

## Article

# Microbial Inoculation Differentially Affected the Performance of Field-Grown Young Monastrell Grapevines Under Semiarid Conditions, Depending on the Rootstock

Pascual Romero <sup>1,\*</sup>, Pablo Botía <sup>1</sup> , Elisa I. Morote <sup>1</sup>, Asunción Morte <sup>2</sup>  and Josefa M. Navarro <sup>1</sup> 

<sup>1</sup> Irrigation and Stress Physiology Group, Instituto Murciano de Investigación y Desarrollo Agroalimentario y Medioambiental (IMIDA), C/Mayor s/n, 30150 Murcia, Spain; pablo.botia@carm.es (P.B.); elisai.morote@carm.es (E.I.M.)

<sup>2</sup> Departamento de Biología Vegetal (Botánica), Facultad de Biología, Campus de Espinardo, Universidad de Murcia, 30100 Murcia, Spain; amorte@um.es

\* Correspondence: pascual.romero@carm.es

## Abstract

A trial was conducted from 2017 to 2023 in a 0.2 ha irrigated vineyard located in a semiarid area of southeastern Spain, using field-grown young vines (0–6 years old) of *Vitis vinifera* L. cv. Monastrell grafted onto three rootstocks: 140Ru, 161-49C, and 110R. The main objective was to evaluate the effect of early co-inoculation in the field using commercial microbial inoculants containing arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and a mycorrhizal helper bacterium (MHB) on young vine performance. We assessed the impact of microbial inoculation and its interaction with the rootstock on soil environment, plant water relations, leaf gas exchange, plant nutrition, growth, yield, and berry quality. Mycorrhizal colonization rates in root samples showed similar values in inoculated and non-inoculated vines across all of the rootstocks; however, inoculated vines grafted onto 140Ru showed significantly higher concentrations of total glomalin in the soil compared to their non-inoculated counterparts. Microbial inoculation altered the soil environment, leading to increased oxygen diffusion rate (161-49C), organic matter decomposition rate (140Ru), soil CO<sub>2</sub> flux (110R, 140Ru), and soil H<sub>2</sub>O flux (110R) values in the rhizosphere of inoculated vines. Additionally, inoculated vines grafted onto 140Ru and 161-49C exhibited improved vegetative and reproductive development, enhancing productive water use efficiency (WUE<sub>yield</sub>), whereas inoculated vines on 110R showed poorer soil–plant water relations, growth, yield, and WUE<sub>yield</sub> compared to non-inoculated vines. Microbial inoculation also led to a significant decrease in must phenolic content, particularly in 140Ru, unlike 110R and 161-49C. These findings indicate that early microbial inoculation had a rootstock-dependent impact on the performance of young grapevines.

**Keywords:** early AMF/bacteria inoculation; leaf gas exchange; plant nutrition; grape quality; water use efficiency; yield response



Academic Editors: Loredana Scalschi, Ana Isabel González-Hernández and Eugenio Llorens

Received: 23 September 2025

Revised: 4 November 2025

Accepted: 6 November 2025

Published: 7 November 2025

**Citation:** Romero, P.; Botía, P.; Morote, E.I.; Morte, A.; Navarro, J.M. Microbial Inoculation Differentially Affected the Performance of Field-Grown Young Monastrell Grapevines Under Semiarid Conditions, Depending on the Rootstock. *Agronomy* **2025**, *15*, 2570. <https://doi.org/10.3390/agronomy15112570>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Plant–microbe interactions in the rhizosphere are crucial for plant health, productivity, and soil fertility [1,2]. Among these microbes, arbuscular mycorrhizal fungi (AMF), from the subphylum *Glomeromycotina*, form spontaneous endomycorrhizal symbioses with grapevine roots. AMF are commonly found in commercial vineyards and have an influence on the surrounding microbial community, forming the mycorrhizosphere. This symbiosis

supports grapevine establishment, growth, photosynthesis, gas exchange, nutrient uptake, drought tolerance, and grape quality [2–6]. These benefits are linked to several mechanisms: (a) improved root water uptake through extended soil exploration by extra-radicular hyphae; (b) enhanced mineral nutrition, especially phosphorus and other nutrients, via both root extension and direct uptake by hyphae; (c) changes in root architecture and anatomy; (d) modulation of physiological processes and secondary metabolite production, including phenolics and stilbenes, as well as increased antioxidant enzyme activity [5,7]; and (e) induction of stress-related hormones, such as ABA, which mediate some plant responses to different stresses [8,9]. AMF hyphae contribute to enhancing soil structure by binding particles and producing glomalin, a stable glycoprotein that enhances soil aggregation and organic matter stabilization [10]. Additionally, AMF can trigger mycorrhiza-induced resistance, improving tolerance to biotic and abiotic stress [6,7]. Recent findings indicate that mycorrhization may impact stomatal anatomy under water-deficit conditions, potentially regulating gene expression related to leaf structure [11].

Under natural conditions, the mycorrhizal complex encompasses not only the plant and symbiotic fungi but also the associated microorganisms, with bacteria—especially rhizobacteria—being the most abundant [12]. These include plant growth-promoting rhizobacteria (PGPR) and mycorrhizal helper bacteria (MHB). PGPR constitute a diverse group found in the rhizosphere, on root surfaces, or in association with roots. Genera such as *Pseudomonas*, *Azospirillum*, *Rhizobium*, and *Bacillus* have been extensively studied for their roles in: (1) enhancing plant nutrition through nitrogen fixation, phosphorus solubilization, and mineral mobilization; (2) supporting plant defense via induced systemic resistance and production of antimicrobial compounds; and (3) modulating hormonal balance and stress responses through phytohormones and stress-related enzymes [1,13,14]. Synergistic interactions among PGPR strains can further enhance these effects [14]. Conversely, MHB contribute to mycorrhizal development by promoting hyphal growth, spore germination, and root colonization [15]. Recent studies indicate that specific MHB strains can alleviate drought stress in *Helianthemum almeriense* colonized by AMF by influencing water relations and hormone levels [12].

Although grapevine is a highly mycotrophic plant, intensive agricultural practices, such as fertilization, tillage, and pesticide use, have reduced soil health and diminished the abundance and effectiveness of AMF and beneficial bacteria [3,6]. The introduction of selected microbial inoculants, particularly when paired with sustainable soil management, has the potential to enhance plant nutrition and survival [16]. These inoculants may also contribute to reducing production costs and minimizing environmental impact by decreasing reliance on chemical fertilizers [1].

Despite evidence of the beneficial roles of AMF and PGPR in grapevine production, their use in conventional and organic viticulture is limited due to concerns about efficiency, costs, and necessity. While controlled experiments often report positive effects on grapevine growth and physiology [6,17], field trials yield inconsistent results. The success of bacterial inoculants depends on factors such as root exudate composition, colonization efficiency, and soil health [1]. AMF performance is affected by a range of environmental and biological variables, including soil pH, nutrient content, moisture, temperature, organic matter, tillage practices, irrigation regime, fungal activity, and host plant identity [3,18–21]. In particular, rootstock genotype and its compatibility with fungal species play a decisive role in establishing effective symbiosis with AMF and in shaping the composition of root-associated microbial communities. Research has shown that although different AMF inocula can colonize a range of grapevine rootstocks, the extent of colonization and its impact on plant performance are strongly influenced by the rootstock genotype [22]. Host identity also affects the structure of AMF and bacterial communities in both the rhizosphere and root

endosphere, with variations in root exudates among grapevine rootstocks being a significant factor driving microbial community dynamics [23,24]. Consequently, grapevines tend to recruit specific fungal and bacterial communities that modulate microbiome function, plant growth, and adaptation to environmental conditions. For example, mycorrhizal inoculation has been reported to promote *Pseudomonas* in 1103P and *Bacillus* in SO<sub>4</sub>; these two genera are recognized for their protective roles against fungal pathogens and soil nematodes [25–27]. Furthermore, the interaction between rootstock genotype and AMF plus PGPB inoculum is crucial for grapevine adaptation to water deficit, both under current and future climate scenarios [11]. Nevertheless, not all of the microbial strains—even within the same species—produce consistent results in the field, underscoring the importance of careful selection. To maximize the benefits of microbial inoculants, a site-specific evaluation considering soil fertility, management practices, irrigation regime, native microbial communities, and grapevine genotype compatibility is essential before application [23,28].

Early field inoculation can promote the development of beneficial interactions between grapevine roots and soil microbes, partly due to reduced microbial competition in young plants [7,9]. For instance, Camprubí et al. [16] showed that early AMF inoculation improved vine establishment and survival in 110R by enhancing plant fitness. Similarly, Torres et al. [22] found that AMF inoculation in two-year-old Merlot vines enhanced vegetative growth, water status, and photosynthesis under deficit irrigation. Rolli et al. [13] also reported that PGPB inoculation increased growth and yield in young grapevines. However, not all of the inoculation efforts are successful. Nerva et al. [7] reported that a commercial AMF inoculant failed to establish in young Pinot noir/3309C vines, regardless of application timing, due to poor inoculant effectiveness, high soil phosphorus levels, and low rootstock compatibility. Similarly, Camprubí et al. [16] found that, whereas several native endophytes and *Glomus intraradices* BEG 72 improved 110R growth under greenhouse conditions with sterile, low-phosphorus soil, only one inoculant showed positive effects on survival and early growth in the field. The presence of AMF alone does not assure a functional symbiosis, and its contribution to vine performance must be assessed under field conditions [6]. In certain scenarios, inoculation may be unnecessary, particularly in soils rich in native AMF populations that can effectively colonize grapevine roots [20].

The main goal of this study was to evaluate the impact of co-inoculating commercial microbial inoculants containing AMF and PGPR, along with the application of MHB, on field-grown Monastrell vines grafted onto 140Ru, 110R, and 161-49C rootstocks. These rootstocks were selected for their widespread use in the study area and their ability to enhance water-use efficiency and grape quality under warm, semiarid conditions. From the early stages of vineyard establishment, we assessed the physiological and agronomic responses of inoculated vines under irrigation. Furthermore, we investigated the influence of the rootstock and its interaction with microbial inoculation in a semiarid environment. Based on prior findings obtained under controlled conditions, we tested two hypotheses: (1) early inoculation with AMF/PGPR improves water and nutrient uptake, growth, and yield in young vines; and (2) the rootstock genotype influences the response of the vine to microbial inoculation.

## 2. Materials and Methods

### 2.1. Field Conditions, Plant Materials, Irrigation, and Soil Treatments

The trial was conducted from 2017 to 2023 in a 0.2-ha plot of young (0–6 years-old) Monastrell (*V. vinifera* L. cv. Mourvedre) trellis vines grafted on three rootstocks, 140Ru, 110R, and 161-49C, in an experimental orchard located in Cehegín (SE Spain, 38°6′38.13″ N, 1°40′50.41″ W, 432 m a. s. l.). Planting took place in March 2017 (Figure 1A). Planting density was 2.8 m between rows and 1.0 m between plants (3571 vines/ha). The soil in the

plot had a clay-loam texture (45% clay, 34% silt, 21% sand) and an organic-matter content of 1.1%, an apparent density of 1.40, a pH of 7.83, an electrical conductivity of  $0.117 \text{ mS cm}^{-1}$ , an active limestone ( $\text{CaCO}_3$ ) percentage of 15.3%, a C/N ratio of 8.9, a total N percentage of 0.072%, an assimilable P of  $22.76 \text{ mg kg}^{-1}$ , a K exchange of  $0.99 \text{ meq/100 g}$ , a Ca exchange of  $13.48 \text{ meq/100 g}$ , a Mg exchange of  $2.85 \text{ meq/100 g}$ , and a cation exchange capacity of  $17.48 \text{ meq/100 g}$ . The soil was highly compacted to a depth of 70 cm due to the previous land use. The climate was Mediterranean semiarid, characterized by hot, dry summers and a low annual rainfall (Table 1). Irrigation water was sourced from a well, with a pH of 7.96 and an electrical conductivity ranging from 0.68 to  $0.81 \text{ dS m}^{-1}$ . The training system was a bilateral cordon trellised to a three-wire vertical system. Vine rows were oriented NW–SE. After pruning, six two-bud spurs (twelve nodes) were left per vine. In May, non-productive green shoots were uniformly removed across all of the treatments, following standard local viticultural practices.

**Table 1.** Annual applied volume of irrigation water and mean values of key climatic parameters for each year of the experiment in the study area.

| Year                                 | Irrigation<br>(mm Year <sup>-1</sup> ) | ETo<br>(mm Year <sup>-1</sup> ) | VPD<br>(kPa) | Rainfall<br>(mm Year <sup>-1</sup> ) | Tmax<br>(°C) | Tmean<br>(°C) | Tmin<br>(°C) | RHmean<br>(%) | Solar Rad.<br>(W m <sup>-2</sup> ) | Daily<br>Sunshine<br>(h) |
|--------------------------------------|----------------------------------------|---------------------------------|--------------|--------------------------------------|--------------|---------------|--------------|---------------|------------------------------------|--------------------------|
| <b>High irrigation conditions</b>    |                                        |                                 |              |                                      |              |               |              |               |                                    |                          |
| 2018                                 | 274.4                                  | 1162                            | 0.92         | 392                                  | 22.46        | 14.98         | 7.59         | 61.79         | 200.07                             | 9.43                     |
| 2019                                 | 283.1                                  | 1173                            | 0.98         | 427                                  | 23.03        | 15.08         | 6.99         | 60.24         | 206.30                             | 9.49                     |
| 2020                                 | 304.0                                  | 1106                            | 0.97         | 393                                  | 23.34        | 15.28         | 8.07         | 63.26         | 202.14                             | 9.42                     |
| <b>Deficit irrigation conditions</b> |                                        |                                 |              |                                      |              |               |              |               |                                    |                          |
| 2021                                 | 103.5                                  | 1100                            | 0.93         | 321                                  | 23.10        | 15.36         | 7.57         | 63.66         | 197.32                             | 9.32                     |
| 2022                                 | 89.4                                   | 1200                            | 1.09         | 300                                  | 25.03        | 16.13         | 7.33         | 61.80         | 191.80                             | 9.45                     |
| 2023                                 | 90.6                                   | 1105                            | 1.09         | 162                                  | 24.55        | 15.81         | 7.04         | 57.25         | 203.98                             | 9.43                     |

Crop evapotranspiration ( $\text{ETc} = \text{ETo} \times \text{Kc}$ ) was estimated using varying crop coefficients ( $\text{Kc}$ ) based on the FAO recommendations and adjusted for the Mediterranean region, as well as reference evapotranspiration ( $\text{ETo}$ ) values (Table 1). The applied  $\text{Kc}$  values were as follows: 0.35 in April, 0.45 in May, 0.52 in June, 0.75 from July to mid-August, 0.60 from mid-August to early September, and 0.45 from mid-September to October.  $\text{ETo}$  was calculated weekly from the mean values of the previous 5 years, applying the Penman-Monteith-FAO method. Daily climatic data were collected from a meteorological station (Campbell scientific Ltd., mod. CR 10X, Logan, UT, USA) located at the experimental vineyard and operated by the Agricultural Information Service of Murcia (SIAM, IMIDA, Murcia, Spain) (Table 1). All of the vines, regardless of the rootstocks, were irrigated with similar annual water volumes from April to October, using high-frequency drip irrigation (2–5 times per week during the late evening, depending on the phenological period). Water was applied by one pressure-compensated emitter per plant ( $4 \text{ L h}^{-1}$ ) with one drip-irrigation line per row. During the first four years (2017–2020), vines were irrigated with high water volumes to ensure a proper vineyard establishment (Table 1). In addition, all of the vines received the same annual dose of organic fertilizer—an amino acid-enriched liquid organic matter (compost,  $50 \text{ L ha}^{-1} \text{ month}^{-1}$ , certified for organic farming)—supplied through the drip irrigation system from April to August. From the fourth year onwards (2021–2023), vines were irrigated solely with water under a controlled deficit irrigation (DI) regime, applying between 90 and  $100 \text{ mm year}^{-1}$  (Table 1). Moreover, no liquid fertilizer was applied through irrigation. Instead, fertilization during this period consisted of a single annual application of solid organic/biodynamic cattle manure in autumn, distributed on both sides of the vine rows and incorporated into the soil via shallow trenching, at a rate of 4 to  $7 \text{ tons ha}^{-1}$ .



