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Soil Traits and Grapevine Rootstock Genotypes Modulate Arbuscular Mycorrhizal Rate and Species in a Mediterranean Environment

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Abstract: The soil microbiota is a key component of agroecosystems, and understanding its traits is crucial for effective agronomic management. Among beneficial microorganisms, arbuscular mycorrhizal fungi (AMFs) are mutually associated with grapevine (*Vitis vinifera* L.), enhancing the ability of this cropping system to adapt to soil conditions and bolstering its resistance and resilience against abiotic stresses, particularly drought, by promoting root growth and enhancing the roots' absorption surface. The objective of this on-field study was to determine AMF species richness and diversity along with their relation to soil chemical, physical, and biological characteristics in two adjacent organic vineyards in Central Italy. The two tested vineyards of the autochthonous cv. Aleatico differed by the presence of grafted (*Vitis berlandieri* × *V. riparia* rootstock; AL-420) or own-rooted (ungrafted *V. vinifera* L.; AL-ORV) vines. To this aim, soil and root samples were collected and geo-referenced. Analysis of the AMF species colonizing roots of both AL-ORV and AL-420 revealed the presence of four species: *Scutellospora alterata*, *Paraglomus laccatum*, *Acaulospora laevis*, and *A. baetica*, with *S. alterata* being the most frequent. Mycorrhization parameters were higher in the roots of grafted plants compared to ungrafted ones. A high beta-glucosidase (BG):N-acetylglusosaminidase (NAG) ratio in two tested vineyards indicated that microbes utilized more cellulose than chitin and peptidoglycan as dominant C resources. A negative correlation between mycorrhization rate (MyCP) and BG was observed, likely because AMFs form mutualistic relationships with plants, depending on the host plant for carbon. Results revealed a positive correlation between the degree of mycorrhizal association and the species involved, with the presence of copper and nickel among metals. Negative correlations were found concerning soil clay content along with beta-glucosidase. In conclusion, the grapevine root system was characterized by a differential symbiotic relationship with AMF species, whose development is influenced by the root genotype, soil texture, and biochemistry. Specifically, the increased frequency of AMFs in relation to copper content strengthens the evidence of their role in maintaining a vine's production capacity in the event of soil contamination by this element.

Keywords: autochthonous grapevine varieties; adaptation strategies; organic farming; soil fertility; soil enzymes; soil metal contamination

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1. Introduction

It is widely recognized that the soil is a key resource for the health of ecosystems and humans. Increasing attention from researchers, institutions, and stakeholders highlights its role in ensuring food security and maintaining essential ecosystem services over time [1]. Maintaining soil health is crucial for preserving countless ecosystem services, meeting

growing food, water, and energy demands, and supporting associated economies. However, some agricultural practices, combined with climate emergencies, are degrading soil health. This degradation reduces land capability, soil resilience [2], and soil biodiversity, limiting crops' ability to adapt to climate change.

In recent years, the European Commission's (EC) "Farm to Fork" and "Biodiversity 2030" strategies have introduced a regulatory framework to create fair, healthy, and environmentally friendly food systems. This framework aims to prevent the adverse effects of intensive agriculture by promoting a circular economy, increasing organic farming, implementing integrated nutrient management plans, and improving pest management [3,4]. The restoration or maintenance of soil fertility using circular and low-impact agronomic techniques is considered fundamental for sustainability in agriculture. The "EU Soil Strategy 2030" outlines targeted measures for climate change mitigation, biodiversity preservation, desertification prevention, soil restoration, soil monitoring, and citizen engagement. Collectively, these initiatives aim to promote healthier soils [5]. Achieving these ambitious goals in agriculture requires adopting sustainable practices, with priorities including enhancing and conserving soil microbial biodiversity and overall biological fertility. The microbial community colonizing roots and soil affects soil properties, nutrient and water absorption, and resistance to harmful organisms [6]. Microbial activity serves as a reservoir for microorganisms that can spread to and inhabit the phyllosphere [7]. Therefore, maintaining soil fertility and microbial biodiversity is crucial for improving agroecosystem resilience and adaptation to climate change challenges [8–10]. Among cropping systems, grapevine is one of the leading agricultural productions in Italy and Europe, representing approximately 21% of the agricultural surface in Europe [11] and sustaining a highly active export sector. According to official data from the Italian National Institute of Statistics [12], Italy has over 680,000 hectares of productive vineyards, with a quarter under organic farming methods, according to the International Organisation of Vine and Wine [13]. Some regions significantly exceed this percentage.

Microbial activity plays a vital role in vine health and growth, influencing grape production and quality and contributing to the distinctive traits of terroirs and wines [14,15]. Beneficial microbial populations in soil could have a high biocontrol potential against grapevine pathogens [16]. Among beneficial microorganisms, arbuscular mycorrhizal fungi (AMFs) are commonly associated with most crops, including grapevine. AMF colonization in grapevine roots increases phosphorus and nitrogen absorption and improves both water uptake, allowing plants to cope with water stress and salt tolerance [17]. Moreover, AMF symbiosis enhances plant defensive capacity, known as "mycorrhiza-induced resistance" (MIR) [18,19], providing systemic protection against a wide range of pathogens, nematodes, and herbivorous arthropods [20–23]. Cultivated vines are usually grafted on American grapevine rootstocks (*V. berlandieri*, *V. riparia*, *V. rupestris*, or hybrids) tolerant to phylloxera aphids. In sandy or well-drained soils, ungrafted *V. vinifera* varieties can be cultivated since phylloxera cannot thrive or has a reduced impact on vine growth and health [24,25]. The choice of rootstock genotypes depends on specific pedoclimatic conditions, as they offer resistance to environmental and pest-related stresses while modulating vine vigor [26]. Additionally, rootstocks shape the distribution and diversity of rhizosphere microbial communities [8]. Variability in the root community of AMFs has been associated with distinct rootstock species and genetic variations among ungrafted plants [27,28].

Results from a previous study conducted in two organic vineyards in central Italy, which aimed to analyze variations in mycorrhizal and plant physiological characteristics, have shown that different soil types and root genotypes significantly influence AMF frequency [10]. The present research was therefore undertaken to assess (i) the AMF species present under organic farming in a volcanic grape-wine growing district, and (ii) the correlation among mycorrhizal species, mycorrhization parameters, and the biological and chemical characteristics of the vineyard soil.

The following highlights summarize the main conclusions: (i) in the Mediterranean basin, soil heterogeneity is confirmed to be a commune trait within the agrosystems; (ii) in-

dependently from the genotype, the grapevine root system was predominantly colonized by *Scutellospora alterata*; (iii) the mycorrhizal species identified were the first time recorded in a vineyard agrosystem; and (iv) mycorrhization was mainly affected by traits of soil texture and soil heavy metal content.

