Applied Soil Ecology 222 (2026) 107008

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Scale-dependent effects of fungicide seed coating on arbuscular mycorrhizal fungi: from spore germination to field performance

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A R T I C L E I N F O

Keywords:
Arbuscular mycorrhizal fungi
Seed coating
Fungicides
Spore germination
Field study
PacBio

A B S T R A C T

Modern agriculture faces the challenge of feeding a growing global population while simultaneously addressing climate change and biodiversity loss. It is therefore essential to transition from high-input systems to more sustainable yet productive agricultural practices. Beneficial soil microorganisms, such as arbuscular mycorrhizal fungi (AMF), contribute to this transition by enhancing plant nutrient uptake and stress resilience. However, seed coatings with fungicides, which are commonly used to protect seedlings in conventional farming, may unintentionally interfere with AMF-plant symbioses.

Here, we investigated the off-target effects of seed-applied fungicides on mycorrhizal symbiosis using a combination of *in vitro*, greenhouse, and field experiments using maize (*Zea mays*) and the AMF species *Rhizoglomus irregulare* as model organisms. *In vitro* assays showed that the fungicide metalaxyl-M significantly inhibited AMF spore germination, while the fungicide fludioxonil had a marginal inhibitory effect. These effects were observed even below the recommended application rate. In a greenhouse experiment with sterilised soil and AMF inoculation, seed coating negatively affected plant biomass and mycorrhizal growth response, with the strongest effects observed four weeks after planting. A second greenhouse experiment using natural soils from nine field sites showed that effects of soil type on plant biomass and AMF colonisation were much stronger than effects of seed coating. Quantitative PCR detected no significant differences in AMF root colonisation between coated and non-coated seeds, suggesting limited effects on overall AMF abundance. Interestingly, PacBio sequencing revealed shifts in AMF community composition at the onset of symbiosis, suggesting that AMF taxa vary in sensitivity to seed coating. Field experiments showed significant early effects of seed coating on plant biomass that diminished at later growth stages, alongside a marginal effect on total AMF abundance.

Overall, this study demonstrates that seed coating with the two fungicides can negatively affect AMF, particularly during early plant development. However, these effects are highly scale-dependent, as they are buffered in natural soils. These findings highlight the need to consider AMF interactions in seed treatment design to support sustainable and resilient agricultural systems.

1. Introduction

Feeding a growing global population is a critical challenge for agricultural production. However, yield gains through conventional intensification are no longer a sustainable option. Although intensification has historically driven major productivity increases (Foley et al., 2005), it has often come at the expense of environmental degradation, including declining soil quality and biodiversity loss (Lehmann et al., 2020; Ramankutty et al., 2018). These trade-offs led to intensification traps (Burian et al., 2024), whereby short-term productivity gains undermine the ecological processes that sustain long-term crop production. Consequently, sustainable agriculture must address the urgent need to reduce environmental impacts while maintaining high productivity. Therefore, the identification of management strategies that maintain yields while supporting soil ecological functioning is a central research priority.

One proposed approach to achieving more sustainable agricultural systems is to integrate the ecosystemic services provided by beneficial

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https://doi.org/10.1016/j.apsoil.2026.107008
Received 12 January 2026; Received in revised form 23 March 2026; Accepted 1 April 2026

soil microorganisms (Bender et al., 2016; Kuila and Ghosh, 2022). Among these, arbuscular mycorrhizal fungi (AMF) are of particular interest due to their widespread occurrence (Kivlin et al., 2011; Lutz et al., 2025; Öpik et al., 2010) and multifunctional roles in agroecosystems (Thirkell et al., 2017). AMF form symbiotic associations with approximately 80% of terrestrial plant species (Parniske, 2008; Trivedi et al., 2020; van der Heijden et al., 2015) and can supply up to 90% of plant phosphorus demand, while also contributing to nitrogen and micronutrient acquisition (Duan et al., 2024; Walder and Van Der Heijden, 2015; Watts-Williams and Cavagnaro, 2014). In addition to improving nutrient uptake, AMF can suppress plant pathogens (Jung et al., 2012; Newsham et al., 1995; Veresoglou and Rillig, 2012; Lutz et al., 2023), enhance tolerance to abiotic stresses such as drought (Augé et al., 2001; Begum et al., 2019), reduce soil nutrient losses and greenhouse gas emissions (Cavagnaro et al., 2015; Zhang et al., 2015), and increase yield in agricultural settings (Lutz et al., 2023). Together, these functions suggest that AMF have the potential to reduce dependence on external inputs while maintaining crop performance.

Despite their potential, AMF are subject to agricultural systems characterised by heavy pesticide use. Fungicides remain a cornerstone of disease control, yet their non-target effects (Johnsen et al., 2001) raise concerns about their compatibility with mycorrhiza-based sustainability strategies. Specifically, several studies demonstrated that fungicides have a negative effect on AMF, either directly or indirectly (Edlinger et al., 2022; Riedo et al., 2021), thereby threatening the ecosystem services provided by AMF. Other studies indicate that the effects of fungicide on AMF are highly inconsistent, as it depends on many parameters, such as the active substance, the mode of action, the mode of application and the dosage (Hage-Ahmed et al., 2019).

Seed-applied fungicides are a particularly relevant but understudied case. They are designed to reduce overall chemical inputs and restrict exposure to localised zones around germinating seeds. Few studies have investigated the impact of fungicide seed coatings on the AMF symbiosis. Jin et al. (2013) evaluated the effects of six commercially available fungicide seed coatings in a greenhouse experiment with pea and chickpea over eight weeks. The effects varied according to the fungicides' mode of action: coatings containing systemic fungicides reduced both mycorrhizal colonisation and host growth, whereas contact fungicides had minimal impact. Cameron et al. (2017), however, reported no significant effects of fungicide seed coatings on AMF colonisation in maize, soybean and oat when inoculated with a four-species AMF mix over six weeks in the greenhouse. Field studies add further complexity. Some studies report substantial reductions in AMF colonisation of maize treated with fludioxonil (Castelli et al., 2014), while others find no measurable impact of fludioxonil or mefenoxam on soybean (Murillo-Williams and Pedersen, 2008). Hardy et al. (2025) found that seed coatings with eight different fungicide treatments delayed AMF colonisation of winter wheat in the greenhouse, but the effect was attenuated under field conditions. Taken together, these studies paint a very varied picture of the effects of seed coatings. However, none of these studies assessed the effects of seed coatings during the pre-symbiotic phase of AMF development, when fungal spores germinate and establish contact with host roots (Hage-Ahmed et al., 2019). The temporal overlap between the peak effects of seed coatings and AMF establishment may disrupt or delay the formation of the AMF symbiosis.

Further, understanding the compatibility between fungicide seed coatings and AMF functioning from *in vitro* to the field is critical for the development of sustainable practices that promote the stimulation of indigenous communities and large-scale field inoculation. So far, it is yet difficult to generalise this effect because most studies are performed in different experimental contexts and at different scales. *In vitro* experiments typically reduce confounding factors but lack ecological realism, whereas field studies provide greater ecological relevance at the cost of increased complexity in disentangling causal effects. We therefore argue that a critical next step is to systematically test seed-applied fungicide effects under standardised biological conditions while explicitly varying environmental complexity.

In summary, the contradictory findings reported in previous studies likely arise from several underlying factors. First, differences in experimental scale - ranging from controlled *in vitro* systems to greenhouse and field experiments - can strongly influence the observed responses, as environmental complexity and biotic interactions increase across these scales. Second, the buffering capacity of soil biota may moderate or mask treatment effects under more complex and biologically active conditions. Third, variation in the composition and diversity of AMF communities across studies may lead to differing plant–fungus interactions and functional outcomes. Together, these factors suggest that understanding the mechanisms behind the reported inconsistencies requires approaches that integrate multiple experimental scales and explicitly consider microbial community composition. Therefore, this study adopts a cross-scale framework combined with molecular community ecology methods to better resolve how AMF communities respond and function under varying environmental contexts. Specifically, we investigated the effects of two widely used seed-applied fungicides, metalaxyl-M and fludioxonil, on the symbiosis between maize (*Zea mays* L.) and the model AMF species *Rhizoglomus irregulare*. This was done across multiple experimental systems with increasing environmental complexity, ranging from *in vitro* experiments to field applications (Fig. 1), and at two plant development time points. We hypothesised that seed-applied fungicides negatively affect AMF establishment and root colonisation, in particular during early plant development, and that the magnitude of these effects decreases with increasing environmental complexity due to buffering biotic and abiotic interactions. By disentangling intrinsic fungicide effects from context-dependent responses, this study aims to provide a more robust assessment of the compatibility of seed-applied fungicides with mycorrhiza-supported sustainable agriculture.

