Effects of pesticides and soil contamination history on plant growth and the performance of arbuscular mycorrhizal fungi

ESS 511 Master's Thesis

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Table of Contents

Abstract 1

Figures 2

Tables 2

1. Introduction 3

1.1 Use of Pesticides and Heavy Metals in Agriculture and Viticulture 3
1.2 Relevance of Soil Microbes and Arbuscular Mycorrhizal Fungi 4
1.3 Pesticide Effect on Soil Microbes and Arbuscular Mycorrhizal Fungi 5
1.4 Adaptation of AMF to Pesticide and Tolerance to Heavy Metals 7
1.5 Knowledge Gaps, Research Questions, and Hypothesis 8

2. Materials and Methods 9

2.1 Study Sites and Soil Sampling 9
2.2 Experimental Setup and Run 9
2.3 Harvests 11
2.4 Physiochemical Soil Analysis 11
  2.4.1 Soil Properties across Management Practices 12
  2.4.2 Pesticide Applied and Detected in Field Soils 13
  2.4.3 Copper Concentration across Management Practices 14
2.5 Total AMF Quantifications by qPCR 14
2.6 Analysis of qPCR and Biomass Data 15
2.7 Statistical Analysis 16

3. Results 17

3.1 Effects of Pesticides on Arbuscular Mycorrhizal Fungi and Plant Biomass 17
3.2 Effects of Inoculation and Pesticides on Plant Biomass and Arbuscular Mycorrhizal Fungi 19
  3.2.1 Plant Dry Biomass 19
  3.2.2 Rhizoglomus irregulare (SAF22) Inoculation across Management Practices 20
  3.2.3 Mycorrhizal Growth Response across Treatments 22
  3.2.4 MGR Relationships with RER, Native AMF, and Plant Biomass 23
  3.2.5 Copper Concentration Relationship with Sensitivity towards Pesticide & RER 23

4. Discussion 24

4.1 Native AMF Root Colonization Plateau 4 Weeks after Plantation 24
4.2 Pesticides reduce Plant Biomass through AMF Symbiosis 25
4.3 Contamination History Negative Effect on Native AMF Colonization 26
4.4 No Adaptation towards Pesticide of Native AMF 27
4.5 Different Effects of Rhizoglomus irregulare Inoculation across Management Practices 28

4.6 Resilience of Rhizoglomus irregulare to Soil Stressors *31*

5. Conclusion and Outlook **32**

Acknowlodgements **34**

References **35**

Supplementary Material **41**

Abstract

Pesticides are widely used in agriculture to protect crops, with fungicides playing a key role in vineyards to control fungal diseases. However, pesticides can negatively impact non-target soil organisms like arbuscular mycorrhizal fungi (AMF), which support plant nutrient uptake, soil structure, and stress resilience. While research has explored the effects of pesticides on AMF, most studies focus on inoculated strains or native communities from non-contaminated soils. However, the effects of pesticides on AMF colonization and sensitivity towards pesticides may be different in contaminated soils. This study investigates how soil contamination history influences AMF's response to pesticide application and the effectiveness of AMF inoculation in soils subjected to different management practices (e.g., conventional and organic management). A greenhouse experiment with red clover was conducted using field soils from grasslands, organic vineyards, and conventional vineyards, representing a contamination gradient. We analyzed the effects of fungicide application and AMF inoculation on plant root colonization and plant biomass over time. Pesticides significantly reduced AMF colonization during the early growth phase, but recovery was observed after four weeks. This disruption likely impaired nutrient exchange, as plant biomass in pesticide-treated soils was reduced at later growing stages. Native AMF colonized roots more effectively in organic soils than in conventional soils, but native AMF from conventional soils showed no adaptation to fresh pesticide applications, suggesting a lack of long-term resilience despite prior exposure. Inoculation with AMF increased plant biomass and root colonization across all management practices. In phosphorus-poor, low-contamination soils, the inoculated strain acted additively, enhancing overall colonization. In phosphorus-rich, highly contaminated soils, it partially replaced native AMF. However, no management differences for mycorrhizal growth response were found. Our findings suggest that previous soil pesticide contamination might impair AMF functionality and competitiveness but not reduce their sensitivity towards fungicides. This should be considered when AMF are involved in developing more sustainable agricultural practices.

Figures

Figure 1: **A)** Mineral Nitrogen, **B)** Organic Carbon, and **C)** Bioavailable Phosphorus 12
Figure 2: Copper Concentration 14
Figure 3: AMF Root Colonization at each Harvest Timepoint. 17
Figure 4: Pesticides Effect on AMF Root Colonization and Plant Biomass. 18
Figure 5: Plant Dry Weight.. 19
Figure 6: Inoculation Effects on AMF.. 21
Figure 7: Mycorrhizal Growth Response. 22

Tables

Table 1: List of Pesticides and Respective Active Ingredients 11
Table 2: Occurrence of Active Ingredients used in the Greenhouse Experiment 13
Table 3: Primers Sequence and AMF Target Species 15

1. Introduction

1.1 Use of Pesticides and Heavy Metals in Agriculture and Viticulture

Pesticides are extensively used in agriculture to protect crops from pests, diseases, and weeds, playing a critical role in maintaining global food security (Popp et al., 2013). They include insecticides, herbicides, and fungicides, each having different targets. Insecticides target harmful insects, herbicides focus on unwanted plants or weeds, and fungicides are used against fungal pathogens, each addressing specific threats to agricultural productivity (Lushchak et al., 2018). According to Oerke (2006), global potential losses to pests without crop protection measures can vary widely between crops, ranging from about 50% for wheat to over 80% for cotton production, and from 30% to 40% for rice, potatoes, and maize. In viticulture, the cultivation of grapevines is associated with intensive pesticide use, mostly fungicides, due to their high vulnerability to fungal pathogens like downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*) (Barata et al., 2012; Dry et al., 2010). With a mean pesticide application rate of about 25 kg/ha, wine grapes are the crop with the highest pesticide use per area and the highest total pesticide use in Switzerland (Agrarbericht, 2024). The frequency and quantity of fungicide applications depend mostly on disease pressure, which, among other factors, is significantly influenced by climate conditions. Downy mildew was classified by Bois et al. (2017) as the most damaging disease in viticulture throughout Europe, followed by powdery mildew, which is also present in Europe but was most commonly reported in North America, Australia, and South Africa. Grey mold (*Botrytis cinerea*) was classified as the third most reported disease in Europe. Powdery mildew thrives in diverse climatic conditions, including intermediate and wet regions, due to its tolerance to a wide range of temperatures. Downy mildew and grey mold are strongly associated with high humidity, with the former prevalent in regions with frequent rainfall or irrigation and the latter favored by factors such as relative humidity and wind speed (Bois et al., 2017). Against these pathogens, a broad range of fungicides is applied in viticulture.

Pesticides work by interfering with biological processes such as enzyme activity, nervous system function, or fungal cell development in target organisms (Casida, 2009). The application of pesticides is typically carried out using various methods, including foliar sprays, soil treatments, and seed treatments, with each method tailored to specific target organisms, crops, and environmental conditions (Collier et al., 2016). While pesticides are essential for protecting crops and ensuring food security, their extensive use has raised significant environmental and health concerns (Sharma et al., 2019). Exposure to pesticides may disrupt soil enzyme activities that drive processes like nitrogen fixation and organic matter breakdown, both of which are crucial for maintaining soil fertility (Arora & Sahni, 2016).

In the comprehensive review conducted by Gunstone et al. (2021), they found that pesticide exposure mostly negatively affected soil invertebrates. The results were highly dependent on the pesticides used, the organism, and the parameters studied (biomass, abundance, growth, structural change, etc.) and therefore cannot be generalized.

In viticulture, organic and conventional vineyards differ significantly in pesticide application and the types of products permitted (Borsato et al., 2020). In conventional viticulture, a broad spectrum of plant production products is used, including synthetic pesticides but also sulfur- and copper-based products, where most Swiss producers follow the "Proof of Ecological Performance guidelines (*Ökologischer Leistungsnachweis*, 10.01.2025). In contrast, organic vineyards are restricted to natural or non-synthetic substances, such as sulfur and copper-based products, and biological control agents that are regulated by the Swiss Organic Farming Ordinance and Bio Suisse standards (*Bio Suisse Standards 2023 - EN V2*).