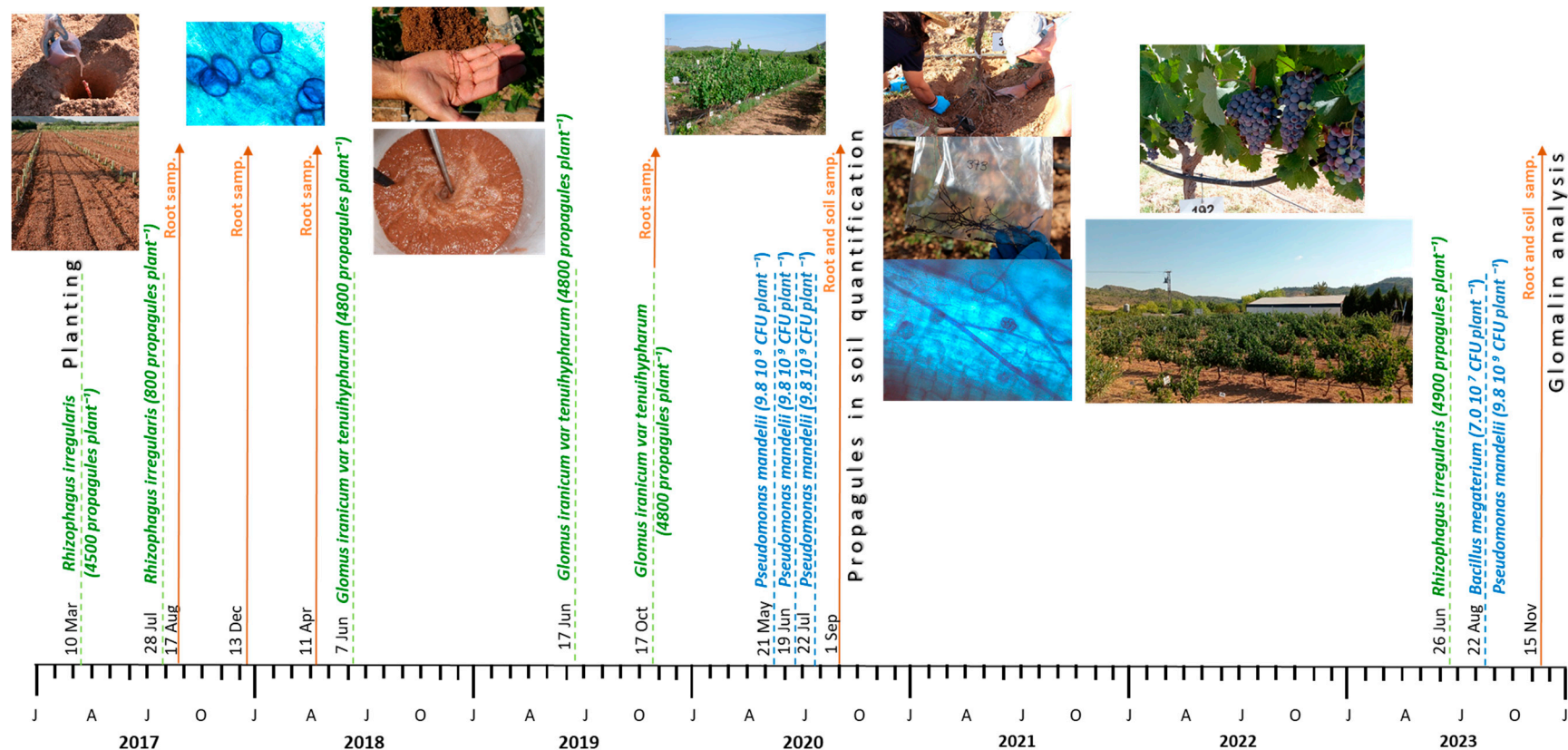
**Figure 1.** Photographs showing various aspects of the experiment conducted between 2017 and 2023 on a newly established vineyard of the Monastrell variety. This study evaluated the effects of different rootstocks (140Ru, 110R, and 161-49C) under varying conditions of mycorrhization. Field microbial inoculation directly into the planting hole (A); plantlets at the beginning of the experiment in 2017–2018 (B); optical microscopy images showing the state of mycorrhization (C); Monastrell plants grafted onto the three studied rootstocks at the start of the experiment (D); various parameters explored during the experiment, including soil and plant gas exchange measurements, monitoring of organic matter degradation, root system sampling, yield control, and grape sampling for quality analysis (E); and the general condition of the vines and clusters of inoculated and non-inoculated vines grafted onto different rootstocks just before harvest (F).

During the early years of vineyard establishment, management practices commonly used by local winegrowers were adopted. Inter-row and under-vine weeds were removed using mechanical methods throughout the growing season. Soil management involved no tillage, and all the vineyard management was conducted in accordance with organic production standards, without the use of unauthorized synthetic herbicides or pesticides. Since 2018, a cover crop of legumes and grasses (vetch, mustard, alfalfa, pea, wheat) has been sown annually in the inter-row spaces each autumn. These cover crops were allowed to grow until spring (April), when they were mowed and incorporated into the soil as green manure using minimal tillage (2–3 cm deep), to preserve the soil structure.

When designing this experiment, several key factors that could promote mycorrhization were initially taken into account to set it up: the low P content in the experimental soil, the selection of microorganisms adapted to a semiarid environment, the use of organic production (with no pesticides, herbicides, inorganic fertilization, or tillage), and the application of a deficit irrigation strategy after the establishment.

## 2.2. Experimental Design and Microbial Inoculation Treatments

From the time of the planting, the vineyard was divided into four subplots, and the experiment followed a randomized block layout with four blocks in total (two blocks per subplot). Two of the subplots were inoculated with a commercial microbial inoculum (I), applied directly into the planting hole at the time of establishment in the field (Figure 1), whereas the other two subplots served as non-inoculated controls (NI). Each subplot contained 24 vines of each rootstock (140Ru, 110R, and 161-49C), totaling 48 vines per rootstock-inoculation combination. Additionally, during the course of the experiment, sequential microbial inoculations were performed using different AMF species (*Rhizophagus irregularis* and *Glomus iranicum*) as well as successive applications of an MHB (*Pseudomonas mandelii* strain 29) and a PGPB (*Bacillus megaterium*) in combination with *R. irregularis*, in the inoculated subplots through the irrigation system. The dates of these applications and their concentrations are shown in Figure 2.



**Figure 2.** Detailed timeline of the field experiment showing applied treatments and specific sampling events. Dashed lines represent microbial inoculations: inoculation with the AMF *Rhizophagus irregularis* (4500, 800, and 4900 propagules plant<sup>-1</sup>), the AMF *Glomus iranicum* var. *tenuihypharum* (4800 propagules plant<sup>-1</sup>), the MHB *Pseudomonas mandelii* strain 29 (9.8·10<sup>8</sup> CFU plant<sup>-1</sup>), and the PGPR *Bacillus megaterium* (7.0·10<sup>7</sup> CFU plant<sup>-1</sup>). Vertical arrows indicate moments of root and soil sampling for various microbiological and root colonization analyses. The photographs visually illustrate the experimental phases: initial soil preparation, treatment applications, and inoculations (**left**); intermediate vegetative growth and sampling during crop development (**center**); final sampling, detailed root samples, microscopic observation of symbiotic structures, and final crop development (**right**). The commercial microbial inocula from Mycosoil and Mycostar (Agrogenia Biotech S.L.) and Mycoup (Symborg) were chosen based on their composition of AMF species typical of an area in the Region of Murcia, as well as on their previous successful application in other crops in the southeastern Spanish Mediterranean region. The last commercial AMF inoculum that was used (*R. irregularis*) also contained PGPB (*B. megaterium*), which solubilizes nutrients such as P and K [29,30]. In addition, the MHB (*P. mandelii* strain 29) had also been isolated and tested by the University of Murcia as a phosphorus solubilizer that promotes mycorrhizal colonization [12,31].

In both subplots (I and NI), five root samplings were conducted (2017, 2018, 2019, 2020, and 2023). For each of the samplings, four representative root samples were collected from different vines of each rootstock–inoculation combination, at a depth of 30–40 cm in the rhizosphere near the plant. Root colonization was assessed using standard staining and microscopy techniques as described by Phillips and Hayman [32] and McGonigle et al. [33], with minor modifications. Two types of organelles were identified: those indicative of active mycorrhization (hyphae, vesicles, and arbuscules inside the root) and those not directly associated with active mycorrhization (spores in the soil, outside the root) but associated with the mycorrhizal environment [34,35].

### 2.3. Extraction and Quantification of Arbuscular Fungal Propagules in Soil

In November 2020, alongside root sampling, soil sampling was conducted to assess the effect of inoculation treatments carried out during that campaign on mycorrhization. AMF propagules were extracted and quantified following the wet sieving and decanting method of Gerdemann and Nicolson [36].

### 2.4. Glomalin Concentration in Soil

In November 2023, soil sampling was conducted to assess glomalin levels at the end of the trial. In the same vines that were used to sample the roots and evaluate mycorrhization status, soil samples were collected from the root environment to measure the concentration of total glomalin (TG). These were extracted using the citrate autoclaving method and quantified via the Bradford assay, following the protocol of Wright and Upadhyayaya [37].

### 2.5. Organic Matter Decomposition

We assessed the rate of organic matter decomposition using standard plant material, according to the Tea Bag Index (TBI) method [38]. Burial of tea bags was carried out between May and June 2019. After 90 days, TBI parameters,  $k$  (decomposition of labile fraction) and  $S$  (stabilization factor), were calculated using the spreadsheet template provided by the TBI research team, available at <http://www.teatime4science.org> (accessed on 5 May 2025).

### 2.6. Soil Gas Exchange and Oxygen Diffusion Measurements

Between July 20 (pre-veraison) and 8 September 2021 (post-veraison), soil  $\text{CO}_2$  and  $\text{H}_2\text{O}$  exchange were monitored in situ with a LI-8100A infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA) equipped with a 20 cm survey chamber. To capture continuous diel patterns, we coupled the analyzer to an automated long-term chamber (model 8100-101). The chamber was mounted on each permanent stainless-steel collar for  $\geq 48$  h at some point between the onset of veraison and two weeks after its completion (mid-ripening). Measurements were taken in the wetted root zone (east-facing wet bulb) on four vines per rootstock  $\times$  inoculation treatment, with sampling days selected randomly but under comparable weather conditions. Fluxes were logged at 60 min intervals, providing high-resolution time series for subsequent analysis.

In 2021, soil oxygen diffusion rate (ODR) was measured with a portable oxygen diffusion meter, model 14.36 (Royal Eijkelpamp, Giesbeek, The Netherlands). This instrument maintains a constant potential of 0.65 V between a cylindrical platinum–iridium micro-electrode (1.2 mm  $\varnothing$ , 6 mm exposed length) and an Ag/AgCl reference electrode, driving the reduction of all of the  $\text{O}_2$  that reaches the Pt surface (constant-current technique). The steady reduction current is internally converted to flux and logged as  $\mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ . Measurements were taken at a depth of 5–10 cm, in the wet root zone, with four replicates per rootstock  $\times$  inoculation treatment, on randomly selected pre- and post-veraison days under comparable weather conditions.

### 2.7. Vine Water Status and Leaf Gas Exchange

Each year, the stem water potential ( $\Psi_s$ ) was determined monthly from fruit set until harvest. Eight healthy, fully exposed, and expanded mature leaves were selected from the main shoots in the middle–upper part of the vine canopy for each rootstock  $\times$  inoculation treatment. All of the leaves were east-facing, enclosed in aluminum foil, and covered with plastic for at least 2 h before midday measurement.  $\Psi_s$  was measured at noon (12:00 p.m.–1:30 p.m.) using a pressure chamber (Model 600; PMS Instrument Co., Albany, OR, USA).

Net leaf photosynthesis was measured every 14 days between 9:00 a.m. and 10:30 a.m. from May to September in 2018, 2020, 2021, and 2023 on selected clear and sunny days. Measurements were taken on east-facing, healthy, fully expanded, mature leaves exposed to sunlight (one leaf on each of 8 representative vines per rootstock, the same vines used to measure  $\Psi_s$ ), located in the outer canopy and growing on the main shoots. Leaf gaseous exchange parameters were measured with a portable photosynthesis measurement system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with a broadleaf chamber (6.0 cm<sup>2</sup>). During the measurements, leaf temperature ranged from 23 to 39 °C, leaf-to-air VPD ranged from 1.4 to 5.3 kPa, and relative humidity ranged from 30 to 50%. The molar air flow rate inside the leaf chamber was 500  $\mu\text{mol mol}^{-1}$ . All of the measurements were taken at a reference CO<sub>2</sub> concentration close to ambient concentration (400  $\mu\text{mol mol}^{-1}$ ) and at a saturating PPFD of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

### 2.8. Leaf Mineral Analysis

Leaf samples were collected in July 2018, 2021, and 2023 for mineral analysis. About 40 leaves were gathered from eight vines per rootstock  $\times$  inoculation treatment. Leaf mineral concentrations were determined using standard digestion and ICP-OES protocols, as well as the Dumas method as described in Romero et al. [39].

### 2.9. Vegetative and Reproductive Development

From 2018 to 2023, the total leaf area (TLA) per vine at the end of July (veraison period) was estimated in 16 to 32 vines (depending on the year) per rootstock  $\times$  inoculation treatment, using a non-destructive method. This method employed a first-order polynomial equation that related the shoot length (SL) to the TLA of the main shoot, previously developed for each of the rootstocks [39]. TLA per plant was calculated by selecting five representative main shoots per vine, measuring their average SL with a tape, and multiplying the average shoot leaf area by the total number of main shoots of the vine. During the winters of 2018 to 2023, pruning weight (PW) measurements were taken in 24 vines per rootstock  $\times$  inoculation treatment, including the same vines used for yield, leaf area, and shoot measurements.

Each year, at harvest (mid-late September), yield response was measured across 24 vines per rootstock  $\times$  inoculation treatment. Harvest date was determined according to the grower's practices in the area, occurring when °Brix reached 23.5–24.0. Yield per vine (kg vine<sup>-1</sup>), number of clusters per vine, berry weight, and cluster weight were calculated. Productive water use efficiency (WUE<sub>yield</sub>) was expressed as the mass of fresh grapes produced per m<sup>3</sup> of applied water per vine.

### 2.10. Berry and Must Quality

Samples of mature berries were collected from each rootstock  $\times$  inoculation treatment at harvest in 2018, 2019, 2020, 2021, 2022, and 2023, and transported to the laboratory. Each sample consisted of 800–900 g of berries, randomly gathered from different clusters on each

of the vines. Berry and must quality parameters were analyzed according to the established protocols for grape composition and phenolic content [39–42].

### 2.11. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA), and means were separated by Duncan's multiple range test, using Statgraphics 2.0 Plus software (Statgraphics Technologies, Inc., The Plains, VA, USA). One- and two-way ANOVAs were used to assess the effects of rootstock and AMF inoculation each year. In the global analysis of technological and phenolic maturity parameters, a three-way ANOVA was employed to evaluate the effects of rootstock, AMF inoculation, and year, and the rootstock  $\times$  AMF inoculation interaction was analyzed. Linear and nonlinear regressions were fitted using SigmaPlot 11.0 (Systat, Richmond, CA, USA), and the best fit for nonlinear models was selected based on Schwarz's Bayesian criterion index (SBC). Due to the non-normal distribution of mycorrhization percentage data, non-parametric statistical tests were applied to assess differences between treatments. The Kruskal–Wallis test was used when comparing more than two treatments, and the Mann–Whitney U test was used for pairwise comparisons. When significant differences were found among more than two treatments, a Duncan post hoc test was performed.

## 3. Results

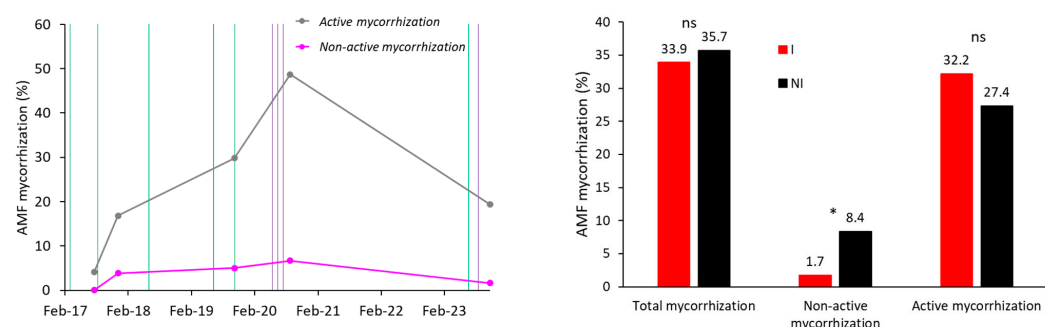
### 3.1. Effect of Microbial Inoculation on Mycorrhization

From 2017 to 2020, mycorrhization levels gradually increased, peaking at nearly 60% (Figure S1). A subsequent decline to approximately 20% by 2023 was likely attributable to the absence of artificial inoculation in 2021 and 2022. Despite this drop, mycorrhization remained significantly higher than at the start of the experiment, suggesting partial microbial stability. Among the rootstocks, 140Ru and 110R exhibited the highest mycorrhization rates (close to 60%), although differences were not statistically significant. Interestingly, I and NI vines exhibited similar total mycorrhization percentages, with marginally higher values observed in NI plants (Figure S2).

Based on their presumed functionality and impact, the percentages of active (hyphae, arbuscules, vesicles) and non-active (spores) mycorrhization were analyzed individually. Active mycorrhization was significantly higher than non-active mycorrhization throughout the experiment (Figure 3). Spore levels increased after the first inoculation and then stabilized at approximately 5%, whereas active mycorrhization increased and was more dynamic, peaking at 50% before declining to 20% after the final inoculation. After seven years, active mycorrhization remained significantly higher compared to the initial levels.

Although the total percentage of mycorrhization in root samples from both I and NI vines was similar, the distribution between active and non-active mycorrhization varied (Figure 3). NI vines had a significantly higher percentage of non-active mycorrhization, whereas I vines showed a higher, but not statistically significant, rate of active mycorrhization. On the other hand, the MHB application revealed a clear impact on non-active mycorrhization (Figure S3). In 2020, following bacterial inoculation, I vines exhibited a significant increase in spore percentage (from 1.7% to 8.2%), whereas NI vines showed a decrease (from 8.4% to 5.2%).

The most consistent indicator of AMF impact was the total glomalin concentration in soil (Table 2). Inoculated vines exhibited 12–13% higher glomalin levels than NI vines. However, a significant  $R \times MI$  interaction showed that only the I vines of the 140Ru rootstock had significantly higher glomalin concentrations than their NI counterparts.



**Figure 3.** (Left): Evolution of the percentage of active mycorrhization (hyphae, arbuscules, and vesicles) and non-active mycorrhization (spores) fractions throughout the experimental period (2017–2023). Values represent the average across the three rootstocks and both I and NI plants. (Right): Average percentage of AMF colonization in Monastrell vines under two different mycorrhizal inoculation conditions (I vs. NI) in October 2019 (after AMF inoculation but without MHB) across all of the rootstocks. Total mycorrhization includes spores and active structures (hyphae, arbuscules, and vesicles). Green and purple lines indicate the day of application of mycorrhizae and bacteria respectively. ns, not significant; \*  $p < 0.05$ , according to the non-parametric Mann–Whitney test at a 95% confidence level.