2. Material and methods

2.1. AMF inoculum, maize varieties, and seed coating

The inoculum of *Rhizoglomus irregulare* strain SAF22 from the Swiss Collection of Arbuscular Mycorrhizal Fungi (van der Heijden, 2024) was produced by the Plant-Soil Interactions group at Agroscope using *Plantago lanceolata* as the host plant. The growth substrate consisted of 15% soil, 65% sand, and 20% oil-binding agent. To generate a non-mycorrhizal control inoculum, a portion of the inoculum was autoclaved at 121 °C and 2 bar for 90 min.

Three maize (*Zea mays* L.) varieties were used: LG31245 (Limagrain), P9610 (Pioneer), and SY Enermax (Syngenta). All varieties are commonly cultivated for both grain and silage production in Switzerland.

The seed coatings included those commonly used for maize, including the fungicide Maxim XL (Syngenta Agro AG), which contains the active ingredients fludioxonil and metalaxyl-M, and Korit 420 FS (Omya AG), which contains the bird-repellent ziram and the biocide and preservative benzisothiazolinone (see Supplementary Table 1).

2.2. Experiment 1: spore germination test

The spore germination assay was adapted from Mallmann et al. (2018) and ISO 10832 (ISO, 2009). To ensure consistency with other experiments in this study, *Rhizophagus irregularis* DAOM 197198 (synonym *Rhizoglomus irregulare*) was used instead of the recommended *Funneliformis mosseae*, constituting a deviation from the ISO standard. Spores were acquired from the commercial product MYCORISE® ASP (Premier Tech), which contains a different strain of the same species used in subsequent experiments, as our AMF strain did not yield enough material to perform the germination experiment with sufficient replicates.

For each replicate, 30 g of autoclaved sand was placed in a sterile

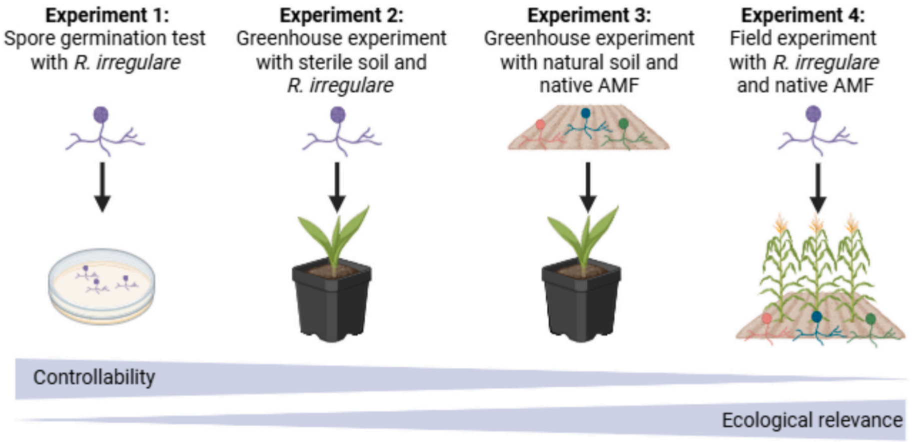


Fig. 1. Overview of the conducted experiments along opposing gradients of controllability and ecological relevance. 1) *In vitro* spore germination test to assess the impact of varying fungicide levels on AMF spore germination of specific AMF taxa. 2) Greenhouse experiment using sterilised soil inoculated with a specific *Rhizoglomus irregulare* strain to examine how seed-applied fungicides influence this controlled symbiosis. 3) Greenhouse experiment using natural soils (conventional, organic, and grassland) to investigate the effects of seed-applied fungicides on native AMF communities. 4) Field experiment evaluating the impact of seed-applied fungicides on local AMF communities and the inoculated *R. irregularis* under real-world conditions. Image created with Biorender.

Petri dish and covered with a nitrocellulose membrane (47 mm diameter, 0.45 µm pore size, 3 mm grid). Approximately 40–50 spores were placed on the membrane, followed by a second membrane and an additional 30 g of sand. The substrate was moistened with 5 mL of Milli-Q water containing the respective fungicide: Metalaxyl-M, fludioxonil and their combination (Supplementary Fig. 1). Each treatment was replicated three times. Petri dishes were sealed with parafilm and incubated at 26 °C for two weeks. Cadmium nitrate [$Cd(NO_3)_2 \cdot 4H_2O$] was used as a positive control, and Milli-Q water served as a negative control (Supplementary Fig. 2A). Spore germination was assessed under a binocular microscope; spores were considered germinated when hyphal length exceeded twice the spore diameter.

2.3. *Experiment 2: greenhouse experiment with sterilised soil*

Soil was collected from a grassland site near Agroscope Reckenholz, sieved through a 5 mm mesh to remove stones and coarse debris, and mixed 1:1 (*v*/v) with sand using a concrete mixer. The soil–sand mixture was sterilised by autoclaving at 121 °C and 2 bar for 90 min. Following sterilisation, the substrate was sealed in bags and stored for four weeks in the dark to allow the breakdown of toxic manganese compounds formed during autoclaving (Boyd, 1971). Pots were sterilised separately by autoclaving at 105 °C and 2 bar for 30 min. AMF inoculum was prepared as described above.

The sterilised substrate was filled into 3 L pots to a total weight of 3.4 kg per pot. Garden fleece was placed at the bottom of each pot to prevent soil loss. Pots were amended with either 5% (v/v) AMF inoculum (resulting in approximately 5000–7000 propagules per pot) or an equivalent volume of autoclaved control inoculum. Maize seeds were coated with Maxim XL and Korit 420 FS. Uncoated seeds were surface-sterilised by washing in 70% ethanol for 5 min, followed by thorough rinsing with autoclaved dH_2O, incubation in 5% bleach with 5 drops/L Triton X-100 for 10 min, and rinsing again with dH_2O. Coated seeds were not sterilised since it would have removed the coating. We acknowledge that the seeds were treated differently; however, because uncoated seeds were sown immediately after sterilisation and all pots were thoroughly watered, any effects of the additional rinsing step are likely negligible.

The experiment was divided into two sub-experiments. In the first, the effects of seed-applied fungicides on the symbiosis between *R. irregulare* SAF22 and three maize varieties (P9610, LG31245, and SY Enermax) were assessed, with plants harvested after three and six weeks. In the second sub-experiment, only variety P9610 was used, and plants were harvested weekly over six weeks to evaluate temporal dynamics while reducing the total number of experimental units. Treatments included controls without AMF inoculation and/or without seed coating. With seven replicates per treatment, the first sub-experiment comprised 168 pots (2 × 2 × 3 × 2 × 7), and the second comprised 112 pots (2 × 2 × 1 × 4 × 7), arranged in 18 blocks in a randomised complete block design (RCBD).

2.4. *Experiment 3: greenhouse experiment with natural soils*

For the greenhouse experiment using natural soils, nine soils were collected across northern Switzerland: three from grasslands, three from organically managed fields, and three from conventionally managed fields, representing a gradient of agriculturally relevant microbial communities. Soil was collected in May and June 2024. For the organically and conventionally managed fields, this occurred just before the maize was sown. Depending on the stage of the crop rotation, the previous crop was either wheat or meadow. At each site, soil was excavated from eight locations per field (approximately 30 × 30 cm wide and 20 cm deep), yielding 140–150 L per field. To minimise microbial disturbance, soils were stored at 4 °C until use and homogenised by sieving through a 5 mm mesh prior to potting. 3 L pots were filled with 2.4 L of soil. The physico-chemial properties of the soils can be found in Supplementary Table 2.