1.2 Relevance of Soil Microbes and Arbuscular Mycorrhizal Fungi

Microbial communities in the soil constitute a significant portion of Earth's genetic diversity and are key players in supporting terrestrial ecosystems (Delgado-Baquerizo et al., 2016). They drive critical processes such as nutrient cycling and can promote plant growth by facilitating nutrient uptake (Van Der Heijden et al., 2008). Among those microbial communities, there are the arbuscular mycorrhizal fungi (hereafter AMF), which belong to the phylum Glomeromycota, which are obligate symbionts that associate with plant roots (Lekberg et al., 2013). They form symbiotic relationships with the roots of 70 to 90% of terrestrial plants (Branco et al., 2022; Shi et al., 2023), and by extending their hyphae, they enhance nutrient acquisition (Khaliq et al., 2022). Inside plant roots, they form specialized structures such as arbuscules, playing a critical role in nutrient and water exchange with the plant (e.g., phosphorus, nitrogen, and micronutrients), which enhances plant growth, resilience, and tolerance to various abiotic and biotic stresses (Augé, 2001; Hodge & Storer, 2015). Mycorrhizal fungi can acquire up to 90% of plant phosphorus (hereafter P) and nitrogen, and certain plants are entirely reliant on nutrients obtained by mycorrhizal fungus (Van Der Heijden et al., 2008). The hyphal network between AMF and plants may also colonize young seedlings, positively influencing their establishment. However, this claim remains unsubstantiated, with some results suggesting no effect or even the opposite (Van Der Heijden & Horton, 2009). A trade-off occurs between plants and AMF, in which AMF obtain carbon compounds, chiefly in the form of sugars and lipids, from the plant in return for the previously mentioned nutrients and water (Roth & Paszkowski, 2017). When nutrients are scarce, plants may preferentially associate with AMF strains that provide higher nutrient returns per unit of carbon invested, thereby optimizing their resource allocation. However, this phenomenon requires further investigation as its generalizability remains uncertain (Weber et al., 2024). Mycorrhizal symbionts get 5–30% of the photoassimilates that plants use, and up to 13 Gt of the CO2 that plants fix on land is temporarily given to the mycelium of mycorrhizal fungi that live underground, highlighting their potential role in carbon cycling and storage (Hawkins et al., 2023; Van Der Heijden et al., 2008).

Arbuscular mycorrhizal fungi also have positive effects on various physical properties of the soil. They enhance soil structure and help the formation of water-stable aggregates, which is crucial for preventing erosion and increasing soil porosity (Rillig & Mummey, 2006). The production of glomalin by AMF hyphae further stabilizes soil particles, promoting a healthy, resilient soil environment (Rillig, 2004). This enhanced structure also increases the soil's capacity to retain water and nutrients, making them more available to plants during periods of stress (Trouvelot et al., 2015). Arbuscular mycorrhizal fungi are found in diverse terrestrial ecosystems, including forests, grasslands, shrublands, and agricultural soils, where their community composition and functions can vary significantly depending on environmental conditions and host plant species (Öpik et al., 2006). Furthermore, AMF contribute significantly to reducing nutrient loss from soils by expanding the nutrient interception zone and mitigating nutrient leaching, especially in systems with high water and nutrient fluxes, such as riparian zones or irrigated farming systems (Cavagnaro et al., 2015). In addition, AMF symbioses can also enhance plant resistance to soil-borne pathogens (Pozo & Azcón-Aguilar, 2007). Arbuscular mycorrhizal fungi interact with wine grapes as well, and a better understanding of this symbiosis could promote more sustainable production.

1.3 Pesticide Effect on Soil Microbes and Arbuscular Mycorrhizal Fungi

Numerous research studies have highlighted adverse impacts of pesticides on soil parameters and their living organisms, yet others have reported minimal or no significant effects, for example, reported by Perucci and Scarponi (1996), where no effect of herbicide application could be detected in microbial biomass in a field experiment even at a 10-fold usual field rate. These contrasting findings indicate variability in the outcomes, influenced by factors such as pesticide type, soil conditions, and experimental designs. In vineyards, the use of fungicides is very common and consequently could lead to concerns about the environment around the parcels, the quality of the soil, and the sustainability of the agroecosystem (Komárek et al., 2010). The response to pesticides is substance- and dose-dependent, with target site, application mode, and soil parameters determining the amount of substance reaching fungal structures and exposure risk (Hage-Ahmed et al., 2019).The use of organic and synthetic pesticides over several years can result in a large accumulation of these substances in the soil (Patinha et al., 2018). Although various regulations have been added in recent years, the use of these products is still a possible risk to biodiversity and different soil parameters.

An investigation by Anderson (1981) examined the impact of different application rates and emphasized the dose dependency on microbial biomass of natural field soils treated in an incubation experiment. At low doses (5 µg/g of a fungicide mixture), microbial biomass was initially diminished to 40% of the control but subsequently recovered to levels comparable to the control samples. The application of a higher dosage (50 µg/g) resulted in a sustained decrease in biomass and modified the relative ratios of bacterial and fungal populations, indicating the importance of the dosage.

Microbial biomass consists of living microorganisms smaller than 5–10 cubic micrometers and represents the biologically active fraction of soil, responding sensitively to management practices and pollution (Powlson, 1994). A study by Piotrowska-Seget et al. (2008) indicated that the fungicide Captan significantly diminished the amounts of culturable bacteria, while total bacterial biomass remained unchanged. This indicates that fungicides may negatively affect non-target soil microorganisms and their functions (Piotrowska-Seget et al., 2008). Fox et al. (2007) demonstrated in their study that a mix of fungicides inhibits the signals of plant-secreted flavonoids that activate the bacterial NodD protein, which in turn induces the expression of nodulation genes essential for nodule formation, essential for the functionality of symbiotic nitrogen-fixing bacteria, potentially resulting in nitrogen-deficient soil from these treated plants, thereby impacting soil fertility.

The effects of fungicides on mycorrhizal fungi are not clear and generalizable yet. Fungicides can influence the establishment of mycorrhiza, particularly when the active ingredient is applied in the soil or on the seed (Jin et al., 2013). However, there is also evidence that certain fungicides may promote mycorrhizal development (Arora & Sahni, 2016). Abd-Alla et al. (2000)'s research highlighted the initial lag phase in the AMF colonization due to pesticide application. Initially, pesticides can reduce AMF's ability to colonize plant roots due to their toxic effects on fungal structures. However, over time, the negative effects of pesticides diminish, allowing AMF to colonize more successfully. Abd-Alla et al. showed that after 60 days, AMF's colonization recovered, especially in cowpea and common bean. This recovery could be attributed to AMF's ability to produce resilient spores or to the natural degradation of pesticides, or perhaps a combination of both.

The application method of the products can significantly influence their impact on AMF. There are fundamentally two categories of fungicide: contact (non-systemic), which is utilized as a protective (preventative) measure prior to the onset of disease or pest infestation. The second category of fungicides is systemic fungicides, which are absorbed by the plant and translocated to the infection site, allowing them to circulate throughout the plant and, possibly, accumulate in the root zone. When fungicides are applied through spraying at recommended rates, there might be little or no observable impact on arbuscular mycorrhizal fungi, as the fungi have no contact with the fungicides (Ayman et al., 1996). Jin et al. (2013), in their greenhouse experiment with pea and chickpea as host plants, found out that systemic fungicides had a significant negative impact on AMF colonization, while contact fungicides only had a minimal effect. In a more recent study conducted by Buysens et al. (2015), a partly contradictory result was found, where different systemic fungicides were tested and had different effects on AMF. Among other systemic fungicides, they also tested azoxystrobin, which did not show any effect on the AMF root colonization success.

Pesticides can affect the root colonization of AMF through direct and indirect mechanisms (Pagano et al., 2023). A possible direct effect is the inhibiting of the phosphorus transport by inhibitor molecules (Zocco et al., 2011). The study of Druille et al. (2013) found that glyphosate decreased the AMF spore viability by 5.8 to 7.7-fold compared to untreated soils, even though the application rate was 26% of the recommended field rate. A decrease in spore viability may affect the AMF diversity, population dynamics, and the symbiosis with plants as well (Smith and Read, 2008). Druille et al. (2013) also found that glyphosate significantly decreased the AMF root colonization; however, they did not study how glyphosate affected the AMF, and an indirect effect through harming the plant could not be excluded. Edlinger et al. (2022) found that land-use intensity and fungicide use significantly reduced the nutrient uptake efficiency of AMF, with cropland soils showing decreased AMF richness and phosphorous transfer compared to grassland soils.

Fungicide sprayed directly on the crops can indirectly affect the AMF. In the study conducted by Okiobe et al. (2022), the fungicide application occurred directly on the tomato, and even though no direct physical contact with the AMF occurred, the AMF root colonization was negatively affected. This can be related to the systemic response in the host plant (Baibakova et al., 2019). A study conducted by Lekberg et al. (2009), more related to ecology than agriculture, indicated that the application of herbicides to eliminate exotic weeds may result in their replacement by less efficient hosts for AMF, thereby altering vegetation composition and necessitating restoration efforts.

Copper-based fungicides, such as Bordeaux mixture, have been used very extensively worldwide for more than 100 years, which has contributed to high amounts of copper in the soil (Merry et al., 1983). Copper contamination can possibly reduce microbial activity and alter their communities and diversities (Smit et al., 2006). Copper in high concentrations might also lead to a decline in phosphorus, zinc, and iron and therefore affect soil fertility (M.O. Azeez et al., 2015). In addition, erosion and runoff in vineyards can lead to the dispersion of copper and plant protection products in general, which can potentially end up in nearby watercourses or non-target soils (Babcsányi et al., 2016; Komárek et al., 2010).

1.4 Adaptation of AMF to Pesticide and Tolerance to Heavy Metals

Arbuscular mycorrhizal fungi have developed specific mechanisms to adapt to pesticides and heavy metals like copper (Hage-Ahmed et al., 2019). Arbuscular mycorrhizal fungi can sequester heavy metals within the roots, reducing toxicity and maintaining symbiotic functions with host plants (Pozo & Azcón-Aguilar, 2007). Giasson et al. (2005) showed that zinc and cadmium were absorbed and translocated to plant roots through the external hyphae of AMF. Arbuscular mycorrhizal fungi have developed ways to deal with copper-contaminated surroundings, namely avoidance and compartmentalization. These techniques encompass confining copper to the cytoplasm, intracellular complexation inside the cytosol, and translocation to the vacuole (Ferrol et al., 2009). However, AMF interactions with metals and their capacity to absorb them seem to be metal- and plant-specific (Giasson et al., 2005b; Lasat, 2002).