**Table 2.** Mean values of total glomalin (TG) in the soil at the end of the experiment in 2023, mean values of oxygen diffusion rate (ODR) in the soil in post-veraison of 2021, and mean values of TBI parameter (decomposition rate constant  $k$ ) measured in 2019. Data were recorded for 140Ru, 110R, and 161-49C rootstocks in inoculated (I) and non-inoculated (NI) treatments.

| Rootstock (R)              |    | TG ( $\mu\text{g g}^{-1}$ Soil DW) | ODR ( $\mu\text{g m}^{-2} \text{s}^{-1}$ ) | $k$   |
|----------------------------|----|------------------------------------|--------------------------------------------|-------|
| 140Ru                      |    | 2267                               | 48.5                                       | 0.019 |
| 110R                       |    | 2194                               | 44.5                                       | 0.019 |
| 161-49C                    |    | -                                  | 44.7                                       | -     |
| Microbial inoculation (MI) |    |                                    |                                            |       |
| I                          |    | 2378                               | 46.9                                       | 0.023 |
| NI                         |    | 2083                               | 45.0                                       | 0.016 |
| R $\times$ MI              |    |                                    |                                            |       |
| 140Ru                      | I  | 2580 b                             | 49.6 b                                     | 0.024 |
|                            | NI | 1953 a                             | 47.5 ab                                    | 0.015 |
| 110R                       | I  | 2176 ab                            | 41.6 ab                                    | 0.021 |
|                            | NI | 2212 ab                            | 47.5 ab                                    | 0.017 |
| 161-49C                    | I  | -                                  | 49.6 b                                     | -     |
|                            | NI | -                                  | 39.9 a                                     | -     |
| ANOVA                      |    |                                    |                                            |       |
| R                          |    | ns                                 | ns                                         | ns    |
| MI                         |    | ns                                 | ns                                         | ***   |
| R $\times$ MI              |    | *                                  | *                                          | ns    |

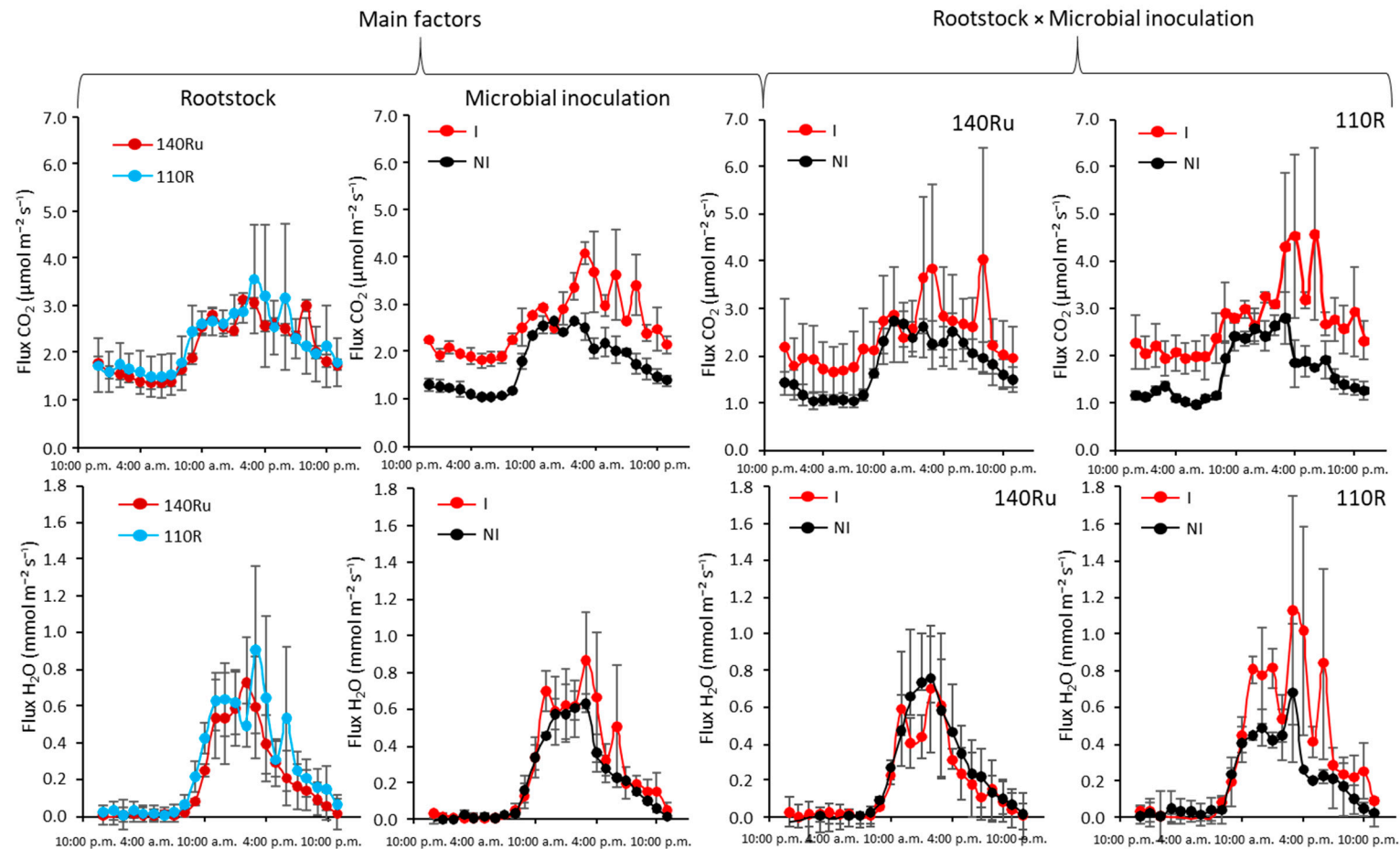
TG data for 161-49C are not available. ns, not significant; \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ . In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

### 3.2. Effect of the Rootstock

#### 3.2.1. Soil Respiration and Organic Matter Cycling

In 2019, no significant differences were found in soil organic matter decomposition during the growing season, based on  $k$  (Table 2) and the stabilization factor ( $S$ , data not shown) parameters of TBI in the rhizosphere of the 110R and 140Ru rootstocks.

Furthermore, no significant differences were identified amongst the rootstocks in ODR in the soil (Table 2) or in soil  $\text{CO}_2$  flux (Figure 4) in the rhizosphere during the 2021 growing season. However, daily soil  $\text{H}_2\text{O}$  efflux (soil evaporation rate) was higher in 110R compared to 140Ru, predominantly during the afternoon and evening hours (3:00 p.m.–10:00 p.m.) (Figure 4).



**Figure 4.** Daily evolution of soil CO<sub>2</sub> (respiration) and H<sub>2</sub>O (evaporation) fluxes measured during the post-veraison period (summer 2021), for vines on rootstocks 140Ru (maroon line) and 110R (blue line), under inoculated (I, red line) and non-inoculated (NI, black line) treatments. Left: Main effect of the rootstock (averaged across inoculation treatments); middle: Main effect of the microbial inoculation (I vs. NI); right: Rootstock × inoculation interaction. Error bars represent  $\pm$  SE.

### 3.2.2. Soil-Plant Water Relations and Leaf Gas Exchange

In 2018, one year after planting, significant differences in leaf gas exchange post-veraison were already observed among the rootstocks, with higher rates of  $A$  and  $g_s$  in 110R compared to 140Ru (Table 3). However, no differences in plant water status were found. In 2020,  $E$ , during the pre-veraison period, and both  $g_s$  and  $E$ , during the post-veraison period, were significantly higher in 161-49C compared to 110R. Consequently, the  $A/E$  (in pre-veraison) and  $A/g_s$  (in post-veraison) were reduced in this rootstock. In 2021, no significant differences in leaf gas exchange were observed amongst the rootstocks, but 161-49C exhibited significantly higher water stress (more negative  $\Psi_s$ ) during post-veraison compared to 110R and 140Ru. In 2023, no significant differences in plant water status or leaf gas exchange were observed among the rootstocks.

**Table 3.** Mean values of leaf gas exchange parameters in the early morning (9:00 a.m.–10:30 a.m.) and midday stem water potential (12:30 p.m.–2:00 p.m.) measured in Monastrell young plants (from 1 to 6 years old) grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI) for each of the years and in two different phenological periods (pre- and post-veraison) during the experimental period (2018–2023).  $A$ , net photosynthesis rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $g_s$ , stomatal conductance rate ( $\text{mol m}^{-2} \text{s}^{-1}$ );  $E$ , transpiration rate ( $\text{mmol m}^{-2} \text{s}^{-1}$ );  $A/g_s$ , intrinsic leaf water use efficiency ( $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ );  $A/E$ , instantaneous leaf water use efficiency ( $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$ );  $\Psi_s$ , midday stem water potential (MPa).

|                                   | Pre-Veraison |       |        |         |       |          | Post-Veraison |          |       |         |       |          |
|-----------------------------------|--------------|-------|--------|---------|-------|----------|---------------|----------|-------|---------|-------|----------|
|                                   | $A$          | $g_s$ | $E$    | $A/g_s$ | $A/E$ | $\Psi_s$ | $A$           | $g_s$    | $E$   | $A/g_s$ | $A/E$ | $\Psi_s$ |
| <b>Rootstock (R)</b>              | <b>2018</b>  |       |        |         |       |          |               |          |       |         |       |          |
| 140Ru                             | 18.8         | 0.160 | 4.6    | 140     | 4.7   | −0.69    | 17.0 a        | 0.239 a  | 5.8   | 78      | 3.0   | −0.75    |
| 110R                              | 18.4         | 0.156 | 4.6    | 136     | 4.5   | −0.68    | 18.3 b        | 0.279 b  | 6.3   | 70      | 3.0   | −0.75    |
| 161-49C                           | 17.9         | 0.137 | 4.1    | 152     | 4.9   | −0.68    | 17.7 ab       | 0.268 ab | 6.3   | 70      | 2.9   | −0.75    |
| <b>Microbial inoculation(MI)</b>  |              |       |        |         |       |          |               |          |       |         |       |          |
| I                                 | 18.7         | 0.158 | 4.6    | 139     | 4.6   | −0.69    | 18.1          | 0.270    | 6.4   | 72      | 2.9   | −0.76    |
| NI                                | 18.0         | 0.144 | 4.2    | 146     | 4.9   | −0.67    | 17.2          | 0.254    | 5.8   | 73      | 3.1   | −0.74    |
| <b>ANOVA</b>                      |              |       |        |         |       |          |               |          |       |         |       |          |
| R                                 | ns           | ns    | ns     | ns      | ns    | ns       | *             | *        | ns    | ns      | ns    | ns       |
| MI                                | ns           | ns    | ns     | ns      | ns    | ns       | *             | ns       | *     | ns      | **    | ns       |
| R × MI                            | ns           | ns    | ns     | ns      | ns    | ns       | ns            | ns       | ns    | ns      | ns    | ns       |
| <b>Rootstock (R)</b>              | <b>2020</b>  |       |        |         |       |          |               |          |       |         |       |          |
| 140Ru                             | 17.2         | 0.241 | 5.5 ab | 74.2    | 3.2 a | -        | 14.4          | 0.168 ab | 4.5 a | 91.9 ab | 3.4   | -        |
| 110R                              | 18.0         | 0.231 | 5.3 a  | 79.4    | 3.5 b | -        | 13.7          | 0.156 a  | 4.3 a | 94.6 b  | 3.4   | -        |
| 161-49C                           | 17.8         | 0.256 | 5.8 b  | 71.4    | 3.1 a | -        | 15.3          | 0.210 b  | 5.3 b | 78.5 a  | 3.0   | -        |
| <b>Microbial inoculation (MI)</b> |              |       |        |         |       |          |               |          |       |         |       |          |
| I                                 | 17.8         | 0.250 | 5.7    | 73.1    | 3.2   | -        | 14.7          | 0.181    | 4.8   | 85.9    | 3.2   | -        |
| NI                                | 17.5         | 0.235 | 5.4    | 76.8    | 3.4   | -        | 14.3          | 0.175    | 4.6   | 90.8    | 3.3   | -        |
| <b>ANOVA</b>                      |              |       |        |         |       |          |               |          |       |         |       |          |
| R                                 | ns           | ns    | *      | ns      | ***   | -        | ns            | **       | **    | *       | ns    | -        |
| MI                                | ns           | ns    | ns     | ns      | ns    | -        | ns            | ns       | ns    | ns      | ns    | -        |
| R × MI                            | ns           | ns    | **     | ns      | **    | -        | ns            | ns       | ns    | ns      | ns    | -        |

Table 3. Cont.

|                                   | Pre-Veraison |       |     |          |     |          | Post-Veraison |       |     |          |     |            |
|-----------------------------------|--------------|-------|-----|----------|-----|----------|---------------|-------|-----|----------|-----|------------|
|                                   | A            | $g_s$ | E   | A/ $g_s$ | A/E | $\Psi_s$ | A             | $g_s$ | E   | A/ $g_s$ | A/E | $\Psi_s$   |
| <b>Rootstock (R)</b>              | <b>2021</b>  |       |     |          |     |          |               |       |     |          |     |            |
| 140Ru                             | 15.0         | 0.164 | 4.0 | 95.4     | 3.8 | −0.97    | 12.5          | 0.117 | 2.9 | 117.7    | 4.6 | −1.28<br>b |
| 110R                              | 14.8         | 0.168 | 4.0 | 93.2     | 3.7 | −1.02    | 12.6          | 0.119 | 2.9 | 115.2    | 4.5 | −1.24<br>b |
| 161-49C                           | 15.0         | 0.156 | 3.9 | 97.1     | 3.9 | −1.07    | 11.4          | 0.115 | 2.8 | 113.3    | 4.3 | −1.39<br>a |
| <b>Microbial inoculation (MI)</b> |              |       |     |          |     |          |               |       |     |          |     |            |
| I                                 | 14.8         | 0.159 | 3.9 | 97.3     | 3.8 | −1.04    | 11.7          | 0.114 | 2.8 | 113.5    | 4.3 | −1.30      |
| NI                                | 14.9         | 0.166 | 4.0 | 93.1     | 3.8 | −1.00    | 12.7          | 0.120 | 2.9 | 117.4    | 4.6 | −1.30      |
| <b>ANOVA</b>                      |              |       |     |          |     |          |               |       |     |          |     |            |
| R                                 | ns           | ns    | ns  | ns       | ns  | ns       | ns            | ns    | ns  | ns       | ns  | *          |
| MI                                | ns           | ns    | ns  | ns       | ns  | ns       | ns            | ns    | ns  | ns       | **  | ns         |
| R × MI                            | ns           | ns    | ns  | ns       | ns  | ns       | ns            | ns    | ns  | ns       | ns  | ns         |
| <b>Rootstock (R)</b>              | <b>2023</b>  |       |     |          |     |          |               |       |     |          |     |            |
| 140Ru                             | 20.3         | -     | 5.6 | -        | 3.7 | −0.81    | 14.0          | 0.148 | 3.9 | 103.0    | 3.8 | −1.11      |
| 110R                              | 20.5         | -     | 5.6 | -        | 3.7 | −0.85    | 13.9          | 0.138 | 4.0 | 105.3    | 3.7 | −1.12      |
| 161-49C                           | -            | -     | -   | -        | -   | -        | -             | -     | -   | -        | -   | -          |
| <b>Microbial inoculation (MI)</b> |              |       |     |          |     |          |               |       |     |          |     |            |
| I                                 | 19.7         | -     | 5.4 | -        | 3.7 | −0.83    | 13.5          | 0.129 | 3.6 | 107.7    | 3.9 | −1.10      |
| NI                                | 21.1         | -     | 5.8 | -        | 3.8 | −0.83    | 14.5          | 0.157 | 4.3 | 100.5    | 3.6 | −1.12      |
| <b>ANOVA</b>                      |              |       |     |          |     |          |               |       |     |          |     |            |
| R                                 | ns           | -     | ns  | -        | ns  | ns       | ns            | ns    | ns  | ns       | ns  | ns         |
| MI                                | ***          | -     | **  | -        | ns  | ns       | *             | **    | **  | *        | ns  | ns         |
| R × MI                            | *            | -     | **  | -        | *   | **       | ns            | ns    | ns  | ns       | ns  | **         |

ns, not significant; \*  $p < 0.1$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$ . In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

### 3.2.3. Leaf Mineral Nutrition

Leaf mineral concentration varied by rootstock from the start of the plantation (Table 4). In 2018, 140Ru showed lower N, P, and K levels, but higher Mg concentrations than 110R and 161-49C; 161-49C had higher Na, and 110R had more B. In 2021, 140Ru vines had higher Na and Mg levels, whereas 161-49C had higher Mn levels. By 2023, 140Ru once again exhibited higher Mg concentrations than 110R.

