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Evaluation of commercial arbuscular mycorrhizal fungal inoculants in grapevine rootstocks through root colonization, community profiles and plant responses to drought

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Abstract

Background: Drought increasingly constrains viticulture and arbuscular mycorrhizal fungal (AMF) inoculants are claimed to enhance grapevine resilience to this stress. However, whether inoculated AMF strains establish in roots, restructure resident communities, and consistently enhance plant performance across different rootstocks and water regimes remains poorly understood. In a factorial glasshouse pot experiment, three ungrafted grapevine rootstocks (SO4, Börner and Vinto) were inoculated with one of three commercial AMF products (A, B or C) or left uninoculated, and were evaluated during establishment, drought and recovery phases. We quantified mycorrhization frequency and intraradical structures by microscopy, characterized root AMF communities by Glomeromycota-targeted partial SSU rRNA gene amplicon

sequencing at establishment, and measured root and shoot dry weight, midday leaf water potential, proline, mineral nutrition, and leaf optical traits.

Results: Inoculation was associated with higher mycorrhization frequency and intraradical structure frequencies than the Control in several rootstock-inoculant comparisons, mainly during establishment; rootstock dependence was supported only for hyphal frequency. Most root profiles were dominated by one virtual taxon (VTX). Exploratory tests detected treatment differences in VTX00114 occurrence (Holm-adjusted $P = 0.018$), but not VTX relative abundance. VTX00114, assigned to *Rhizoglomus irregulare*, was detected in 50.0% and 33.3% of roots inoculated with Inoculants A and B, respectively, but not in Control roots or those receiving Inoculant C. In Börner, all inoculants increased root dry weight relative to the Control under well-watered conditions, whereas Inoculant C decreased root dry weight under drought (supported by an inoculation × water interaction). In SO4, Inoculants A and B were associated with reduced shoot dry weight during establishment. Drought-affected plants exhibited more negative leaf water potentials, and drought-stressed Control plants accumulated higher proline concentrations than well-watered Controls. However, no consistent inoculation-related improvements in leaf water potential, proline, mineral nutrition or optical traits were observed.

Conclusions: Inoculation-associated mycorrhization and community patterns were not consistently accompanied by improved plant performance. Root colonization, community profiles, inoculant establishment, and functional benefit must be evaluated as distinct outcomes. Furthermore, responses under glasshouse pot conditions should be interpreted within specific product × rootstock × water combinations, as they may not directly translate to field vineyard conditions.

Keywords: Vitis, drought stress, microbial inoculants, root colonization, amplicon sequencing

Background

Climate change is intensifying drought across major viticultural regions, compromising yield, berry quality, and the long-term viability of established vineyards [1–3]. Because most commercial *Vitis vinifera* L. vineyards rely on grafted vines, rootstock selection is a key adaptation strategy, as rootstocks influence scion water use, nutrient acquisition and vigor [4, 5]. Complementary to genotype-based adaptation, manipulation of the root microbiome, particularly through inoculation with arbuscular mycorrhizal fungi (AMF), has attracted growing interest as a potentially sustainable approach to enhancing grapevine tolerance to abiotic stress [6].

AMF form symbioses with most terrestrial plants, extending the effective absorptive surface of the root system and potentially improving water and nutrient acquisition, osmotic adjustment, and antioxidant capacity under stress [7–10]. Meta-analyses indicate that AMF can improve stomatal conductance, biomass production, and nutrient uptake under water deficit [11, 12], while direct evidence shows that extraradical hyphae transport water to hosts and can enhance soil-plant hydraulic conductance [13, 14]. In grapevine, mycorrhization has been associated with improved water status and mineral nutrition, and commercial AMF inoculants are marketed for viticultural use [15–17]. However, arbuscular mycorrhizal symbiosis spans a mutualism-parasitism continuum determined by host genotype, fungal identity, and environmental conditions [18–21], and severe water limitation may shift the balance between its costs and benefits [21].

AMF inoculation must be distinguished from naturally occurring mycorrhizal symbiosis. Grapevine roots in non-sterile soils are commonly colonized by resident AMF communities shaped by soil conditions, management, and host genotype [15, 22–25]. Externally applied

propagules must be viable and of sufficient quality [26], compatible with the host and soil environment, and able to colonize roots in the presence of resident fungi [23]. Competition and priority effects may therefore restrict their establishment [23, 27]. Total microscopic colonization cannot distinguish resident from introduced AMF, so demonstrating establishment of an inoculant requires molecular tracking capable of distinguishing introduced fungi from resident populations [27, 28]. The rationale for inoculation is consequently not that vineyard soils necessarily lack AMF, but that selected viable propagules may augment resident communities during stages or under conditions in which colonization or function is limiting [23, 26, 29]. Nursery and early-establishment stages may be relevant intervention windows because early mycorrhizal associations can persist after transplantation [30, 31]. Outcomes nevertheless vary: inoculants may increase colonization and plant traits without detectably restructuring resident AMF communities, colonize roots without improving field growth, or fail to establish [27, 28, 31].

Commercial formulations containing AMF may also differ in their declared taxa, propagule densities, and additional components. This compositional variability is biologically relevant because AMF species and isolates vary in colonization strategy, resource exchange, and effects on host performance [21, 26]. *Rhizophagus irregularis* and *Funneliformis mosseae* are among the AMF most frequently used in grapevine experiments and commercial formulations, although grapevine studies have also evaluated multispecies products containing taxa such as *Claroideoglomus etunicatum*, *Septoglomus deserticola*, and *Gigaspora margarita* [28, 29]. Across grapevine research, positive, neutral, and negative responses have been reported depending on the AMF-host combination, water regime, and trait evaluated [29]. Commercial

inoculants have generally underperformed relative to inoculants formulated for research purposes [32], and in a meta-analysis only about 12% of commercial-inoculant trials combined sufficient AMF colonization with increased crop growth [33]. Total colonization, establishment of introduced fungi, changes in resident AMF communities, and functional plant benefits should therefore be treated as related but distinct measures of success.

Host genotype adds a further source of variation. Under common field conditions, rootstock genotype can have a greater effect than scion genotype on bacterial and fungal community assembly in the grapevine rhizosphere and root endosphere [24]. Consistent with this broader influence, rootstocks can recruit distinct root AMF assemblages despite similar levels of microscopic colonization, whereas scion effects appear limited [25]. This genotype-dependent variation may also have functional consequences, as rootstocks differ in growth and drought-related traits [5], and root-derived AMF communities can differentially affect biomass and nutrient acquisition [34]. Water availability adds another layer of contingency because AMF effects on growth and physiology under water deficit can differ among grapevine genetic backgrounds [35, 36]. It therefore remains unclear whether commercial inoculant effects are general or depend on the specific combination of product, rootstock genotype, and water regime.

We therefore conducted a factorial glasshouse pot experiment in non-sterile substrate, comprising three rootstocks (Börner, SO4 and Vinto), three commercial inoculants (A, B and C), an uninoculated Control and two water regimes, evaluated across establishment, drought and recovery phases. We combined microscopy, amplicon sequencing of root AMF communities, and growth and physiological measurements to assess inoculation-associated changes in root mycorrhization, AMF community composition and plant performance in the presence of resident AMF. We

hypothesized that (i) commercial inoculation increases total AMF colonization and alters root AMF community composition; and (ii) the effects of inoculation on plant growth, water status, and stress-related physiological traits vary among inoculants and rootstock genotypes and between water regimes. We also examined descriptively whether changes in colonization or community composition were accompanied by improvements in plant performance.

Methods

Plant material and experimental design

The study was conducted from May 2024 to October 2024 in a glasshouse at Hochschule Geisenheim University (HGU), Geisenheim, Germany. The fully factorial pot design combined three ungrafted grapevine rootstocks, four inoculation treatments, and two water regimes applied after establishment. The inoculation treatments consisted of an uninoculated Control and three commercial inoculants containing AMF, designated A, B, and C (Additional file 1: Supplementary Table S1). Products are anonymized because the study was designed to evaluate inoculation outcomes under defined conditions, not to rank or endorse commercial products; product identities and lot numbers are available in confidence to the editor and reviewers and, for replication purposes, from the corresponding author upon reasonable request. The rootstocks represented contrasting interspecific *Vitis* backgrounds. Börner originated from *Vitis riparia* 183 Gm × *Vitis cinerea* Arnold. Selection Oppenheim 4 (SO4) originated from *Vitis berlandieri* × *Vitis riparia*. Vinto originated from Berlandieri Rességuier #1 × Börner. Dormant, virus-tested cuttings were collected from propagation fields maintained by the Department of Plant Breeding at Hochschule Geisenheim University (Rheingau, Germany) in February 2024. Preparation and storage of the cuttings are described in Supplementary Methods S1.1.

During the initial propagation period, from day 0 to day 40, the cuttings were grown in propagation substrate (“Typ Vermehrung”, Einheitserde/Patzer Erden, Germany). On day 40, plants were transplanted into 5-L pots containing a non-sterilized experimental substrate composed of 45% nutrient-poor substrate (“Nullerde”, Einheitserde/Patzer Erden, Germany), 45% ED73 soil substrate (“Typ ED-73”, Einheitserde/Patzer, Germany), and 10% perlite (v/v/v). Consequently, inoculant effects were evaluated in the presence of a resident microbial community rather than under AMF-free conditions. The propagation and experimental substrates were not assessed for AMF propagules before use. Substrate chemical characteristics are provided in Supplementary Table S2.