2. Materials and Methods

2.1. Study Site

The study was carried out in the 2022 growing season in two vineyards of the "Azienda Agricola Le Coste" in Northern Latium (Central Italy), located on the hill slopes of a volcanic lake, Lake Bolsena (42°36′00″ N, 11°56′00″ E). The two neighboring vineyards being studied are located within a Land unit, based on the zonation classification [29] characterized as having 'medium and low warmth exposure' on soil predominantly composed of ignimbrite, a volcanic material known for its inconsistent nature and various textures, such as ashes and lapilli. As indicated in previous research [30], the soil composition falls between the 'sandy-loam' or 'sandy' soil types, locally referred to as '*lapillo*'. The whole area experiences a Mediterranean climate with low annual precipitation, leading to severe drought during summer (from June to August). Moreover, the area's bioclimatic indices exhibit an increasing pattern in extreme heat events [31]. The two vineyards were selected because they offered an opportunity to compare grapevine–AMF interaction under identical microclimatic conditions. Located next to each other, these vineyards grow the same grapevine cultivar (cv. Aleatico) but differ in their root systems—one using grafted vines and the other using own-rooted vines. This region boasts a rich history of cultivating traditional grapevine cultivars, notably with the autochthonous 'Aleatico', an aromatic grape variety holding a PDO (Protected Designation of Origin), the 'Aleatico di Gradoli'. The two tested vineyards were planted in 2004 and trained using the traditional "alberello vine" system, with planting distances of 0.5 m × 1.5 m. The cv 'Aleatico' grapevines were either grafted onto *V. berlandieri* × *V. riparia* rootstock (AL-420 A), or ungrafted (referred to as own-rooted vines, AL-ORV) (Figure 1). Both vineyards, referred to as AL-ORV and AL-420 A, followed standard organic viticulture and regenerative soil management practices. They utilized roller crimpers, which mechanically roll over cover crops or weeds, crimping the stems and laying them flat on the ground. This process creates a mulch layer on the soil surface that suppresses weed growth by preventing weed seeds from germinating and blocking sunlight from reaching existing weeds. Roller crimpers are often used in no-till or reduced-tillage systems. Their respective soils were characterized by a medium-high organic matter content (2.49% and 2.63%, respectively) but significantly differed in the C/N ratio (4.7 and 7.27, respectively). Furthermore, the soil of the former vineyard exhibited a notably higher clay content (40%) and significantly lower levels of sand (9%) and silt (22%) compared to the latter one [10].

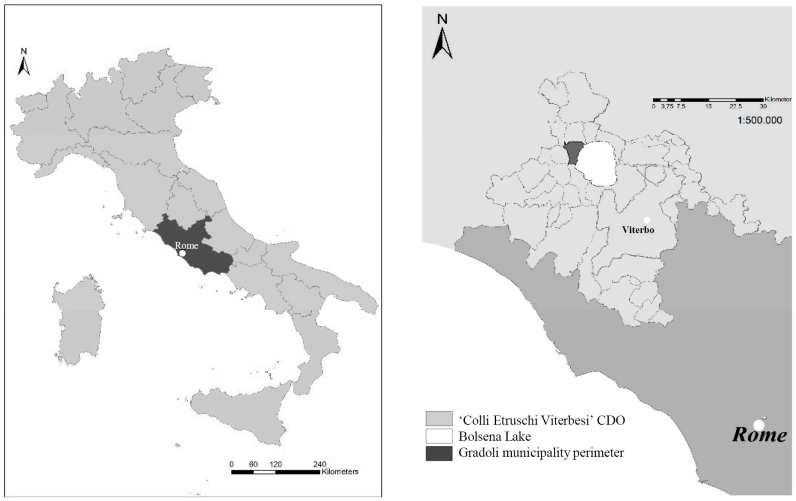

Figure 1. *Cont.*

Figure 1. Location of the two investigated vineyards in Central Italy (Gradoli Municipality): grapevine cv 'Aleatico' grafted onto *Vitis berlandieri* × *V. riparia* rootstock (AL-420A) and own-rooted (AL-ORV). Source of map: Google Earth.

2.2. Sampling Plan

This study is based on data collected throughout the 2022 growing season. Soil sampling was carried out in April 2022 when grapevines were at the growing shoot stage after bud break (phenological stage BBCH 19—ten visible leaves), when active root regeneration still occurs [32]. Grapevine rootlets and soil samples were collected at a depth of 20 cm (after removing the grass sward) and at a distance of 20 cm from the vine trunk, on 10 vines per vineyard [10]. Soil samples were collected using manual augers and then stored for use in the soil physical, chemical, and biological fertility assessment, as reported by Biasi et al. (2023) [10]. Grapevine young white roots belonging to the two different genotypes (*V. berlandieri* × *V. riparia* and *V. vinifera*) were separated from the soil by sieving and then directed to mycorrhization analysis *in planta*. Each soil and root sample was geo-referenced by GPS for data mapping and spatialization within the vineyards.

2.3. Isolation and Molecular Identification of AMFs from Grapevine Root System

Grapevine young white roots (420 A rootstock and *Vitis vinifera* roots) free of soil were washed several times with water, and 1 cm long roots were soaked in KOH (10%) for 5 min at 92 °C. Roots were then washed with distilled water, stained with 5% ink–vinegar solution for 5 min at 92 °C, and destained by rinsing in tap water [33]. Root fragments (30 replicates per vine) were mounted on slides with a drop of glycerol and observed under an optical microscope (Axioskop Zeiss, Oberkochen, Germany). AMF structure and mycorrhization rate were determined as reported by Biasi et al. (2023) [10].

Afterward, only AMF-colonized grapevine young white roots were ground with a mortar and pestle in liquid nitrogen. DNA was extracted from lyophilized samples using the Nucleospin Plant II kit according to the manufacturer's protocols (Macherey-Nagel, Düren, Germany). For DNA amplification, a two-step procedure (nested PCR) was performed [34]. The first step was conducted with the universal primers NS5 and ITS4 (Sigma-Aldrich, St. Louis, MI, USA) with an annealing temperature of 51 °C. Aliquots of 2 µL were run on an agarose gel to estimate the quantity of PCR products. The second step was performed using the products of the first PCR as a template with various combinations of Glomales-specific primers (GIGA 5.8, ARCH1311, ACAU1660, Sigma-Aldrich) and universal primers (ITS1F, ITS4, Sigma-Aldrich) with an annealing temperature of 61 °C for 5 cycles and then 60 °C for 25 cycles. PCR reactions were preheated to 61 °C during sample loading (hot start), and the reaction volume was 25 µL. Samples were purified using Macherey-Nagel's clean-up kit according to the manufacturer's instructions, and DNA was quantified with Qubit™ 1X ds DNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). BLAST analyses were conducted on the sequences to find the closest matches in GenBank.