**Table 4.** Leaf mineral concentration in Monastrell plants at veraison grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI) in 2018, 2021, and 2023. N, P, K, Ca, Mg, and Na in % in DW, Fe, Cu, Mn, Zn, and B in  $\text{mg L}^{-1}$ .

|                                   | N           | P       | K      | Ca   | Mg     | Na      | Fe   | Zn   | Mn  | Cu   | B    |
|-----------------------------------|-------------|---------|--------|------|--------|---------|------|------|-----|------|------|
| <b>Rootstock (R)</b>              | <b>2018</b> |         |        |      |        |         |      |      |     |      |      |
| 140Ru                             | 3.0 a       | 0.158 a | 0.62 a | 2.15 | 0.45 b | 0.044 a | 75.6 | 19.1 | 147 | 11.1 | 38.3 |
| 110R                              | 3.2 b       | 0.179 b | 0.73 b | 1.94 | 0.36 a | 0.047 a | 76.5 | 18.3 | 133 | 11.5 | 49.1 |
| 161-49C                           | 3.2 b       | 0.178 b | 0.75 b | 2.08 | 0.35 a | 0.059 b | 68.1 | 19.2 | 153 | 12.3 | 43.3 |
| <b>Microbial inoculation (MI)</b> |             |         |        |      |        |         |      |      |     |      |      |
| I                                 | 3.2         | 0.175   | 0.72   | 2.03 | 0.39   | 0.047   | 70.5 | 18.7 | 146 | 11.6 | 42.8 |
| NI                                | 3.1         | 0.169   | 0.67   | 2.08 | 0.38   | 0.053   | 76.4 | 19.1 | 143 | 11.6 | 44.3 |
| <b>ANOVA</b>                      |             |         |        |      |        |         |      |      |     |      |      |
| R                                 | **          | **      | **     | ns   | ***    | **      | ns   | ns   | ns  | ns   | ns   |
| MI                                | ns          | ns      | ns     | ns   | ns     | ns      | ns   | ns   | ns  | ns   | ns   |
| R × MI                            | ns          | ns      | ns     | ns   | ns     | ns      | ns   | **   | ns  | ns   | ns   |

Table 4. Cont.

|                            |         | N    | P     | K    | Ca       | Mg      | Na       | Fe   | Zn      | Mn     | Cu       | B      |
|----------------------------|---------|------|-------|------|----------|---------|----------|------|---------|--------|----------|--------|
| Rootstock (R)              |         | 2021 |       |      |          |         |          |      |         |        |          |        |
|                            | 140Ru   | 2.52 | 0.142 | 0.65 | 1.64     | 0.33 b  | 0.053 b  | 84.7 | 33.8    | 133 ab | 14.4     | 27.7 a |
|                            | 110R    | 2.47 | 0.139 | 0.65 | 1.62     | 0.28 a  | 0.040 a  | 83.7 | 32.9    | 125 a  | 13.1     | 31.5 b |
|                            | 161-49C | 2.43 | 0.138 | 0.69 | 1.67     | 0.26 a  | 0.037 a  | 82.5 | 35.3    | 145 b  | 13.9     | 25.6 a |
| Microbial inoculation (MI) |         |      |       |      |          |         |          |      |         |        |          |        |
|                            | I       | 2.48 | 0.140 | 0.68 | 1.67     | 0.30    | 0.033    | 80.6 | 36.6    | 136    | 13.7     | 27.8   |
|                            | NI      | 2.47 | 0.139 | 0.65 | 1.62     | 0.29    | 0.054    | 86.7 | 31.4    | 132    | 13.9     | 28.7   |
| R × MI                     |         |      |       |      |          |         |          |      |         |        |          |        |
| 140Ru                      | I       | 2.51 | 0.144 | 0.69 | 1.74 bc  | 0.34 d  | 0.032 a  | 80.7 | 35.7    | 135 bc | 14.5 bc  | 27.9   |
|                            | NI      | 2.53 | 0.139 | 0.62 | 1.54 a   | 0.32 cd | 0.075 c  | 88.6 | 31.9    | 131 ab | 14.4 bc  | 27.4   |
| 110R                       | I       | 2.41 | 0.139 | 0.64 | 1.68 abc | 0.31 cd | 0.033 a  | 79.3 | 36.0    | 135 bc | 13.9 abc | 31.6   |
|                            | NI      | 2.53 | 0.139 | 0.65 | 1.57 a   | 0.26 ab | 0.047 b  | 88.2 | 29.8    | 115 a  | 12.2 a   | 31.3   |
| 161-49C                    | I       | 2.52 | 0.137 | 0.70 | 1.59 ab  | 0.24 a  | 0.033 a  | 81.8 | 38.0    | 139 bc | 12.6 ab  | 23.9   |
|                            | NI      | 2.35 | 0.139 | 0.67 | 1.76 c   | 0.29 bc | 0.040 ab | 83.3 | 32.5    | 151 c  | 15.2 c   | 27.3   |
| ANOVA                      |         |      |       |      |          |         |          |      |         |        |          |        |
|                            | R       | ns   | ns    | ns   | ns       | ***     | ***      | ns   | ns      | ***    | ns       | ***    |
|                            | MI      | ns   | ns    | ns   | ns       | ns      | ***      | **   | ***     | ns     | ns       | ns     |
|                            | R × MI  | ns   | ns    | ns   | ***      | ***     | ***      | ns   | ns      | **     | ***      | ns     |
| Rootstock (R)              |         | 2023 |       |      |          |         |          |      |         |        |          |        |
|                            | 140Ru   | 2.27 | 0.111 | 0.64 | 1.63     | 0.35    | 0.017    | 65.2 | 28.3    | 133    | 18.3     | 48.9   |
|                            | 110R    | 2.25 | 0.111 | 0.62 | 1.60     | 0.29    | 0.016    | 63.4 | 28.0    | 119    | 20.2     | 48.2   |
| Microbial inoculation (MI) |         |      |       |      |          |         |          |      |         |        |          |        |
|                            | I       | 2.26 | 0.116 | 0.68 | 1.63     | 0.34    | 0.016    | 66.0 | 28.7    | 134    | 23.4     | 52.9   |
|                            | NI      | 2.26 | 0.108 | 0.58 | 1.59     | 0.30    | 0.018    | 62.5 | 27.6    | 119    | 15.2     | 44.1   |
| R × MI                     |         |      |       |      |          |         |          |      |         |        |          |        |
| 140Ru                      | I       | 2.27 | 0.116 | 0.66 | 1.72 b   | 0.38 b  | 0.016    | 65.5 | 26.9 a  | 141    | 19.3 a   | 57.3   |
|                            | NI      | 2.27 | 0.107 | 0.62 | 1.53 a   | 0.32 a  | 0.019    | 64.8 | 29.7 ab | 126    | 17.4 a   | 40.4   |
| 110R                       | I       | 2.26 | 0.115 | 0.71 | 1.54 a   | 0.29 a  | 0.015    | 66.6 | 30.4 b  | 127    | 27.4 b   | 48.6   |
|                            | NI      | 2.25 | 0.109 | 0.54 | 1.65 ab  | 0.29 a  | 0.017    | 60.2 | 25.5 a  | 111    | 13.0 a   | 47.8   |
| ANOVA                      |         |      |       |      |          |         |          |      |         |        |          |        |
|                            | R       | ns   | ns    | ns   | ns       | ***     | ns       | ns   | ns      | ns     | ns       | ns     |
|                            | MI      | ns   | ***   | **   | ns       | **      | ns       | ns   | ns      | ns     | ***      | ns     |
|                            | R × MI  | ns   | ns    | ns   | *        | *       | ns       | ns   | ***     | ns     | **       | ns     |

ns, not significant; \*  $p < 0.1$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.001$ . In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

### 3.2.4. Vegetative and Reproductive Development

In 2018, one year after planting, clear differences in vegetative and reproductive growth were observed among the rootstocks (Table 5, Figure S4). Vines on 140Ru showed greater shoot growth, TLA, and yield (number of clusters) than those on 110R and 161-49C. This trend persisted through 2023, with 140Ru generally exhibiting higher shoot growth, TLA, pruning weight, yield, cluster number, cluster weight, and PW. Since 2018, 140Ru also displayed the highest  $WUE_{yield}$ , whereas 161-49C showed the lowest (Figure 5).

**Table 5.** Mean values of vegetative (main shoot length; total leaf area, TLA; winter pruning weight, PW), and reproductive parameters of Monastrell plants grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI) for each of the years during the experimental period (2018–2023).

|                            | Main Shoot<br>Length (cm) | TLA<br>(m <sup>2</sup> Vine <sup>-1</sup> ) | PW<br>(kg Vine <sup>-1</sup> ) | Yield<br>(kg Vine <sup>-1</sup> ) | Number of<br>Clusters<br>Vine <sup>-1</sup> | Cluster Weight<br>(g) |
|----------------------------|---------------------------|---------------------------------------------|--------------------------------|-----------------------------------|---------------------------------------------|-----------------------|
| Rootstock (R)              |                           | 2018                                        |                                |                                   |                                             |                       |
| 140Ru                      | 101 b                     | 1.42 b                                      | 0.24                           | 0.79 b                            | 4.4 b                                       | 184                   |
| 110R                       | 78 a                      | 1.11 a                                      | 0.22                           | 0.35 a                            | 2.4 a                                       | 147                   |
| 161-49C                    | 77 a                      | 1.15 a                                      | 0.25                           | 0.32 a                            | 2.1 a                                       | 129                   |
| Microbial inoculation (MI) |                           |                                             |                                |                                   |                                             |                       |
| I                          | 88                        | 1.20                                        | 0.28                           | 0.47                              | 2.9                                         | 153                   |
| NI                         | 82                        | 1.26                                        | 0.19                           | 0.50                              | 3.0                                         | 154                   |

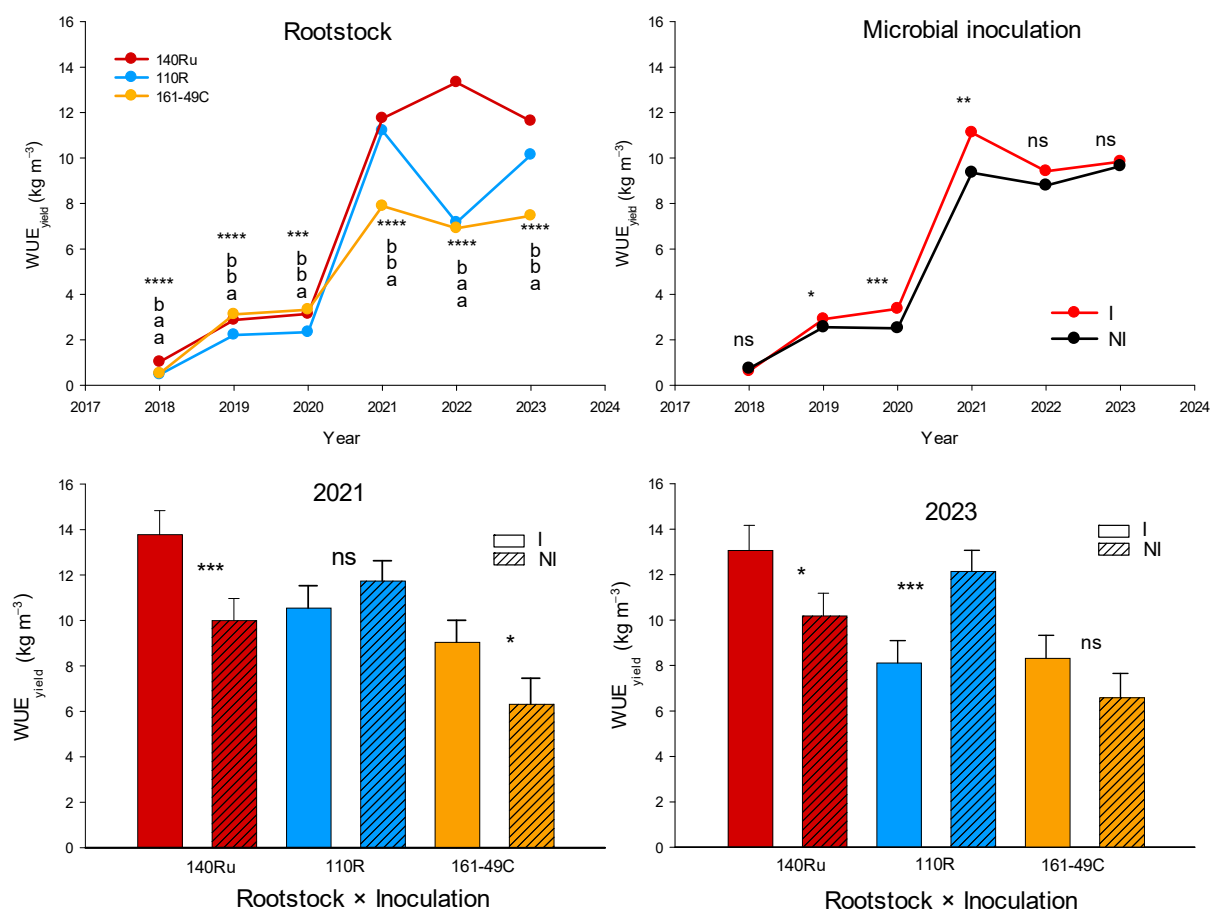
Table 5. Cont.

|                                   | Main Shoot Length (cm) | TLA (m <sup>2</sup> Vine <sup>−1</sup> ) | PW (kg Vine <sup>−1</sup> ) | Yield (kg Vine <sup>−1</sup> ) | Number of Clusters Vine <sup>−1</sup> | Cluster Weight (g) |
|-----------------------------------|------------------------|------------------------------------------|-----------------------------|--------------------------------|---------------------------------------|--------------------|
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | **                     | ***                                      | ns                          | ***                            | ***                                   | ns                 |
| MI                                | ns                     | ns                                       | ***                         | ns                             | ns                                    | ns                 |
| R × MI                            | ns                     | ns                                       | ns                          | ns                             | ns                                    | ns                 |
| <b>Rootstock</b>                  | <b>2019</b>            |                                          |                             |                                |                                       |                    |
| 140Ru                             | 68                     | 1.75                                     | 0.19 b                      | 2.27 b                         | 17.4                                  | 131 ab             |
| 110R                              | 67                     | 1.61                                     | 0.15 a                      | 1.75 a                         | 15.1                                  | 118 a              |
| 161-49C                           | 78                     | 2.08                                     | 0.17 ab                     | 2.42 b                         | 16.4                                  | 146 b              |
| <b>Microbial inoculation (MI)</b> |                        |                                          |                             |                                |                                       |                    |
| I                                 | 76                     | 2.00                                     | 0.18                        | 2.33                           | 16.4                                  | 144                |
| NI                                | 66                     | 1.62                                     | 0.16                        | 1.96                           | 16.1                                  | 119                |
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | ns                     | ns                                       | **                          | ***                            | ns                                    | ***                |
| MI                                | *                      | ns                                       | ns                          | *                              | ns                                    | ***                |
| R × MI                            | ns                     | ns                                       | ns                          | ns                             | ns                                    | ns                 |
| <b>Rootstock (R)</b>              | <b>2020</b>            |                                          |                             |                                |                                       |                    |
| 140Ru                             | 94 b                   | 2.88 b                                   | 0.41                        | 2.68 b                         | 11.3 b                                | 212 b              |
| 110R                              | 83 a                   | 2.37 a                                   | 0.40                        | 2.02 a                         | 9.8 a                                 | 188 a              |
| 161-49C                           | 95 b                   | 2.97 b                                   | 0.45                        | 2.81 b                         | 11.6 b                                | 221 b              |
| <b>Microbial inoculation (MI)</b> |                        |                                          |                             |                                |                                       |                    |
| I                                 | 96                     | 3.02                                     | 0.44                        | 2.87                           | 12.0                                  | 219                |
| NI                                | 85                     | 2.46                                     | 0.40                        | 2.14                           | 9.7                                   | 195                |
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | ***                    | ***                                      | ns                          | ***                            | *                                     | ***                |
| MI                                | ***                    | ***                                      | ns                          | ***                            | ***                                   | ***                |
| R × MI                            | ns                     | ns                                       | ns                          | ns                             | ns                                    | ns                 |
| <b>Rootstock (R)</b>              | <b>2021</b>            |                                          |                             |                                |                                       |                    |
| 140Ru                             | 113 b                  | 3.72 b                                   | 0.41 b                      | 3.37 b                         | 17.8 b                                | 178 b              |
| 110R                              | 95 a                   | 2.95 a                                   | 0.36 ab                     | 3.17 b                         | 18.5 b                                | 161 b              |
| 161-49C                           | 91 a                   | 2.98 a                                   | 0.33 a                      | 2.09 a                         | 15.9 a                                | 115 a              |
| <b>Microbial inoculation (MI)</b> |                        |                                          |                             |                                |                                       |                    |
| I                                 | 103                    | 3.17                                     | 0.39                        | 3.10                           | 17.7                                  | 159                |
| NI                                | 97                     | 3.26                                     | 0.34                        | 2.67                           | 17.2                                  | 144                |
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | ***                    | *                                        | ***                         | ***                            | **                                    | ***                |
| MI                                | ns                     | ns                                       | **                          | **                             | ns                                    | **                 |
| R × MI                            | ns                     | *                                        | **                          | **                             | ns                                    | ns                 |
| <b>Rootstock (R)</b>              | <b>2022</b>            |                                          |                             |                                |                                       |                    |
| 140Ru                             | 83 b                   | 2.86                                     | 0.38 b                      | 3.35 b                         | 16.0 b                                | 188                |
| 110R                              | 77                     | 2.76                                     | 0.27 a                      | 1.77 a                         | 11.4 a                                | 297                |
| 161-49C                           | 66 a                   | 2.42                                     | 0.26 a                      | 1.71 a                         | 10.5 a                                | 142                |
| <b>Microbial inoculation (MI)</b> |                        |                                          |                             |                                |                                       |                    |
| I                                 | 74                     | 2.77                                     | 0.31                        | 2.36                           | 13.0                                  | 263                |
| NI                                | 77                     | 2.59                                     | 0.29                        | 2.20                           | 12.3                                  | 155                |
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | ***                    | ns                                       | ***                         | ***                            | ***                                   | ns                 |
| MI                                | ns                     | ns                                       | ns                          | ns                             | ns                                    | ns                 |
| R × MI                            | **                     | **                                       | **                          | ns                             | ns                                    | ns                 |

Table 5. Cont.

|                                   | Main Shoot Length (cm) | TLA (m <sup>2</sup> Vine <sup>-1</sup> ) | PW (kg Vine <sup>-1</sup> ) | Yield (kg Vine <sup>-1</sup> ) | Number of Clusters Vine <sup>-1</sup> | Cluster Weight (g) |
|-----------------------------------|------------------------|------------------------------------------|-----------------------------|--------------------------------|---------------------------------------|--------------------|
| <b>Rootstock (R)</b>              |                        |                                          |                             |                                |                                       |                    |
| <b>2023</b>                       |                        |                                          |                             |                                |                                       |                    |
| 140Ru                             | 99                     | 2.66                                     | 0.37                        | 2.95 b                         | 13.7 b                                | 191 c              |
| 110R                              | 73                     | 1.84                                     | 0.30                        | 2.57 b                         | 14.2 b                                | 162 b              |
| 161-49C                           | -                      | -                                        | -                           | 1.96 a                         | 11.7 a                                | 140 a              |
| <b>Microbial inoculation (MI)</b> |                        |                                          |                             |                                |                                       |                    |
| I                                 | 86                     | 2.48                                     | 0.33                        | 2.52                           | 12.7                                  | 169                |
| NI                                | 87                     | 2.02                                     | 0.34                        | 2.47                           | 13.7                                  | 160                |
| <b>ANOVA</b>                      |                        |                                          |                             |                                |                                       |                    |
| R                                 | ***                    | *                                        | *                           | ***                            | **                                    | ***                |
| MI                                | ns                     | ns                                       | ns                          | ns                             | ns                                    | ns                 |
| R × MI                            | ns                     | ns                                       | *                           | ***                            | **                                    | ***                |

ns, not significant; \*  $p < 0.1$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$ . In each column and for each factor or interaction, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level. (-) represents data not taken.