Inoculation and experimental phases

At transplanting on day 40, plants showed uniform vegetative growth and were at a comparable developmental stage. Commercial AMF-containing inoculants were applied using a two-step procedure. First, the roots of each plant assigned to an inoculation treatment were immersed for approximately 5 s in a 10 g L^{-1} suspension of the corresponding product in distilled water. Second, the same inoculant was placed adjacent to each plant’s root system at a rate of 4.5, 6, or 4 g $plant^{-1}$ for Inoculants A, B, and C, respectively (Supplementary Table S1). These amounts were selected according to manufacturer-recommended application rates and label-declared product concentrations. Material retained during the root dip was not quantified separately. Control plants received neither the root dip nor inoculant. All plants received the same foliar fertilization program, with three applications each of an NPK, a Mg–S and a Ca–N foliar fertilizer between days 77 and 98 (Supplementary Methods S1.2).

153 The experiment comprised three sequential phases: establishment, drought, and recovery (Figure
154 1). During establishment, 240 plants were distributed among five spatial blocks, with four plants
155 per rootstock × inoculation combination in each block and randomized plant positions. On day
156 120, one plant per combination was destructively sampled from each of the same four blocks,
157 yielding 48 samples; the fifth block was not sampled. Within each treatment combination and
158 block, plants for destructive sampling were randomly selected. The remaining plants were mixed
159 across their original blocks and redistributed into four physical blocks. Water regimes followed a
160 fixed alternating arrangement, with two blocks per regime, to reduce accidental watering of
161 drought-treated plants. Within each block, positions of rootstock × inoculation combinations
162 were randomized. Each block contained four plants per combination, giving eight plants per
163 rootstock × inoculation × water-regime combination. At each drought endpoint, two plants per
164 combination were sampled from each of the two blocks within each water regime using the same
165 selection procedure. The remaining four plants per rootstock × inoculation × water-regime
166 combination were retained for recovery monitoring and the final destructive harvest. During
167 recovery, they were regrouped by rootstock × inoculation × previous-water-regime combination
168 in rows. Nominal numbers available for nondestructive monitoring declined from 20 during
169 establishment to eight during drought and four during recovery. The plant was the biological
170 sampling unit.

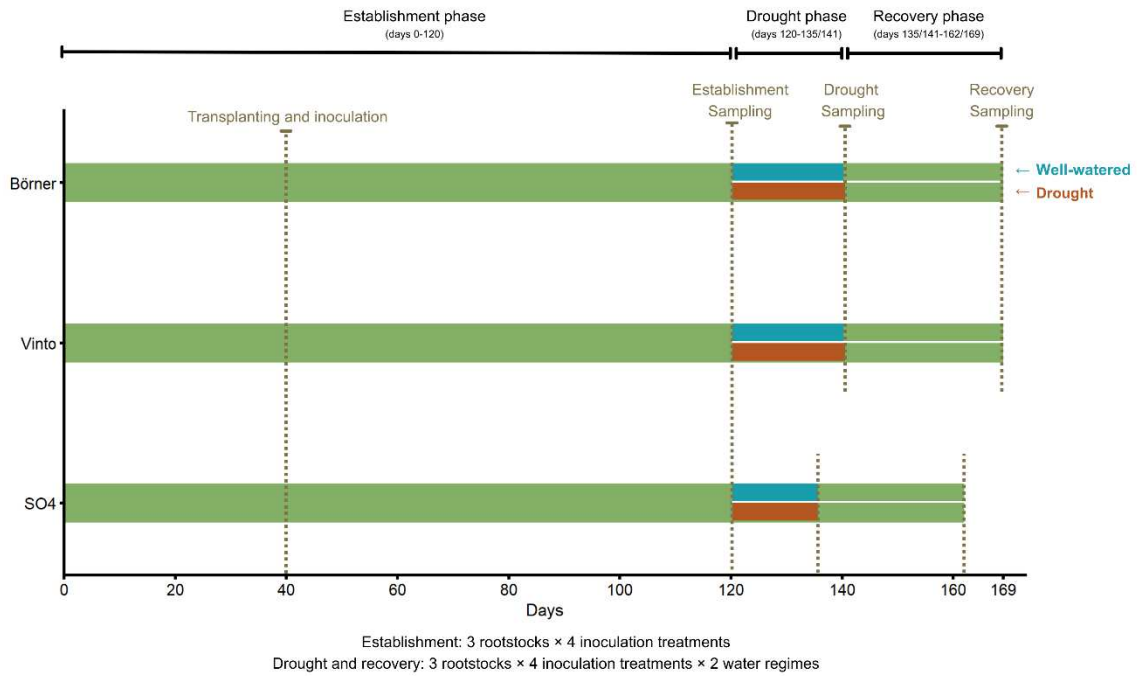


Figure 1. Experimental design and sampling timeline. Three ungrafted grapevine rootstocks received four inoculation treatments and were evaluated during establishment, drought, and recovery. Green indicates establishment and rewatered recovery periods, blue indicates well-watered conditions, and orange indicates drought. Dotted lines mark transplanting and inoculation or destructive sampling. Drought-treated plants retained for recovery were rewatered after the corresponding drought harvest. Rootstock-specific sampling dates and nominal sample sizes are provided in Supplementary Table S3.

Substrate moisture was monitored using a handheld soil meter (Uzoli SGS13, Uzoli, China), which reports relative readings on a 0–90% scale. During establishment, plants were watered every three days to maintain readings of approximately 85%. Before differential watering, all pots were adjusted to approximately 90%. Readings were then allowed to decline progressively to 20–30% in drought-treated pots, while well-watered pots were maintained at 80–90%.

Drought progression differed among rootstocks, and destructive sampling occurred on day 135 for SO4 and day 141 for Börner and Vinto. After the corresponding harvest, remaining drought-

185 treated plants were rewatered, while well-watered plants continued to receive regular watering.
186 Recovery harvests occurred on day 162 for SO4 and day 169 for Börner and Vinto. Water
187 regime during recovery therefore denotes previous treatment history. No mortality occurred, and
188 no plants were excluded from analysis. Nominal sample sizes and measurement dates are
189 summarized in Supplementary Table S3.

190 **Mycorrhization frequency and intraradical AMF structures**

191 At each destructive harvest, roots were separated from the substrate and washed thoroughly to
192 remove adhering particles. A subsample for microscopy was stored in 50% (v/v) ethanol under
193 refrigeration until processing. Root colonization was assessed by staining and light microscopy
194 following modifications of Brundrett et al. [37], McGonigle et al. [38] and Moukarzel et al. [39].
195 Root segments (1 cm) were cleared with KOH, bleached with H_2O_2, acidified and stained with
196 methylene blue (Supplementary Methods S1.3).

197 For each plant, three slides were prepared, each containing seven 1-cm root segments. Five
198 predefined points per segment were examined with a 40× objective, giving a nominal 35
199 observation points per slide and 105 points per plant. Each point was scored as positive or
200 negative for AMF colonization and for the presence of hyphae, arbuscules, vesicles and spores.
201 Mycorrhization frequency (F%) and each structure-specific frequency were calculated as the
202 percentage of positive observation points. Because the scoring system recorded incidence at each
203 point, the endpoint represents frequency rather than colonization intensity within a positive root
204 segment. The plant was the biological replicate (n = 4 per treatment combination), and the
205 observation points constituted technical subsampling within a plant.

206 **Biomass, mineral nutrition, leaf water potential and proline content**

At each destructive harvest, shoots and roots were separated and oven-dried for 48 h at 65 °C to constant mass. Oven-dried foliar material was finely milled and analyzed for P, K, Ca, Mg, Fe, Zn, Mn, Cu, C and N. Foliar samples were subjected to microwave digestion, after which P, K, Ca, Mg, Fe, Zn, Mn and Cu were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) using a SPECTRO ARCOS spectrometer (SPECTRO Analytical Instruments, Kleve, Germany). Total C (TC) and total N (TN) contents were determined by dry combustion using an elemental analyzer (Vario MAX Cube, Elementar Analysensysteme, Germany). Each foliar sample represented one plant and was analyzed in technical duplicate. The two measurements were averaged to obtain one value per element per plant for statistical analysis. The C:N ratio was calculated from the corresponding C and N concentrations.

Midday leaf water potential was measured using a Scholander-type pressure chamber. Proline content was assessed as a descriptive drought-response endpoint using freeze-dried, finely ground leaf tissue. Proline was extracted from 200 mg of lyophilized leaf tissue and quantified with an amino acid analyzer (S433, Sykam Chromatographie Vertriebs GmbH, Germany) after post-column ninhydrin derivatization, using external calibration [40, 41] (Supplementary Methods S1.5). Results were expressed as mg kg^{-1} dry weight.