2.4. Soil Physiochemical Characteristics and Quality Assessment

A set of soil physicochemical characteristics, including texture by particle size analysis, bulk density, acidity (pH), total organic carbon (TOC), and total nitrogen (N) parameters,

have been reported for the two vineyards under different rhizosphere conditions in Biasi et al. (2023) [10]. New soil determinations were conducted to estimate the nutrient potential of soils by analyzing both macro- and micronutrients, including available phosphorus (P), potassium (K), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), magnesium (Mg), calcium (Ca), and boron (B). Additionally, soil quality was assessed by measuring heavy metals and metalloids (HMs) such as cadmium (Cd), lead (Pb), chromium (Cr), arsenic (As), and nickel (Ni) (Table 1).

Table 1. Soil macro- and micromineral nutrients, heavy metals, and metalloids (HMs) assessed in sampled soils of the two studied vineyards under different rhizosphere (own-rooted—AL-ORV—and grafted—AL-420A). Units, standard range values, and methodological references are reported.

Mineral Elements		Range Values mg kg^{-1}	Reference
Nitrogen (%)	N	1–5	[35]
Phosphorus Olsen (mg kg^{-1})	P	20–60	[36]
Available Potassium (mg kg^{-1})	K	40–500	[36]
Copper (mg kg^{-1})	Cu	10–120	[36]
Zinc (mg kg^{-1})	Zn	10–150	[36]
Iron(mg kg^{-1})	Fe	50–150	[36]
Manganese (mg kg^{-1})	Mn	750–1000	[36]
Molybdenous (mg kg^{-1})	Mo	0.1–5	[36]
Calcium (mg kg^{-1})	Ca	10–250 (0.85–20 in non-calcareous soil)	[36]
Magnesium (mg kg^{-1})	Mg	Up to 400 (0.5–50 in non-calcareous soil)	[36]
Boron (mg kg^{-1})	B	5–30	[37]
HMs			
Cadmium (mg kg^{-1})	Cd	0.1–5	[36]
Lead (mg kg^{-1})	Pb	5–120	[36]
Chromium (mg kg^{-1})	Cr	10–150	[36]
Nichel (mg kg^{-1})	Ni	5–120	[36]
Arsenic (mg kg^{-1})	As	1–70	[38]

Soil samples were extracted using the Mehlich 3 method [39,40] and analyzed using a simultaneous plasma emission spectrophotometer (Plasma Optical Emission Spectroscopy (ICP-OES) ICP-OES Iris; Thermo Optek, Milano, Italy). Total phosphate (P_Olsen) was measured using the perchloric acid ($HClO_4$) digestion method [41]. The soil's water content at the wilting point (SWW) and field capacity (SWFC) were measured using a porous ceramic plate, where water from moist samples was extracted by increasing air pressure in the equipped extractor. The retention curve method was employed to determine SWW and SWFC [42]. Soil enzyme activity was evaluated by employing an extraction/desorption method [30], utilizing fluorescent analogs of individual enzyme substrates on microplates (Table 2).

Table 2. Set of enzymatic activities assessed in the sampled soils of the two vineyards under different rhizosphere (own-rooted—AL-ORV—and grafted—AL-420A). Methodological references are reported.

Enzyme	Biogeochemical Cycle	Substrate	Soil Function	Reference
Arylsulfatase (AS)	S	Organic S compounds	S source for plants, nutrient for microbial biomass	[43]
Leucine-aminopeptidase (LA)	N	amino acid residues	N source for plants, nutrient for microbial biomass	[44]
N-acetylglusosaminidase (NAG)	N	chitin, peptidoglycan		[45]
Beta-glucosidase (BG)	C	Cellobiose, cellotriose	C energy source for the growth and activity of soil microbes	[46]
Alkaline Phosphatase or Acid Phosphatase (AP)	P	Organic phosphorus	P source for plants, nutrient for microbial biomass	[43]

2.5. Data Statistical Analysis and Data Spatialization

Statistical analyses were performed using XLSTAT. One-way analysis of variance (ANOVA) and the Newman–Keuls method were used to identify significant differences (p-value < 0.05) among chemical, AMF, and enzymatic traits in the tested vineyards (AL-420A and AL-ORV). Two-way ANOVA was used to determine the effect of AMF species (the most common one) and rootstock species (*Vitis vinifera* L. for AL-ORV and *V. berlandieri* × *V. riparia* for AL-420A) and their interaction (AMF prevalent species x *Vitis* species) on soil quality indicators. These indicators included soil biological activity (enzymes), physical-chemical traits (sand, clay, silt contents, TOC, N, and C/N), hydrological parameters (soil moisture at field capacity and wilting point), essential nutrients (N, P, K), micromineral nutrients (Cu, Zn, Fe, Mn, Mo, Mg, Ca, B), soil heavy metals and metalloids (HMs: Cadmium, Cd; Crome, Cr; Lead, Pb; Nickel, Ni; Arsenic, As), mycorrhizal colonization rates (MyCP), and AMF structure. AMF structure was represented by frequency of mycorrhizal colonization (F—%), intensity of mycorrhizal colonization (M—%), and frequency of arbuscules (A—%) and vesicles (V—%) in mycorrhizal root fragments.

Pearson correlation analysis was used to explore the relationships between the new set of parameters, and the results were presented as a heatmap. The dataset was standardized before performing Principal Component Analysis (PCA) by subtracting the mean and dividing by the standard deviation for each variable's value. Multicollinearity was tested, and redundant variables were removed (N, Cd, Cr, Na). Statistical analysis identified soil parameters that better explain the interaction among soil physical and chemical traits, enzyme activity, micromineral nutrients, soil heavy metal content, mycorrhization potential, AMF species diversity, and structural traits. Five parameters were selected for their correlation with AMF species (mycorrhization rate and clay, beta-glucosidase, Cu, and Ni content) and mapped. Mapping was carried out using support vector machines combined with ordinary kriging [47] available in QGIS software (QGIS Development Team, 2024, QGIS version 3.32.3).

3. Results

3.1. Vineyards' Soil Traits and Data Spatialization

Soil micromineral nutrients and heavy metals in the tested vineyards were assessed, and mean values were determined. Generally, both vineyard types, hosting either AL-420A or AL-ORV, had medium concentrations of available nutrients (Table 3). The former displayed significantly higher availability of P (+69%), Cu (+64%), Ni (+100%), and Zn (+45%), whereas the latter showed higher K (+31%). Both vineyards were characterized by a

markedly low content of heavy metals, with the soil hosting AL-420 attaining a significantly higher concentration of Pb (+57%) compared to those hosting AL-ORV (Table 4).

Table 3. Mineral nutrient levels in soils of the two studied vineyards under different rhizosphere (own-rooted—AL-ORV—and grafted—AL-420A). Letters represent statistical significance for comparing vineyards ($p \leq 0.01$, and $p \leq 0.05$; ns—not significant).