There are different strategies adapted by AMF to deal with pesticides. The most common ones are morphological adaptation, compartmentation, transformation, protective chemicals (antioxidants, chaperones, and trehalose), pollutant transport, and modifications in gene expression (Lenoir et al., 2016).

A hormetic response, where AMF functionality increases under low levels of stress (e.g., pesticide application), has been demonstrated in a recent study (Jakobsen et al., 2021). However, it can be concluded that the ability of AMF to tolerate heavy metals and resist pesticides depends greatly on the species and strain of AMF, the pesticides and metals present in the soil, and other physicochemical factors (Hage-Ahmed et al., 2019).

1.5 Knowledge Gaps, Research Questions, and Hypothesis

Although there is an increasing amount of research on the effects of pesticides on AMF, substantial gaps persist in understanding how different histories of pesticide exposure affect AMF colonization and functioning. Most investigations employ inoculated AMF strains or native communities from unpolluted soils, which are not adapted to pesticide treatment and may therefore exhibit increased sensitivity. Conversely, native symbionts in soils exposed to regular pesticide exposure may have evolved adaptation mechanisms, making them less susceptible to further pesticide treatments. This master's thesis overall research questions were how pesticide application and soil contamination history influence AMF root colonization and host plant biomass, particularly in terms of the response of native and conditioned AMF. Additionally, it examined how copper concentration and pesticides affect the AMF.

In this study, soil samples were collected from 12 grasslands, 12 organically managed vineyards, and 12 conventionally managed vineyards, forming a contamination gradient from low to high. A greenhouse experiment was then conducted to assess the research gaps. The soils were treated with a pesticide application. Red clover (*Trifolium pratense L.)* was chosen as the experimental plant, and *Rhizoglomus irregulare* (hereafter *R. irregulare*) SAF22 was used as inoculum to address the following hypotheses:

- Fresh pesticide application negatively impacts AMF root colonization
- Native AMF will be less effective in colonizing roots in highly contaminated soils
- Conditioned native AMF (AMF in conventional soils) will respond less sensitively to fresh pesticide application compared to unconditioned native AMF, which will show a lag phase in root colonization when growing under pesticide conditions
- The incoculation effect will be weaker in highly contaminated soils
- Copper concentration and pesticide application will influence the relative establishment of *R. irregularis* (RER), with potential interactions between these factors affecting the strain's ability to establish

2. Materials and Methods

2.1 Study Sites and Soil Sampling

We conducted a greenhouse experiment using 36 soils from three different management systems, forming a pesticide contamination gradient from low (grasslands) to medium (organic vineyards) to high (conventional vineyards). We sampled 12 soils from each management system. The grasslands were selected near the sampled vineyards and were managed as grassland since there are records, with no history of plant protection products application. The organic parcels were managed organically at least for the last 10 years with no application of synthetic pesticides but the use of other plant protection products. The conventional vineyards were yearly treated with synthetic pesticide until the present. Those selected soils were from the canton of Zurich. We sampled all soils in March 2024 over a period of 17 days. Using a spade, we collected 5 cubes measuring 25 x 25 x 20 cm from the center of each inter-row. We conducted the soil sampling within a 10x10 meter square, ensuring it was at least 10 meters away from the parcel border. We evenly distributed the five sampling locations within the square and collected them in five consecutive inter-rows, maintaining a minimum distance of 5 meters between them. We selected the sampling location at each position to ensure it was as representative as possible for the surrounding vegetation. We collected each soil cube in a plastic bag and stored it undisturbed at 4°C until the conclusion of the sampling campaign. We then prepared the soils for sieving. The preparation included the manual break-up of the soil cubes and the removal of stones, roots, and coarse plant debris. Next, we dried the soils at 7°C for 72 hours, sieved them to 5 mm, and stored them at 4°C.

2.2 Experimental Setup and Run

The experimental setup was conducted over four days, with each day dedicated to one block of nine soils, starting on the 15th of April and continuing until the 18th of April 2024. The management practices were evenly and randomly distributed across 4 blocks. All soil samples were subjected to the same treatments.

The experiment can be divided into two subexperiments. In the first one, we aimed to look at the development of AMF over time under pesticide application compared to a control. We prepared the pots for 4 harvest points (see chapter 2.3), giving a total of 8 pots per soil: 4 pots with fresh pesticide application and 4 without pesticide application. For this experiment, a total of 288 pots were prepared.

With the second subexperiment, we aimed to look at the AMF inoculation success, which was only conducted for the final harvest point. Next to the pesticide treatment, we inoculated half of the pots with the *Rhizoglomus irregulare* isolate BEG21 (accession SAF22) from the Swiss Collection of Arbuscular Mycorrhizal Fungi. This AMF inoculum was initially isolated from a Swiss grassland in 1994 and further cultivated in a greenhouse at Agroscope in Reckenholz, Zürich, using *Plantago lanceolata* as a host plant for AMF propagation. This widespread AMF species is common in various ecosystems, particularly in agricultural fields (Öpik et al., 2006). *Rhizoglomus irregulare* is a host-generalist AM species well-suited to various soil conditions and intensive practices, supports plant nutrition, and has been shown to establish well and form symbiosis with red clover. Half of the pots were inoculated with the AMF spores and the other half with a control without the spores. The total number of pots for this second subexperiment was 144.

The experiment was performed using 1L pots, previously sterilized. The pesticides and AMF inoculum were added before filling the soil into the pots to assure a homogeneous distribution across all pots of a treatment. First, 12.28 ml of a pesticide (reported in **Table 1**) mixture composed of 9 commonly used fungicides was applied with a spray bottle. The quantity was chosen based on the lowest recommended single dose, and the active ingredients were chosen based on the target diseases (p*owdery mildew, downey mildew , and Botrytis cinerea)*. For the pots without the pesticide treatment, the same amount of deionized water was added. Then, the inoculum was added as a mass percentage: 5% inoculum and 95% soil. A soil-specific mass of fresh soil was added to the pots to reach a dry mass of 600 g. In each pot, 12 seeds of red clover were planted at 1 cm depth with the help of toothpicks. Prior to planting, seeds were sterilized. They were soaked in 70% ethanol for 5 minutes, followed by two rinses with sterile water. They were then immersed for 10 minutes in a 5% bleach solution, with Triton X-100 (5 drops per liter) added to reduce surface tension around the seeds. Afterwards, the seeds were rinsed twice more with sterile water. After the seeds were planted, the pots were watered to 60% of each soil's water holding capacity (WHC) and covered with a drilled plastic foil for 3 days to keep moisture. All pots were placed randomly within blocks on tables in a greenhouse at the Research Institute of Agroscope, Reckenholz (**Suppl. Figure 1**). The temperature in the greenhouse was set to 22°C during the day and 18°C at night with 16 hours of light daily. Additional light was provided when the natural light intensity fell below 300 $Wm^{-2.}$. The pots were moved 3 times a week to avoid any differences in growth due to the positioning of the pots inside the greenhouse chamber. Pots were watered to 60% WHC two to seven times a week, depending on watering needs. After 9 days, plants were thinned out to four well-positioned, healthy seedlings in each pot. The continuous removal of unwanted weeds from the pots was carried out consistently until the time of harvest.

About 4 weeks after planting, plants got infected with powdery mildew and had to be treated with wettable sulphur ("Netzschwefel Stulln" from Andermatt Biocontrol, Switzerland) (**Suppl. Figure 2**).

***Table 1** List of pesticides and respective active ingredients applied to our pots (d. mildew stands for downey mildew and p. mildew for powdery mildew).*

Product	**Active Ingredient & % of final mixture**	**kg/ha**	**Systemic**	**Target disease**
Cantus	Boscalid (12%)	1.20	Yes	botrytis
Flint	Trifloxystrobin (2%)	0.24	Yes	d. mildew
Pergado	Mandipropamid (31%)	3.20	Yes	d. mildew
Ridomil Vino	metalaxyl-M (22%)	2.25	Yes	d. mildew
Slick	Difenoconazol (1%)	0.15	Yes	p. mildew
Switch	Cyprodinil (12%)	1.20	Yes	botrytis
Switch	Fludioxonil (12%)	1.20	No	botrytis
Talendo	Proquinazid (4%)	0.40	No	p. mildew
Vivando	Metrafenone (3%)	0.32	Yes	p. mildew

2.3 Harvests

The first subexperiment was split into four harvest time points that were one, two, four, and eight weeks after planting, respectively. For the second sub-experiment, there was only one harvest after week 8. For both subexperiments, the harvest protocol was the same. The plants were first separated manually from the soil, and the remaining soil was washed away from the roots using water. The plants and roots were then tapped dry with a paper towel, and the shoots were removed and placed in a paper bag. The roots were then weighed and cut into pieces of approximately 1-2 cm. A representative sub-sample of roots was collected in Eppendorf tubes for DNA extraction, and the remaining roots were again weighed and put in paper bags. Shoots and roots were dried at 60°C for 48 hours and then weighed using a scale (Mettler Toledo AB104-S/FACT). The Eppendorf tubes containing roots for DNA extraction were stored at -20°C.