Leaf optical traits

Leaf reflectance indices, including NDVI, OSAVI, PRI, RDVI, and SIPI (Additional file 2: Supplementary Data Table S4E), were obtained using a PolyPen RP 410 UVIS spectrometer (Photon Systems Instruments, Czech Republic) and its built-in calculation formulas. Leaf chlorophyll (Chl), epidermal flavonol (Flav), anthocyanin (Anth) and nitrogen balance (NBI) indices were measured using a Dualex Scientific Dx4.5 leaf-clip sensor (FORCE-A, France).

Measurements were conducted repeatedly during establishment, drought and recovery phases on common calendar days. The selected endpoint days were 100, 134 and 162 for Dualex and 115, 135 and 162 for PolyPen. Endpoint dates and nominal sample sizes are reported in Supplementary Table S3; endpoint-specific record counts are reported in Additional file 2: Supplementary Data Tables S4D and S4E. These variables were classified as exploratory endpoints and were used for repeated physiological screening.

Root AMF community sequencing

Root AMF communities were characterized at the establishment harvest on day 120. Subsamples of washed roots were stored at −80 °C, ground under liquid nitrogen, and subjected to genomic DNA extraction using the DNeasy Plant Pro Kit (Qiagen, Hilden, Germany), with 30 mg of ground tissue per extraction. The analysis included 48 root samples, representing four biological replicates per rootstock × inoculation treatment combination. DNA from each commercial inoculant was extracted in triplicate from 25 mg of product material using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Product extracts were processed using the same amplification and sequencing workflow as root extracts and served as descriptive references.

AMF DNA was amplified using AML1/AML2, targeting an approximately 795-bp fragment of the Glomeromycota SSU rRNA gene [42]. DNA quality control, PCR conditions, amplicon purification and quantification, and library preparation are described in Supplementary Methods S1.7. PCR negative controls yielded no visible amplicon. Purified amplicons were barcoded using the Native Barcoding Kit 96 V14 (SQK-NBD114.96; Oxford Nanopore Technologies, UK) [43], pooled in equimolar amounts, and sequenced on an R10.4.1 PromethION flow cell

using a PromethION 2 Solo device. Raw FASTQ files were deposited in the European Nucleotide Archive (ENA) under project accession PRJEB128143 [44].

Sequencing was conducted using MinKNOW version 26.01.15 with live basecalling using the super-accuracy (SUP) model version 5.2.0. FASTQ files were concatenated for each sample, and complete amplicon sequences were extracted using a custom Python script. Reads were clustered and polished using NGSpeciesID version 0.3.1 [45]. The resulting consensus sequences were combined for each sample and assigned to AMF virtual taxa (VTX) using BLASTN version 2.13.0 [46] against the MaarjAM database [47]. VTX were used as taxonomic units to describe each sample's AMF community profile. Taxonomic affiliations were additionally compared with EUKARYOME [48], whose nomenclature was followed for concordant AMF assignments where available. Count and taxonomic assignment data were compiled using custom Python scripts into a sample-by-VTX read-count matrix. Per-sample VTX-assigned read counts and taxonomic affiliations are provided in Supplementary Data Table S4G. Information on the analysis scripts and repository is provided in Supplementary Methods S1.8.

Statistical analysis

Statistical analyses were conducted in R version 4.6.0 [49]. Continuous outcomes were analyzed using Gaussian linear models; microscopy percentages underwent empirical-logit transformation with a continuity adjustment. Establishment models included rootstock, inoculation treatment, and their interaction. Drought models included inoculation treatment, water regime, and their interaction and were fitted separately within each rootstock to accommodate rootstock-specific sampling dates. Plants were the biological sampling units, whereas water regimes were assigned at the physical-block level. Recovery outcomes were summarized descriptively following

regrouping by treatment combination. Proline was also descriptive because well-watered measurements were available only for Control plants. Primary endpoints were mycorrhization frequency, root and shoot dry weight, and leaf water potential; secondary endpoints were arbuscule, vesicle, and hyphal frequencies. Other endpoints were considered exploratory. Further details on endpoint classifications, transformations, and model specifications are provided in Supplementary Methods S2.1. Sampling days and sample sizes are provided in Supplementary Table S3.

Observed data are summarized using arithmetic means and SDs or medians, as specified. Planned contrasts between estimated marginal means (EMMs) compared each inoculant with the Control using emmeans [50]; microscopy contrasts were estimated on the empirical-logit scale. For primary and secondary endpoints, *P*-values were Holm-adjusted within each family of three inoculant-versus-Control comparisons, and 95% confidence intervals were Bonferroni-adjusted within the same family. Planned contrasts were assessed independently of omnibus interaction significance; statements of treatment dependence required support from the relevant interaction. In exploratory analyses, Benjamini–Hochberg adjustment was applied to Holm-adjusted contrast *P*-values and, separately, to omnibus *P*-values across the complete families specified in Supplementary Methods S2.2.

Establishment optical models included block as a fixed effect; ambiguous PolyPen block assignments were handled as described in Supplementary Methods S2.4. Because sample-to-block identifiers were unavailable for destructive samples, factorial treatment models were fitted without a block term, and conditional Gaussian variance bounds were used to assess the sensitivity of planned contrasts and global interaction tests to the unrecorded block structure.

Assuming the reported balanced sampling, additive block effects and homogeneous residual variance, these bounds give upper limits for *P*-values from block-adjusted models, but they do not account for block × treatment interactions or bias from non-random allocation. During drought, they were applied to inoculant-versus-Control contrasts within each water regime, and they were not applied to the water-regime main effect, leaf water potential or recovery. Figure asterisks indicate conditional Holm-adjusted *P*-value upper bounds below 0.05; M indicates nominal global interaction support under the corresponding bound, not a product-specific interaction test (Supplementary Methods S2.3; Supplementary Data Table S4B).

Model assumptions were checked with residual and influence diagnostics, and robustness was examined using HC3 covariance estimates [51, 52], an arcsine-square-root transformation for microscopy proportions and, for Dualex, exclusion of the block with uncertain treatment allocation (Supplementary Methods S2.4–S2.6; Supplementary Data Tables S4A–S4B and S4D–S4E).

Root-community analyses used the 48 individual root profiles, with relative abundances calculated from the total assigned-VTX read count per sample. Community profiles were summarized descriptively using VTX richness, dominance and mean relative abundances, with individual profiles provided in Supplementary Data Table S4G. Differences in the relative abundance of individual VTX among inoculation treatments and among rootstocks were assessed using Kruskal–Wallis tests. VTX occurrence, defined as at least one assigned read in the processed count matrix, was additionally compared using Fisher's exact tests. Holm adjustment was applied across estimable taxa separately for each grouping factor and test type. Bray–Curtis-based PERMANOVA and PERMDISP [53–55] were conducted as supplementary multivariate

analyses, with methodological details and results provided in Supplementary Methods S2.7 and Supplementary Table S5C. All community tests were interpreted as exploratory because sample-to-block linkage was unavailable, and PERMANOVA results were interpreted alongside the assessment of multivariate dispersion. The count matrix, individual profiles and complete test results are provided in Supplementary Data Table S4G.

During the preparation of this manuscript, the authors used OpenAI Codex (OpenAI; accessed August–October 2026) and Claude (Anthropic) to assist with R-code development, statistical workflow and language editing. All AI-assisted outputs were critically reviewed and verified by the authors, who take full responsibility for the accuracy, originality, and integrity of the final manuscript.

Results

Drought and recovery patterns in leaf water status and proline

At the final drought endpoint, midday leaf water potential (Ψleaf; arithmetic mean ± SD of observed plant-level values pooled across inoculation treatments) was −1.73 ± 0.32 MPa in drought-stressed SO4 plants compared with −0.56 ± 0.13 MPa in well-watered plants, −2.58 ± 0.38 versus −0.48 ± 0.10 MPa in Börner, and −1.73 ± 0.18 versus −0.40 ± 0.04 MPa in Vinto (Supplementary Figure S1A). These observed differences were consistent with the imposed water regimes (Supplementary Table S5A).