Mineral Elements		AL-ORV		AL-420A	
Total Nitrogen (%)	N	0.5	ns	0.34	ns
Phosphorus Olsen (mg kg^{-1})	P	30.16	ns	24.56	ns
Available Phosphorus (mg kg^{-1})	P	27.10	b	65.65	a
Available Potassium (mg kg^{-1})	K	1495.87	a	1185.46	b
Copper (mg kg^{-1})	Cu	39.70	b	65.17	a
Zinc (mg kg^{-1})	Zn	13.97	b	20.32	a
Iron (mg kg^{-1})	Fe	179.12	ns	195.12	ns
Nickel (mg kg^{-1}))	Ni	0.06	b	0.12	a
Manganese (mg kg^{-1})	Mn	61.60	b	84.07	a
Molybdenous (mg kg^{-1})	Mo	0.44	ns	0.65	ns
Calcium (mg kg^{-1})	Ca	1040.82	ns	1142.57	ns
Magnesium (mg kg^{-1})	Mg	392.05	ns	367.69	ns
Boron (mg kg^{-1})	B	trace	-	trace	-

Table 4. Heavy metals in soils of the two studied vineyards under different rhizosphere (own-rooted—AL-ORV—and grafted—AL-420A). Letters represent statistical significance for comparing vineyards ($p \leq 0.01$, and $p \leq 0.05$; ns—not significant).

Heavy Metals		AL-ORV (mg kg^{-1})		AL-420A (mg kg^{-1})	
Cadmium	Cd	0.05	ns	0.06	ns
Lead	Pb	6.19	b	9.73	a
Chromium	Cr	0.07	a	0.0002	b
Nickel	Ni	0.06	b	0.12	a
Arsenic	As	trace	-	trace	-

In both vineyards, appreciable amounts of NAG, BG, and AP enzymatic activity were detected (Table 5). A significantly higher content of NAG and BG was recorded in the AL-ORV soil, with values 25% and 30% higher, respectively, than those in the soil hosting AL-420A. The BG:NAG ratio varied significantly from 5.8 for the AL-420A to 5.4 for the AL-ORV soil root zone.

Table 5. Enzymatic activity in soils of the two studied vineyards under different rhizosphere (own-rooted—AL-ORV—and grafted—AL-420A). Letters represent statistical significance for comparing vineyards ($p \leq 0.01$, and $p \leq 0.05$; ns—not significant).

Enzymatic Activity		AL-ORV μmol g^{-1} h^{-1}		AL-420A μmol g^{-1} h^{-1}	
Arylsulfatase	AS	0.11	ns	0.13	ns
Leucine-aminopeptidase	LA	0.04	ns	0.04	ns
N-acetylglusosaminidase	NAG	0.35	a	0.27	b
Beta-glucosidase	BG	1.92	a	1.54	b
Alkaline Phosphatase or Acid Phosphatase	AP	1.08	ns	0.98	ns

A previous comparison of the soil chemical and physical characteristics between the two vineyards under investigation [10] revealed significant differences in the average composition of their topsoil. Specifically, differences were observed in sand, loam, and clay content, as well as in their C/N ratio and water content at field capacity (SWFC%).

The present study reports the spatial distribution of values related to soil texture and bulk density that is functional for correlating AMF typology with rhizosphere characteristics (Figure 2).

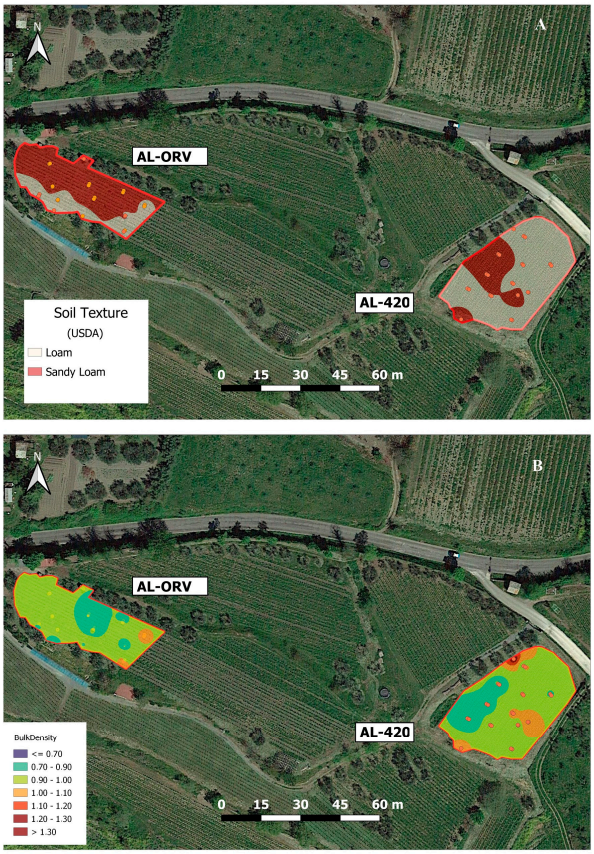

Figure 2. Spatialization of soil texture and soil bulk density in the two vineyards. Soil texture (**A**) and soil bulk density (**B**). Dots represent the sites of soil and root sampling (10 replications per vineyard). cv 'Aleatico' grafted onto *Vitis berlandieri* × *V. riparia* rootstock, AL-420A; cv 'Aleatico' own-rooted, AL-ORV. Source of map and data: Google Earth.

3.2. AMF Identification

The microscopic investigation applied to the grapevine fine root samples allowed the detection of mycorrhizal structures in both grafted and ungrafted vines. Root colonization of the vines varied from 61.7% to 99.3% (Table S1), with a significant difference in the mycorrhization rate (MyCP—%) between AL-ORV and AL-420A, being generally higher on rootstock. Morphological and molecular analysis (Figure 3) revealed that the endomycorrhizal representative species in the two vineyards belong to the Acaulosporaceae family (*Acaulospora laevis* and *Acaulospora baetica*), the Gigasporaceae family (*Scutellospora alterata*), and the Paraglomeraceae family (*Paraglomus laccatum*) (Table S1). *S. alterata* (Gene bank accession PQ101123) and *P. laccatum* (Gene bank accession PQ106507) were detected in 70% and 20% of the analyzed grafted and ungrafted grapevine roots, respectively. *A. baetica* (Gene bank accession PQ106255) was found in one sample of ungrafted roots (AL-ORV), whereas *A. laevis* (Gene bank accession PQ104883) was found in one sample of grafted roots (AL-420A).

Independently from the genotype, the grapevine root system was predominantly colonized by *Scutellospora alterata*.