Maize seeds (variety P9610) were either uncoated or coated with Maxim XL alone or in combination with Korit 420 FS using a seed-coating machine. A volume of 1.7 L per 100 kg of seeds (equivalent to 2.55 mL per 150 g) was applied for 1 min. Plants were harvested five or nine weeks after planting. Each treatment was replicated six times, resulting in 324 pots (3 × 9 × 6), arranged in 12 blocks following an RCBD.

2.5. *Experimental conditions and harvest procedures (experiments 2–3)*

All greenhouse experiments were conducted at Agroscope Reckenholz under controlled conditions with a minimum temperature of 20 °C during the day and 16 °C at night. Artificial lighting was provided for 16 h per day and switched off for 8 h; lights were automatically disabled if temperatures exceeded 25 °C. Three seeds were sown per pot, and one week after emergence, seedlings were thinned to one plant per pot. Water-holding capacity (WHC) was determined for each soil, and pots were maintained at approximately 60–70% WHC. Irrigation was applied two to three times per week.

Aboveground biomass was harvested by cutting plants at soil level and determining fresh weight. Roots were rinsed with tap water, cut into ~1 cm fragments, and thoroughly mixed. Subsamples were stored in 2 mL tubes for DNA extraction. Remaining root material was dried at 60 °C for 72 h, as was shoot biomass. Total belowground dry biomass was extrapolated based on fresh-to-dry weight ratios.

2.6. Experiment 4: field experiment

The field experiment was conducted on a conventional field that also served as the source of natural soil for the greenhouse experiment ("Con3"). Inoculation and reseeding were performed shortly after the farmer's original sowing. For each plot, a furrow (3 m × 15 cm × 15 cm) was excavated, and seeds were temporarily removed. A total of 1.7 kg of either AMF inoculum (SAF22) or autoclaved control inoculum was mixed with the excavated soil and returned to the furrow, resulting in an inoculum concentration of approximately 5% (*v*/v), following Lutz et al. (2023). This equates to around 20,000 propagules per metre inoculated. Twenty seeds per plot (10 coated and 10 uncoated) were sown at standard spacing (15 cm) and depth (5 cm). The maize variety P9610 was used throughout (Supplementary Fig. 3).

Harvests were conducted five and sixteen weeks after sowing. The first time point was selected to capture the early stages of AMF symbiosis establishment, whereas the second corresponds to the typical maize growing season in Switzerland and represents the maturity stage just prior to silage harvest. At the first harvest, three plants per plot were pooled due to field variability. At the final harvest, all remaining plants per plot were combined. Shoots were weighed, shredded, homogenised, and dried at 60 °C for 72 h. Cobs were weighed separately and dried for an extended period. Fine roots were cleaned and collected for further analyses.

2.7. Determination of root colonisation (experiments 2–4)

DNA was extracted from homogenised, lyophilised root samples using the NucleoSpin® 96 Plant II Kit (Macherey-Nagel). Lyophilised samples were combined with three to four 1-mm diameter glass beads and homogenised using a FastPrep-24™ 5G instrument (MP Biomedicals). Approximately 20–30 mg of the resulting ground tissue was transferred to 1.5-mL Eppendorf tubes for DNA extraction. Cell lysis was performed using the FastPrep-24™ 5G, followed by centrifugation. The lysates were applied to a 96-well binding plate, washed three times according to the manufacturer's protocol, and DNA was eluted by centrifugation. Eluted DNA was diluted 1:10 with deionised water in a 96-well PCR plate. DNA concentration was quantified using PicoGreen staining (Lumiprobe Pico488) measured with a Varian Eclipse™ fluorescence spectrophotometer. Finally, DNA samples were normalised to a concentration of 1 ng/μL using a PIPETMAX® 268 automated pipetting system (Gilson®).

Quantitative PCR (qPCR) was performed using isolate-specific primers targeting *Rhizoglomus irregulare* SAF22 (Rirre_22KS-F and Rirre_Alk-R) for inoculated samples. For samples containing natural AMF communities, total AMF abundance was quantified using the primer pair AMG1F and AM1. Primer sequences are provided in Supplementary Table 3. All reactions were run in technical triplicate on a 384-well plate using a CFX Opus 384 Real-Time PCR System and analyzed with CFX Maestro Software (Bio-Rad). Each 10-μL reaction contained 5 μL of template DNA (1 ng/μL), 2 μL of 5× HOT FIREPol® EvaGreen® qPCR Mix Plus, 0.5 μL of each primer, and 2 μL of sterile deionised water. A 10-fold serial dilution of standard DNA (1×10^{-3} to 1×10^{-9} ng/μL), amplifiable by both primer pairs, was used to generate standard curves. The qPCR cycling program consisted of an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing for 30 s at either 62 °C (Rirre_22KS-F/Rirre_Alk-R) or 63 °C (AMG1F/AM1), and elongation at 72 °C for 20 s. A melting curve analysis was performed at the end of each run to verify amplification specificity.

We acknowledge that qPCR quantifies AMF abundance rather than the proportion of root length colonised and therefore does not provide a direct measure of colonisation intensity. However, qPCR allows high-throughput, strain-specific assessment across our large sample set, enabling robust comparisons of AMF abundance among treatments.

2.8. AMF community sequencing and bioinformatics (experiment 3)

AMF communities were characterised by sequencing a long rDNA fragment spanning the SSU–ITS–LSU regions using a modified two-step PCR protocol based on Kolaříková et al. (2021). A two-step PCR approach was employed using the primer pairs NS31/LSUmAr and AML1/LSUmAr for PCR1, followed by NS31_Glo3/LSUmBr for PCR2 (Kolaříková et al., 2021; Krüger et al., 2012; Lee et al., 2008; Simon et al., 1992). In PCR2, universal M13 primer tails and a 5AmMC6 modification were added to prevent ligation of carry-over amplicons from PCR1 to SMRTbell adapters during downstream library preparation. PCR1 was performed separately for each primer pair, and the resulting products were pooled prior to PCR2, which was conducted in triplicate. Each 10 μL PCR reaction contained 1× Kapa HiFi HotStart DNA Polymerase ReadyMix (Roche, Basel, Switzerland), 500 nM of each primer, and either 4 ng of template DNA (PCR1) or 4 μL of pooled PCR1 product (PCR2). In contrast to the original protocol, PCR1 products were not diluted but purified using self-made SPRI beads (GDC, Zurich, Switzerland) after both PCR steps.

Index PCR was subsequently performed in a 20 μL reaction volume using custom M13 barcodes as described by Stalder et al. (2025). Each reaction contained 1× Kapa HiFi HotStart ReadyMix (Roche), 300 nM of each forward and reverse index primer, and 6 μL of purified PCR2 product. PCR conditions are provided in Supplementary Table 4. Index PCR products were visualised on a 1% agarose gel, and fragments ranging from 1500 to 3000 bp were excised and purified using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel) according to the manufacturer's instructions. DNA concentrations were quantified using the Qubit™ dsDNA Quantification Assay Kit Q32853 (Thermo Fisher Scientific, Waltham, MA, USA). Samples were pooled using a Liquid Handling Station (BRAND, Wertheim, Germany). The pooled library was diluted to a final concentration of 8 ng/μL and sequenced on a PacBio Revio Sequencer using a SMRT Cell 25 M with a 12 h movie (<5 kb libraries; Pacific Biosciences, Menlo Park, CA, USA). Library preparation and sequencing were performed at the Functional Genomics Centre Zurich.

Raw sequencing data were demultiplexed using the PacBio *lima* tool v2.13.0 with the specified adapter and barcode sequences. Default parameters were applied, with the additional options —different (retaining only barcode pairs composed of different barcodes) and —split-names (naming output files according to resolved barcode-pair identifiers). Primer sequences were removed using *cutadapt* v4.8 (Martin, 2011), and sequence quality was assessed with *FastQC* v0.12.1 (Andrews, 2010). Trimmed reads were imported into *QIIME2* v2022.11.1-2 (Caporaso et al., 2010) using the Single-EndFastqManifestPhred33V2 format and denoised with the DADA2 *denoise-ccs* plugin (Callahan et al., 2016), employing pseudo-pooling (—pooling-method pseudo) and pseudo chimera detection (—p-chimera-method pseudo), while all other parameters were left at default settings. The resulting high-quality amplicon sequence variants (ASVs) were subsequently clustered into operational taxonomic units (OTUs) at a 97% sequence similarity threshold. Taxonomic assignment of representative OTU sequences was performed using the QIIME 2 *feature-classifier classify-sklearn* method (Bokulich et al., 2018) with the EUKARYOME reference database (v1.9.3) (Tedersoo et al., 2024). The OTU table, taxonomy assignments, and representative sequences were exported for downstream analyses in R (R Core Team, 2020). The OTU and taxonomy tables, as well as associated metadata and representative sequences can be found in the Supplementary Material 2.