2.4 Physiochemical Soil Analysis

Soil properties were analyzed from the 36 untreated field soils. Pesticide residues were measured using the method presented by Rösch et al. (2023). It consists of an adapted “quick, easy, cheap, effective, rugged, and safe (QuEChERS)” extraction (Lehotay et al., 2007) followed by chemical analysis through liquid chromatography coupled to triple quadrupole mass spectrometry with electrospray ionization (LC-ESI-MS/MS). Total copper and other heavy metals were measured using the HNO3 extraction method, and bioavailable phosphorus was measured using the CO2-P method, both according to the reference methods of the Swiss Federal Agricultural Research Station (Schweizerische Referenzmethoden der Forschungsanstalten Agroscope). Mineral nitrogen was determined by the mineral nitrogen compounds (NO3—N, NO2—N, and NH4—N) in the CaCl2 extract. Organic carbon, pH, and silt/clay/sand contents were also measured according to the reference method mentioned above.

2.4.1 Soil Properties across Management Practices

The mineral nitrogen was significantly higher in grassland soils compared to conventional vineyards (**Figure 1A**). The organic carbon (Corg) content did not significantly differ across the management practices (**Figure 1B**). Bioavailable phosphorus (P) content was significantly higher in conventional soils compared to grasslands, which showed the lowest P content (**Figure 1C**).

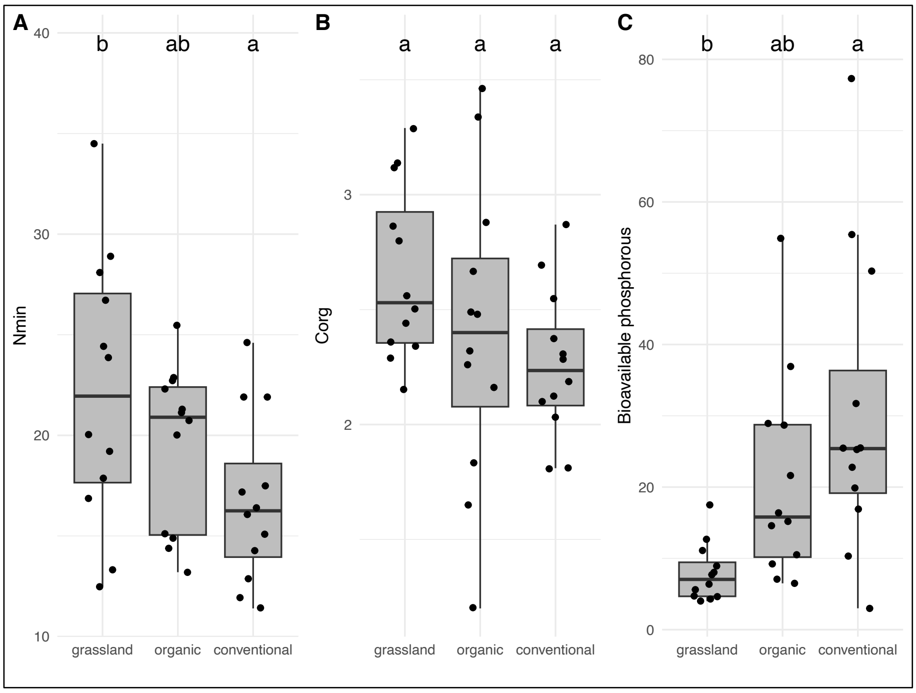

Figure 1 A) *Mineral nitrogen (Nmin),* ***B)*** *Organic carbon (Corg), and* ***C)*** *Bioavailable phosphorus across management practices. Different letters (a, b) indicate significant variations across groups.*

The mean pH value was consistent across all management practices, with a mean of 7.3. The soil's structures were assessed as the silt, clay, and sand proportions; here again, very similar proportions across management practices (almost 40% sand, 35% silt, and 25% clay). The mean of potassium in grassland soils was 1.98 mg/kg, in organic soils 5.17 mg/kg, and in conventional soils 5.53 mg/kg.

Two grassland soils were excluded from the analysis because the plants performed significantly worse in these soils compared to other grassland soils. In one soil, the mineral bioavailable phosphorus and mineral nitrogen levels were very low compared to the median of all grasslands (Nmin of the interested soil = 12.5, median = 21.9; bioav. P of the interested soil = 4, median = 7.05). The second soil had relatively high sand content (68% against the median of 36%) and resulted in low levels in a few metals like iron (6200 against the median of 12013) and zinc (28.5 against the median of 51). A visualization of the plant performance in these soils is provided in the appendix (**Suppl. Figure 3**), where it can also be observed that 3 out of 4 red clover plants died for no observable reason in the second-mentioned soil.

2.4.2 Pesticide Applied and Detected in Field Soils

All the active ingredients applied in the greenhouse experiment were detected in the field soils. These include Boscalid, Cyprodinil, Fludioxonil, Metalaxyl, Difenoconazole, Proquinazid, Mandipropamid, Metrafenone, and Trifloxystrobin. Cyprodinil and fludioxonil were the most common across all management practices (in 23 and 28 soils, respectively), which were also present in all conventional soils and mainly present in organic soils. Fludioxonil was found in 6 grassland soils as well. Metalaxyl and Metrafenone were the most applied in 2023 in the chosen parcels for our experiment with 7 and 6 soils, respectively (**Table 2**).

Table 2 *Occurrence of active ingredients used in the greenhouse experiment across the 36 field soils (12 soils per management practices) and applied in 2023 (data from 1 parcel missing).*

Component Groups/Management practices	***Boscalid***	***Cyprodinil***	***Difenoconazole***	***Fludioxonil***	***Mandipropamid***	***Metalaxyl***	***Metrafenone***	***Proquinazid***	***Trifloxystrobin***
Grassland	3	2	0	6	0	0	1	0	3
Organic	5	9	0	10	0	5	0	0	1
Conventional	8	12	10	12	9	7	8	9	9
TOTAL	16	23	10	28	9	12	9	9	13
Applied in conventional soils in 2023	3	1	3	1	5	7	6	5	5

The pesticide concentration median for grasslands was 0.6 mg/kg, with a median of 2 active ingredients detected. For organic soils, the median concentration was 2.2 mg/kg, with 6 active ingredients. Lastly, for conventional soils, the median concentration was 60.4 mg/kg, with 15 active ingredients.

2.4.3 Copper Concentration across Management Practices

Total copper concentration followed a contamination gradient, with conventional vineyards having significantly higher concentrations than grasslands. The variability across conventional vineyards was much higher compared to the other management practices. The median copper concentration in grasslands was 41.15 mg/kg, in organic soils 192.05 mg/kg, and in conventional soils 358.95 mg/kg (**Figure 2**).

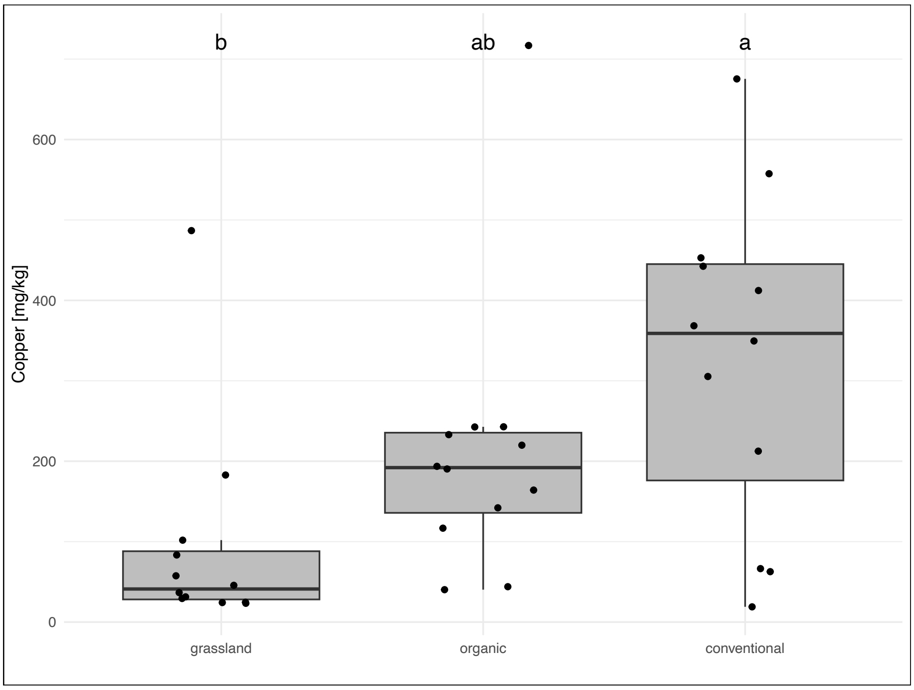

Figure 2 *Copper concentration across management practices. Different letters (a, b), indicate significant variations across groups.*

2.5 Total AMF Quantifications by qPCR

Abuscular mycorrhizal roots from both subexperiments were lyophilized using Christ® Beta 1-8 for 24 hours; the roots were then homogenized with a 3 mm glass bead using the FastPrep-24 5G instrument (MP Biomedicals, USA). Approximately 20 mg of roots were then weighed and used for the DNA extraction (if total root biomass < 25 mg, the whole roots were used for DNA, and the weight was noted). The DNA extraction was performed using the NucleoSpin 96 Plant II DNA Kit (Macherey-Nagel) according to the manufacturer's protocol, including a negative control for each extraction. The DNA concentration was measured using a fluorescence spectrometer (Cary Eclipse Varian, Agilent Technologies, Inc.), and samples were then diluted to a final concentration of 1 ng/µl with molecular-grade ddH2O using the Gilson® PIPETMAX® 268 pipetting robot for quantitative PCR analysis. Arbuscular mycorrhizal fungi root colonization was quantified through absolute quantification using quantitative polymerase chain reaction (qPCR) on the extracted root DNA. This approach was chosen over microscopy due to its efficiency and reduced observer bias. The primers AMG1F and AM1 were used to amplify the 18S rRNA gene, detecting a broad range of AMF species. For detecting *R. irregulare* specifically, the forward primer Rirre_22KS-F (Bender et al., 2019a) and the reverse primer Rirre_Alk-R (Alkan et al., 2006) were used, amplifying a sequence coupling part of the SSU and the ITS1 regions (ITS1-SSU). Details and sequences of the primers used are given in **Table 3**.