At the recovery measurement, Ψleaf (arithmetic mean ± SD) in previously drought-stressed plants increased to −0.69 ± 0.14 MPa in SO4, −1.16 ± 0.30 MPa in Börner, and −0.76 ± 0.20 MPa in Vinto. The corresponding values in continuously well-watered plants were −0.51 ± 0.10,

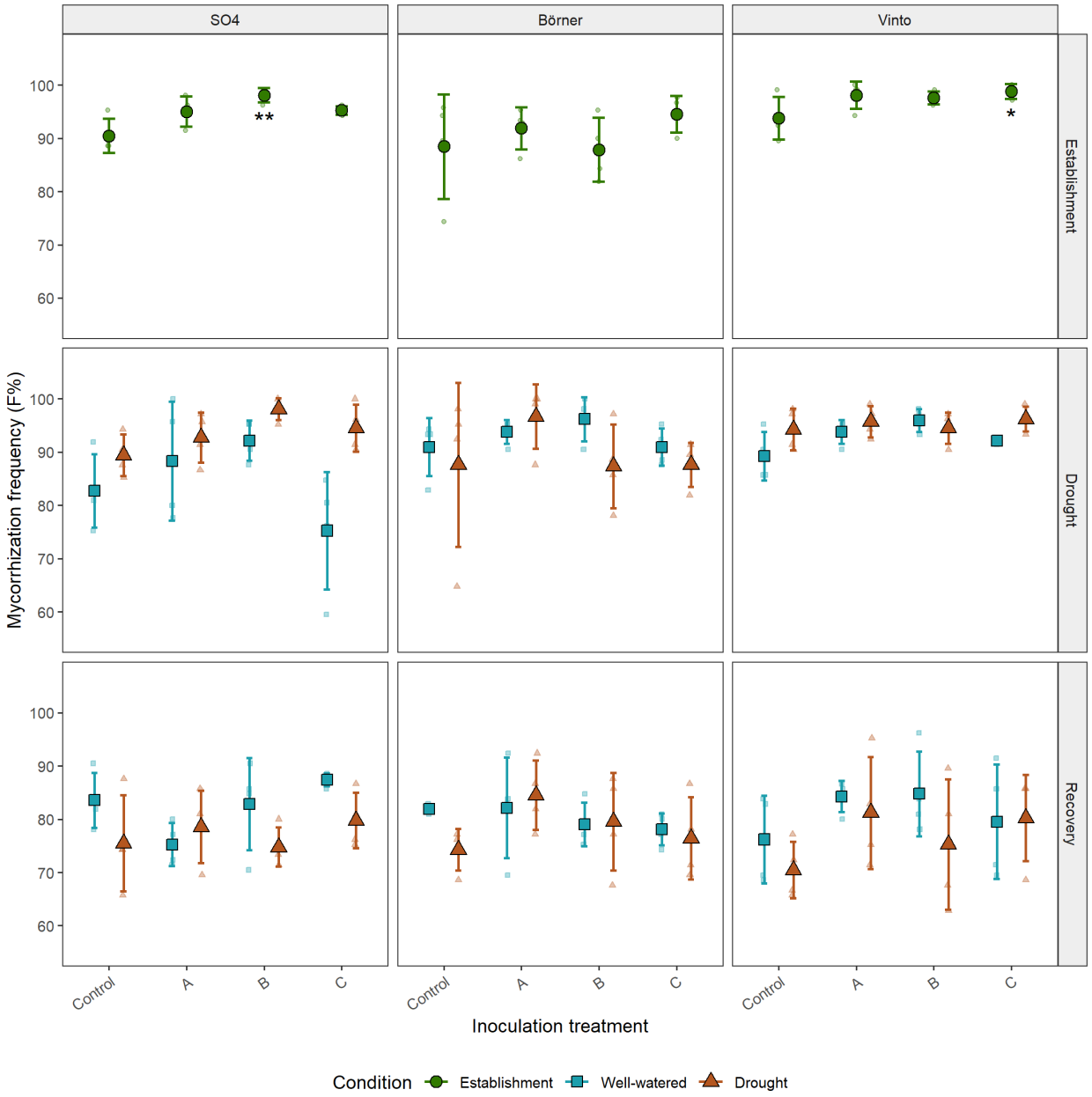
338 −0.75 ± 0.19, and −0.94 ± 0.15 MPa, respectively (Supplementary Figure S1A). These
339 descriptive comparisons indicate a substantial reversal of the drought-associated decline in leaf
340 water potential. Differences between observed arithmetic means for the two water-treatment
341 histories varied among rootstocks: previously drought-stressed SO4 and Börner plants had more
342 negative mean leaf water potentials than continuously well-watered plants, whereas Vinto
343 showed the reverse pattern. Across these measurements, no consistent improvement in leaf water
344 status was apparent in inoculated plants relative to the corresponding Controls.

345 At the final drought endpoint, median proline concentrations were higher in droughted than in
346 well-watered Control plants for SO4 (4.23 versus 0.30 mg kg^{-1} DW), Börner (152.66 versus 0.35
347 mg kg^{-1} DW) and Vinto (31.18 versus 3.66 mg kg^{-1} DW), consistent with proline accumulation
348 accompanying the imposed water deficit. Across the observed treatment combinations, Börner
349 had the highest concentrations and greatest dispersion, Vinto was intermediate, and SO4 had the
350 lowest concentrations. No consistent descriptive response to inoculation was evident (n = 4 per
351 observed combination; Supplementary Figure S1B).

352 **Inoculation was associated with higher root mycorrhization in several rootstock-inoculant**
353 **comparisons**

354 Inoculation was associated with increased mycorrhization frequency (F%) in several rootstock-
355 inoculant comparisons, with the clearest effects occurring during establishment (Figure 2).
356 Inoculant B increased F% in SO4 (from 90.5 to 98.1%), and Inoculant C increased F% in Vinto
357 (from 93.8 to 98.8%), relative to their respective Controls. The corresponding EMM contrasts on
358 the empirical-logit scale remained significant in the conditional block-sensitivity analysis, with
359 upper bounds for the Holm-adjusted P-values of 0.0097 and 0.0255, respectively (Supplementary

Table S6). Positive EMM contrasts were also observed for Inoculant A in Vinto during establishment and, under drought, for Inoculant B in SO4 and Inoculant A in Börner; these comparisons did not meet the significance criterion in the sensitivity analysis. The rootstock × inoculation interaction for F% at establishment did not meet the conditional criterion (*P* upper bound ≤ 0.134; Supplementary Table S5B), so these within-rootstock contrasts do not establish rootstock-dependent responses.



366

367 **Figure 2. Mycorrhization frequency (F%) across experimental phases, rootstocks and inoculation treatments.**

368 Small symbols represent individual plants; large symbols represent observed arithmetic means, and error bars

369 indicate ±1 SD (n = 4 per experimental combination). Green circles, teal squares, and brown triangles represent

370 establishment, well-watered, and drought conditions, respectively. During recovery, drought denotes the preceding

371 water treatment. Asterisks identify establishment and drought EMM contrasts on the empirical-logit scale with

372 conditional P-value upper bounds below 0.05, with Holm adjustment within each rootstock, phase and water-regime

family (* $P < 0.05$ and ** $P < 0.01$). Recovery is descriptive. The conditional calculation and its assumptions are described in Methods; complete results are provided in Supplementary Data Table S4B.

At establishment, the intraradical structures that differed from the Control varied among rootstocks. EMM contrasts indicated higher arbuscule frequency with Inoculant B in SO4 and with Inoculant C in Vinto (Holm-adjusted $P = 0.0048$ and 0.0169, respectively, in the model without block adjustment). In Börner, all three inoculants increased hyphal and vesicle frequencies, whereas no significant increase in arbuscule frequency was detected. Inoculant A also increased hyphal frequency in Vinto. These increases relative to the Control remained significant in the conditional block-sensitivity analysis. The rootstock × inoculation interaction for hyphal frequency was nominally significant ($P = 0.0089$) and also retained support in that analysis (conditional upper bound $P \leq 0.0151$; Supplementary Table S5B and Supplementary Data Table S4B). The corresponding interactions for arbuscule and vesicle frequencies did not meet the conditional criterion ($P \leq 0.132$ and ≤ 0.186, respectively). The positive arbuscule response estimated for Inoculant A in Vinto (Holm-adjusted $P = 0.0394$) did not meet the significance criterion in the conditional sensitivity analysis. Support for the Vinto responses and the Börner vesicle responses varied across sensitivity analyses; the Börner–Inoculant A vesicle contrast was not significant under either HC3 or arcsine-square-root analysis. The SO4–Inoculant B arbuscule response and the Börner hyphal responses were the most consistently supported across the microscopy sensitivity analyses. During drought, Inoculant B was associated with higher arbuscule frequency in droughted SO4 (conditional Holm upper bound $P = 0.0191$) but lower hyphal frequency in well-watered SO4 (EMM contrast = −2.20 on the empirical-logit scale; upper bound $P = 0.0038$). Neither corresponding inoculation × water interaction met the conditional criterion ($P \leq 0.142$ and ≤ 0.285, respectively; Supplementary

Table S5B). Full estimates, confidence intervals, model diagnostics, and microscopy sensitivity results are provided in Supplementary Data Tables S4A and S4B.

Across treatments, F% remained high but declined descriptively from establishment to the drought and recovery phases. The drought-phase measurements did not show a consistent reduction in F%, suggesting that root occupation by AMF persisted during the imposed water deficit. Control F% was already high at establishment, averaging approximately 88–94%, consistent with the use of non-sterile substrate and the presence of resident AMF. During recovery, vesicle frequency was descriptively higher with Inoculant C than with the Control in continuously well-watered Vinto (EMM contrast = +1.58 on the empirical-logit scale; Supplementary Table S6). Because F% records the occurrence of colonization at observation points rather than its intensity within colonized root segments, these results demonstrate widespread root occupation but not necessarily equivalent intraradical fungal biomass. Microscopy therefore indicates higher total mycorrhization in several rootstock-product combinations but cannot determine whether the observed structures originated from resident or introduced fungi.