2.9. Statistical analysis

The data handling, manipulation and statistical analysis were performed with R under version 4.4.0 (R Core Team, 2020). For all analysis of variance (ANOVA) and linear regression models, the normality assumption for the data and residuals was tested using the Shapiro-Wilk test from the *stats* package (R Core Team, 2020), while the assumption of homogeneity of variances was assessed using Levene's test from the *car* package v3.1.2 (Fox and Weisberg, 2018). If normality assumptions were in doubt, graphical analyses such as Q-Q plots and histograms were employed to evaluate data distribution. When assumptions were violated, data were log10 transformed or the non-parametric factorial test implemented in the *ARTool* package v0.11.2 was applied (Wobbrock et al., 2011).

Where appropriate, mixed linear models (MLM) were employed to account for fixed and random effects, using the *nlme* package v1.5.1 for model fitting (Pinheiro J et al., 2025). The block variable was typically included as a random effect to account for any differences occurring between blocks. Additionally, in the greenhouse experiment with natural soils, the soil variable was included as a random effect to account for variation among soil types.

The mycorrhizal growth response (MGR) was calculated following Köhl et al. (2016) to quantify the biomass response to AMF inoculation. For each inoculated plant (I), biomass was compared to the mean biomass of control plants (C_mean) within the same seed-coating treatment.

AMF community sequencing data were imported into the *phyloseq* package v1.50.0 (McMurdie and Holmes, 2013) in R. Replicates were merged using the *merge_replicates* function (fun = sum). Merged samples were rarefied to an even sequencing depth corresponding to the sample with the lowest read count (*i.e.*, 355 sequences). Sequences assigned to the phylum Glomeromycota were extracted using the *subset_taxa* function. Community composition was visualised using bar plots generated with the *plot_bar* function in *phyloseq* and *ggplot2* v4.0.0 (Wickham, 2011). Differences in beta dispersion were assessed using Bray–Curtis distances calculated with the *phyloseq::distance* function. To assess effects of the different soils and seed coatings on AMF community composition, permutational multivariate analysis of variance (PERMANOVA) was used in the *adonis2* function of the *vegan* package v2.7.2 (Oksanen et al., 2022) and 999 permutations. To assess OTUs that were differentially abundant between seed coating treatments, differential abundance analysis between coated and uncoated samples was conducted using *DESeq2* v1.46.0 (Love et al., 2014) (test = "Wald", fitType = "parametric") and contrasts were extracted with the *results* function. All plots were created using the package *ggplot2*. Partial redundancy analysis (pRDA) was performed on a Hellinger-transformed OTU table using the *rda* function of the *vegan* package, with "Soil" included as a conditioning variable. Significant OTUs were fitted onto the constrained ordination using the *envfit* function, with a significance threshold of $p < 0.05$.

2.10. Accession numbers

The sequencing data has been deposited in the NCBI database under the accession number PRJNA1395854.

3. Results

3.1. Spore germination test (experiment 1)

The results of the spore germination test indicate the proportion of germinated spores after a two-week incubation with each treatment. The combination of the fungicides fludioxonil and metalaxyl-M significantly inhibited spore germination on average by 27% ($F_{(4,10)} = 6.797$, $p = 0.007$; Supplementary Table 5). This inhibitory effect was observed even at concentrations four times lower than the recommended dose in the commercial product Maxim XL (Syngenta) (Fig. 2C). Similar effects were observed when fludioxonil and metalaxyl-M were applied individually (Fig. 2A and B and Supplementary Table 5). Furthermore, the commercial fungicide mixture, used in the subsequent experiments (Maxim XL and Korit 420 FS), also showed a trend toward reduced spore germination ($F_{(4,10)} = 3.098$, $p = 0.067$; Fig. 2D). Similar responses to the cadmium–nitrate positive control confirmed the reliability of our methods (Supplementary Fig. 2A).

Notably, strong inhibition occurred even at the recommended application dose (Fig. 2), and in most cases, inhibition was evident at lower concentrations. However, germination rates were already low at minimal doses and did not decline further at higher concentrations, producing a plateau rather than a clear dose–response relationship. This suggests that future experiments should explore even lower fungicide concentrations to determine minimal inhibitory thresholds.

3.2. Greenhouse experiment with sterilised soil (experiment 2)

The objective of this experiment was to evaluate the effect of seed-applied fungicides on the inoculated AMF (SAF22). The impact of AMF inoculation on plant dry weight was assessed using the mycorrhizal growth response (MGR), a ratio that quantifies the difference in plant biomass between inoculated and non-inoculated (control) plants (Fig. 3). At three weeks, all maize varieties showed a slight negative MGR when treated with seed-applied fungicides compared to untreated control plants (Fig. 3A). The largest difference was observed in variety LG31245, where the mean MGR was −7.00% with fungicide treatment, while plants without seed coating showed a mean MGR of 5.79% ($t_{(12)} = -1.504$, $p = 0.158$; Supplementary Table 6). At six weeks, the effect of seed coating on the MGR was marginally significant ($F_{(1,36)} = 4.011$, $p = 0.053$). Additionally, an interaction between maize variety and seed coating treatment indicated a trend toward differential varietal responses to seed coating ($F_{(2,36)} = 2.43$, $p = 0.102$; Supplementary Table 6). The shoot and root dry weight analysis showed that the AMF inoculation increased the shoot biomass and that seed coating had a significant negative effect on the shoot and root biomass of P9610 variety only (Supplementary Fig. 4 and Supplementary Table 7). Root colonisation was only observed in AMF-inoculated plants, proving that the control inoculum worked as intended (Supplementary Fig. 5A). Among these, no significant difference in AMF colonisation was found between seed coating and non-coating treatments (Supplementary Table 8).

3.3. Timeseries experiment (experiment 2)

To explore temporal patterns, plant shoots and roots of the variety P9610 were havested each week. Plants without seed-applied fungicides consistently exhibited a more positive MGR than those treated with fungicide (Fig. 4). This trend was supported by a two-way ANOVA, which revealed a significant main effect of seed-applied fungicides on MGR ($F_{(1,54)} = 4.895$, $p = 0.03$; Supplementary Table 9). However, a statistically significant difference between the seed coating treatments was only observed after 4 weeks ($t_{(12)} = -2.216$, $p = 0.047$). At this timepoint, the MGR was −8.62% in plants with seed coating and 8.52% in those without. Notably, the negative effect of seed coating was observed not only in AMF-inoculated plants but also in those treated with the control inoculum, suggesting that the fungicides may have a generally detrimental effect on plant growth (Supplementary Fig. 6). Root colonisation increased during the early timepoints, peaked at three weeks, and subsequently declined (Supplementary Fig. 7). As in the main experiment, seed-applied fungicides did not significantly affect root colonisation (Supplementary Table 10).

