values next: Fp, ns, non-significant.

Supplementary Table 2. Analyses of variance (ANOVAs) showing the effects vine variety, grapevine age, and their interaction on three diversity indices of arbuscular mycorrhizal fungi.

	Shannon	Simpson	Inverse Simpson
Variety	0.411ns	0.444ns	0.479ns
Age	0.205ns	0.152ns	0.042ns
Variety X Age	0.417ns	0.613ns	0.252ns

F value is given, with *p* values next: Fp, ns, non-significant.

Supplementary Figure 1. Non-metric multidimensional scaling (NMDS) of arbuscular mycorrhizal fungal (AMF) communities from soils of different vineyard varieties across Chile.