**Figure 5.** Evolution of productive water use efficiency ( $WUE_{yield}$ ) in Monastrell vines grafted onto different rootstocks and under two microbial inoculation conditions (I vs. NI) during the experimental period (2018–2023). Values of  $WUE_{yield}$  for each of the rootstocks and for both inoculated and non-inoculated plants in 2021 and 2023 (one-way analysis). ns, not significant; \*  $p < 0.1$ , \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.001$ . Different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

### 3.2.5. Berry Quality Response

During the six-year period, 140Ru exhibited inferior overall berry technological and phenolic quality, with increased berry weight, pH, malic acid, and K content, while displaying reduced levels of CI, anthocyanins per TSS, TPI, and extractable anthocyanins (Tables 6 and 7). In contrast, 110R and 161-49C demonstrated better quality, with lower pH along with higher TSS, anthocyanins, TPI, extractable polyphenols, and must nutrient content.

**Table 6.** Technological maturity parameters of Monastrell grape must at harvest, grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and subjected to two different microbial inoculation conditions (I vs. NI) from 2018 to 2023. Mean values represent the average of five years, except for the sugar content, which uses the average of three years. Parameters include berry weight (g), CI (color intensity), TSS (total soluble solids, °Brix), TA (titratable acidity, g tartaric acid L<sup>−1</sup>), MI (maturity index, TSS/TA), pH, malic acid, tartaric acid (g L<sup>−1</sup>), glucose and fructose (g L<sup>−1</sup>), and anthocyanins/TSS (mg L<sup>−1</sup> °Brix<sup>−1</sup>).

| Rootstock (R)                     | Berry Weight | CI     | TSS     | TA      | MI    | pH      | Malic Acid | Tartaric Acid | Glucose | Fructose | Anthocyanins/TSS |
|-----------------------------------|--------------|--------|---------|---------|-------|---------|------------|---------------|---------|----------|------------------|
| 140Ru                             | 1.88 b       | 3.99 a | 21.83 a | 3.60 b  | 70 a  | 4.20 b  | 2.00 b     | 3.26          | 146 ab  | 156 ab   | 19.1 a           |
| 110R                              | 1.84 b       | 4.66 b | 22.65 b | 3.43 a  | 77 b  | 4.21 b  | 1.64 a     | 3.37          | 160 b   | 168 b    | 23.6 b           |
| 161-49C                           | 1.68 a       | 4.38 b | 21.59 a | 3.52 ab | 71 a  | 4.09 a  | 1.54 a     | 3.27          | 137 a   | 144 a    | 26.2 b           |
| <b>Microbial inoculation (MI)</b> |              |        |         |         |       |         |            |               |         |          |                  |
| I                                 | 1.84         | 4.33   | 22.09   | 3.47    | 73    | 4.17    | 1.71       | 3.30          | 151     | 159      | 22.1             |
| NI                                | 1.77         | 4.36   | 21.97   | 3.56    | 72    | 4.16    | 1.74       | 3.31          | 144     | 153      | 23.8             |
| <b>Year</b>                       |              |        |         |         |       |         |            |               |         |          |                  |
| 2018                              | 1.87 bc      | 4.64 b | 22.70 c | 4.23 c  | 54 a  | 4.17 ab | 3.03 d     | 3.89 c        | -       | -        | 11.1 a           |
| 2020                              | 1.92 bc      | 4.16 b | 24.98 d | 4.27 c  | 58 a  | 4.18 ab | 1.74 c     | 3.64 c        | -       | -        | 16.2 a           |
| 2021                              | 1.47 a       | 4.25 b | 19.10 a | 3.89 b  | 54 a  | 4.06 a  | 1.59 bc    | 3.06 b        | 165 b   | 182 b    | 35.7 b           |
| 2022                              | 1.96 c       | 3.56 a | 23.13 c | 2.64 a  | 114 c | 4.16 ab | 0.85 a     | 1.27 a        | 143 a   | 146 a    | 16.1 a           |
| 2023                              | 1.79 b       | 5.12 c | 20.92 b | 2.54 a  | 83 b  | 4.26 b  | 1.43 b     | 4.65 d        | 135 a   | 139 a    | 35.8 b           |
| <b>ANOVA</b>                      |              |        |         |         |       |         |            |               |         |          |                  |
| R                                 | ***          | ***    | ***     | **      | ***   | *       | ***        | ns            | *       | *        | ***              |
| MI                                | ns           | ns     | ns      | ns      | ns    | ns      | ns         | ns            | ns      | ns       | ns               |
| Year                              | ***          | ***    | ***     | ***     | ***   | ***     | ***        | ***           | ***     | ***      | ***              |
| R × MI                            | ns           | ns     | ns      | ns      | ns    | ns      | ns         | ns            | ns      | ns       | ns               |

ns, not significant; \*, \*\*, and \*\*\* indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

**Table 7.** Phenolic maturity parameters and mineral nutrition of the must made from Monastrell grapes at harvest, grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and subjected to two different microbial inoculation conditions (I vs. NI) from 2018 to 2023. Mean values represent the average of five years: total phenol index (TPI), extractable polyphenols (mg L<sup>−1</sup>), extractable anthocyanins (mg L<sup>−1</sup>), total tannins (mg g<sup>−1</sup> FW), K, Mg, P, and Zn (mg L<sup>−1</sup>).

| Rootstock (R)                     | TPI     | Extractable Polyphenols | Extractable Anthocyanins | Total Tannins | K      | Mg      | P     | Zn      |
|-----------------------------------|---------|-------------------------|--------------------------|---------------|--------|---------|-------|---------|
| 140Ru                             | 13.6 a  | 30.9 a                  | 230 a                    | 2.28 a        | 2167 c | 101.9 b | 185 a | 0.28 a  |
| 110R                              | 13.9 ab | 31.4 a                  | 270 b                    | 1.93 a        | 2045 b | 98.8 ab | 187 a | 0.29 a  |
| 161-49C                           | 15.4 b  | 42.3 b                  | 284 b                    | 2.80 b        | 1943 a | 94.8 a  | 209 b | 0.35 b  |
| <b>Microbial inoculation (MI)</b> |         |                         |                          |               |        |         |       |         |
| I                                 | 13.5    | 32.1                    | 250                      | 2.11          | 2056   | 99.0    | 189   | 0.29    |
| NI                                | 15.1    | 37.7                    | 273                      | 2.56          | 2048   | 98.0    | 198   | 0.32    |
| <b>Year</b>                       |         |                         |                          |               |        |         |       |         |
| 2018                              | 19.9 d  | 39.8 b                  | 137 a                    | 2.06 ab       | 2804 e | 113.6 c | 223 b | 0.31 b  |
| 2020                              | 12.2 b  | 24.4 a                  | 193 b                    | 1.53 a        | 2259 d | 105.8 b | 250 c | 0.44 d  |
| 2021                              | 13.5 b  | 43.1 b                  | 335 c                    | 3.30 c        | 1350 a | 85.8 a  | 159 a | 0.28 b  |
| 2022                              | 9.8 a   | 24.1 a                  | 207 b                    | 2.36 b        | 2014 c | 104.3 b | 175 a | 0.39 c  |
| 2023                              | 16.1 c  | 42.9 b                  | 436 d                    | 2.43 b        | 1831 b | 82.9 a  | 161 a | 0.099 a |

Table 7. Cont.

| Rootstock (R) | TPI | Extractable Polyphenols | Extractable Anthocyanins | Total Tannins | K   | Mg  | P   | Zn  |
|---------------|-----|-------------------------|--------------------------|---------------|-----|-----|-----|-----|
| ANOVA         |     |                         |                          |               |     |     |     |     |
| R             | *   | *                       | **                       | **            | *** | **  | *** | **  |
| MI            | *   | *                       | *                        | *             | ns  | ns  | *   | *   |
| Year          | *** | ***                     | ***                      | ***           | *** | *** | *** | *** |
| R × MI        | ns  | ns                      | ns                       | ns            | ns  | *   | ns  | ns  |

ns, not significant; \*, \*\*, and \*\*\* indicate significant differences at the 0.05, 0.01, and 0.001 levels of probability, respectively. In each column and for each factor, different letters indicate significant differences according to Duncan's multiple range test at a 95% confidence level.

Some differences among rootstocks in berry technological and phenolic parameters were observed at an early stage (Tables S1 and S2). In 2018, vines on 140Ru showed lower TSS, MI, tartaric acid, total sugars, must percentage, anthocyanins,  $A_{520}$ , and CI, but higher  $L^*$ ,  $a^*$ , and  $C^*$  values than 110R and 161-49C. Conversely, 161-49C displayed lower acidity and higher tartaric acid and MI levels than the other rootstocks.

### 3.3. Effect of Microbial Inoculation and Its Interaction with the Rootstock

#### 3.3.1. Soil Respiration and Organic Matter Cycling

During the post-veraison period in 2021, a significant interaction between rootstock and microbial inoculation was observed, with inoculated 161-49C vines showing a markedly higher ODR than NI vines. Conversely, no significant differences in ODR were detected between I and NI vines for 110R and 140Ru.

The decomposition rate ( $k$ ) of standardized OM litter exhibited a significant increase in I treatments (Table 2). A more detailed analysis by rootstock revealed that this increase was significant only in 140Ru vines, but not in 110R (Table 2).

Repeated microbial inoculation resulted in a substantial increase in rhizospheric  $CO_2$  flux throughout the growing season in both 110R and 140Ru rootstocks relative to NI vines (Figure 4). Furthermore, microbial inoculation enhanced the daily soil water flux within the rhizosphere of 110R vines, notably during the afternoon and evening hours. Conversely, in 140Ru vines, no significant differences in water flux were observed between I and NI vines.

#### 3.3.2. Soil-Plant Water Relations and Leaf Gas Exchange

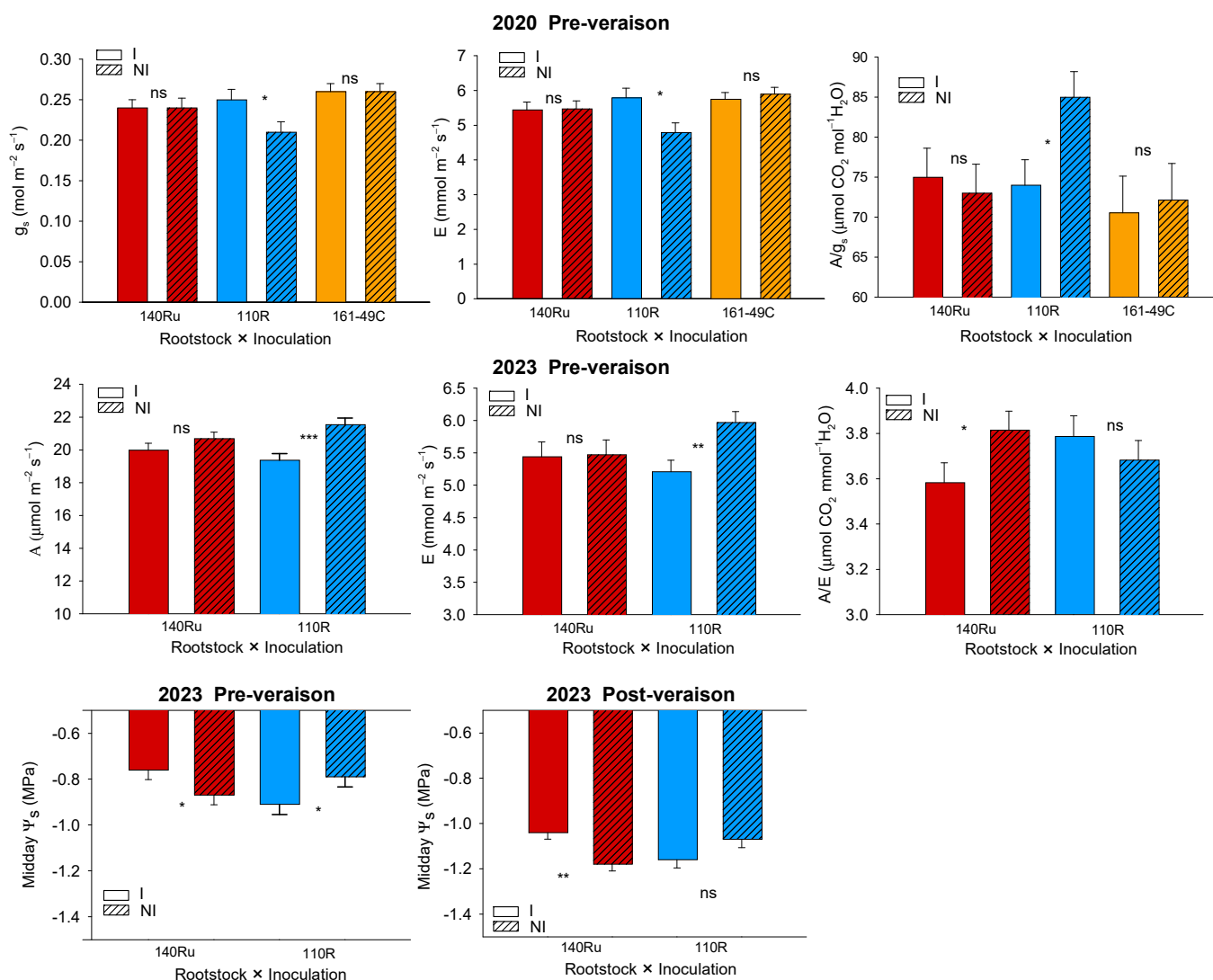
During the pre-veraison period of 2018, there were no significant differences between I and NI vines in leaf gas exchange or in  $\Psi_s$ , and no interaction with the rootstock was detected (Table 3). However, during the post-veraison period of the same year, both  $A$  and  $E$  were significantly higher in I vines than in NI vines. As a result,  $A/E$  was significantly lower in I vines (Table 3).

In 2020, a significant  $R \times MI$  interaction was observed. In 110R vines, the I treatment significantly enhanced  $E$  and decreased  $A/g_s$  during the pre-veraison period (Figure 6). However, no significant differences were identified in the other rootstocks.

In 2021, once again, the pre-veraison period demonstrated no significant differences in  $\Psi_s$  between I and NI vines, nor any interaction with the rootstock (Table 3). However, during the post-veraison period,  $A/E$  was significantly lower in I vines, following a trend similar to that observed in 2018 (Table 3).

The most noticeable effects were observed in 2023. Microbial inoculation significantly decreased  $A$  and  $E$  during the pre-veraison period, as well as  $A$ ,  $g_s$ , and  $E$  during the post-veraison period, and, consequently,  $A/g_s$  increased (Table 3). A rootstock-specific analysis revealed that, in 110R, inoculation significantly reduced  $A$  and  $E$  during the pre-veraison period, whereas no such effect was observed in 140Ru (Figure 6). Similarly, during the

pre- and post-veraison periods in 2023, inoculated 110R vines exhibited more negative  $\Psi_s$  values than NI 110R vines. Conversely, inoculated 140Ru vines demonstrated lower water stress levels (less negative  $\Psi_s$  and lower  $A/E$ ) than non-inoculated 140Ru vines (Figure 6).