3.4. Greenhouse experiment with natural soils (experiment 3)

To evaluate the effect of seed-applied fungicides on naturally

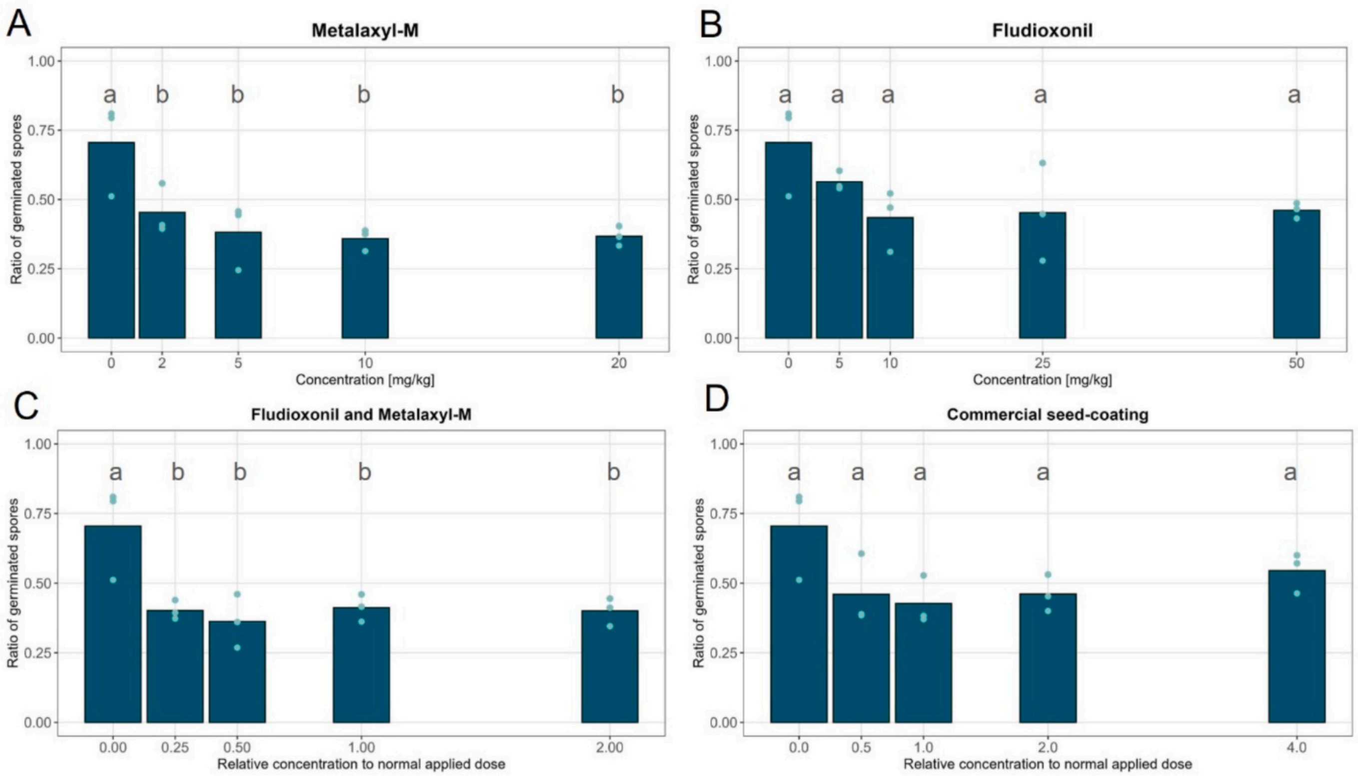


Fig. 2. Results of the spore germination test (experiment 1). The results for the *two* individual substances (A) fludioxonil and (B) metalaxyl-M, (C) their mixture and (D) the commercial seed coating consisting of Maxim XL and Korit 420 FS, are shown. Bar heights represent the mean germination ratio, and light blue points indicate the ratios from individual tests. The x-axis shows the absolute (A, B) or relative (C, D) concentration compared to the recommended applied dose. Pairwise *t*-tests were conducted to compare the different groups, and statistically significant differences are denoted by different letters (a, b). Data represent three replicates per treatment group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

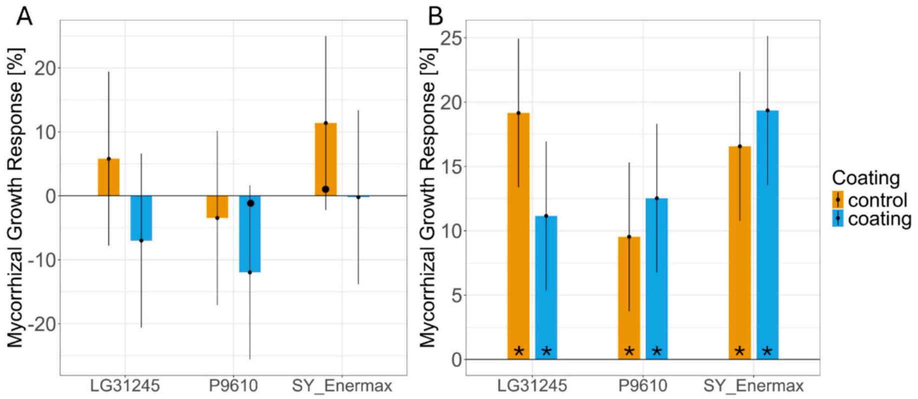


Fig. 3. Mycorrhizal growth response for the greenhouse experiment with sterile soil (experiment 2). Results are shown for harvests after three (A) and six (B) weeks of growth. Bar heights correspond to the mean mycorrhizal growth response (MGR) of the respective group, and the error bars indicate the standard error. The x-axis shows different maize varieties, both with and without a seed coating, and the y-axis shows the MGR as a percentage. Significant deviations from zero (one-sample *t*-test) are indicated in the graph as * ($p < 0.05$) and . ($p < 0.1$). Data represent seven replicates per treatment group.

occurring AMF communities in different soil samples, the same measurements as in the greenhouse experiment with sterilised soil were conducted. No effect of the individual soil samples was detected on either shoot or root dry biomass (Supplementary Fig. 8 and Supplementary Table 11). Additionally, the inclusion of the bird-repellent Korit 420 FS showed no noticeable effect.

The different soil types appeared to have the strongest influence on root colonisation. Notably, samples from grassland soils exhibited the highest AMF copy numbers. In contrast, seed coating treatments did not result in significant differences in colonisation across most soils (Supplementary Fig. 9). A one-way ANOVA, accounting for the random effects of block and soil, confirmed that seed coating had no significant effect on AMG1 copy numbers ($F_{(2,104)} = 0.011$, $p = 0.989$; Supplementary Table 12).

Furthermore, it was tested whether soil properties could better explain root colonisation than seed-applied fungicides. Among the most influential soil variables, simple linear regressions were conducted using pH (slope = −0.384, $p = 0.006$, R2 = 0.041) and bioavailable phosphorus (slope = −0.046, $p < 0.0001$, R2 = 2.26, Supplementary Table 13, Supplementary Fig. 10).