A master mix was prepared consisting of 7 µl of 5x HOT FIREPol® EvaGreen® qPCR Mix Plus, 1.75 µl of each primer, and 7 µl of sterile, deionized H2O, which was combined with 17.5 µl of normalized DNA samples (1 ng/µl). Each sample was prepared in triplicate, with a volume of 10 µl per well. A tenfold dilution series of standard DNA (190 bp from mycorrhiza fungi for total AMF community; single spore sequences OTU16 of SAF22, 1100 bp), was employed to establish the standard curve. The quantitative PCR was performed using a CFX Opus 384 Real-Time PCR cycler with CFX Maestro Software (Bio-Rad). The protocol included an initial denaturation phase followed by 44 cycles of denaturation, primer annealing, elongation, and melting curve analysis. The data was input into LinRegPCR to ascertain the Ct-value (cycle threshold), which was subsequently used alongside the standard curve to compute the copy number in each sample. The mean of the triplicates was utilized to subsequently determine the copy number per nanogram of DNA.

Table 3 *Primers sequence and AMF target species*

Target Species	Primers	Sequences 5' – 3'
Whole AMF community	*AMG1F* *AM1*	*ATAGGGATAGTTGGGGGCAT* *GTTTCCCGTAAGGCGCCGAA*
SAF22	*Rirre_22KS-F.* *Rirre_Alk-R*	*GAGACCATGATCAGAGATCAGGT* *GGTCATTTAGAGGAAGTAAAAGTCGTAAC*

The data were then processed with R Studio, and a minimum acceptable amplification efficiency of 1.8 and a maximum Cq deviation of 0.6 between replicates were chosen. For all data analysis regarding the AMF root colonization, the copy number per milligram of DNA was used. The efficiencies of the total AMF community (tot.AMF), quantified using the primers AMG1F and AM1, and *R. irregularis*, quantified using the primers Rirre_22KS-F and Rirre_ALK-R, showed differences between the standards and the samples. To account for these discrepancies and ensure reliable quantification, the One Point Calibration (OPC) method was preferred over the Standard Curve (SC) method (Brankatschk et al., 2012).

2.6 Analysis of qPCR and Biomass Data

To assess the relative abundance of *R. irregulare* within the total AMF community, the percentage of *R. irregulare* copy numbers in total AMF copy numbers was calculated for each sample. To evaluate the establishment success of *R. irregulare*, the ratio between the percentage of *R. irregularis* in inoculated samples and its percentage in non-inoculated samples was calculated. This ratio, referred to as the

Relative Establishment Ratio of *R. irregularis* (hereafter RER), provides a normalized measure of the relative establishment success of *R. irregularis* within the AMF community compared to non-inoculated conditions.

The Mycorrhizal Growth Response (MGR) variable was calculated to evaluate the plant growth response to AMF inoculation. This variable compares the biomass of inoculated clover plants with the biomass of the non-inoculated ones. The formula for MGR quantifies the relative impact of AMF inoculation on plant biomass, serving as an indicator of the symbiotic benefit provided by the inoculum.

$$If\ M_{Inoculated} > M_{Non-inoc}, MGR = \left(1 - \left(\frac{M_{Non-inoc}}{M_{Inoculated}}\right)\right) x\ 100\ [\%]$$

$$If\ M_{Inoculated} < M_{Non-inoc}, MGR = \left(-1 + \left(\frac{M_{Inoculated}}{M_{Non-inoc}}\right)\right) x\ 100\ [\%]$$

$M_{Inoculated}$: biomass of the plants inoculated

$M_{Non-inoc}$: biomass of the plants non-inoculation

2.7 Statistical Analysis

R version 4.2.2 (R Core Team, 2022) was used for statistical analyses. For all statistical tests, a significance threshold of $p < 0.05$ was applied. Significance levels were denoted as follows: $p < 0.001$ = '***', $p < 0.01$ = '**', $p < 0.05$ = '*' and $p < 0.1$ = '.' . Data visualization was performed using the ggplot function within the package "ggplot2" version 3.4.4. In order to analyze the effect of the treatments on the dependent variables, the linear fixed model (LMM) was used with the lmer function from the lme4 package (Bates et al., 2015). The soil IDs (the soils in each management practice) and the experimental blocks were treated as random effects. The statistical significance of fixed effects was assessed using Type III ANOVA with Satterthwaite's approximation for degrees of freedom. Model assumptions, including normality of residuals and homoscedasticity, were checked graphically. After the ANOVA, a post-hoc Tukey's honest significance test (Tukey's test) from 'stats' was used to compare groups. The non-parametric Spearman's rank correlations were used to assess different relationships. For statistical analysis, the log10 of the copy numbers per ng DNA were used.

3. Results

3.1 Effects of Pesticides on Arbuscular Mycorrhizal Fungi and Plant Biomass

The aim of this sub-experiment was to evaluate the effect of pesticide application on plant biomass and AMF root colonization over time across three management practices: grassland, organic, and conventional. The experiment included four harvest timepoints, conducted at one, two, four, and eight weeks after planting.

Arbuscular mycorrhizal fungi root colonization was detected as early as one week after planting in all management practices, with a steep increase observed until week four. However, between the third and fourth harvests (weeks four and eight), no further increase in AMF root colonization occurred; instead, a decline was observed, with colonization rates at T4 being lower than at T3 (**Figure 3**). In contrast, plant shoot dry biomass showed no plateau or decrease after week four, continuing to increase over time (**Suppl. Figure 4**). A mixed-effects ANOVA revealed that management practices significantly affected AMF copy numbers ($p = 0.025$), with conventional soils being slightly but significantly less colonized than organic soils (**Suppl. Table 1b**). Timepoints had a strongly significant effect on AMF root colonization ($p < 0.001$), and colonization was significantly lower in pesticide-treated plants compared to untreated plants ($p < 0.001$). The interaction between pesticide treatment and timepoints also significantly affected AMF copy numbers ($p < 0.001$). All other interactions were not significant (**Suppl. Table 1a**).

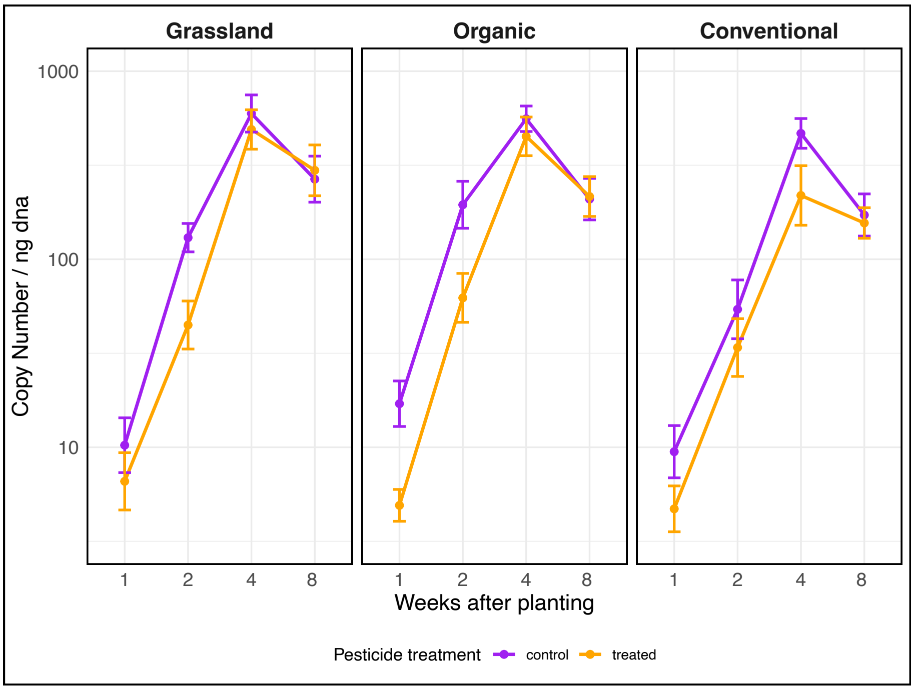

Figure 3 *Mean LOG Copy Number per ng DNA ± SE AMF root colonization per sampling timepoint, soil management, and pesticide treatment.*

The sensitivity to pesticide application was calculated as the ratio of AMF copy numbers per ng DNA in pesticide-treated plants to those in control plants (no pesticide application). A one-sample t-test was used to determine whether this ratio significantly differed from 1. Arbuscular Mycorrhizal Fungi sensitivity to pesticides was highest during the first two weeks, with ratios significantly different from 1 (week 1: $p < 0.001$; week 2: $p = 0.014$), indicating a pronounced negative impact of pesticides during the initial growth phase. By week 4, the sensitivity had decreased, and by week 8, the negative impact of pesticides had diminished further and was no longer detectable (**Figure 4, panel A).** The sensitivity of shoot biomass to pesticide application was similarly calculated as the ratio of shoot dry biomass in pesticide-treated plants to that in control plants. An opposite trend was observed compared to AMF root colonization. At week 1 and 2, the ratio was close to 1, with week 1 showing a slight but significant difference ($p = 0.037$). However, at week 4 and week 8, the ratio decreased significantly ($p < 0.001$), indicating that pesticide treatment negatively affected plant biomass during later growth stages (**Figure 4, panel B**).