Root AMF community profiles and taxon occurrence during establishment

All 48 establishment root samples yielded AMF amplicon data. Observed VTX richness was low, with a median of two VTX per sample (range: 1–5), and 45 of the 48 samples had more than 90% of assigned reads belonging to a single VTX. Control roots were consistently dominated by *Glomus* sp. VTX00309, with arithmetic mean relative abundances of 99.8–99.9% across rootstocks (Figure 3).

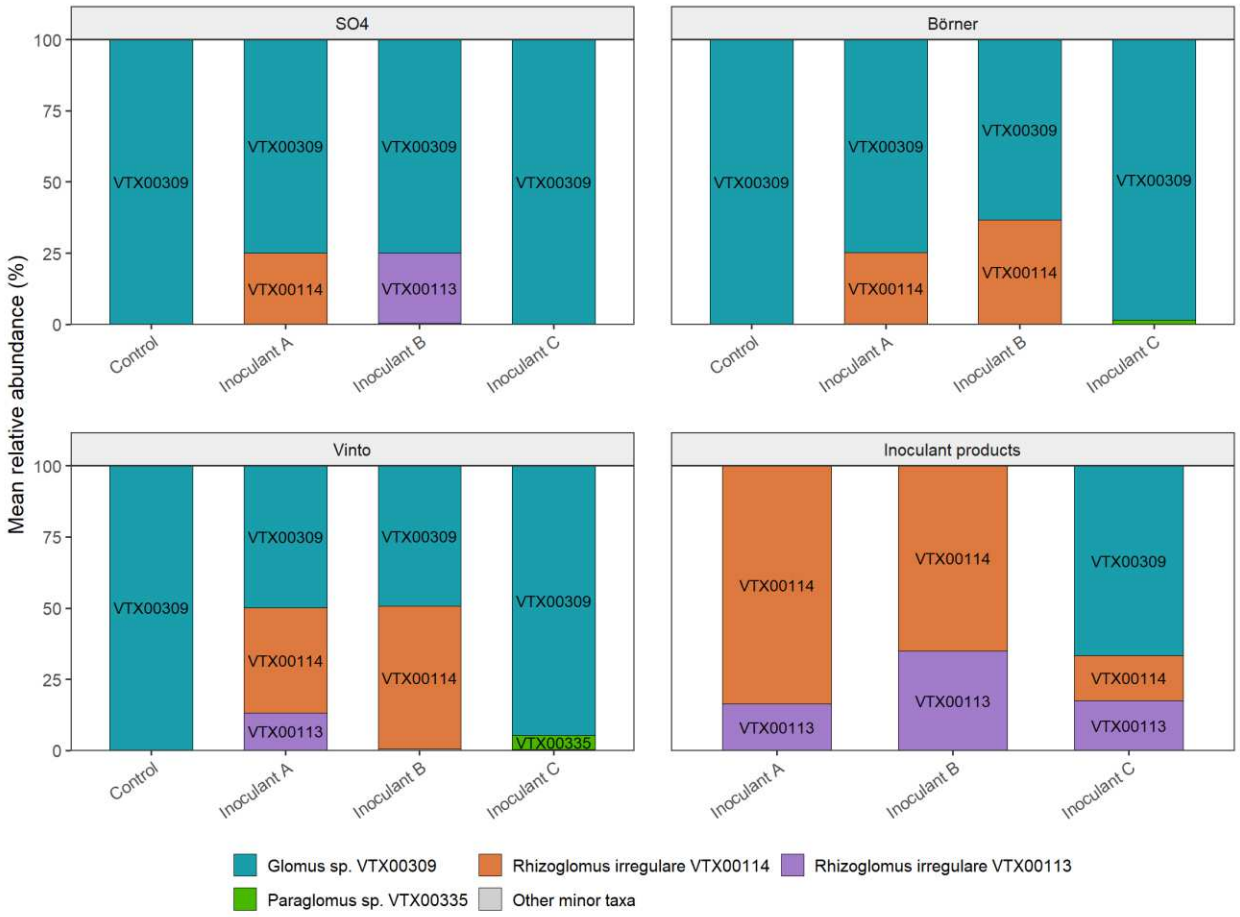


Figure 3. Root AMF community composition during establishment. Stacked bars show the arithmetic mean relative abundance of AMF virtual taxa (VTX) for each rootstock × inoculation treatment combination ($n = 4$ root samples per combination). Product profiles represent the arithmetic mean of three DNA extracts per inoculant and are shown as descriptive references. Bars summarize arithmetic means across individual samples and do not necessarily represent the composition of an individual root sample or product extract. Minor VTX are grouped as "Other minor taxa." Product samples were excluded from inferential analyses of root communities. Individual root profiles and assigned-VTX read counts are provided in Supplementary Data Table S4G.

No individual VTX differed significantly in relative abundance among inoculation treatments after Holm adjustment (Kruskal–Wallis tests, all adjusted $P \geq 0.071$; Supplementary Table S5C). However, the occurrence of VTX00114 differed among inoculation treatments (Holm-adjusted

Fisher's exact $P = 0.018$). VTX00114 was not detected in Control or Inoculant C roots but occurred in 6/12 Inoculant A-treated roots (50.0%) and 4/12 Inoculant B-treated roots (33.3%). No estimable VTX differed significantly in relative abundance or occurrence among rootstocks after Holm adjustment (all adjusted $P = 1.000$). Individual profiles and complete test results are provided in Supplementary Data Table S4G.

The mean reference profiles of Inoculants A and B predominantly comprised VTX00114 and VTX00113, both assigned to *Rhizoglomus irregulare* by EUKARYOME (Figure 3). These VTX were recovered from subsets of the corresponding treated roots, providing treatment-associated taxonomic correspondence without confirming commercial isolate identity. Inoculant C reference extracts were heterogeneous: two were dominated by VTX00309, whereas the third consisted almost entirely of VTX00113 and VTX00114 (Supplementary Data Table S4G). These latter VTX were not detected in C-treated roots. Because VTX00309 was also abundant in Control roots, its occurrence in C-treated roots could not distinguish fungi originating from the product from resident fungi. Establishment of fungi from Inoculant C therefore remained unresolved.

Supplementary PERMANOVA indicated an exploratory association between inoculation treatment and root AMF community profiles ($R^2 = 0.188$, $P = 0.026$), accompanied by differences in multivariate dispersion among treatments (PERMDISP $P = 0.015$). The multivariate result may therefore reflect differences in both average composition and within-treatment variability. Neither rootstock nor the rootstock × inoculation interaction was significant in PERMANOVA ($P = 0.516$ and 0.901, respectively; Supplementary Table S5C; Supplementary Figure S2).

Growth responses varied among rootstock and water-regime combinations

In Börner, EMM contrasts indicated higher root dry weight with all three inoculants than with the Control under well-watered conditions (A +4.38 g, B +5.31 g, C +6.43 g; conditional Holm upper bounds $P = 0.025$, 0.016 and 0.006, respectively; the Bonferroni interval for Inoculant A included zero), whereas under drought it was lower with Inoculant C (−5.51 g; $P = 0.019$; Figure 4; Supplementary Table S6). The drought reduction with Inoculant A (−4.33 g) was not supported. The inoculation × water interaction was supported ($F(3,24) = 8.39$; conditional $P \leq 0.0011$; Supplementary Table S5B), although it does not establish a separate interaction for each inoculant. The interaction partly reflected the Control response: drought-stressed Control plants had higher observed root dry weight than well-watered Controls (18.3 ± 2.8 versus 14.8 ± 3.3 g, arithmetic mean ± SD), whereas all inoculated treatments had lower root dry weight under drought than under well-watered conditions (Figure 4). Across rootstocks, the rootstock × inoculation × water interaction was also nominally significant ($F(6,72) = 3.52$, $P = 0.0041$; Supplementary Table S5B), but it is interpreted cautiously because rootstock and drought sampling day were partly confounded.