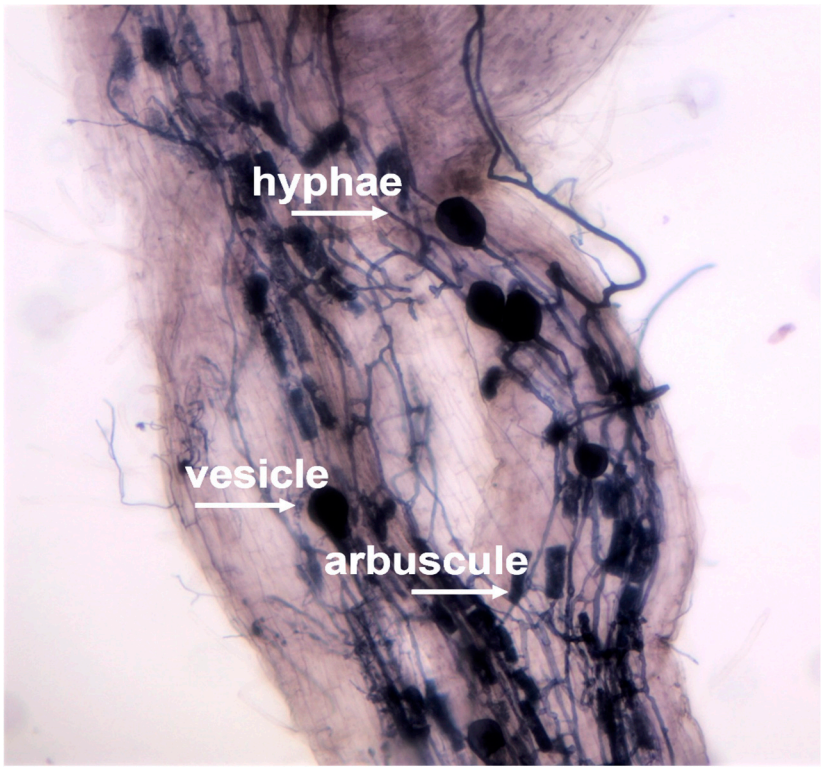

Figure 3. Representative mycorrhizal structures of *Scutellospora alterata* AMF, the most frequent AMF in the study site. The arrows show the typical morphology of the fungi, based on hyphae, vesicles, and arbuscules.

3.3. Correlation Among Soil Traits and AMF Specificities

The heat map of Pearson's correlation coefficients is shown in Figure 4, for selected variables. Clay content positively correlated with soil moisture at field capacity (+0.58) and negatively correlated with AMF species (−0.76) and mycorrhization rate (−0.52). Clay content also had a positive correlation (0.45) with BG, which showed a negative relationship with AMF species (−0.56) and the C/N ratio (−0.50). BG was positively correlated with AP (0.61) and NAG (0.90), and negatively correlated with MyCP (−0.66), A (−0.74), and M (−0.72).

Among micromineral nutrients important for plant growth and soil heavy metal content, Ni, Pb, and Cu exhibited significant negative correlations with clay (−0.62, −0.70, and −0.70, respectively), BG (−0.57, −0.58, and −0.66, respectively), and NAG, while showing positive correlations with clay, AMF species (0.7, 0.9, and 0.8, respectively), and particularly with relative arbuscular richness (A-%) (0.7, 0.54, and 0.68, respectively). Additionally, Pb and Cu were negatively correlated with NAG (−0.54 and −0.55, respectively) and positively correlated with the intensity of mycorrhizal root colonization (M—%) (0.54 and 0.49, respectively). Correlations among all the tested variables are reported in Figure S1.

The AMF frequency, i.e., the mycorrhization rate, was highly related to soil traits and root genotypes (Table S1 and Table 6). The AMF species exhibited a significant association with clay content, AP activity, and Cu, Ni, and Pb concentrations (Table 6). Hydrological soil traits, such as soil moisture at field capacity, showed a significant relationship with the interaction between AMF and *Vitis* species. Additionally, (MyCP) and arbuscule (A) frequency were found to be associated with AMF species.

Figure 5 shows the most correlated soil variables to the mycorrhization rate. The Ordinary Kriging (OK) plugin was used to map soil properties and analyze and interpret their spatial variation. The spatialization of specific soil variables (Figure 5) exhibited abrupt variations, offering crucial categorical insights into interpreting the diversity of AMF species and frequency based on soil physiochemical characteristics. The mycorrhization rate (MyCP) was inversely correlated to clay content and BG activity. In particular, a gradient

for BG activities was found extending from the perimeters of the vineyards towards their central areas. The decrease in BG activities corresponded to an increase in Cu and Ni content, while simultaneously correlating with an increase in mycorrhization rates. In AL-ORV, where *Acaulospora baetica* was detected, the lowest level of clay and the highest concentrations of Cu and Ni were recorded. Conversely, in AL-420A, *Acaulospora laevis* was detected in similar clay content but with more moderate concentrations of Cu and Ni and activity of the BG enzyme.

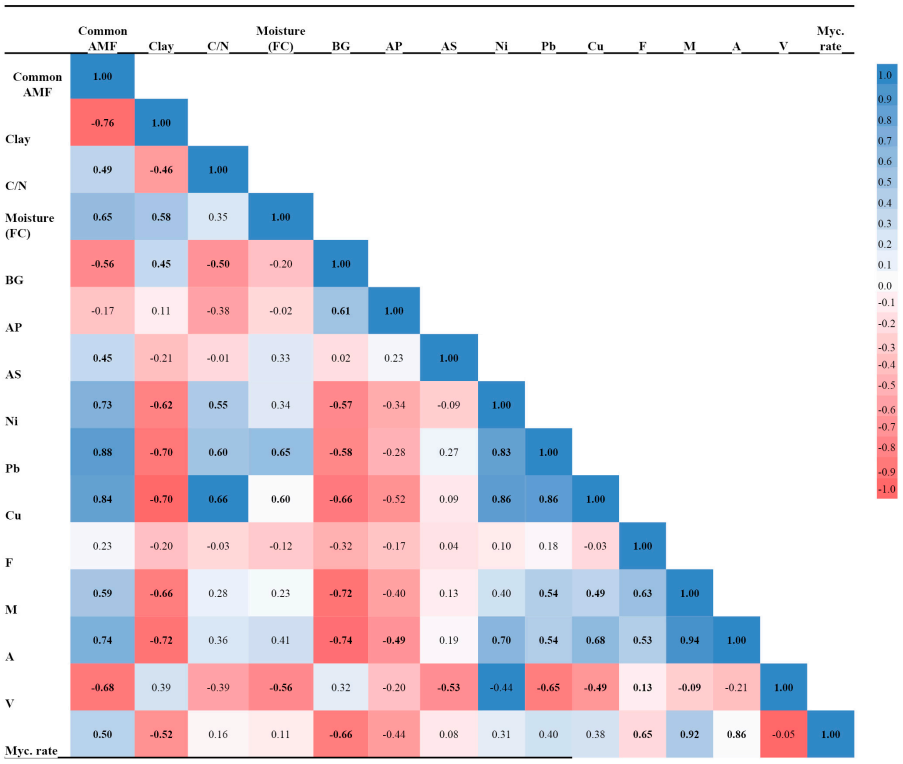

Figure 4. Heatmap of Pearson correlations among selected soil physical-chemical properties, soil enzymatic activity, hydrological parameters, and AMF presence and frequency (mycorrhization rate) in the rhizosphere of the tested vineyards. Abbreviations: arbuscular mycorrhizal fungi-AMF; clay%-Clay; ratio between carbon and nitrogen-C/N; moisture% at field capacity-FC; soil enzymes (μmol g^{-1} h^{-1}): beta-glucosidase-BG; alkaline phosphatase or acid phosphatase-AP; arylsulfatase-AS; N-acetylglusosaminidase-NAG; heavy metals (mg kg^{-1}): nickel-Ni; lead-Pb; copper-Cu); mycorrhization traits and rate (%): frequency of mycorrhizal colonization-F; intensity of mycorrhizal colonization-M; frequency of arbuscules-A and vesicles-V; mycorrhizal colonization-Myc rate or MyCP. Data refer to soil and rootlets samples in the vineyard with grafted vines of cv 'Aleatico' (AL-420A) and in the vineyard with own-rooted vines (AL-ORV).