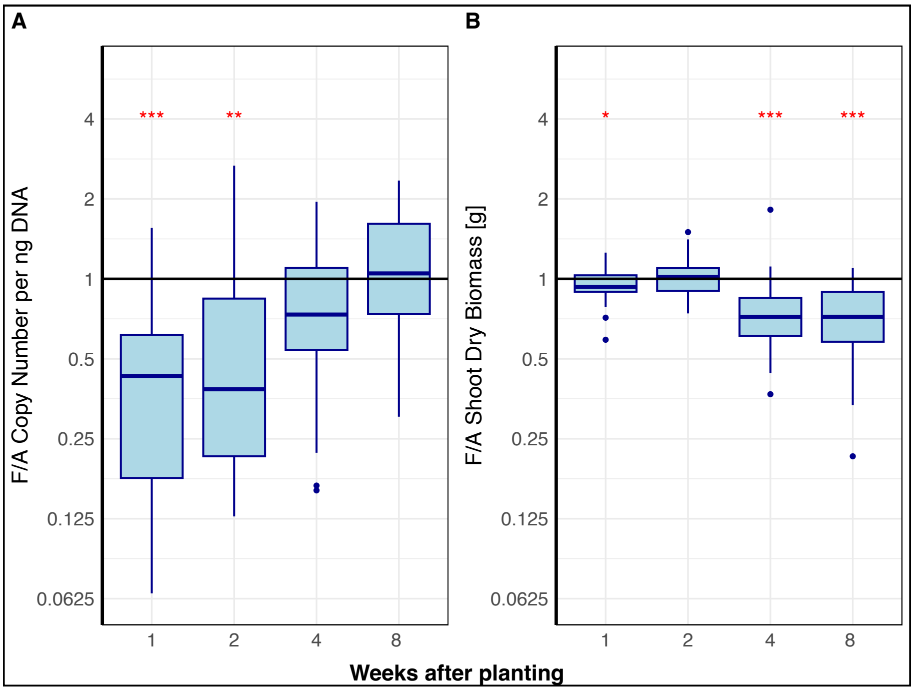

__Figure 4__ Ratios between pesticide-treated vs. non-treated pots for A) AMF root colonization (copy number per ng DNA), and B) shoot dry biomass grouped by harvest timepoint. "F" (Fresh) refers to samples from pesticide-treated pots, while "A" (Aged) refers to those from control pots (no pesticide treatment, only aged residues). The asterisks indicate the level of significant difference of the ratio to 1, based on a t-Test (for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$).*

3.2 Effects of Inoculation and Pesticides on Plant Biomass and Arbuscular Mycorrhizal Fungi

The aim of this experiment was to evaluate the effects of inoculation and pesticide treatment on AMF root colonization and plant biomass across three management practices: grassland, organic, and conventional. For the following statistical analysis and visualizations, the data used were from the last harvest at week 8.

3.2.1 Plant Dry Biomass

Soil management practices had a significant effect on plant dry biomass ($p = 0.005$). Organically managed soils yielded the highest biomass (**Suppl. Table 2b**) with a mean of 1.89 g across the four treatments (pesticide + inoculation), followed by conventional with 1.26 g (66% of organic soils) and grassland with a mean of 1.17 g (62% of organic soils). Pesticide treatment also had a significant effect on the plant biomass ($p < 0.001$), as well as inoculation ($p = 0.003$), decreasing and increasing the biomass, respectively. All interactions between these factors were not significant (**Suppl. Table 2a**). Across the three management practices, the pesticide application resulted in a 24.7% reduction in biomass (median of individual reduction across all soils). The *R. irregulare* inoculation resulted in an increase in biomass of 9.35% (median of individual increase across all soils) (**Figure 5**).

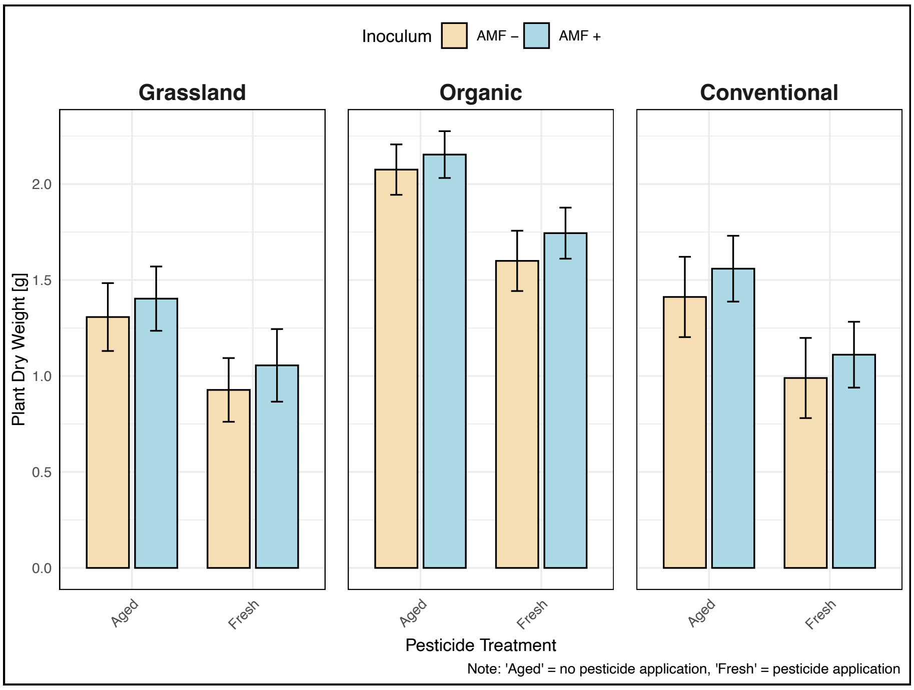

Figure 5 *Mean ± SD of dry plant biomass across management practices, pesticide treatment, and R. irregulare inoculation.*

3.2.2 Rhizoglomus irregulare (SAF22) Inoculation across Management Practices

For all management practices, a significant increase in total AMF colonization was observed in inoculated plants ($p < 0.001$), and the difference in total copy number across management practices was also significant ($p = 0.008$) (**Suppl. Table 3a**). Total copy numbers in grassland soils were significantly higher than in conventional soils (**Suppl. Table 3b**). Although the interaction between management practices and inoculation had no significant effect on total AMF copy numbers ($p = 0.068$, **Suppl. Table 3a**), a slight trend was evident, with the increase through inoculation varying across management practices (grassland > organic > conventional). Pesticide application did not significantly decrease the total copy number.

Before inoculation, the native *R. irregulare* (SAF) copy numbers accounted for less than 3% of the *R. irregulare* copy numbers after inoculation, confirming the success of inoculation.
Management practices also significantly affected the SAF copy number ($p = 0.046$) (**Suppl. Table 4a**), with organically managed soils being slightly more colonized than conventional soils (**Suppl. Table 4b**). The interaction between inoculation and management practices significantly influenced the SAF copy number ($p < 0.001$), with inoculated SAF copy numbers being significantly higher in grassland soils than in conventional soils (**Suppl. Table 4c**). Although pesticide application did not significantly affect the SAF copy number overall, the interaction of pesticide and inoculum was significant ($p = 0.004$) (**Suppl. Table 4a**).

Figure 6 shows the relative increase in total AMF colonization through *R. irregulare* inoculation and the relative contribution to the inoculated strain. The inoculation led to a greater increase in AMF colonization in grasslands, where it acted in an additive manner. Conversely, in organic and conventional soils, the inoculated AMF strain partly replaced the native AMF population. In all management practices, inoculation resulted in an overall increase in total AMF copy numbers. In grasslands, inoculation increased whole AMF community colonization by a factor of 2.5 in pesticide-free samples and by a factor of 2 in pesticide-treated samples. For organic soils, the increase was approximately 1.9 in pesticide-free samples and 1.6 in treated samples. In conventional soils, the increase was about 1.4 in pesticide-free samples and 1.6 in treated samples. These results highlight the variability in inoculation effectiveness across different management practices.