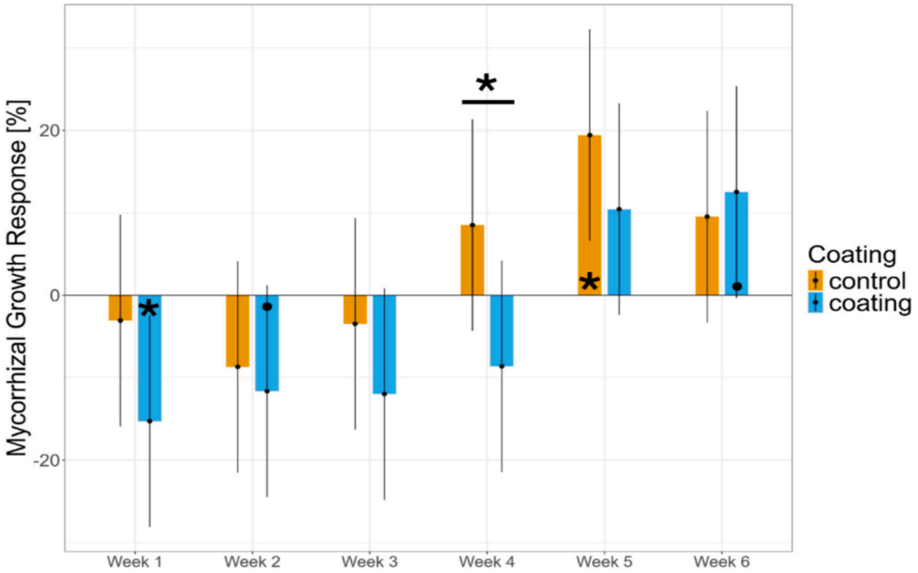
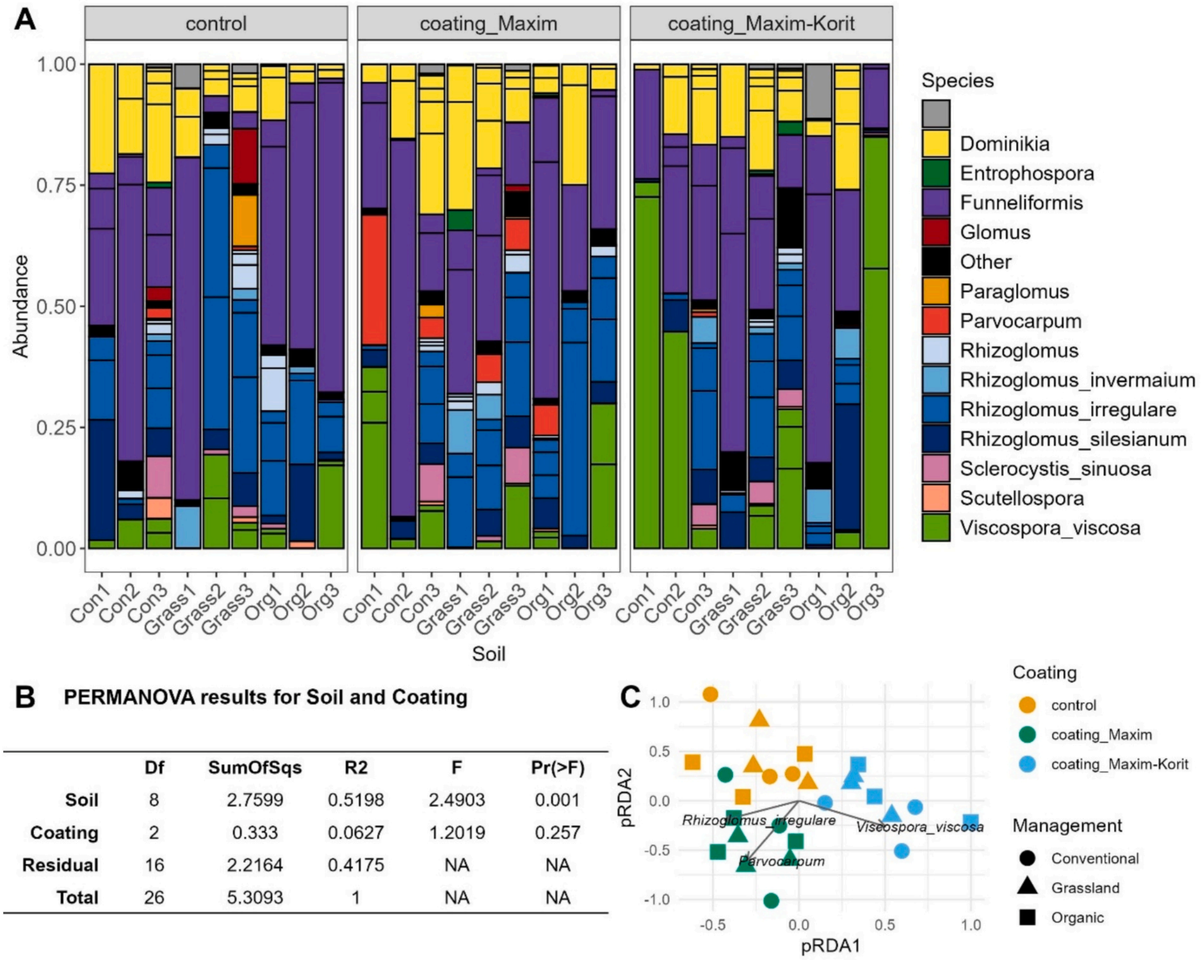


Fig. 4. Mycorrhizal growth response of the time series experiment (experiment 2). The x-axis shows the different treatment groups and their respective harvest timepoints, categorised by the presence or absence of an initial seed coating. Bar heights represent the mean mycorrhizal growth response (MGR) for each group, with error bars indicating the standard error. Significant deviations from zero (one-sample *t*-test) are denoted by * for $p < 0.05$ and by . for $p < 0.1$. Significant differences between treatment groups at the same timepoint are marked with an asterisk. Data represent seven replicates per treatment group.



PERMANOVA results for Soil and Coating

	Df	SumOfSqs	R2	F	Pr(>F)
Soil	8	2.7599	0.5198	2.4903	0.001
Coating	2	0.333	0.0627	1.2019	0.257
Residual	16	2.2164	0.4175	NA	NA
Total	26	5.3093	1	NA	NA

Fig. 5. AMF community composition (experiment 3). A) Relative abundance bar plot of AMF species across soils and seed coatings. Colours represent different species, and black horizontal lines indicate OTUs within each species. B) PERMANOVA results showing the effect of soil and coating on AMF community composition. Soil had a significant effect, whereas seed coating did not. C) Partial redundancy analysis (pRDA) accounting for soil effects, highlighting three ASVs (arrows) that differ significantly among coatings. Species labels are indicated next to the corresponding arrows. Data represent six replicates per treatment group.

A three-way ANOVA including seed coating, pH, and bioavailable P was performed (Supplementary Table 14). Both bioavailable P ($F_{(1,45)} = 17.161$, $p = 0.0001$) and pH ($F_{(1,45)} = 4.183$, $p = 0.047$) had significant effects on AMG1 copy numbers. Significant interactions were found between seed coating and pH ($F_{(2,98)} = 3.707$, $p = 0.028$), as well as a three-way interaction with bioavailable P ($F_{(2,98)} = 6.391$, $p = 0.003$). These results suggest that soil pH modulates the effect of seed coating on AMF colonisation. Overall, this experiment indicates that the effects of fungicide seed coating on AMF abundance are lower compared to the effects of soil type.

3.5. *AMF community composition in greenhouse experiment with natural soils (experiment 3)*

We intentionally sequenced the early harvest time point (5 weeks) to capture potential effects at the initiation of symbiosis, recognising that not all samples might yield sufficient DNA for sequencing at this early stage. Of the 163 original samples, 133 produced enough DNA for sequencing and subsequent bioinformatic processing (Supplementary Table 15). However, due to the low sequencing output, replicates were merged to reach a sufficient sequencing depth (Supplementary Fig. 11). The AMF communities in the roots were dominated by species belonging to the genera *Funneliformis*, *Rhizoglomus*, *Dominikia*, and *Viscospora* (Fig. 5A). PERMANOVA revealed that soil type was the primary factor explaining variation in community composition ($F = 2.49$, $p = 0.001$), whereas seed coating had no significant effect ($F = 0.257$, $p = 0.257$) (Fig. 5B). Differential abundance analysis revealed marginal differences in abundance for only two taxa across seed coating treatments. *Parvocarpum* sp. was more abundant in Maxim XL–treated seeds than in the control ($\log_2 FC = 3.145$, p.adjusted = 0.013), while *Viscospora viscosa* was marginally more abundant in the Maxim XL + Korit treatment compared to the control ($\log_2 FC = 2.747$, p.adjusted = 0.135). Consistent with these results, partial redundancy analysis (pRDA) accounting for soil effects identified three OTUs that were significantly more abundant in coated than in uncoated samples (Fig. 5C). *Viscospora viscosa* (OTU ID: f04629383f04f16119624c23279ae0c; $R = 0.319$, $p = 0.008$) was enriched in samples coated with Maxim XL and Korit, while *Parvocarpum* sp. (OTU ID: a56528467e11442767b59da2cc5fedcc; $R = 0.461$, $p = 0.001$) and *Rhizoglomus irregulare* (OTU ID: 33455e6cf387b3c1535a4e472112986f; $R = 0.225$, $p = 0.046$) were also more abundant in samples coated with Maxim XL only.

3.6. *Field Experiment (experiment 4)*

The biomass of maize plants grown under field conditions was assessed at two time points, five and sixteen weeks after sowing. At the first harvest, plants from non-treated seeds exhibited significantly lower shoot dry weight (Supplementary Fig. 12). In contrast, these plants showed higher root dry weight, an effect that was further enhanced by AMF inoculation. Two-way ANOVA confirmed a significant effect of seed coating on both shoot dry weight ($F_{(1,21)} = 9.900$, $p = 0.005$) and root dry weight ($F_{(1,21)} = 23.59$, $p = 0.001$), with opposite directions of response (Supplementary Table 16).