Table 6. Results of ANOVA showing the statistics and the significance of the effect of soil properties on AMF species. Abbreviations as in Figure 4. Asterisks represent statistical significance for comparing vineyards (** $p \leq 0.01$, and * $p \leq 0.05$).

	AMF Species		
VARIABLES	**F**	**ProbF**	**Sign.**
Clay	6.01	0.04	*
AP	8.44	0.02	*
Mycorrhization rate	7.90	0.02	*
Cu	7.50	0.02	*
Ni	23.66	0.00	**
Pb	11.84	0.01	**

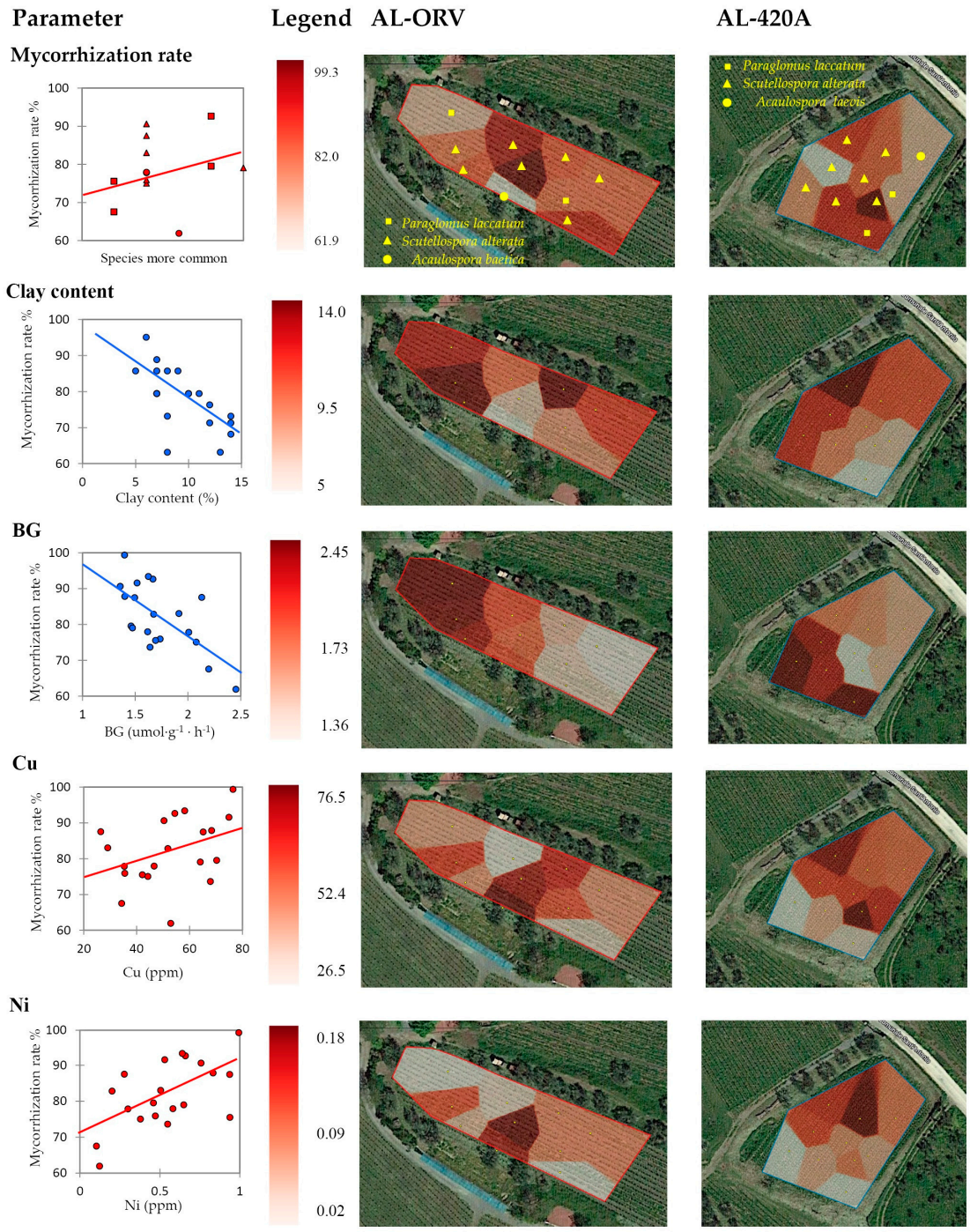

Figure 5. Correlation among mycorrhization rate (MyCP- %) of the more common species of AMF in the vineyards, and selected soil properties (clay content-%), enzymes (beta-glucosidase-BG.µmol g^{-1} h^{-1}) and heavy metals (copper-Cu, and nickel-Ni-mg kg^{-1}) (**left**). The spatial distribution of the AMF species and variation of each independent variable for the two tested vineyards (own-rooted-AL-ORV, and grafted-AL-420A) are reported in the images (**right**). Spatialization was performed using the Ordinary Kriging (OK) plugin.

The result of PCA showed that the first two PCs explain 68.2% of the total variance. PCA revealed the correlations of the main components with each variable, facilitating the interpretation of the newly generated variables. According to the factor loadings, the first PC, which explains 41.2% of the total variance, has higher positive correlations with AMF species and their traits (A, M, MyCP), clay content, TOC, C/N ratio, hydrological

parameters, BG and AP activities, and all soil chemical microelements (excluding P Olsen and Mg). In contrast, the second PC, which explains 17.8% of the total variance, is strongly correlated with sand and silt content, AS activities, AMF traits (F and V), and P Olsen

The PCA biplot in Figure 6 shows both the PC scores and the loadings of variables. PCA resulted in a clear separation between ten soil samples for each vineyard that clustered in two different quadrants: quadrant II for AL-ORV (grey circles) and quadrant IV for AL-420A (black triangles). The traits grouped in the same quadrant of AL-ORV were strongly associated with it: sand and clay contents (53.1%, 11.5%), the bioavailability of inorganic ortho-phosphate (P Olsen) and potassium (K), and the frequency in vesicles (%). The AL-420A group consists of soils rich in TOC, available P, soil microelements such as Zn, Cu, Ni, and Pb, higher volumetric soil moisture at field capacity, and mycorrhization rate (MyCP).