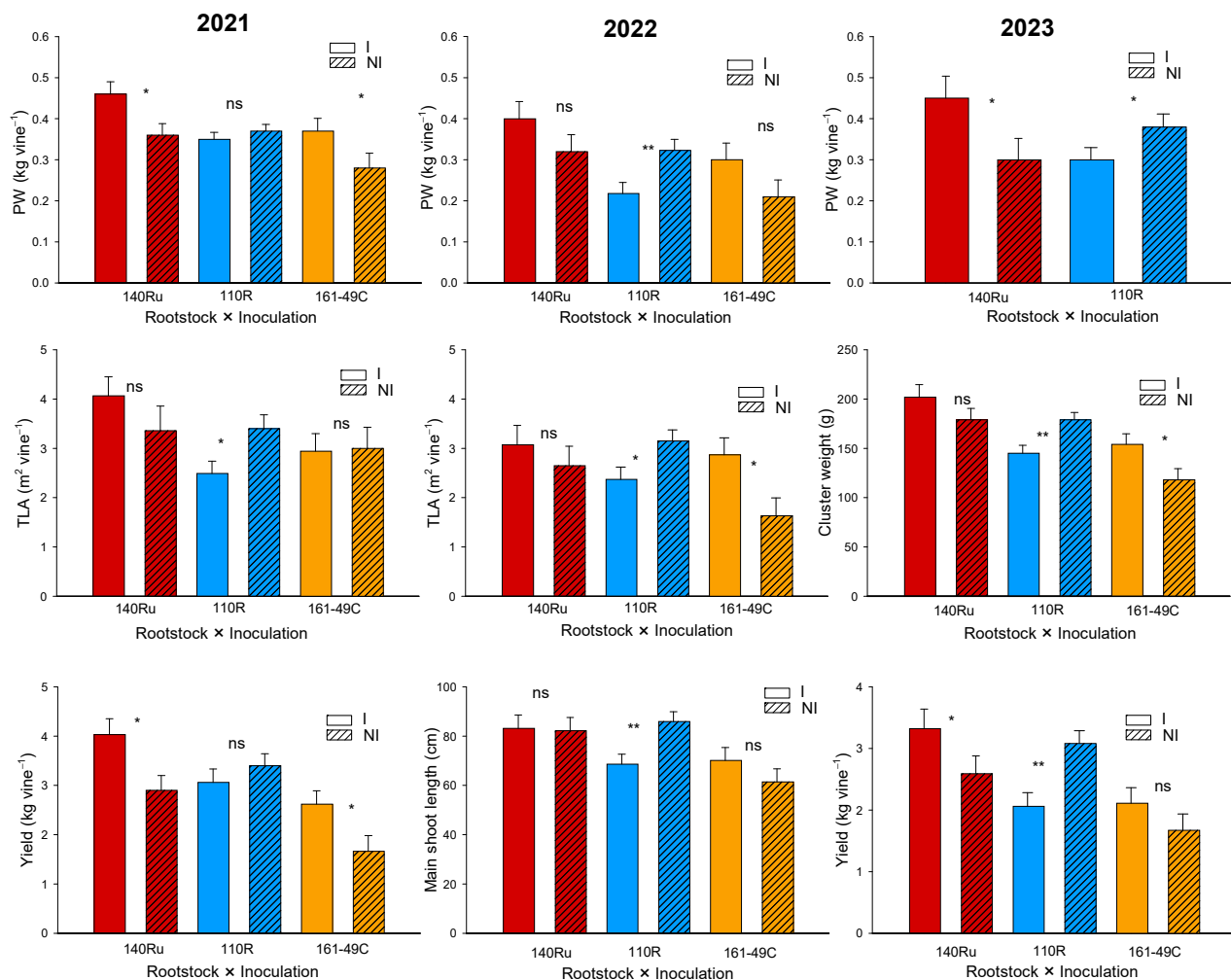
**Figure 6.** Significant differences in Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI), in leaf gas exchange parameters ( $A$ , leaf photosynthesis rate;  $g_s$ , stomatal conductance rate;  $E$ , leaf transpiration rate;  $A/g_s$ , intrinsic leaf water use efficiency, for years 2020 and 2023, and midday stem water potential [ $\Psi_s$ ], for the pre- and post-veraison periods of 2023). For each of the rootstocks, one-way ANOVA was performed for each of the physiological parameters. ns, not significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ . Mean values for each of the rootstocks were compared using Duncan's multiple range test at a 95% confidence level.

### 3.3.3. Leaf Mineral Nutrition

Inoculation had no effect on leaf mineral content in 2018 (Table 4). Nevertheless, in 2021, I vines exhibited lower Na and Fe levels and higher Zn concentrations than NI vines. A significant  $R \times MI$  interaction was also observed: in 110R, inoculation increased Mg and Mn values and decreased Na concentration; in 161-49C, it decreased Ca, Mg, and Cu levels; meanwhile, in 140Ru, it increased Ca and decreased Na levels. In 2023, microbial inoculation resulted in increased P, K, Mg, and Cu concentrations compared to NI vines. The interactions with rootstocks were also evident: in 110R, inoculation increased Zn and Cu levels, whereas in 140Ru, it led to increased concentrations of leaf Ca and Mg (Table 4).

### 3.3.4. Vegetative and Reproductive Development

Microbial inoculation enhanced several growth and yield parameters, including shoot length (2019, 2020), TLA (2020), PW (2018, 2021), yield, cluster weight (2019–2021), and the number of clusters per vine (2020), compared to NI vines (Table 5). However, rootstock-specific analysis revealed contrasting responses (Figure 7). In 110R, inoculation led to a significant reduction in PW (2022, 2023), TLA (2021, 2022), shoot length (2022), yield (2023), and  $WUE_{yield}$  (2023 Figure 5). In contrast, 140Ru vines responded positively, with increases in PW, yield (2021, 2023), and  $WUE_{yield}$  (2021, 2023). Similarly, in 161-49C, inoculation increased TLA (2021), PW, yield (2021),  $WUE_{yield}$  (2021), and cluster weight (2023) (Figure 7).



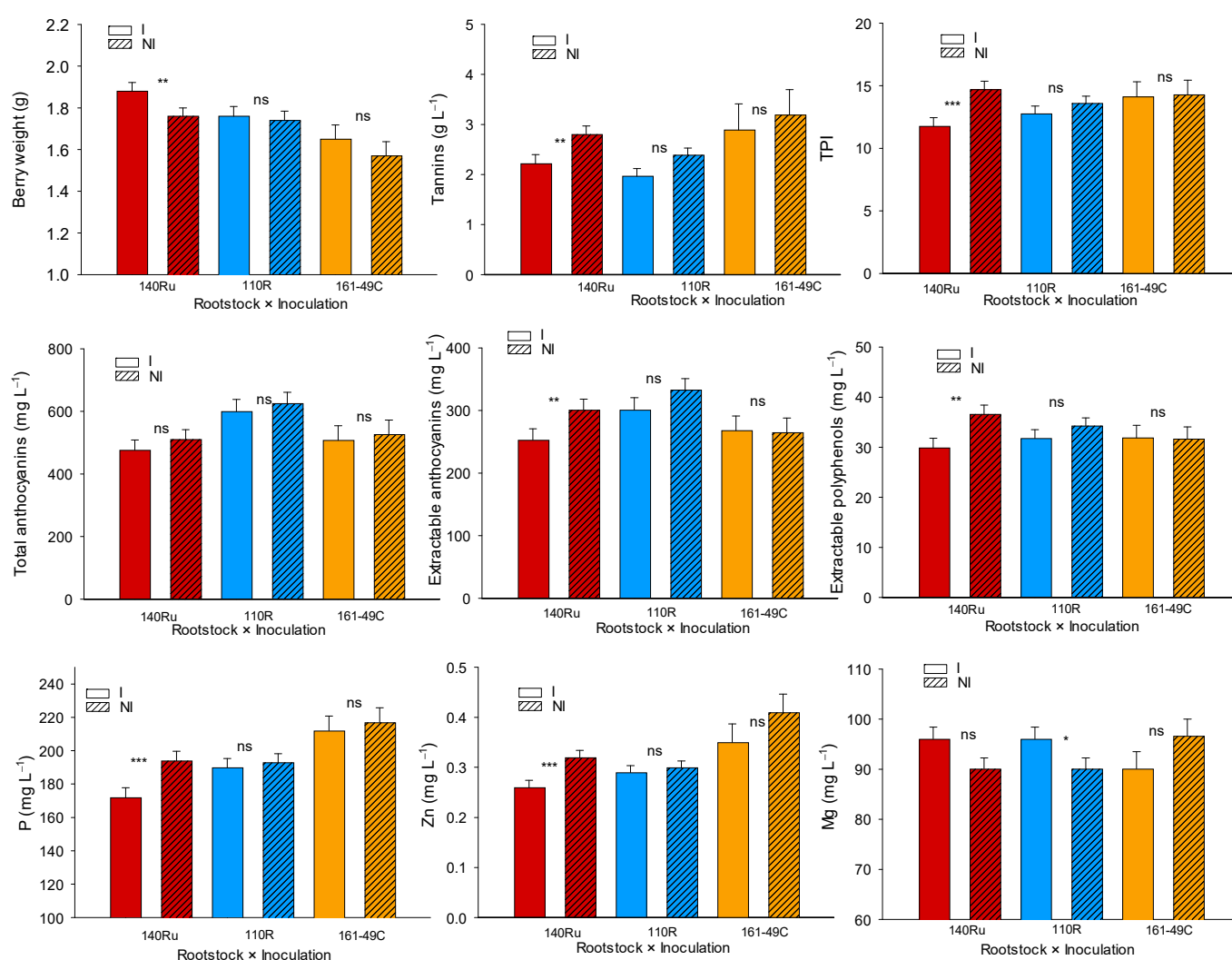
**Figure 7.** Significant interactive effects between rootstock and microbial inoculation on vegetative (main shoot length; total leaf area, TLA; winter pruning weight, PW), and reproductive parameters in Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI), for the years 2021, 2022, and 2023. For each of the rootstocks, a one-way ANOVA was performed for each vegetative and reproductive parameter. ns, not significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ . Mean values for each of the rootstocks were compared using Duncan's multiple range test at a 95% confidence level.

In general, inoculation did not significantly influence vine mortality over a five-year period (data not shown), with comparable rates observed between I and NI treatments (32–35%). However, in 110R, mortality was higher in I vines (36%) than in NI (24%), and in 161-49C, mortality was slightly lower in I (39%) than in NI (46%). Nevertheless, these differences were not statistically significant.

### 3.3.5. Berry Quality Response

Inoculation also altered the technological and phenolic composition of the berries at harvest, although these effects were contingent upon both the year and the rootstock (Tables S1 and S2). The early response, evaluated in 2018, indicated that inoculated vines had a significant reduction in TSS, total soluble sugars, TPI, extractable polyphenols, extractable anthocyanins, and tannins, whereas berry weight increased in comparison to the NI treatment.

Over the six-year period, microbial inoculation did not significantly affect the technological quality parameters of the berries (Table 6). However, it caused a significant decrease in berry polyphenolic content (TPI, extractable polyphenols, and anthocyanins), total tannins, and must nutrient content (P and Zn) (Table 7), particularly in 140Ru vines (Figure 8). In contrast, in 110R and 161-49C, microbial inoculation had no significant impact on berry phenolic quality or must nutrient concentration, except for an increased Mg concentration in the must in 110R (Figure 8).



**Figure 8.** Mean values of berry weight, berry phenolic quality parameters, and must mineral content in Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI), during the experimental period (2018–2023). ns, not significant; \*  $p < 0.1$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.01$ . Significant differences between the mean values for each of the rootstocks were determined using Duncan's multiple range test at a 95% confidence level.

## 4. Discussion

### 4.1. Effect of the Rootstock on the Performance of a Young Vineyard

Although 140Ru is frequently associated with elevated vigor in mature vines [39,43], its early effects on young vines are less comprehensively documented. In this study, a pronounced rootstock effect was observable as early as one year post-planting (2018), with vines grafted onto 140Ru exhibiting increased shoot growth, TLA, and yield (Table 5, Figure S4). This trend persisted throughout the initial five growing seasons, during which 140Ru consistently yielded greater shoot biomass and higher production than 110R or 161-49C, thereby indicating that the early differences were sustained over time. The larger leaf area associated with 140Ru may have contributed to this outcome by increasing the photosynthetic surface, thus potentially compensating for its lower photosynthesis rates and reduced leaf nutrient content (N, P, K) relative to the other rootstocks (Tables 3 and 4). The persistence of this pattern in young, productive vines suggests a possible linkage to genetic differences among rootstocks [44], with 140Ru demonstrating higher root water uptake and transport capacity [39,45–47]. These traits likely supported a more effective utilization of soil water, resulting in higher  $WUE_{yield}$  (Figure 5). Quantitatively,  $WUE_{yield}$  in 140Ru was approximately 15–20% higher than in 161-49C during the most productive years. This was further evidenced by a lower daily soil water flux in 140Ru, indicating decreased soil evaporation and more efficient water uptake in comparison to 110R (Figure 4). Leaf water potentials remained comparable across rootstocks during the initial three years, suggesting that the disparities in performance were not attributable to plant water status but rather to variations in water use efficiency.

Rootstock influenced early berry quality in young vines. Vines grafted onto 161-49C and 110R demonstrated superior berry quality, evidenced by increased CI, MI, anthocyanin/TSS ratio, polyphenol content, as well as nutrient and sugar levels in the must (Tables 6 and 7). These rootstocks also yielded berries with lower weight, pH, and malic acid concentration compared to 140Ru. These findings are consistent with previous studies on mature vines [39,44,48]. The reduced berry size observed in 161-49C and 110R likely contributed to a higher concentration of skin-based phenolic compounds. Conversely, the larger berries of 140Ru appeared to dilute these compounds, providing a plausible explanation for the lower anthocyanin and tannin concentrations despite its higher yield [49]. Additionally, berries from 140Ru accumulated greater amounts of K, resulting in increased must pH, which illustrates a trade-off between yield and color intensity that has also been reported in Tempranillo vines grafted onto 1103P versus 161-49C [48].

Five years after planting (2022), vine mortality (vines that dried up, failed to establish) and those that exhibited minimal growth and remained unproductive varied according to the rootstock: 27% in 140Ru, 30% in 110R, and 46% in 161-49C. Water stress is unlikely to explain this decline, as vines were fully irrigated during the first three years and subjected to moderate deficit irrigation in the subsequent three years. No differences in leaf water potential were observed during the early years (Table 3). Similar declines in young vines grafted onto 161-49C have been reported in vineyards in Languedoc, as well as in Germany and Italy [50]. Although the precise cause remains undetermined, affected 161-49C vines have exhibited low root carbon reserves, elevated levels of tyloses (resulting in over 40–50% xylem embolism), the presence of polyphenols and necroses (indicators of stress), and cambium dysfunction [50].

These findings indicate that more vigorous and water-efficient rootstocks, such as 140Ru and 110R, may be more suitable for establishing new vineyards under semiarid conditions and in the context of climate change. From a practical standpoint, growers must consider the trade-off between early vigor and yield (favored by 140Ru) and improved berry composition (favored by 161-49C). In future scenarios characterized by increased

drought and heat events, ensuring rapid canopy development and sustained vine function may take precedence over maximizing phenolic content, particularly when quality can be partially preserved through deficit irrigation and canopy management. Consequently, the selection of rootstocks becomes a critical factor, as low-vigor rootstocks such as 161-49C may encounter greater challenges in establishment, exhibiting early decline, and result in higher vine mortality, often leading to costly replanting.

#### 4.2. Effect of Microbial Inoculation and Its Interaction with the Rootstock in the Soil Environment

Root colonization analysis indicated a gradual increase in mycorrhizal colonization over time. In young plants, the association with soil microbiota is not yet fully established, and microbes can colonize plant niches more easily. This helps explain why mycorrhizal colonization tends to be higher in young grapevines compared to older ones [6], as reduced microbial competition facilitates the establishment of AMF in root cells [7]. Colonization rose from below 10% at planting to peak values of approximately 50–60% by late 2020 (Figure 3 and Figure S1), which reflects the high receptivity of young vines and the effect of three consecutive annual AMF inoculations (2017–2019).

The presence of mycorrhizal colonization in NI vines indicates the existence of natural fungal communities prior to the start of the trial, thereby suggesting spontaneous mycorrhization. Over time, the levels of colonization in both I and NI treatments followed a comparable trajectory, stabilizing and subsequently declining after two years in the absence of inoculation (2021–2022). These findings imply that: (a) periodic inoculation is necessary to sustain high colonization levels, as artificially increased colonization tends to decline without reinforcement; (b) the colonization observed in NI plants may have been partially attributable to cross-contamination from the inoculum, given that all of the vines were located within the same vineyard but in separated subplots; and (c) the similar colonization patterns in I and NI vines might indicate that the introduced AMF did not fully integrate with native microbial communities and, consequently, did not enhance colonization beyond natural levels.

AMF colonization capacity varies depending on fungal species, host plant, and environmental conditions [51]. Recent research has demonstrated that AMF inoculation under field conditions does not consistently replace native AMF or enhance root colonization [6]. Nevertheless, glomalin concentrations were markedly higher in I vines, particularly in 140Ru (Table 2), aligning with previous findings in inoculated seedlings [52]. This suggests that inoculation may have bolstered native colonization [53]. The application of *P. mandelii* strain 29, an MHB, may also have played a role in increasing colonization, as documented in earlier studies [12]. On average, inoculated soils contained approximately 12–13% more total glomalin than NI soils, with an increase of about 25% in 140Ru, indicating a significant rootstock  $\times$  inoculation interaction (Table 2).

Although the presence of glomalin and structures such as arbuscules, vesicles, hyphae, and spores indicates AMF colonization of grapevine roots, their detection alone does not confirm functional effectiveness or a positive impact on plant performance [6,51,54]. For instance, spore density in the rhizosphere was not correlated with root colonization under different soil conditions [34,35], highlighting the necessity to distinguish between active (arbuscules, vesicles, and hyphae) and latent, non-active (spores) mycorrhization when evaluating inoculants in field studies. Notably, following the application of *P. mandelii* strain 29 in 2020, spore density in inoculated roots increased, suggesting stimulation of AMF sporulation (Figure S3). Our data confirmed that I vines contained a higher proportion of active fungal structures than NI vines, whereas NI vines harbored more dormant spores. Conversely, the commercial inoculum applied at the conclusion of the experiment (Figure 2) contained both AMF and the PGPB *B. megaterium*, a common combination that enhances

nutrient uptake, root growth, and stress tolerance [6]. The presence of *B. megaterium* likely contributed to the improvements that were observed in soil and plant performance. To confirm that the established symbiosis was genuinely functional and advantageous, it was imperative not only to assess mycorrhizal colonization levels but also to analyze soil conditions together with physiological and agronomic parameters in the plant.

In 161-49C, AMF inoculation may have enhanced soil aeration, as evidenced by an increased oxygen diffusion rate (Table 2). Mycorrhizal hyphae are recognized for inducing soil structural modifications, such as increased porosity, aggregate stability, and pore connectivity, which facilitate gas exchange [52,55–58]. Consistent with this, inoculation significantly elevated daily soil CO<sub>2</sub> flux in 140Ru and 110R vines (Figure 4), despite no increase in O<sub>2</sub> diffusion was observed (Table 2). Litter decomposition, evaluated through the TBI study, revealed that the decomposition rate constant (*k*) was approximately 10% higher in inoculated soils, indicating a more rapid turnover of labile organic matter. Consequently, the daily soil CO<sub>2</sub> efflux recorded in 2021 was also higher in inoculated vines of 140Ru and 110R, with the most notable differences occurring during afternoon peaks (Figure 4).