At the last harvest, plants inoculated with AMF tended to exhibit higher shoot dry weight in treated and non-treated seeds (Supplementary Fig. 12). This pattern was supported by two-way ANOVA (Supplementary Table 16), which revealed marginal but non-significant effects of AMF inoculation ($F_{(1,19)} = 2.691$, $p = 0.117$) and seed coating ($F_{(1,19)} = 2.196$, $p = 0.155$). Given the inherent variability of field experiments, these trends are noteworthy.

To assess the effect of seed-applied fungicides on AMF inoculation success, mycorrhizal growth response (MGR) values were calculated based on shoot, root, and cob biomass (Fig. 6A). Among these metrics, only root MGR showed a larger difference between seed coating treatments, although this effect was not statistically significant ($F_{(1,21)} = 2.162$, $p = 0.152$; Supplementary Table 17).

Root colonisation by the inoculated SAF22 strain, as well as total AMF colonisation, was quantified at both harvests (Fig. 6B). Nearly all plots inoculated with SAF22 displayed high levels of root colonisation. Notably, several plots receiving the control inoculum also showed relatively high SAF22-assigned colonisation (Supplementary Fig. 13), likely reflecting amplification of closely related *Rhizoglomus irregulare* strains by the primer used. As expected, SAF22-specific copy numbers were significantly higher in roots from plots inoculated with the SAF22-containing inoculum (Supplementary Fig. 14; Supplementary Table 18). Moreover, the negative impact of seed coating was more pronounced when SAF22-specific colonisation was considered, especially at the later harvest ($F_{(1,21)} = 3.735$, $p = 0.067$).

At the first harvest, SAF22-inoculated plants exhibited higher total AMF root colonisation, but this difference diminished by the later time point. A two-way ANOVA incorporating harvest time revealed a significant effect of SAF22 inoculation on total AMF colonisation at both time points (Supplementary Table 19), whereas seed coating and the interaction between seed coating and inoculation were not statistically significant. At the later harvest, although differences were not statistically significant (seed coating: $F(1,21) = 1.735$, $p = 0.202$; interaction: F

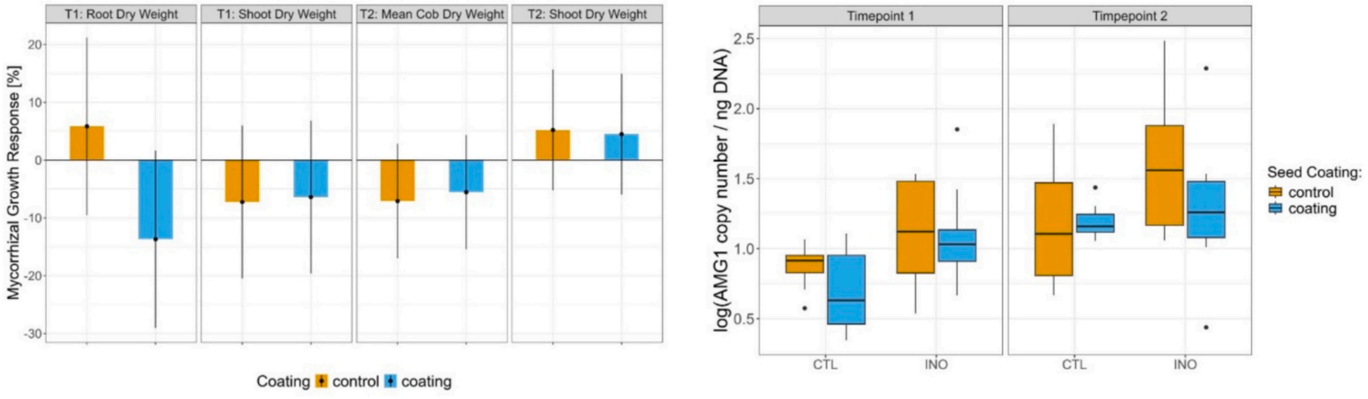


Fig. 6. Results of the field trial (experiment 4). a) Mycorrhizal growth response (MGR) calculated at two time points, presented in separate plots. The first time point (T1) was five weeks after sowing, and the second time point (T2) was 16 weeks after sowing. MGR was calculated for plants with and without an initial seed coating. Bar heights represent the mean MGR for each group, and error bars indicate the standard error. No significant deviations from zero (one-sample *t*-test) or significant differences between treatment groups were detected. B) Log10-transformed AMG1 copy numbers per ng DNA at the two harvesting timepoints: Timepoint 1 (five weeks after planting) and Timepoint 2 (16 weeks after planting). The x-axis indicates whether the plants were inoculated with AMF (INO) or with a control inoculum (CTL). Data represent eight replicates per treatment group.

(1,21) = 1.823, $p = 0.191$), the results hint that seed coating may have slightly reduced root colonisation when SAF22 was applied. This pattern could reflect suppression of locally adapted AMF species by the high concentration of SAF22, particularly under seed coating conditions. Overall, these findings suggest that seed-applied fungicides may be more detrimental to SAF22 than to the broader AMF community, with overall AMF colonisation potentially maintained through compensation by less sensitive AMF taxa.

4. Discussion

4.1. Seed coating effects are strongest at the smallest experimental scale

In the *in vitro* spore germination test (SGT), both fungicides inhibited spore germination across all treatments, with effects that were significant or marginally significant.

A spore was classified as germinated when hyphal length reached at least twice the spore diameter, a less restrictive criterion than the five-fold standard defined in ISO 10832 (ISO, 2009; Malfatti et al., 2021; Mallmann et al., 2018), which typically yields lower germination rates. Importantly, the plateau does not compromise our conclusions, as a similar pattern was observed in the cadmium-nitrate positive control, indicating a methodological rather than treatment-specific constraint.

Both fludioxonil (contact fungicide) and metalaxyl-M (systemic fungicide) inhibited spore germination, but no additive effect was detected when the two were combined. The inhibitory effect of metalaxyl-M aligns with findings by Mallmann et al. (2018), though in our study, inhibition occurred at lower concentrations, suggesting species- and strain-specific differences in sensitivity.

Despite the strong *in vitro* inhibition, these effects were not replicated in subsequent greenhouse or field experiments, supporting our second hypothesis that fungicide effects diminish with increasing system complexity. The discrepancy likely reflects fundamental differences in fungicide exposure: *in vitro* spores are uniformly exposed to high concentrations, whereas exposure in environmental conditions is spatially restricted near the seed coat. Since fludioxonil is a contact fungicide and root growth beyond the seed zone, AMF may not be exposed to biologically relevant fungicide concentrations.

Additionally, sterile conditions of the SGT prevent microbial degradation and soil-buffering processes, representing a worst-case scenario. Environmental factors such as pH, organic matter, and microbial community composition, absent in the SGT, can strongly influence fungicide persistence and bioavailability (Sweeney et al., 2022). Overall, while SGTs are valuable for screening of potentially harmful compounds, their results should be interpreted with caution to avoid overestimating effects under field conditions. Incorporating unsterilised or amended soils, as suggested by Sweeney et al. (2022), or defined microbial consortia from different management systems, could improve ecological realism in future studies.

4.2. Seed coating delays early mycorrhizal growth biomass benefits

AMF inoculation with SAF22 significantly promoted plant growth under greenhouse conditions, particularly at later harvests (weeks 4–6; Fig. 4). In contrast, at the earliest harvest (week 3), the mycorrhizal growth response (MGR) was consistently higher in non-coated plants across all maize varieties, indicating that seed coating delayed the onset of AMF-mediated benefits (Fig. 3). This pattern was maintained throughout the time series (weeks 1–5), with non-coated plants exhibiting higher MGR at all time points except week 6. By the final harvest, this difference had disappeared, likely because root systems had reached near-maximal expansion, reducing the relative contribution of AMF to plant growth. Together, these results suggest that seed coating may influence the early stages of AMF symbiosis under greenhouse conditions, thereby supporting our first hypothesis, although these effects were not consistently observed under field conditions. This is in line with the findings of Hardy et al. (2025) who found that seed coatings had the greatest impact on AMF colonisation in winter wheat after five weeks.