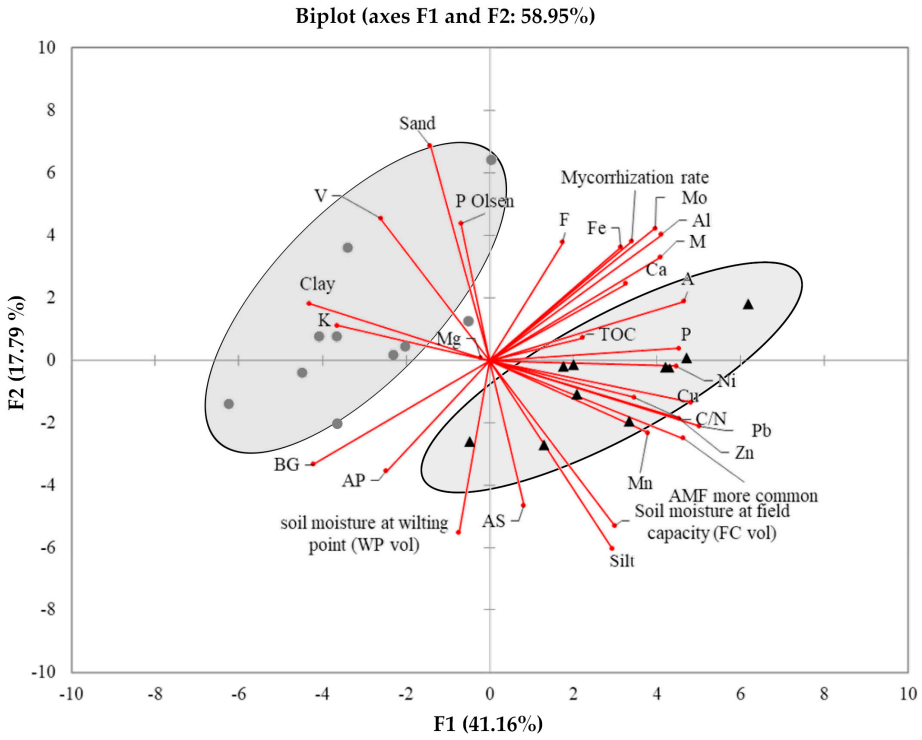

Figure 6. Principal components analysis (PCA) performed using soil physical-chemical properties, soil enzymatic activity, hydrological parameters, and AMF morphology and potential in the rhizosphere of the two studied vineyards. Samples from grafted cv 'Aleatico' (AL-420A), black triangles; samples from own-rooted cv 'Aleatico' (AL-ORV), grey circles. AMFs more common in vineyards (%); clay, silt, and sand content (%); ratio between carbon and nitrogen-C/N; total organic carbon-TOC (%); soil moisture (vol.) at field capacity-FC and at wilting point-WP; soil enzymes (μmol g^{-1} h^{-1}): beta-glucosidase-BG; alkaline phosphatase or acid phosphatase-AP; arylsulfatase-AS; mineral elements (% or ppm): nitrogen-N; phosphorus Olsen-P Olsen; available Potassium-K; zinc-Zn; iron-Fe; manganese-Mn; molybdenous-Mo; calcium-Ca; magnesium-Mg; boron-B; heavy metals (mg kg^{-1}): cadmium-Cd; nickel-Ni; lead-Pb; copper-Cu; chromium-Cr; arsenic-As; mycorrhization traits and rate (%): frequency of mycorrhizal colonization-F; intensity of mycorrhizal colonization-M; frequency of arbuscules-A, and vesicles-V; mycorrhizal colonization-Mycorrization rate or MyCP.

4. Discussion

Agrobiodiversity is essential for sustainable viticulture, as it encompasses not only species directly related to food production but also all biological systems such as soil microbiota. It spans multiple scales, from ecosystems to species and genes, and faces significant threats of depletion at every level. Understanding the nature and features of some agrobiodiversity components, such as AMFs, represents a strategy to preserve

this precious environmental heritage.The initial stages of this research aimed to study the interactions between AMFs and grapevine roots in different soil conditions. The goal was to evaluate the mycorrhizal potential of the soil in an organic vineyard across two plots: one with grafted vines and one with own-rooted vines. Overall, the findings confirm that organically managed vineyards, identified as having a high fertility level, exhibit substantial mycorrhizal potential [10].

Previous research has shown that rootstock species and environmental conditions influence the distribution and composition of soil microbial communities, including arbuscular mycorrhizal fungi (AMFs) [8].

Variations in the AMF root community may be linked to different rootstock species and the genotype of the ungrafted plant [27]. Implementing our research on the grapevine–AMF relationship involved assessing the colonization of vines by AMFs in georeferenced samples, identifying the most represented species, and correlating the level of symbiosis with soil chemical and physical parameters. The observations in tested vineyards confirm that mycorrhization parameters are higher in the roots of grafted plants (i.e., on American grapevine species) compared to ungrafted ones (i.e., on European grapevine species) [10]. Specifically, the AL-420 grafted vines exhibited significantly greater colonization by AMFs than the AL-ORV ungrafted plants. This variation can be attributed to the capability of different plant species within the same *genus* to recruit beneficial microorganisms involved in the rhizosphere microbiota composition. These findings corroborate previous observations indicating that environmental and genetic components of the vineyards influence the colonization rate and composition of AMFs [48–52]. These findings, therefore, could support the hypothesis of the existence of a *situ*-specific selection of AMFs depending on the local conditions.

An analysis of the AMF species colonizing roots in the soils of AL-ORV and AL-420 revealed the presence of four species: *Scutellospora alterata*, *Paraglomus laccatum*, *Acaulospora laevis*, and *A. baetica*. Among these, *S. alterata* was the most frequently found in the root samples from both georeferenced vineyard plots. *Paraglomus laccatum* was identified in a few samples from both plots, while *A. laevis* and *A. baetica* were detected in one case in AL-ORV and AL-420, respectively. *Scutellospora alterata* (Gigasporales, Glomeromycota) was recently isolated as a new species from soils of the semi-arid Caatinga biome in northeast Brazil [53], *Paraglomus laccatum* (Blaszk.) Renker, Blaszk & Buscot (Syn. *Glomus laccatum*) has been associated with AMF colonizing the roots of grass species and other wild plants in Poland [54]. *Acaulospora* spp. (class Glomeromycetes) is a widespread genus considered stress tolerators [55,56], with several species recently described from colder and tropical environments [57] in acidic and salt soils [58,59], concurring to increase the effectiveness in P uptake by enhancing the secretion of acid phosphatase in the mycorrhizal roots [60]. *Acaulospora baetica* is recently described as a new species found around the roots of endangered and/or endemic plants in the Sierra Nevada and Sierra de Baza (Andalucía, southern Spain) [61]. To our knowledge, this is the first report on the colonization of these mycorrhizal species in grapevine. The rate of mycorrhizal species colonization is influenced by the interaction of multiple factors related to microclimate, soil characteristics, and the host plant. We have previously reported that the ungrafted vines (AL-ORV) showed better leaf resilience traits such as higher average chlorophyll (CHL) content throughout the growing season, stomatal conductance (gs), and a higher average of AMF storage organs (namely vesicles), while the grafted vines (AL-420A), more sensitive to climate conditions and, therefore, exhibiting lower gs and CHL content, presented higher AMF frequency, which was likely linked to the need of improved uptake and transport of water from the bulk soil to the vine [8,62]. The rate of mycorrhization (MyCP) negatively correlated with the observed clay content. Both sampling sites featured sandy soil of volcanic origin and inherently low clay content. Several studies have emphasized the significant role played by clay, along with organic matter and glomalin produced by AMFs, in shaping soil structure by fostering the creation of aggregates [63]. In both vineyards, we observed a high content of organic matter in the soils, which likely acted as a key factor in influencing mycorrhizal