The combined application of AMF, MHB, and PGPB appears to enhance the soil microenvironment, thereby supporting both root and microbial activity within vineyards [22,59,60]. The observed increase in CO<sub>2</sub> flux and elevated *k* values in I vines suggest augmented microbial activity and organic matter decomposition, which are essential processes in soil nutrient cycling and CO<sub>2</sub> release [38,61]. Notably, higher levels of total glomalin, active mycorrhization, and *k* in inoculated 140Ru vines point to a superior compatibility of this rootstock with the applied inoculum. In contrast, in 110R, microbial inoculation significantly increased daily soil water flux (Figure 4), suggesting greater surface water loss and potentially decreased water uptake efficiency in inoculated vines compared to NI.

#### 4.3. Effect of the Microbial Inoculation and Its Interaction with the Rootstock on Young Vine Performance

There is clear evidence indicating that both AMF and PGPB improve plant growth and productivity in various crops, including grapevines [1,3]. Although numerous studies demonstrate benefits from early inoculation under controlled conditions [16,51], fewer field-scale studies exist, particularly with direct in-field inoculation of young vines [7,13]. Most prior research employed pre-inoculated plant material containing AMF and/or PGPB in nursery settings [6,20]. In contrast, the present study applied microbial inoculants directly in the vineyard, bypassing nursery-stage inoculation. This field-based inoculation methodology consistently improved growth and yield parameters over seven seasons, aligning with previous field research [13,51,62]. However, the magnitude of these effects varied depending on the specific parameter, rootstock, and year. Under non-imposed DI conditions (2018–2020), AMF-inoculated vines exhibited increased leaf gas exchange rates (*A*, *E*), enhanced plant growth (PW, TLA, shoot length), and higher berry weight. These findings contrast with studies reporting adverse vegetative effects in the absence of water stress [22]. Under DI conditions (2021–2023), inoculation enhanced yield and shoot growth, with variability across different rootstocks. Specifically, vines grafted onto 140Ru and 161-49C demonstrated enhanced vegetative and reproductive traits, including higher WUE<sub>yield</sub> (Figure 5). Conversely, 110R vines exhibited reductions in TLA, yield, and WUE<sub>yield</sub>, potentially attributable to suboptimal soil-plant water relations. In 2023 (the driest season), inoculated 110R vines displayed midday  $\Psi_s$  values 0.1–0.2 MPa lower than their NI counterparts, with decreases of 10–15% in *A*, *g<sub>s</sub>*, and *E*, suggesting a possible imbalance between carbon-cost and benefit, increased water stress, and poor inoculum compatibility. Soil surface H<sub>2</sub>O flux was approximately 12% higher in inoculated 110R plots (Figure 4), indicating accelerated soil drying and earlier onset of stress. Prior research has

demonstrated that AMF-colonized vines exhibit improved WUE in other rootstocks such as 1103P and SO4 [7], whereas 110R displays pronounced AMF-rootstock specificity [16]. Only select AMF strains (e.g., *G. intraradices* BEG 72) enhanced 110R performance, whereas other native strains proved ineffective. Contrary to the outcomes observed in inoculated plants grafted onto 110R, vines inoculated onto 140Ru experienced reduced water stress and superior photosynthetic performance, attributable to larger leaf areas (Figure 8), which is consistent with previous studies [22,53]. These findings support the hypothesis that grapevine response to AMF is genotype-dependent. Certain rootstocks (e.g., 1103P) respond more favorably to specific AMF strains, whereas others show limited responsiveness [23]. Additional research indicates that AMF inoculation can influence bunch and berry weight to different extents across varieties [63], emphasizing the necessity for a precise inoculation strategy that takes rootstock-AMF compatibility into consideration. This underscores the premise that microbial inoculation effectiveness in vineyards cannot be generalized to all cases and should be optimized through targeted matching of rootstock and AMF genotypes.

The AMF/PGPB inoculation enhanced P status across all of the rootstocks compared to NI controls (e.g., in 2023; Table 4), aligning with previous studies using diverse AMF strains [4]. Leaf concentrations of other nutrients (mainly K, Mg, Zn, and Cu) also increased in I vines; however, the effects varied according to the rootstock and the year (Table 4). Specific interactions were identified: inoculated 140Ru vines exhibited increased Ca and Mg levels; 110R vines demonstrated higher Mg, Zn, Mn, and Cu values; and 161-49C vines showed slight reductions in Ca, Mg, and Cu, indicating a potential decrease in nutrient uptake under inoculation for this rootstock. Although leaf N concentration did not differ significantly, the increased TLA and yield in I vines—particularly 140Ru and 161-49C—suggest greater total N per plant, which implies enhanced N uptake and assimilation [51,64]. Must analysis revealed lower P and Zn in inoculated 140Ru berries, likely attributable to yield dilution effects, whereas Mg increased in inoculated 110R berries (Figure 8). These results support the hypothesis that AMF and other beneficial microbes (such as *Pseudomonas* spp., *Bacillus* spp.) promote grapevine nutrition by improving the availability and facilitating the translocation of key nutrients (N, P, K, Ca, Mg) [64,65]. This is probably mediated by the extensive soil exploration by AMF hyphae [3] and the inclusion of an MHB (*P. mandelii* strain 29), recognized for its phosphorus solubilization capabilities [12,31]. Additionally, this may have contributed to a higher microbial activity and faster decomposition/mineralization of organic matter (higher soil CO<sub>2</sub> flux and k; Figure 4, Table 2), thereby augmenting nutrient cycling. Furthermore, AMF inoculation could potentially upregulate the expression of nutrient transporter genes in grapevines [66]. Conversely, the literature presents inconsistent findings [5,22,65], indicating that the efficacy of nutrient enhancement by AMF is highly contingent upon both the inoculum and the plant genotype [66].

Despite year-to-year and rootstock variability (Tables S1 and S2), AMF/PGPB inoculation influenced grape berry metabolism, as previously reported [7,22,63]. In early years (2018–2020), inoculated vines exhibited lower TSS, sugar content, and MI, along with higher berry weight (Table S1). However, over five years, inoculation had no significant effect on most technological traits (Table 6). In contrast, phenolic content was consistently reduced in inoculated vines, particularly in 140Ru, which showed fewer total phenolics, extractable anthocyanins, and tannins compared to NI vines (2018–2023; Figure 8; Table 7). This reduction may reflect lower stress levels in inoculated plants and/or a dilution effect caused by increased berry size. No significant differences in berry weight or phenolic content were observed in 110R and 161-49C vines. These findings support a dilution-based hypothesis: improved water and nutrient status in inoculated vines (notably 140Ru) led to higher vigor and larger berries with a lower skin-to-pulp ratio, thereby reducing phenolic

concentration per unit of juice. This contrasts with studies under controlled conditions where AMF increased phenolic traits [4,5,67]. Our results align more closely with those of Antolín et al. [63], who observed AMF effects on berry traits to be variety-dependent, with some demonstrating increased anthocyanins and total soluble solids, whereas others (berry color, total phenolic and anthocyanin content) showed reductions.

All these results suggest that: (a) microbial inoculation exerted varying effects on soil conditions and vine performance depending on the rootstock; (b) interactions between commercial AMF/PGPB inoculants and native soil/root microbial communities were either synergistic or antagonistic, thereby influencing vine growth and yield contingent upon the rootstock; and (c) these effects are intricately linked to rootstock-specific rooting patterns and microbial compatibility. Growth promotion (140Ru, 161-49C) or suppression (110R), yield, and early plant fitness in inoculated vines may originate from differences in rooting patterns, which affect mycorrhizal responsiveness [6] and the composition of native AMF/bacterial communities associated with each of the rootstocks, as well as their compatibility with introduced foreign AMF/bacteria [7]. Recent investigations demonstrate that rootstocks shape the grapevine microbiome and influence AMF/bacterial community structure [2,24,68], chiefly through root exudate composition [69]. Generally, rootstocks perform better in conjunction with their native AMF communities [62]. The relative abundance of specific AMF species is critical, as some promote growth and nutrient uptake, whereas others may compete for root colonization or soil niches, thereby reducing benefits [62]. To advance understanding in this area, future research should aim to identify the native AMF and bacterial genera or species associated with each of the rootstocks under the specific soil and climatic conditions of this study.

## 5. Conclusions and Future Remarks

Rootstocks exhibiting high vigor and water efficiency, such as 140Ru and 110R, demonstrated superior early survival rates and fewer grafting issues than the low-vigor 161-49C under semiarid conditions and amidst climate change scenarios. Extended multi-season monitoring confirmed that rootstock selection constitutes a critical factor for the swift establishment of vineyards in hot and dry environments. 140Ru consistently exhibited the highest early shoot growth, leaf area, and cumulative yield. This performance is presumably linked to its deeper, more conductive root system, which enhances water uptake and hydraulic conductance [70], thereby compensating for its lower foliar nutrient levels through an expanded photosynthetic surface. Conversely, 161-49C, despite producing grapes of higher quality, experienced approximately 50% vine loss or severe decline by year 5, aligning with reports of graft failure and early decline in arid climates.

Microbial co-inoculation enhanced early vine performance in 140Ru and 161-49C, but not in 110R. Vines inoculated on 140Ru and 161-49C exhibited larger canopies, increased PW, and higher yields, along with improved midday  $\Psi_s$  and elevated  $WUE_{yield}$  under deficit irrigation conditions. Conversely, in 110R, inoculation marginally decreased  $A$ ,  $\Psi_s$ , TLA, yield, and  $WUE_{yield}$ , suggesting poor compatibility with the microbial inoculum and possible carbohydrate competition. This underscores the necessity for a tailored inoculation strategy based on rootstock-AMF affinity. Additionally, inoculation increased berry weight in 140Ru; however, it decreased the phenolic content in the must, likely due to a dilution effect associated with a larger berry size. Sugar and acidity levels in the must remained unaffected across all of the rootstocks.

Based on these results, the integration of microbial inoculants (AMF, MHB, PGPB) into vineyard management should be undertaken cautiously. Their effectiveness relies on rootstock-microbe compatibility, and sustained benefits require repeated applications, making cost-benefit analysis crucial. There is limited knowledge on whether native AMF/PGPB

populations are adequate for colonization in new vineyards or which factors influence their establishment. Future research should focus on long-term soil management strategies to support native beneficial microbes. Agroecological practices such as reduced pesticide use, mulching, cover cropping, and agroforestry may enhance microbial diversity and colonization while delivering additional ecological benefits. In semiarid regions, these approaches could also prove to be more economically sustainable over time.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15112570/s1>, Figure S1. Evolution of the percentage of root mycorrhization in Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) during the experimental period (2017–2023). Figure S2. Evolution of the percentage of root mycorrhization in Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI) during the experimental period (2017–2023). Figure S3. Evolution of the percentage of non-active mycorrhization (spores) fraction during the experimental period (2017–2023), including the three rootstocks. Figure S4. Evolution of main shoot growth of Monastrell vines grafted onto three different rootstocks (140Ru, 110R, and 161-49C) and under two different microbial inoculation conditions (I vs. NI) during 2018. Table S1. Technological maturity parameters of Monastrell grape must at harvest, grafted onto different rootstocks (140Ru, 110R, and 161-49C) and two different microbial inoculation treatments (I vs. NI), from 2018 to 2023. Table S2. Phenolic maturity parameters of the must made from Monastrell grapes at harvest grafted onto three different rootstocks (140Ru, 110R, and 161-49 C) and under two different microbial inoculation treatments (I vs. NI) from 2018 to 2023.

**Author Contributions:** Conceptualization: P.R. and J.M.N.; methodology: P.R., J.M.N., P.B., E.I.M. and A.M.; data curation: P.R., J.M.N. and P.B.; formal analysis: P.R., J.M.N., P.B. and E.I.M.; investigation: P.R., J.M.N. and P.B.; writing—original draft preparation: P.R.; writing—review: P.R., J.M.N., P.B. and A.M.; editing: J.M.N.; funding acquisition: P.R., J.M.N. and P.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación through the project UPGRAPE “PID2021-123305OB-C33”, cofinanced by the European Union; and the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain), Subprograma Nacional de Recursos y Tecnologías Agrarias, through projects “FEDER 1420–13” and “FEDER 1420–24”, co-financed by the European Regional Development Fund (FEDER).

**Data Availability Statement:** Data will be made available upon request.

**Acknowledgments:** We want to thank Francisco Javier Martínez, Ana Verónica Martínez, Mariano Saura, Eva María Arques, Francisco Hernandez, Leandro Olivares, Fabian Soto, and Juan Antonio Palazón for their support in laboratory and field tasks. During the preparation of this manuscript, the authors used Microsoft Copilot (GPT-4 architecture, July 2025 version) to assist with the revision and refinement of the English language. This assistance was limited to language editing and did not involve content generation or interpretation of scientific results. The final version of this manuscript was additionally reviewed by Andrés Paredes, a professional English translator specialized in scientific texts.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|      |                                      |
|------|--------------------------------------|
| I    | inoculated                           |
| NI   | non-inoculated                       |
| AMF  | arbuscular mycorrhizal fungi         |
| MHB  | mycorrhizal helper bacteria          |
| PGPR | plant growth-promoting rhizobacteria |

|               |                                         |
|---------------|-----------------------------------------|
| PGPB          | plant growth-promoting bacteria         |
| DI            | deficit irrigation                      |
| ODR           | oxygen diffusion rate                   |
| TBI           | Tea Bag Index                           |
| S             | stabilization factor                    |
| k             | decomposition rate constant             |
| TG            | total glomalin                          |
| $\Psi_s$      | midday stem water potential             |
| A             | net photosynthesis rate                 |
| $g_s$         | stomatal conductance rate               |
| E             | transpiration rate                      |
| $A/g_s$       | intrinsic leaf water use efficiency     |
| A/E           | instantaneous leaf water use efficiency |
| $WUE_{yield}$ | productive water use efficiency         |
| VPD           | vapor pressure deficit                  |
| TLA           | total leaf area                         |
| PW            | winter pruning weight                   |
| SL            | shoot length                            |
| PW            | pruning weight                          |
| MI            | maturity index                          |
| TA            | titratable acidity                      |
| $A_{520}$     | absorbance at 520 nm                    |
| CI            | color index                             |
| SMI           | seed maturity index                     |
| AEI           | anthocyanin extractability index        |
| TSS           | total soluble solid                     |
| TPI           | total polyphenol index                  |

## References

- De Souza, R.; Ambrosini, A.; Passaglia, L.M.P. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Bio.* **2015**, *38*, 401–419. [\[CrossRef\]](#)
- Balestrini, R.; Sillo, F.; Boussageon, R.; Wipf, D.; Courty, P.E. The hidden side of interaction: Microbes and roots get together to improve plant resilience. *J. Plant Interact.* **2024**, *19*, 2323991. [\[CrossRef\]](#)
- Trouvelot, S.; Bonneau, L.; Redecker, D.; van Tuinen, D.; Adrian, M.; Wipf, D. Arbuscular mycorrhiza symbiosis in viticulture: A review. *Agron. Sustain. Dev.* **2015**, *35*, 1449–1467. [\[CrossRef\]](#)
- Torres, N.; Goicoechea, N.; Morales, F.; Antolín, M.C. Berry quality and antioxidant properties in *Vitis vinifera* cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop Pasture Sci.* **2016**, *67*, 961–977. [\[CrossRef\]](#)
- Torres, N.; Antolín, M.C.; Goicoechea, N. Arbuscular mycorrhizal symbiosis as a promising resource for improving berry quality in grapevines under changing environments. *Front. Plant Sci.* **2018**, *9*, 897. [\[CrossRef\]](#)
- Velaz, M.; Santesteban, L.G.; Torres, N. Mycorrhizae and grapevines: The known unknowns of their interaction for wine growers' challenges. *J. Exp. Bot.* **2025**, *76*, 3001–3015. [\[CrossRef\]](#)
- Nerva, L.; Balestrini, R.; Chitarra, W. From plant nursery to field: Persistence of mycorrhizal symbiosis balancing effects on growth-defence tradeoffs mediated by rootstock. *Agronomy* **2023**, *13*, 229. [\[CrossRef\]](#)
- De Corato, U. Soil microbiota manipulation and its role in suppressing soil-borne plant pathogens in organic farming systems under the light of microbiome-assisted strategies. *Chem. Biol. Technol. Agric.* **2020**, *7*, 17. [\[CrossRef\]](#)
- Nerva, L.; Giudice, G.; Quiroga, G.; Belfiore, N.; Lovat, L.; Perria, R.; Volpe, M.G.; Moffa, L.; Sandrini, M.; Gaiotti, F.; et al. Mycorrhizal symbiosis balances rootstock-mediated growth-defence tradeoffs. *Biol. Fertil. Soils* **2021**, *58*, 17–34. [\[CrossRef\]](#)
- Fall, A.F.; Nakabonge, G.; Ssekandi, J.; Founoune-Mboup, H.; Apori, S.O.; Ndiaye, A.; Badji, A.; Ngom, K. Roles of arbuscular mycorrhizal fungi on soil fertility: Contribution in the improvement of physical, chemical, and biological properties of the soil. *Front. Fungal Biol.* **2022**, *3*, 723892. [\[CrossRef\]](#)
- Kozikova, D.; Pascual, I.; Goicoechea, N. Arbuscular mycorrhizal fungi improve the performance of Tempranillo and Cabernet Sauvignon facing water deficit under current and future climatic conditions. *Plants* **2024**, *13*, 1155. [\[CrossRef\]](#)
- Guarnizo, A.L.; Navarro-Ródenas, A.; Calvo-Polanco, M.; Marqués-Gálvez, J.E.; Morte, A. A mycorrhizal helper bacterium alleviates drought stress in mycorrhizal *Helianthemum almeriense* plants by regulating water relations and plant hormones. *Environ. Exp. Bot.* **2023**, *207*, 105228. [\[CrossRef\]](#)