In the field, a contrasting pattern emerged in biomass allocation between shoots and roots. After five weeks, plants grown from non-coated seeds exhibited lower shoot dry weight but higher root dry weight, an effect that was further amplified by AMF inoculation (Fig. 6). This reversal suggests that seed coating influenced early biomass partitioning rather than overall plant growth. One possible explanation is that seed coating reduced pathogen pressure, allowing coated plants to allocate more resources to shoot growth, whereas uncoated plants may have invested more heavily in root biomass as a compensatory response to pathogen stress. In this context, AMF symbiosis may confer additional protection against pathogens (Newsham et al., 1995), potentially explaining the stronger inoculation effects observed in non-coated plants. However, it remains unclear whether these patterns are driven primarily by the direct suppression of soil-borne pathogens by the fungicide or by indirect effects mediated through interactions between AMF and pathogens. For example, Afek (1990) reported increased AMF colonisation following metalaxyl application in non-fumigated soil but no effect in fumigated soil, suggesting that the observed response was likely mediated by pathogen presence rather than a direct stimulation of AMF. Because pathogen abundance was not measured in our study, these mechanisms remain speculative, but they highlight the importance of biotic interactions when evaluating seed coating effects under realistic field conditions.

By the final harvest, seed coating had no detectable effect on shoot or cob biomass. Over the 16-week growing period, early treatment effects likely diminished due to environmental variability, plant growth dilution, and fungicide degradation or leaching, rendering the influence of seed-applied fungicides negligible at later developmental stages. However, as this study was conducted at a single field site, broader conclusions regarding the effects of seed-applied fungicides on AMF under field conditions remain limited. Future studies should therefore examine the effects of seed-applied fungicides across a wider range of field sites and explicitly integrate AMF and pathogen community analyses to better resolve context-dependent outcomes. Experimental approaches that combine controlled mechanistic studies with field-scale experiments will be particularly important to clarify how fungicide seed coatings influence AMF establishment and plant–fungal interactions in agricultural systems.

4.3. Seed coating has limited effects on AMF root colonisation

Seed coating had little to no effect on AMF root colonisation across maize varieties in the greenhouse experiments. Time-series qPCR analyses showed no differences in AMF copy number between seed-coating treatments at the different time points. These findings are consistent with previous studies reporting no effects of metalaxyl in fumigated soil (Afek, 1990) or of fungicide seed treatments in maize (Cameron et al., 2017). In contrast, other greenhouse studies using *Rhizoglomus irregulare* in different host crops have reported reduced AMF colonisation following metalaxyl-M application (Hernández-Dorrego and Mestre Parés, 2010; Jin et al., 2013), indicating strong crop- and context-dependent responses.

This context dependency likely also explains the mixed results reported from field studies. In our field experiment, no effects of seed coating on AMF root colonisation were detected using either general AMF or SAF22-specific qPCR. Similarly, Murillo-Williams and Pedersen (2008) found no effect in soybean, whereas Castelli et al. (2014) reported reduced colonisation in maize.

Despite clear effects of seed coating on plant growth at early time points, the absence of detectable changes in AMF abundance was unexpected and raises questions about the sensitivity of qPCR in this context. While qPCR provides quantitative estimates of fungal abundance, it does not capture functional or structural aspects of colonisation. Microscopic analyses could reveal changes in hyphal density, arbuscule formation, or vesicle abundance that remain undetected by molecular methods. In addition, plant nutrient analyses - particularly phosphorus uptake - could help determine whether seed coatings affect the functional efficiency of the mycorrhizal symbiosis rather than its overall abundance.

4.4. Seed coating effects are highly context-dependent

Soil-specific responses to fungicides were highly variable. In our greenhouse experiment using natural soils, some soils exhibited positive responses while others showed negative effects, likely reflecting differences in both abiotic and biotic conditions. Such variability is not surprising, as natural soils are inherently complex systems where multiple interacting and largely uncontrollable factors can obscure treatment effects. Although soil nutrient variables were included in explanatory models, inference was limited by the small number of fields ($n = 9$), and pH and bioavailable phosphorus explained only a minor fraction of the observed variation.

Host-specific responses further complicated interpretation. In the maize variety P9610, seed coating significantly reduced plant biomass irrespective of AMF inoculation, although the negative effect was more pronounced in the presence of AMF. These results are consistent with broader evidence that fungicides can be phytotoxic. For example, fludioxonil has been shown to reduce CO_2 assimilation (Dias, 2012), and metalaxyl-M has been associated with reduced biomass in citrus and pepper (Singh et al., 2007). Similarly, Jin et al. (2013) reported contrasting biomass responses in pea and chickpea treated with metalaxyl-M and fludioxonil. Notably, in pea, biomass reductions occurred in the presence of native AMF and were more pronounced when the community was diluted by inoculation with a commercial *Glomus irregulare* strain, whereas in chickpea, negative effects were observed only with the commercial AMF inoculum.

In contrast, Cameron et al. (2017) detected no biomass effects in maize treated with fungicide seed coatings and inoculated with a mixture of four AMF species, highlighting that plant responses depend strongly on AMF community composition. Together, these findings suggest that higher AMF diversity may buffer against the negative effects of seed-applied fungicides, which could explain the absence of biomass effects in our greenhouse experiment using natural soil containing native AMF communities. This interpretation is further supported by the relatively small differences in AMF community composition observed between coating treatments, underscoring a potential community-level buffering effect. Nevertheless, a small number of AMF taxa differed significantly in abundance between coating treatments (*e.g. Viscospora viscosa*, *Parvocarpum* sp., and *Rhizoglomus irregulare*; Fig. 5), potentially indicating greater resilience to fungicide exposure. However, the resilience of these taxa cannot be inferred conclusively and should be tested in dedicated experiments. While our experimental design captures natural variation among soils, future studies could benefit from explicitly quantifying microbial community composition to more directly test the role of microbial diversity as a buffering mechanism.

5. Conclusions

Overall, our results highlight that the effects of fungicide seed coatings on AMF are highly context-dependent and vary strongly across experimental systems. While both fungicides clearly inhibited *Rhizoglomus irregulare* spore germination *in vitro*, these effects did not translate into consistent reductions in AMF colonisation or maize growth in greenhouse and field experiments conducted in natural soils. This discrepancy likely reflects the greater ecological complexity of soil environments, where fungicide exposure is spatially restricted, microbial degradation occurs, and diverse native AMF communities may buffer potential negative effects.

Our findings suggest that fungicides may influence early stages of AMF development under controlled conditions and may transiently affect the timing of mycorrhizal growth responses. The relevance of these mechanisms under agricultural conditions remains uncertain and likely depends on soil properties, microbial community composition, pathogen pressure, and host genotype. Here we emphasise the importance of considering AMF interactions when designing seed treatments, since protecting these beneficial fungi can help to create more sustainable and resilient agricultural systems.

Glossary

AMF	Arbuscular Mycorrhizal Fungi
MGR	Mycorrhizal Growth Response
qPCR	Quantitative Polymerase Chain Reaction
SGT	Spore Germination Test
WHC	Water Holding Capacity

CRediT authorship contribution statement

Selim Walliser: Writing – original draft, Investigation, Conceptualization. **Raphaël Boussageon:** Writing – review & editing, Investigation. **Alain Valzano-Held:** Investigation. **Marcel G.A. van der Heijden:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Stefanie Lutz:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT for language editing and refinement. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Jürg Hiltbrunner from the Extension Arable Crops group at Agroscope for sharing his expertise on seed coating, and for providing seeds and fungicides. We would also like to thank the Seed Quality group at Agroscope for allowing us to use their seed coating machine.

This study was financially supported by the Swiss National Science Foundation through grants awarded to MvdH (Implementation Networks Grant Scheme, 40IN40_215832; 10.003.819) and SL (National Research Programme 84, 408440_226950).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107008.

Data availability

All data is available as part of this manuscript and the supplementary material. The sequencing data has been deposited in the NCBI database under the accession number PRJNA1395854.

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