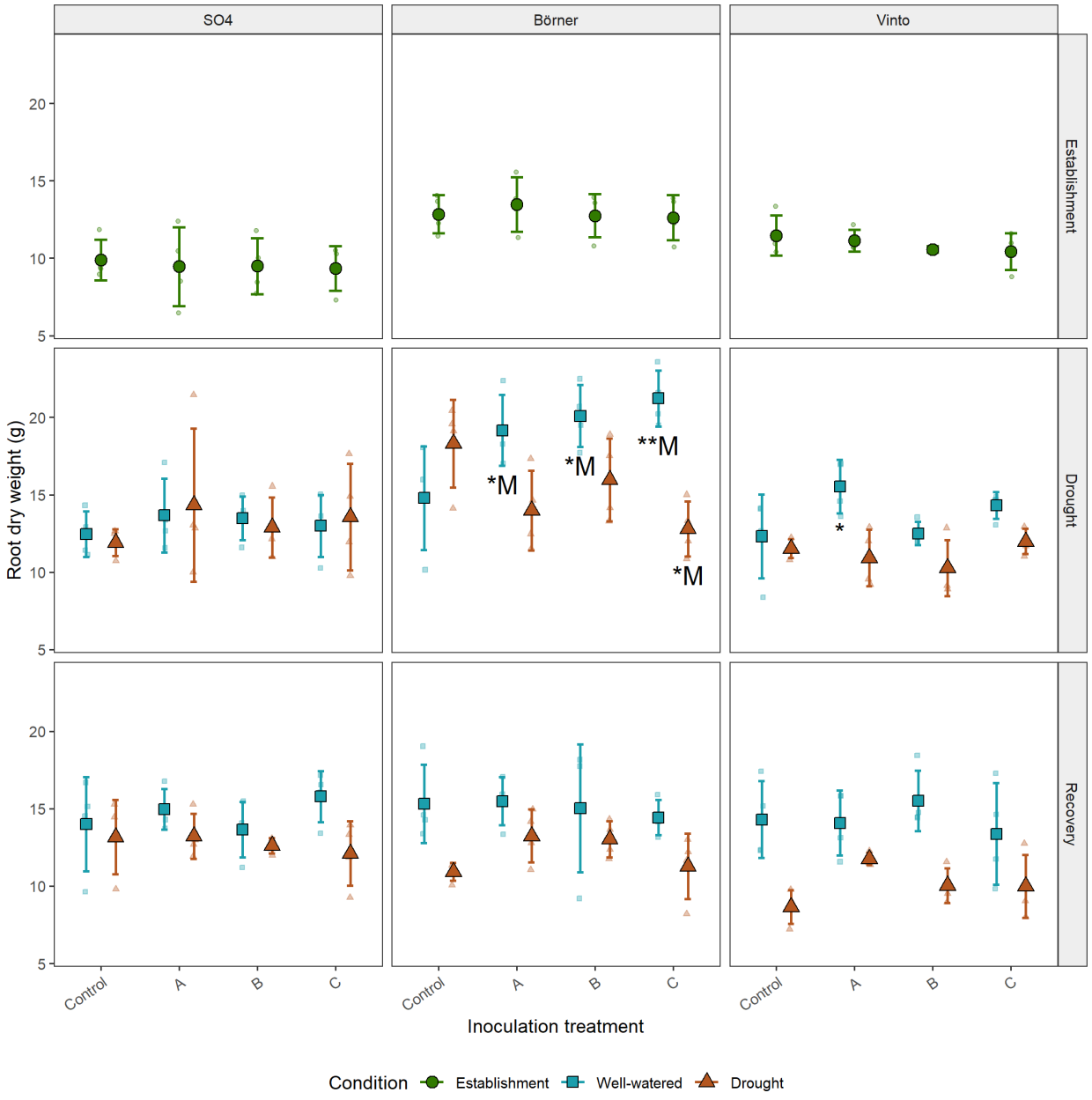


Figure 4. Root dry weight across experimental phases, rootstocks and inoculation treatments. Small symbols represent individual plants; large symbols and error bars indicate observed arithmetic means ±1 SD ($n = 4$ per combination). Green circles, teal squares, and brown triangles denote establishment, well-watered, and drought conditions, respectively; during recovery, drought denotes previous treatment history. Asterisks indicate conditional Holm-adjusted P-value upper bounds below 0.05 for planned inoculant-versus-Control EMM contrasts within each rootstock, phase, and water-regime family (* $P < 0.05$; ** $P < 0.01$). M additionally denotes nominal support for the

corresponding global interaction under the conditional block bound; it is not a product-specific interaction test. Recovery is descriptive. See Supplementary Methods S2.3 and Supplementary Data Table S4B.

Additional differences from the corresponding Control were observed in Vinto and SO4. In well-watered Vinto, the root dry-weight EMM contrast for Inoculant A versus the Control was +3.22 g (Holm-adjusted $P = 0.0215$). During establishment, shoot dry-weight EMM contrasts in SO4 were −9.04 and −6.93 g for Inoculants A and B versus the Control, respectively (Holm-adjusted $P = 0.006$ and 0.030; Supplementary Table S6). All three contrasts remained significant in the conditional block-sensitivity analysis. However, neither the inoculation × water interaction for root dry weight in Vinto ($P = 0.127$) nor the rootstock × inoculation interaction for shoot dry weight during establishment ($P = 0.061$) was significant (Supplementary Table S5). These contrasts therefore describe differences from the corresponding Control without establishing water- or rootstock-dependent responses. During recovery, observed arithmetic mean shoot dry weight was descriptively higher by 8.87 g for Inoculant C than for the Control in continuously well-watered Börner plants (41.87 ± 3.31 versus 33.00 ± 5.34 g, arithmetic mean ± SD; $n = 4$ per group).

Nutritional and leaf optical responses

Foliar nutrient concentrations showed no consistent response to inoculation. Across P, K, Ca, Mg, Fe, Zn, Mn, Cu, C, N and the C:N ratio, no planned inoculant-versus-Control contrast or inoculation-related omnibus interaction remained significant in the exploratory models without block adjustment after Benjamini–Hochberg correction; the minimum adjusted q values were 0.203 for contrasts and 0.215 for omnibus tests (Supplementary Figure S3A; Supplementary Data Table S4C).

Leaf optical responses were similarly limited. At establishment, the block-adjusted model estimated a lower anthocyanin index in Vinto treated with Inoculant A relative to the Control (EMM contrast = −0.02265, Bonferroni-adjusted 95% CI = −0.0351 to −0.0102, $q = 0.1074$; Supplementary Figure S3B). This contrast did not remain significant after correction across the complete exploratory family under either the Gaussian model or the HC3 sensitivity analysis (HC3 Holm-adjusted $P = 0.0689$; $q = 1.000$). The rootstock × inoculation interaction was not significant after correction ($q = 0.283$). No establishment contrast for chlorophyll, flavonols, NBI, or any PolyPen reflectance index remained significant after correction under either covariance method (Supplementary Table S5D; Supplementary Data Tables S4D and S4E). Overall, the exploratory nutritional and optical measurements did not indicate a consistent physiological benefit of inoculation.

Multi-trait overview of inoculation responses

Across the primary and secondary endpoints, the factorial models identified 28 planned inoculant-versus-Control EMM contrasts that were significant after Holm adjustment during establishment and drought (Supplementary Table S6). Twenty remained significant in the conditional block-sensitivity analysis: 13 of the 54 establishment contrasts and seven of the 108 drought contrasts (Figure 5). Most of these 20 contrasts involved microscopy endpoints: 13 concerned mycorrhization frequency or intraradical AMF structures and represented related components of the same assessment rather than independent functional benefits. The remaining seven involved biomass: two reductions in shoot dry weight in SO4 during establishment and five root dry-weight responses during the drought phase, comprising four in Börner and one in Vinto. Eight of the 20 contrasts belonged to two models with nominal global interaction support,

indicated by M: four establishment hyphal contrasts and four drought-phase root dry-weight contrasts in Börner. These counts summarize separate Holm-adjusted families of three inoculant-versus-Control comparisons.