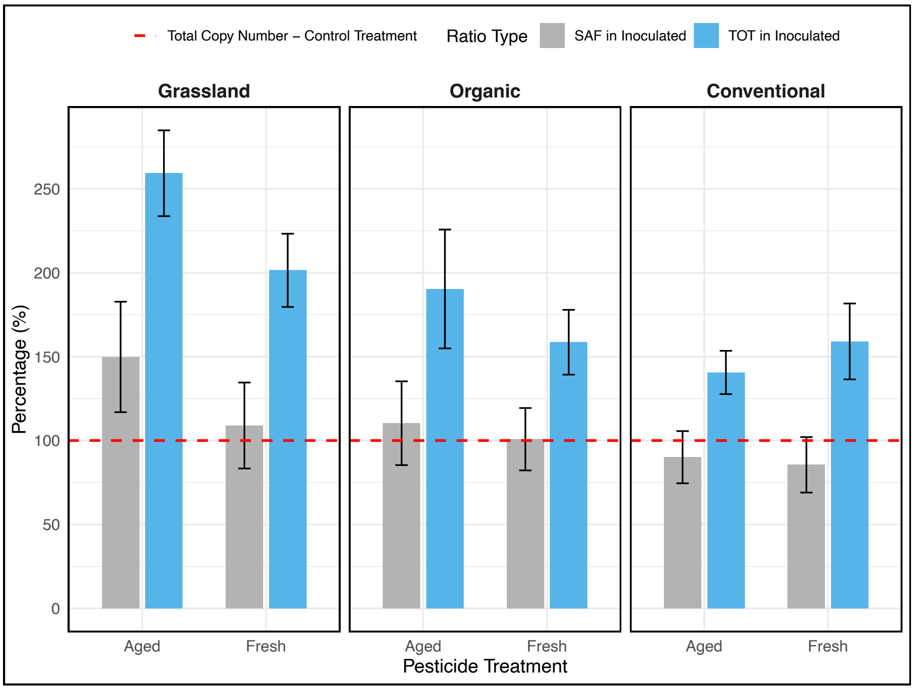

Figure 6 *Mean ± SE of proportion in % of total AMF (copy number per ng DNA) colonization of inoculated plant roots compared to the non-inoculated control in blue and the proportion in % of SAF (copy number per ng DNA of R. irregulare) compared to the non-inoculated control in grey. "Fresh" refers to samples from pesticide-treated pots, while "Aged" refers to those from control pots (no pesticide treatment, only aged residues). The red dotted line indicates the mean total copy number per ng DNA in the control treatment (no pesticide and no inoculation) for each management practice, which was set as 100% for better comparison.*

3.2.3 Mycorrhizal Growth Response across Treatments

The mycorrhizal growth response (MGR) was predominantly positive, with 50 out of 68 cases showing an increase in plant biomass due to AMF inoculation. In grassland soils without pesticide application, the MGR was significantly different from 0 (p = 0.012), as was the case for untreated organic soils (p = 0.006) and pesticide-treated conventional soils (p = 0.016). Among the management practices, conventional soils without pesticide application exhibited the lowest mean MGR (~6.5%), while pesticide-treated conventional soils showed the highest mean MGR (~17.5%). Grassland soils displayed intermediate MGR values, with mean responses of ~8% and ~10% for treated and untreated conditions, respectively. Similarly, organic soils showed comparable MGR values under both treated and untreated conditions (**Figure 7**).

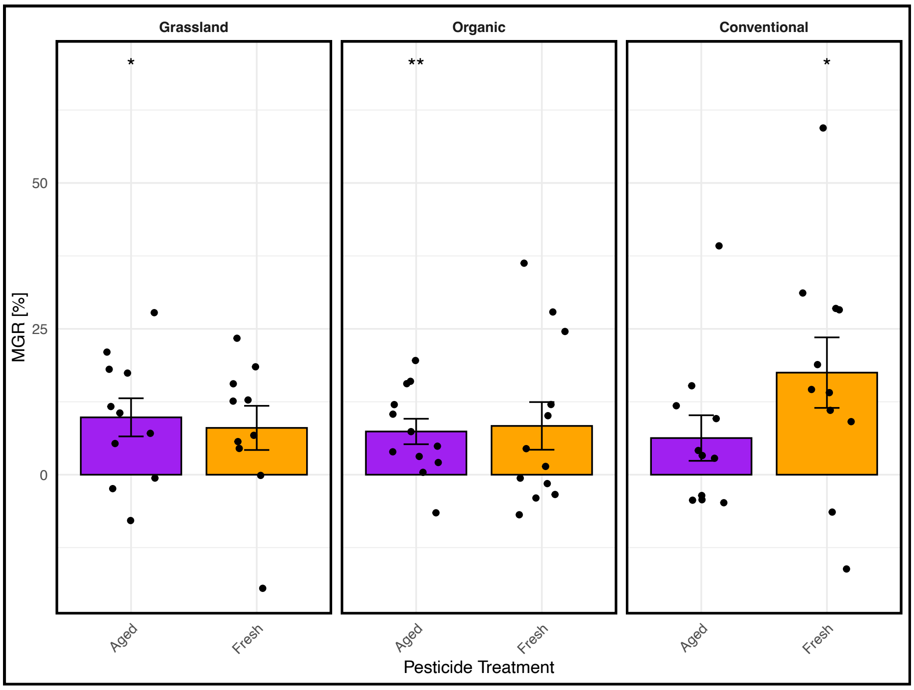

***Figure** 7 Mean ± SE of mycorrhizal growth response (MGR) by management practice and pesticide treatment.* "Fresh" refers to samples from pesticide-treated pots, while "Aged" refers to those from control pots (no pesticide treatment, only aged residues). The asterisks indicate the level of significant difference of the ratio to 1, based on a t-test (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$).

3.2.4 MGR Relationships with RER, Native AMF, and Plant Biomass

The management practices, the pesticide application, and the interaction of both did not have any significant effect on RER (**Suppl. Table 5**). The relationship between MGR and RER was not significant, and no trend was observable (Spearman's rho =0.107; p = 0.39) (**Suppl. Figure 5**).

The management practices and pesticide application and the interaction of both did not have any significant effect on MGR (**Suppl. Table 6**). The MGR was negatively correlated with the native AMF copy number (Spearman's rho = -0.25; p = 0.042) (**Suppl. Figure 6)**.

The plant with lower biomass showed a higher MGR, and no trend of differences across management practices and pesticide treatments was observed (Spearman's rho = -0.545; p < 0.001) (**Suppl. Figure 7**).

3.2.5 Copper Concentration Relationship with Sensitivity towards Pesticide & RER

The sensitivity to pesticide was calculated as the ratio of the AMF copy number per ng DNA from pesticide-treated plants to the AMF copy number per ng DNA from control plants (no pesticide application) as in **Figure 4**. The relationship between copper concentration and sensitivity to pesticide was not significant (Spearman's rho = 0.136; p = 0.258) (**Suppl. Figure 8**) and neither was the relationship between copper concentration and RER (Spearman's rho = -0.088; p = 0.428) (**Suppl. Figure 9**). The copper concentration interaction with pesticide treatment did not significantly affect RER, nor the 2 factors alone (**Suppl. Table 7**).

4. Discussion

4.1 Native AMF Root Colonization Plateau 4 Weeks after Plantation

After one week, the roots of red clover were already colonized by the native AMF, highlighting their efficiency in establishing symbiosis early in the growth of the host plant (**Figure 3**). This result aligns with findings from numerous studies, such as Jansa et al. (2008) and Werner & Kiers (2015), who demonstrated the ability of different AMF species to rapidly colonize host plants within days. This confirms that our experimental setup was successful, as the plants grew as expected and allowed for effective observation of AMF colonization. The temporal evolution of AMF root colonization (**Figure 3**) showed that it reached a plateau 4 weeks after planting and then gradually declined. The difference in AMF copy number could be explained by the size and development of the roots. At week 4, the roots were mainly finer than at week 8, where they were already thicker, and the finer ones were probably more difficult to properly remove from the pot and collect for DNA extraction. Another possible reason for this decrease in AMF colonization could be the interaction with rhizobia. As the host plant, red clover is known to form a symbiosis with rhizobia (N-fixing bacteria), there could be a synergistic effect of AMF and rhizobia. It has been shown that rhizobia and AMF might compete and influence each other when they colonize the same plant. A study by Larimer et al. (2014) found that rhizobia inoculation decreased the AMF root colonization. On the other hand, a study conducted by Kavadia et al. (2021) reported that AMF colonization generally increased in cowpea roots when AMF and rhizobia were inoculated, compared to only AMF inoculation, indicating a beneficial effect of rhizobia on AMF root colonization. They found that the co-existence of AMF and rhizobia was highly dependent on the AMF species. When different AMF species and rhizobia were inoculated together, different results were observable, with some fungi not being able to colonize roots and therefore being outcompeted. The species that successfully colonized roots even in the presence of rhizobia was *R. irregulare*, which, as demonstrated by Werner & Kiers (2015), this fungus was more competitive compared to other species. Even though the mechanism behind these complex interactions is not well understood, some studies tried to tackle these interactions and found that the host plant might produce signaling molecules during the formation of symbiosis with rhizobia that might favor the proliferation of specific AMF species (Abdel-Lateif et al., 2012). In our experiment, the *R. irregulare* was present in the native AMF community, but in very low proportion to the whole community (less than 3%) (**Suppl. Figure 10**). Therefore, this might suggest that the rhizobia could have negatively affected AMF colonization; however, this is just a hypothesis that arises, and the mechanisms between this interaction, which might be species-specific, are not well studied and understood yet.