colonization. A C/N ratio lower than 9, particularly for AL-ORV, indicates soil with a low N content and higher N-mineralization rates [64,65].

The results from this study reveal a correlation between the degree of mycorrhizal root symbiosis and the species involved, with certain chemical and physical characteristics of the soil and its functionality (enzyme activities). The BG:NAG ratio is considered a good indicator of resource allocation of soil microbes to acquire energy and nutrients and gives information on the source of the C resources (substrate) for microbes [66,67]. A high BG:NAG ratio in the two tested vineyards indicated that microbes utilized more cellulose than chitin and peptidoglycan (the targeted compounds by NAG) as dominant C resources, showing a higher BG activity and a higher BG:NAG ratio. In addition, BG was used as an enzyme for the early indication of changes in organic matter status and its turnover [68]. The major activity (25%) of BG in AL-ORV leads to a greater C resource for microbes and AMFs that improves the life cycle of AMFs and leads to the final stage of formation of nutrient storage vesicles and daughter spores after successful arbuscular formation enables carbon uptake in exchange for mineral nutrients delivered by the fungus [69].

We found a negative correlation between MyCP and BG; this should not be a surprise if we consider that AM fungi are plant mutualists that rely on the plant host for carbon, and they typically do not produce enzymes for carbon degradation and acquisition [70]. In addition, they are commonly viewed as crucial for soil nutrient absorption, in particular for improving P uptake of the host plant [71].

Our findings showed no relationship between AM fungal colonization rate and AP, related to the P geobiochemical cycle, and negative ones with BG and NAG, respectively related to the cycle of C and N, respectively. Their activity serves as an index of soil microbial activity and quality [72]. In particular, AL-ORV showed low soil Olsen-P levels for native AM fungal colonization while AL-420A showed medium-high levels. Deng et al. [73] showed that AM fungal colonization in wheat and maize linearly declined as soil P increased until critical levels of soil Olsen-P; once reached this level, a linear-plateau relationship was obtained between AM fungal colonization and soil Olsen-P and only small increases were seen for AM mycorrhization rate. Nevertheless, the symbiotic relationship between the plant and AMFs is hindered or inhibited when the available P level is elevated [74]. AMFs enhanced soil enzyme activity optimally at smaller soil available P [71], in fact, on AL-420A under low level of soil Olsen-P, the enzymatic activities of BG and NAG were increased by 30% and 25% compared to grafted vines.

Finally, our findings also showed the possible relationship between AMFs and heavy metals in soils. Copper (Cu) has been widely employed as a fungicide, notably in vineyards, to prevent infection by downy mildew (*Plasmopara viticola*) and other pathogens, under organic farming. Cu is a significant micronutrient for plants because they tend to uptake it and store the surplus within their tissues. However, the extensive use of copper has led to its accumulation in vineyard soils and groundwater, posing a risk to the functionality of soil microbiota and vine health [75]. In Europe, compared to the average soil Cu concentration of 16.85 mg/kg, vineyards have the highest mean soil Cu concentration among all land use categories, at 49.26 mg/kg [76]; this is probably also because of the high incidence of organic farming in viticulture. This result proves that while the Cu concentration in the soil hosting the ungrafted plants is in line with the European average, it is significantly higher in the soil for the grafted plants and that mycorrhization frequency had a positive correlation with the Cu and Ni content of the soil. It has been reported that in vineyards with long-term foliar application of Cu-based fungicides, some AMF species have developed strategies to tolerate high soil Cu levels, including releasing organic substances, such as glomalin, which can form complexes with Cu, thereby restricting its bioavailability [75]. Furthermore, AMFs can compartmentalize and store Cu, reducing its toxic effects on plant metabolism and benefiting both symbionts [77]. Therefore, the present results show that measures promoting soil fertility and vine mycorrhization increase and potentially enhance Cu tolerance by plants in contaminated soils [78].

5. Conclusions

Organic viticulture guarantees sustainability in one of the most connotative and iconic Mediterranean landscapes. The vineyard agro-system represents one of the most environmentally friendly land uses, where grape and wine quality meets the quality of the environment and the landscape, which melds into the *terroir* concept. Organic farming is pivotal in preserving soil's global fertility, mainly its biological fertility. In this context, the grapevine root system forms symbiotic relationships with certain mycorrhizal species, whose development is influenced by root genotype and soil texture and biology. Each environment selects its own soil microbiota and microbiota frequency. The higher frequency of mycorrhizae in relation to recorded copper content underscores their potential role in maintaining the productive capacity of vines in contaminated soil. To verify the adaptive capacity to copper contamination of different rootstocks colonized by these mycorrhizae, further studies under controlled conditions are necessary.

From a practical standpoint, understanding the specificity of local arbuscular mycorrhizal fungi presents an opportunity for nurseries to produce mycorrhized plantlets with species adapted to the soil into which they will be transplanted. This provides winegrowers with propagation material that is in symbiosis with native mycorrhizal species, offering durability and valuable ecosystem services.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture14081425/s1: Table S1. Mycorrhization rate and commune mycorrhizal species sampled from own-rooted (AL-ORV) and grafted (AL-420A) grapevine roots; Figure S1. Heatmap of Pearson correlations among all the soil physical-chemical properties, soil enzymatic activity and hydrological parameters, and AMF presence and frequency (mycorrhization rate) in the rhizosphere of the tested vineyards. Abbreviations as in Figures 4–6.

Author Contributions: R.B. and G.C. conceptualization; R.B., G.C. and E.B. methodology design; A.C., E.B. and S.V. sampling; S.V., M.M. and R.F. soil determination; E.B. and A.B. data analysis; A.C. and G.C. mycorrhizal species identification; R.B., G.C. and E.B. manuscript writing; R.B. funding responsibility. All authors have read and agreed to the published version of the manuscript.

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