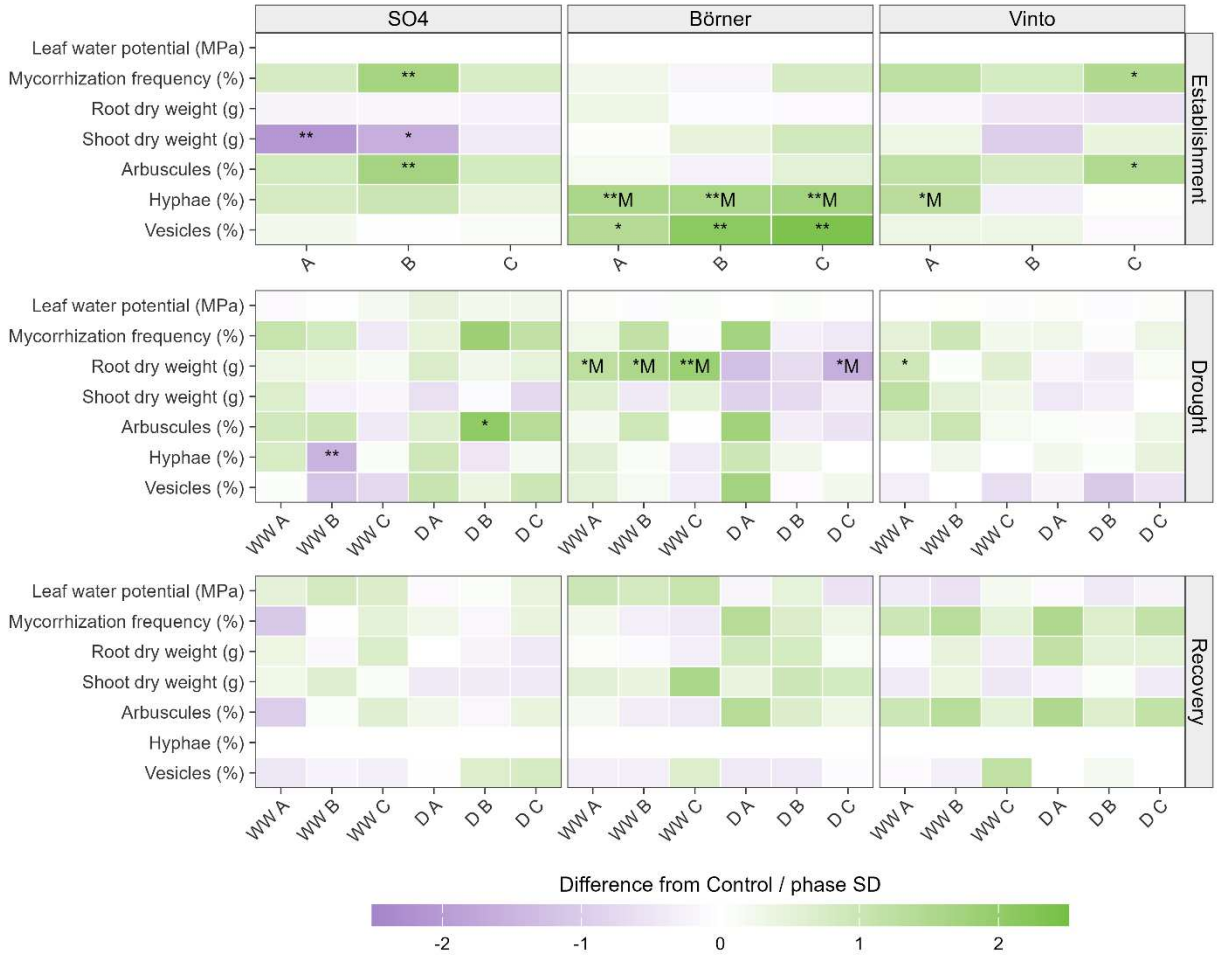


Figure 5. Inoculant-versus-Control differences across primary and secondary endpoints, experimental phases, and rootstocks. Fill color represents the difference between the observed arithmetic means of each inoculated treatment and its matched uninoculated Control, standardized by the phase-specific SD for each endpoint. For microscopy endpoints, these means and SDs were calculated after empirical-logit transformation; biomass and leaf water potential were summarized on their original measurement scales. The phase-specific SD is a scaling factor, not a measure of uncertainty in the difference. Purple and green indicate lower and higher values relative to the Control, respectively. Panels are grouped by experimental phase (Establishment, Drought, and Recovery) and

rootstock (SO4, Börner, and Vinto). Blank leaf-water-potential cells during Establishment indicate that this endpoint was not measured. Blank hyphal-frequency cells during Recovery indicate undefined standardized differences because all recorded frequencies were zero and the phase-specific standard deviation was therefore zero. A–C denote Inoculants A–C; WW denotes well-watered plants; D denotes drought-stressed plants during the Drought phase and previously drought-stressed plants during Recovery. Asterisks indicate significance based on conditional upper bounds for Holm-adjusted *P* values, with correction applied to the three inoculant-versus-Control EMM contrasts within each endpoint, rootstock, phase, and, where applicable, water regime (* $P < 0.05$; ** $P < 0.01$). M additionally indicates nominal support from the corresponding global interaction in the conditional analysis, rather than a product-specific interaction test. All leaf-water-potential comparisons and all Recovery comparisons are descriptive. Complete estimates, intervals, and sensitivity analyses are provided in Supplementary Data Table S4B; selected contrasts are presented in Supplementary Table S6.

Discussion

Inoculation-associated patterns in AMF community composition and mycorrhization

A central finding of this study is that inoculation-associated mycorrhization and community patterns were not consistently accompanied by improved plant performance. Hypothesis (i) was only partly supported. Inoculation was associated with mostly positive differences from the Control in total mycorrhization frequency and intraradical structures in particular rootstock-inoculant comparisons, especially during establishment. Because Control F% was already 88–94%, the supported increases in total mycorrhization frequency amounted to only a few percentage points on the observed scale, and their biological relevance is uncertain. The structures contributing to these differences varied among rootstocks, with hyphae and vesicles in Börner and arbuscules in SO4 and Vinto; however, only hyphal frequency showed interaction support for rootstock dependence. These microscopy results indicate treatment-associated

differences in root occupation by AMF, but colonization in a non-sterile substrate can include resident fungi and cannot, by itself, demonstrate establishment of taxa present in commercial products [26–28].

Amplicon sequencing provided complementary evidence on community profiles and taxonomic correspondence, but also requires cautious interpretation. Individual profiles complement the arithmetic means by showing variation among roots and product reference extracts. No individual VTX differed significantly in relative abundance among inoculation treatments after Holm adjustment, whereas VTX00114 occurrence differed among treatments. VTX00114 and VTX00113, both assigned to *Rhizoglomus irregulare* by EUKARYOME, dominated the reference profiles of Inoculants A and B. VTX00114 occurred in 50.0% and 33.3% of the corresponding treated roots, respectively, but was not detected in Control roots, providing evidence of treatment-associated taxonomic correspondence. Inoculant C reference extracts were heterogeneous: two were dominated by VTX00309 (assigned to *Glomus* sp.), which was also widespread in Control roots, whereas the third consisted almost entirely of VTX00113 and VTX00114, which were not recovered from C-treated roots. Establishment of fungi originating from Inoculant C therefore remained unresolved. The supplementary PERMANOVA also indicated a treatment association, although unequal multivariate dispersion limits interpretation of this pattern as a shift in average community composition.

Taxonomic inference remains constrained by the resolution of the partial SSU marker and reference-database coverage [42, 47, 48]. Consequently, correspondence at the VTX level cannot confirm the identity or establishment of individual commercial isolates. Non-detection likewise does not demonstrate absence or failure to establish, because low-abundance taxa may escape

amplification, sequencing or taxonomic assignment. These qualified findings agree with reports that commercial AMF products can increase colonization without measurably restructuring resident grapevine communities or improving plant performance [27, 28, 31], and that colonization and growth promotion do not consistently coincide [26, 32, 33]. Product reference profiles also departed from the declared compositions: not all declared taxa were recovered, and one Inoculant C reference extract yielded *R. irregulare*-assigned VTX not listed on its label. These discrepancies could reflect low abundance, amplification or database bias, differences between declared and actual product content [33], or cross-contamination during sample processing.

No rootstock association or rootstock × inoculation interaction was detected in the exploratory community analysis. Field studies nevertheless show that rootstock genotype can shape root and rhizosphere microbial communities and recruit distinct AMF assemblages despite comparable microscopic colonization [24, 25, 39]. Rootstock-associated AMF communities may also differ functionally in their effects on grapevine biomass and nutrient acquisition [34]. The shared propagation history and substrate, low VTX richness and single establishment endpoint may have limited detection of rootstock-associated differences in this experiment. Roots were dominated by *Glomus* sp. VTX00309, whose source was not determined. This background differs from the more diverse AMF assemblages reported in vineyard roots [25, 39], where competition between introduced and resident fungi may also differ. Nevertheless, subsequent growth patterns varied among rootstock and water-regime combinations.

Growth responses were context dependent, and water status did not improve

Hypothesis (ii) was supported only in part. Plant growth showed contrasting patterns among treatment combinations. In Börner, root dry weight was higher than in the Control for all three inoculants under well-watered conditions but lower under drought. Conditional support was retained for all three well-watered increases and the drought reduction with Inoculant C, whereas the drought reduction with Inoculant A did not retain support. This pattern is compatible with the mutualism-parasitism continuum, in which the net return from AM symbiosis depends on host genotype, fungal identity, nutrient demand, and the environment [18, 19, 21]. However, carbon limitation cannot be inferred because photosynthesis and carbon transfer were not measured, leaving the mechanism underlying the response to the commercial formulations unresolved. Interpretation also requires caution because droughted Börner Controls had higher mean root dry weight than well-watered Controls (18.3 versus 14.8 g; $n = 4$), contributing to the observed contrasts and interaction. Differential water depletion during drought induction is another possible explanation, although endpoint Ψleaf was similar in drought-stressed inoculated plants (−2.52 to −2.61 MPa) and Controls (−2.60 MPa). This comparison was based on three plants per treatment combination at one time point, and pot-level water use was not recorded. No treatment differences in root dry weight were detected at establishment, and allocation of water regimes to physical blocks leaves a possible spatial contribution to the interaction.

Recent grapevine evidence reinforces the need for this conditional interpretation. AMF benefits under water deficit have been reported for particular rootstocks and isolates [16, 35], but the magnitude and direction of responses vary with rootstock-scion background, AMF identity, water regime, and measured trait [29, 36]. The lower shoot dry weights in SO4 with Inoculants A

and B during establishment and the root response in well-watered Vinto were supported within those treatment combinations, but their interaction results did not establish broader rootstock or water dependence. In SO4 at establishment, Inoculant B was associated with higher F% and arbuscule frequency but lower shoot dry weight, illustrating that greater colonization did not translate into a growth benefit; this co-occurrence does not demonstrate a colonization cost. The Börner recovery shoot difference was descriptive. The conditional root-growth interaction in Börner supports further evaluation of specific product × rootstock × water combinations, without establishing a general ranking of the inoculants.