13. Rolli, E.; Marasco, R.; Saderi, S.; Corretto, E.; Mapelli, F.; Cherif, A.; Borin, S.; Valenti, L.; Sorlini, C.; Daffonchio, D. Root-associated bacteria promote grapevine growth: From the laboratory to the field. *Plant Soil* **2017**, *410*, 369–382. [\[CrossRef\]](#)
14. Khabbaz, S.E.; Ladhalakshmi, D.; Babu, M.; Kandan, A.; Ramamoorthy, V.; Saravanakumar, D.; Al-Mughrabi, T.; Kandasamy, S. Plant growth promoting bacteria (PGPB)—A versatile tool for plant health management. *Can. J. Pestic. Pest Manag.* **2019**, *1*, 1–25. [\[CrossRef\]](#)
15. Sangwan, S.; Prasanna, R. Mycorrhizae helper bacteria: Unlocking their potential as bioenhancers of plant-arbuscular mycorrhizal fungal associations. *Microb. Ecol.* **2022**, *84*, 1–10. [\[CrossRef\]](#)
16. Camprubí, A.; Estaún, V.; Nogales, A.; García-Figueroles, F.; Pitet, M.; Calvet, C. Response of the grapevine rootstock Richter 110 to inoculation with native and selected arbuscular mycorrhizal fungi and growth performance in a replant vineyard. *Mycorrhiza* **2008**, *18*, 211–216. [\[CrossRef\]](#)
17. Rolli, E.; Marasco, R.; Vigani, G.; Ettoumi, B.; Mapelli, F.; Deangelis, M.L.; Gandolfi, C.; Casati, E.; Previtali, F.; Gerbino, R.; et al. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ. Microbiol.* **2015**, *17*, 316–331. [\[CrossRef\]](#)
18. Liu, M.; Shen, Y.; Li, Q.; Xiao, W.; Song, X. Arbuscular mycorrhizal fungal colonization and soil pH induced by nitrogen and phosphorus additions affects leaf C:N:P stoichiometry in Chinese fir (*Cunninghamia lanceolata*) forests. *Plant Soil* **2021**, *461*, 421–440. [\[CrossRef\]](#)
19. Wahab, A.; Muhammad, M.; Munir, A.; Abdi, G.; Zaman, W.; Ayaz, A.; Khizar, C.; Reddy, S.P.P. Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants* **2023**, *12*, 3102. [\[CrossRef\]](#)
20. Schreiner, R.P.; Moyer, M.M.; East, K.E.; Zasada, I.A. Managing arbuscular mycorrhizal fungi in arid Columbia Basin vineyards of the Pacific Northwest United States. *Am. J. Enol. Viticult.* **2023**, *74*, 0740020. [\[CrossRef\]](#)
21. Holland, T.C.; Bowen, P.; Bogdanoff, C.; Hart, M.M. How distinct are arbuscular mycorrhizal fungal communities associating with grapevines? *Biol. Fertil. Soils* **2014**, *50*, 667–674. [\[CrossRef\]](#)
22. Torres, N.; Yu, R.; Kurtural, S.K. Arbuscular mycorrhizal fungi inoculation and applied water amounts modulate the response of young grapevines to mild water stress in a hyper-arid season. *Front. Plant Sci.* **2021**, *11*, 622209. [\[CrossRef\]](#)
23. Giovannini, L.; Pagliarani, C.; Moine, A.; Giannetti, G.; Carra, A.; Nerva, L.; Chitarra, W.; Gambino, G.; Sbrana, C.; Balestrini, R. The impact of the symbiont genotype on the effect of arbuscular mycorrhizal symbiosis on grapevine growth and physiology. *Plant Biosyst.* **2025**, 1–13. [\[CrossRef\]](#)
24. Darriaut, R.; Antonielli, L.; Martins, G.; Ballestra, P.; Vivin, P.; Marguerit, E.; Mitter, B.; Masneuf-Pomarède, I.; Compant, S.; Ollat, N.; et al. Soil composition and rootstock genotype drive the root associated microbial communities in young grapevines. *Front. Microbiol.* **2022**, *13*, 1031064. [\[CrossRef\]](#)
25. Niem, J.M.; Billones-Baaijens, R.; Stodart, B.; Savocchia, S. Diversity profiling of grapevine microbial endosphere and antagonistic potential of endophytic pseudomonas against grapevine trunk diseases. *Front. Microbiol.* **2020**, *11*, 477. [\[CrossRef\]](#)
26. Petit, E.; Gubler, W.D. Influence of *Glomus intraradices* on Black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. *Plant Dis.* **2006**, *90*, 1481–1484. [\[CrossRef\]](#)
27. Li, H.Y.; Yang, G.D.; Shu, H.R.; Yang, Y.T.; Ye, B.X.; Nishida, I.; Zheng, C.C. Colonization by the arbuscular mycorrhizal fungus *Glomus versiforme* induces a defense response against the root-knot nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene VCH3. *Plant Cell Physiol.* **2006**, *47*, 154–163. [\[CrossRef\]](#)
28. Lailheugue, V.; Darriaut, R.; Tran, J.; Morel, M.; Marguerit, E.; Lauvergeat, V. Both the scion and rootstock of grafted grapevines influence the rhizosphere and root endophyte microbiomes, but rootstocks have a greater impact. *Environ. Microbiome.* **2024**, *19*, 24. [\[CrossRef\]](#)
29. Pandian, K.; Samiyappan, K.; Rangasamy, A.; Raju, I.; Chitraputhirapillai, S.; Bose, J. *Bacillus megaterium*-embedded organo biochar phosphorous fertilizer improves soil microbiome and nutrient availability to enhance black gram (*Vigna mungo* L.) growth and yield. *Soil Sci. Plant Nutr.* **2024**, *24*, 2048–2063. [\[CrossRef\]](#)
30. Zhao, Y.; Zhang, M.; Yang, W.; Di, H.J.; Ma, L.; Liu, W.; Li, B. Effects of microbial inoculants on phosphorus and potassium availability, bacterial community composition, and chili pepper growth in a calcareous soil: A greenhouse study. *J. Soils Sediments* **2019**, *19*, 3597–3607. [\[CrossRef\]](#)
31. Navarro-Ródenas, A.; Berná, L.M.; Lozano-Carrillo, C.; Andriano, A.; Morte, A. Beneficial native bacteria improve survival and mycorrhization of desert truffle mycorrhizal plants in nursery conditions. *Mycorrhiza* **2016**, *26*, 769–779. [\[CrossRef\]](#)
32. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–161. [\[CrossRef\]](#)
33. McGonigle, T.P.; Miller, M.H.; Evans, D.G.; Fairchild, G.L.; Swan, J.A. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **1990**, *115*, 495–501. [\[CrossRef\]](#)

34. Han, X.; Xu, C.; Wang, Y.; Huang, D.; Fan, Q.; Xin, G.; Müller, C. Dynamics of arbuscular mycorrhizal fungi in relation to root colonization, spore density, and soil properties among different spreading stages of the exotic plant three flower beggarweed (*Desmodium triflorum*) in a *Zoysia tenuifolia* lawn. *Weed Sci.* **2019**, *67*, 730–742. [\[CrossRef\]](#)
35. Sharma, K.; Singh, M.; Srivastava, D.K.; Singh, P.K. Exploring the diversity, root colonization, and morphology of arbuscular mycorrhizal fungi in Lamiaceae. *J. Basic Microbiol.* **2025**, *65*, e2400379. [\[CrossRef\]](#)
36. Gerdemann, J.W.; Nicolson, T.H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **1963**, *46*, 235–244. [\[CrossRef\]](#)
37. Wright, S.F.; Upadhayaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil.* **1998**, *198*, 97–107. [\[CrossRef\]](#)
38. Keuskamp, J.A.; Dingemans, B.J.J.; Lehtinen, T.; Sameel, J.M.; Hefting, M.M. Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems. *Methods Ecol. Evol.* **2013**, *4*, 1070–1075. [\[CrossRef\]](#)
39. Romero, P.; Botía, P.; Navarro, J.M. Selecting rootstocks to improve vine performance and vineyard sustainability in deficit irrigated *Monastrell* grapevines under semiarid conditions. *Agric. Water Manag.* **2018**, *209*, 73–93. [\[CrossRef\]](#)
40. Romero, P.; Gil-Muñoz, R.; del Amor, F.; Valdés, E.; Fernández-Fernández, J.I.; Martínez-Cutillas, A. Regulated deficit irrigation based upon optimum water status improves phenolic composition in *Monastrell* grapes and wines. *Agric. Water Manag.* **2013**, *121*, 85–101. [\[CrossRef\]](#)
41. Saint-Cricq, N.; Vivas, N.; Glories, Y. Maturité phénolique: Définition et contrôle. *Rev. Fr. d'Oenolog.* **1998**, *173*, 22–25.
42. Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdied, D. *Handbook of Enology Volume 2: The Chemistry of Wine and Stabilization and Treatments*, 3rd ed.; John Wiley & Sons, Ltd.: Chichester, UK, 2006; p. 456.
43. Wooldridge, J.; Louw, P.J.E.; Conradie, W.J. Effects of rootstock on grapevine performance, petiole and must composition, and overall wine score of *Vitis vinifera* cv. Chardonnay and Pinot noir. *S. Afr. J. Enol. Vitic.* **2010**, *31*, 45–48. [\[CrossRef\]](#)
44. Santesteban, L.G.; Rekarte, I.; Torres, N.; Galar, M.; Villa-Llop, A.; Visconti, F.; Intrigliolo, D.S.; Escalona, J.M.; Herralde, F.; Miranda, C. The role of rootstocks for grape growing adaptation to climate change. Meta-analysis of the research conducted in Spanish viticulture. *OENO One* **2023**, *57*, 283–290. [\[CrossRef\]](#)
45. Romero, P.; Botía, P.; Morote, E.; Navarro, J.M. Optimizing deficit irrigation in *Monastrell* vines grafted on rootstocks of different vigour under semiarid conditions. *Agric. Water Manag.* **2024**, *292*, 108669. [\[CrossRef\]](#)
46. Lovisolo, C.; Lavoie-Lamoureux, A.; Tramontini, S.; Ferrandino, A. Grapevine adaptations to water stress: New perspectives about soil/plant interactions. *Theor. Exp. Plant Physiol.* **2016**, *28*, 53–66. [\[CrossRef\]](#)
47. Tramontini, S.; van Leeuwen, C.; Domec, J.C.; Destrac-irvine, A.; Basteau, C.; Vitali, M.; Mosbach-Schulz, O.; Lovisolo, C. Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant Soil* **2013**, *368*, 215–230. [\[CrossRef\]](#)
48. Pou, A.; Rivacoba, L.; Portu, J.; Mairata, A.; Labarga, D.; García-Escudero, E.; Martín, I. How rootstocks impact the scion vigour and vine performance of *Vitis vinifera* L. cv. Tempranillo. *Aust. J. Grape Wine Res.* **2022**, *2022*, 9871347. [\[CrossRef\]](#)
49. Navarro, J.M.; Botía, P.; Romero, P. Changes in berry tissues in *Monastrell* grapevines grafted on different rootstocks and their relationship with berry and wine phenolic content. *Plants* **2021**, *10*, 2585. [\[CrossRef\]](#)
50. Spilmont, A.S.; Sereno, C.; El khoti, N.; Torregrosa, L. The decline of the young vines grafted onto 161-49C. *Acta Hort.* **2016**, *1136*, 251–264. [\[CrossRef\]](#)
51. Berdeja, M.P.; Reynolds, N.K.; Pawlowska, T.; Vanden Heuvel, J.E. Commercial bioinoculants improve colonization but do not alter the arbuscular mycorrhizal fungal community of greenhouse-grown grapevine roots. *Environ. Microbiome* **2025**, *20*, 15. [\[CrossRef\]](#)
52. Zhang, Z.; Mallik, A.; Zhang, J.; Huang, Y.; Zhou, L. Effects of arbuscular mycorrhizal fungi on inoculated seedling growth and rhizosphere soil aggregates. *Soil Tillage Res.* **2019**, *194*, 104340. [\[CrossRef\]](#)
53. Nicolás, E.; Maestre-Valero, J.F.; Alarcón, J.J.; Pedrero, F.; Vicente-Sánchez, J.; Bernabé, A.; Gómez-Montiel, J.A.; Hernández, J.A.; Fernández, F. Effectiveness and persistence of arbuscular mycorrhizal fungi on the physiology, nutrient uptake and yield of Crimson seedless grapevine. *J. Agric. Sci.* **2015**, *153*, 1084–1096. [\[CrossRef\]](#)
54. Peng, Y.-Y.; Liu, C.-Y.; Hao, Y. Differential effects of arbuscular mycorrhizal fungi on rooting and physiology of ‘Summer Black’ grape cuttings. *Horticulturae* **2025**, *11*, 825. [\[CrossRef\]](#)
55. Rillig, M.C.; Mummey, D.L. Mycorrhizas and soil structure. *New Phytol.* **2006**, *171*, 41–53. [\[CrossRef\]](#)
56. Leifheit, E.F.; Veresoglou, S.D.; Lehmann, A.; Morris, E.K.; Rillig, M.C. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation—A meta-analysis. *Plant Soil* **2014**, *374*, 523–537. [\[CrossRef\]](#)
57. Bitterlich, M.; Sandmann, M.; Graefe, J. Arbuscular mycorrhiza improves substrate hydraulic conductivity in tomato by altering pore connectivity. *Front. Plant Sci.* **2018**, *9*, 301. [\[CrossRef\]](#)
58. Hao, J.; Cui, B.; Liu, B.; Wu, Y.; Zhang, C. Effects of biochar and arbuscular mycorrhizal fungi on winter wheat growth and soil N<sub>2</sub>O emissions in different phosphorus environments. *Front. Plant Sci.* **2022**, *13*, 1069627. [\[CrossRef\]](#)

59. Ganugi, P.; Soverini, M.; Di Lorenzo, M.; Fiorini, A.; Tittarelli, A. A 3-year application of different mycorrhiza-based plant biostimulants distinctively modulates photosynthetic performance, leaf metabolism, and fruit quality in grapes (*Vitis vinifera* L.). *Front. Plant Sci.* **2023**, *14*, 1236199. [[CrossRef](#)]
60. Aguilera, P.; Barra, P.J.; Pollak, B.; Varela, S. Application of arbuscular mycorrhizal fungi in vineyards: Water and biotic stress under a climate change scenario. *Front. Microbiol.* **2022**, *13*, 826571. [[CrossRef](#)] [[PubMed](#)]
61. MacDonald, E.; Martin, E.; Brummell, M.E.; Bieniada, A.; Elliott, J.; Engering, A.; Tasha-Leigh, G.; Saraswati, S.; Touchette, S.; Turmel-Courchesne, L.; et al. Using the Tea Bag Index to characterize decomposition rates in restored peatlands. *Boreal Environ. Res.* **2018**, *23*, 221–235.
62. Moukarzel, R.; Ridgway, H.J.; Waller, L.; Guerin-Laguette, A.; Cripps-Guazzone, N.; Jones, E.E. Soil arbuscular mycorrhizal fungal communities differentially affect growth and nutrient uptake by grapevine rootstocks. *Microb. Ecol.* **2023**, *86*, 1035–1049. [[CrossRef](#)]
63. Antolín, M.C.; Izurdiaga, D.; Urmeneta, L.; Pascual, I.; Irigoyen, J.J.; Goicoechea, N. Dissimilar responses of ancient grapevines recovered in Navarra (Spain) to arbuscular mycorrhizal symbiosis in terms of berry quality. *Agronomy* **2020**, *10*, 473. [[CrossRef](#)]
64. Berdeja, M.P.; Ye, Q.; Bauerle, T.L.; Vanden Heuvel, J.E. Commercial bioinoculants increase root length colonization and improve petiole nutrient concentration of field-grown grapevines. *HortTechnology* **2023**, *33*, 48–58. [[CrossRef](#)]
65. Nogales, A.; Santos, E.S.; Abreu, M.M.; Arán, D.; Victorino, G.; Pereira, H.S.; Lopez, C.M.; Viegas, W. Mycorrhizal inoculation differentially affects grapevine's performance in copper contaminated and non-contaminated soils. *Front. Plant Sci.* **2019**, *9*, 1906. [[CrossRef](#)] [[PubMed](#)]
66. Balestrini, R.; Salvioli, A.; Dal Molin, A.; Novero, M.; Gabelli, G.; Paparelli, E.; Marroni, F.; Bonfante, P. Impact of an arbuscular mycorrhizal fungus versus a mixed microbial inoculum on the transcriptome reprogramming of grapevine roots. *Mycorrhiza* **2017**, *27*, 417–430. [[CrossRef](#)] [[PubMed](#)]
67. Bruisson, S.; Maillot, P.; Schellenbaum, P.; Walter, B.; Gindro, K.; DeglèneBenbrahim, L. Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection. *Phytochemistry* **2016**, *131*, 92–99. [[CrossRef](#)] [[PubMed](#)]
68. Moukarzel, R.; Ridgway, H.J.; Gurein-Laguette, A.; Jones, E.E. Grapevine rootstocks drive the community structure of arbuscular mycorrhizal fungi in New Zealand vineyards. *J. Appl. Microbiol.* **2021**, *131*, 2941–2956. [[CrossRef](#)]
69. Marastoni, L.; Lucini, L.; Miras-Moreno, B.; Marco Trevisan, M.; Segá, D.; Zamboni, A.; Varanini, Z. Changes in physiological activities and root exudation profile of two grapevine rootstocks reveal common and specific strategies for Fe acquisition. *Sci. Rep.* **2020**, *10*, 18839. [[CrossRef](#)]
70. Romero, P.; Acosta, J.M.N.; Pérez-Pérez, J.G.; Dodd, I.C.; Antolinos, V.; Marín, J.L.; del Amor Saavedra, F.; Molina, E.M.; Ordaz, P.B. The rootstock imparts different drought tolerance strategies in long-term field-grown deficit irrigated grapevines. *Sci. Hortic.* **2025**, *346*, 114167. [[CrossRef](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.