Plant water status responded clearly to drought and rewatering, while inoculation produced no consistent descriptive benefit at the sampled endpoints. Drought-treated plants had more negative midday leaf water potentials in all rootstocks, and droughted Controls had higher proline concentrations than well-watered Controls. Leaf water potential increased after rewatering, with recovery varying among rootstocks. This differs from studies and meta-analyses in which AMF improved stomatal conductance, hydraulic conductance, or plant water status under water limitation [11, 13, 14, 16]. Confinement of roots and extraradical mycelium within 5-L pots may have restricted the advantage of hyphal access to water beyond the root depletion zone, although access to water in small soil pores could still have occurred. This restriction does not exclude other mechanisms of stress alleviation, including stomatal regulation, aquaporin-mediated water transport, osmotic adjustment, antioxidant protection, hormonal signaling, or improved mineral nutrition [9–11, 13, 14]. However, proline and foliar nutrient concentrations showed no consistent inoculation response, while gas exchange, transpiration, aquaporin activity, antioxidant responses, and hormonal signaling were not measured. These endpoint measurements

636 therefore do not exclude transient or unmeasured effects on other components of the drought
637 response.

638 **Nutritional and optical responses provided no evidence of a generalized benefit**

639 Foliar nutrient concentrations and the Dualex and PolyPen optical indices showed no consistent
640 inoculation effect after multiplicity correction; the lower anthocyanin estimate in Vinto did not
641 remain significant after correction and was not part of a broader pattern. Two features of the
642 experimental system may have limited the scope for a nutritional benefit. First, the high
643 mycorrhization frequency of Control roots suggests that resident AMF may already have
644 provided much of the nutrient-acquisition function that inoculation could add. Second, observed
645 arithmetic mean foliar N concentrations of approximately 0.6–1.3% of dry weight suggest that N
646 supply may have limited growth, although published sufficiency ranges depend on tissue type
647 and developmental stage and may not apply directly to whole leaves of young ungrafted
648 rootstocks [56, 57]. Under N limitation, improved P or K acquisition need not translate into
649 growth or physiological benefits, and constrained photosynthesis may reduce the carbon
650 available to the fungal partner [18, 21, 58, 59]. Neither mechanism was tested here.
651 Together with the growth and water-status results, these data show that fungal responses and
652 plant benefits were not consistently coupled: most conditionally supported contrasts concerned
653 related microscopy endpoints, whereas growth responses were confined to particular treatment
654 combinations. Increased colonization alone therefore does not identify the product most likely to
655 benefit a given rootstock under a particular water regime, and product evaluation requires
656 functional as well as microscopic and taxonomic criteria.

Implications for evaluating AMF inoculants and study limitations

Our results identify several requirements for evaluating commercial AMF inoculants. First, total mycorrhization, community profiles, establishment of introduced fungi, and plant benefit should be treated as distinct outcomes. Microscopy indicated treatment-related increases in mycorrhization but could not distinguish introduced from resident AMF, while partial SSU amplicon sequencing described community profiles and provided treatment-associated taxonomic correspondence without resolving individual commercial isolates. Future evaluations would therefore benefit from authenticated and molecularly traceable isolates, supported by isolate-specific markers or sufficiently resolved genomic reference information [26–28]. Providing such markers, or reference sequences for the declared isolates, would also allow growers and researchers to verify product establishment, which remained unresolved here for Inoculant C because the VTX dominating two of its three reference extracts also dominated the resident background. Complementary long-read sequencing of longer ribosomal amplicons could improve taxonomic resolution. For example, Kolaříková et al. [60] used PacBio sequencing of an approximately 2.5-kb amplicon spanning the partial SSU, the complete ITS region and the partial LSU to distinguish closely related AMF taxa and to improve the taxonomic characterization of AMF communities in roots. Their published protocol used two initial primer-pair reactions followed by a nested PCR with tagged primers, adding preparation steps relative to the single-round amplification used here.

Second, the restricted rooting volume used here may have limited the capacity of extraradical hyphae to provide access to water or nutrients beyond the root depletion zone. Future glasshouse experiments could use larger containers or hyphae-accessible compartments to evaluate this

679 mechanism more directly [13, 14]. Repeated measurements of gas exchange, whole-plant water
680 use, chlorophyll fluorescence, and hydraulic traits could also identify transient physiological
681 responses not captured by endpoint biomass and leaf water potential. Finally, the context-
682 dependent growth responses observed here illustrate the importance of testing defined product ×
683 rootstock × water combinations before field validation. Glasshouse experiments can identify
684 promising combinations and potential trade-offs, but multi-site and multi-season vineyard trials
685 remain necessary to determine whether establishment and plant responses persist after
686 transplantation [27, 30, 31].

687 These recommendations should be read alongside the limitations of the present study. The scope
688 of inference reflects a single-season glasshouse experiment with four plants per destructive-
689 sampling cell, three per leaf-water-potential cell, and two physical blocks per water regime
690 during drought. Because each water regime was applied to only two physical blocks and sample-
691 to-block identifiers were not retained, a block × inoculation interaction cannot be separated from
692 an inoculation × water interaction. The water-dependent root response in Börner could therefore
693 partly reflect spatial differences between blocks and should be confirmed in an experiment in
694 which water regimes are randomized at the plant level. Missing sample-to-block identifiers
695 required conditional bounds for eligible destructive endpoints and exploratory interpretation of
696 community tests. Recovery was descriptive after treatment-based regrouping. Sequencing was
697 restricted to establishment samples, so changes in community profiles and persistence of the
698 detected taxa during drought and recovery were not assessed. Drought sampling dates also
699 differed among rootstocks. The conditional bounds rely on balanced sampling, additive block
700 effects and homogeneous residual variance; they do not eliminate potential bias from non-

701 random irrigation allocation. Responses represent the commercial formulations and their
702 application procedure, since other product components and the root dip were not controlled
703 separately (Supplementary Table S1). Microscopy measured colonization frequency rather than
704 intensity. The findings consequently apply to the tested products, rootstocks, and cultivation
705 conditions, with field transfer requiring validation.

706 Conclusions

707 Commercial AMF inoculation was associated with higher grapevine root mycorrhization in
708 several product-rootstock comparisons, although rootstock dependence was supported only for
709 hyphal frequency. These differences occurred primarily during establishment, and one reduction,
710 in hyphal frequency with Inoculant B in well-watered SO4, occurred during the drought phase.
711 Most root profiles were dominated by one VTX. VTX00114 occurred in 50.0% and 33.3% of
712 roots receiving Inoculants A and B, respectively, and was not detected in Control roots or those
713 receiving Inoculant C. However, sequencing could not confirm the establishment of individual
714 commercial isolates or track Inoculant C against the resident background.

715 Plant benefits were more limited and varied among rootstock and water-regime combinations. In
716 Börner, all three inoculants were associated with higher root dry weight than the Control under
717 well-watered conditions, whereas Inoculant C was associated with lower root dry weight under
718 drought, and the inoculation × water interaction was supported. In SO4, Inoculants A and B were
719 associated with lower shoot dry weight during establishment, and in well-watered Vinto,
720 Inoculant A was associated with higher root dry weight, without supporting interactions. No
721 consistent descriptive improvement in leaf water status was observed with inoculation at the

sampled endpoints. Mineral nutrition and leaf optical traits likewise showed no consistent improvement with inoculation.

The results therefore do not support a general drought-adaptation benefit from commercial AMF inoculation under the pot-based glasshouse conditions tested. Because rooting volume, substrate conditions, and environmental variability differ substantially from those in vineyards, these findings should not be directly extrapolated to field conditions. Future evaluations should test specific product × rootstock × environment combinations and assess colonization, establishment, community restructuring, and plant performance as distinct but complementary outcomes. Commercial products containing authenticated and molecularly traceable AMF strains, together with multi-site and multi-season field validation, will be needed before their use can be recommended as a reliable viticultural strategy.

Declarations

Ethics approval and consent to participate

Not applicable. The rootstock cuttings were obtained from propagation fields of the Department of Plant Breeding, Hochschule Geisenheim University. Collection and use of the plant material complied with relevant institutional, national and international guidelines and legislation, and no specific permissions were required.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Authors' contributions

BVA: Writing original draft, Conceptualization, Methodology, Investigation, Formal analysis, Writing & editing. TS: Writing & editing, Conceptualization, Resources. MS: Writing & editing, Investigation, Formal analysis. TP: Writing & editing, Investigation. DF: Writing & editing, Conceptualization, Supervision, Project administration, Funding acquisition. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The phenotypic measurements, processed AMF community data and statistical results supporting this study are provided in Supplementary Data Table S4. Raw amplicon sequencing FASTQ files were deposited in the European Nucleotide Archive under project accession PRJEB128143. The sequence-processing commands and custom analysis scripts are provided in the AMF_analysis_pipeline repository [61].

Additional files

Additional file 1. Supplementary methods, tables and figures (.docx). Additional experimental methods (Supplementary Methods S1) and detailed statistical methods (Supplementary Methods S2), Supplementary Tables S1–S3 and S5–S6, a description of Supplementary Data Table S4, and Supplementary Figures S1–S3.

Additional file 2. Supplementary Data Table S4 (.xlsx). Treatment summaries, model diagnostics, planned contrasts, sensitivity analyses, multiplicity inputs, AMF community data, and source measurements (S4A–S4H).

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