The plateau in AMF root colonization can be explained by the trade-off between nutrient acquisition and energy costs in the symbiosis. When mineral nutrients are abundant, the host plant regulates its resource allocation, reducing the supply of photosynthate to the fungi (Jiang et al., 2018; Verlinden et al., 2018). This regulation occurs because symbiosis with AMF, while beneficial, can impose high energy costs on the host plant (Smith & Smith, 2011), leading colonization to reach a maximum at some point. The AMF colonization peak usually occurs at the initial plant growth phase but is highly dependent on plant species (Vázquez-Santos et al., 2024). Red clover typically begins flowering approximately between 40 to 70 days after seeding, and some of our plants started flowering towards the end of the experiment. At this stage the plant shifts energy from vegetative growth to reproductive development, which could result in reduced biomass accumulation (Black et al., 2009). Therefore, this could potentially explain the maximum level of AMF colonization at week 4. The pot size could potentially also explain the AMF colonization dynamics and the plateau after week 4. In the meta-analysis conducted by Qin et al. (2022), they showed that the pot sizes had an influence on the AMF colonization, since the roots could be more pot-bound and therefore less colonizable by AMF.

4.2 Pesticides reduce Plant Biomass through AMF Symbiosis

In this study we focused on the effect of a fresh pesticide application on the AMF's capacity to colonize roots. As shown in **Figure 4A**, the pesticide strongly and significantly affected the AMF colonization in the initial growth phase, showing a more prominent effect at this early stage. Four weeks after plantation, the effect diminished, and AMF in pesticide-treated pots could colonize the red clover roots similarly as in the control pots. The decreasing effect of pesticide can be explained by the pesticide dissipation and the less bioavailability (L. Chen & Zhang, 2010; Zhang et al., 2015). Not only the dissipation of pesticide but also the capacity of soil microorganisms to react to the toxicity of pesticide can explain the recovery shown after 4 weeks. Soil microbes significantly contribute to the degradation of pollutants and pesticides (Pino & Peñuela, 2011; Zhao et al., 2009). Nonetheless, as indicated in the meta-analysis by Verma et al. (2014), the capacity of microbes to degrade pesticides is significantly influenced by the specific microbial strain and the type and combination of pesticides used. Consequently, it can be deduced that the microbial community may have affected the diminished sensitivity to pesticide reported in our experiment; nevertheless, a conclusive determination cannot be made. One of the most interesting results of this study is the indirect effect of pesticide application on plant biomass. As shown in **Figure 4B**, the plant biomass reaction to the pesticide occurred later compared to AMF, where we observed a significant negative effect of pesticide on plant biomass. This led us to conclude that the pesticide most certainly did not affect the biomass directly but through harming the AMF, which were then less effective in providing nutrients to the plants, resulting in less yield after week 4 compared to the control plants. The pesticides likely disrupted AMF functionality, impairing their ability to transport nutrients to the plant. This disruption, in turn, may have gradually led to nutrient deficiencies in the plant, culminating in the observed reduction in biomass after several weeks. The lag in this response underscores the time required for the pesticide's initial effects on AMF to manifest as measurable changes in plant growth. This hypothesis is supported by later studies that could correlate the use of pesticides to the impact of phosphorus transport to the plant, which could be the reason for less yield under pesticide treatment (Edlinger et al., 2022; Sallach et al., 2021). This conclusion can be made based on the several studies indicating the positive effect of AMF on host plant biomass (Erman et al., 2011; Kebede et al., 2024; H. Zhang & Xiao, 2024; Zhu et al., 2017), and therefore when AMF underperformed due to the pesticide application, this can result in less biomass production since the symbiosis might have been influenced. The mycorrhizal dependency, presented by van der Heijden et al. (2003), resulted being very high for legumes in the study conducted by Romero et al. (2023), consolidating our conclusion that red clover biomass decrease might have been affected by the decrease in AMF colonization due to the pesticide application and might also partly explain the higher biomass yielded in organic soils compared to the conventional soils (**Figure 5**).

4.3 Contamination History Negative Effect on Native AMF Colonization

In our study, one of the main goals was to determine whether the native AMF's ability to colonize roots is influenced by the contamination histories of the soils. As hypothesized, we found that the native AMF copy numbers in conventional soils were slightly but significantly lower than those in organic soils, as shown in **Supplementary Table 1b**. While grassland soils did not exhibit the highest native AMF colonization, they showed a trend toward higher colonization rates compared to conventional soils, although this difference was not statistically significant ($p = 0.07$). The organic soils in our experiment also yielded the highest red clover aboveground biomass (**Figure 5**), which aligns with the highest native AMF root colonization in organic soils; they yielded more than grasslands, which surprisingly had the lowest yield. Grassland field soils showed the lowest levels of bioavailable phosphorus (**Figure 1**) and potassium, both potentially contributing to the surprisingly low yield, given that both are essential macronutrients for plant growth (Delgado et al., 2024). Microbial activity may also explain this low yield, as it plays a critical role in nutrient cycling, including the decomposition of organic matter and the release of essential nutrients like phosphorus and nitrogen. Differences in microbial activity between soils could potentially impact nutrient availability, therefore influencing plant biomass production (Babalola, 2019; Timea et al., 2012). Conventional soils in our study were found to have high pesticide concentrations, which may have influenced the ability of native AMF to colonize the plants. Several studies support the finding that management practices can impact the ability of AMF to colonize roots. For example, van Geel et al. (2015) observed higher AMF abundance in organically managed soils compared to conventionally managed soils. Similarly, Wahdan et al. (2024) found that AMF spore abundance and AMF diversity were significantly greater in organic soils. However, evidence for contrary results also exists; Chen et al. (2022) observed that AMF colonization was higher in conventional soils compared to organic soils. The studies mentioned utilized different experimental methods, observed different AMF species, and measured distinct response variables. These differences in approaches and goals make direct comparisons between the results indicative but not fully comparable. While synthetic pesticide contamination could potentially explain the lower colonization success of AMF in conventional soils compared to organic soils in our experiment, other management practices may also contribute to the observed differences in native AMF colonization. Tillage, which directly impacts the mycelium (Verbruggen and Kiers, 2010), could influence AMF dynamics in vineyards. However, very few studies have been conducted on this topic, and surprisingly, a study by Lumini et al. (2010) found that tilled vineyards harbored higher AMF diversity than vineyards with soil cover. In Swiss vineyards, tillage is not a common practices, therefore this might not explain the difference in colonization between management practices, but worth mentioning since weed control in vineyards might affect the AMF dynamics in the soils. High fertilizer input is also known to reduce AMF colonization in many agroecosystems, including vineyards (Karagiannidis and Nikolaou, 1999). Weed control and cover cropping can also potentially affect AMF diversity and colonization in vineyards. However, there is no clear evidence of either a negative or positive impact. Baumgartner et al. (2010) found no correlation between mycorrhizal colonization and weed frequency, whereas Radić et al. (2012) showed that selected neighboring weeds can influence arbuscular mycorrhizal formation.

4.4 No Adaptation towards Pesticide of Native AMF

The hypothesis that conditioned native AMF would exhibit lower sensitivity to a fresh pesticide application compared to unconditioned native AMF was rejected. We did not observe any significant differences across the management practices, as there was no interaction between pesticide application and management (**Suppl. Table 1a & 3a**). This suggests that prior exposure to pesticide might not confer increased tolerance or adaptive response in native AMF communities present in our soils to subsequent pesticide application. As reported by Lenoir et al. (2016), AMF have developed various strategies to cope with pesticides. However, these strategies appear to be highly dependent on the specific AMF strain, the active ingredient, and the mode of action of the pesticide. In our experiment we utilized active ingredients commonly used in conventional vineyards, and all of them were also applied in 2023 in several soils analyzed as shown in **Table 2**. However, no evidence of native AMF adaptation was observable. The time between the last pesticide application in the fields and the fresh application and consequent AMF quantification (in this case, qPCR) could be an important factor when assessing the adaptation capabilities of AMF to pesticides. Since the last pesticide application in the vineyards occurred six months prior to our experiment, any adaptability or resistance the AMF might have developed may have diminished over time. This suggests that adaptation to pesticides might be temporary. Given that some of the morphological adaptations could be energetically costly to maintain, the AMF may reduce or cease these adaptive strategies in the absence of ongoing pesticide exposure. Studies on the adaptation capabilities of AMF to pesticides and/or heavy metals vary widely in their time horizons. Several studies have examined effects and adaptation within short-term periods, ranging from a few days to a month (Calonne et al., 2010; Lenoir et al., 2016). Considering that such adaptation strategies may be temporary, future research should explore the long-term adaptation potential of AMF to contaminants.

One possible explanation for this lack of adaptive response is that AMF colonization and resilience depend heavily on the community structure and diversity of AMF present in the soil. Long-term pesticide exposure can reduce AMF diversity, leading to the dominance of a few tolerant strains, which may not necessarily enhance community-level adaptability. Studies have shown that AMF diversity is crucial for maintaining ecosystem functions, including nutrient uptake and stress tolerance (van der Heijden et al., 1998). If pesticide exposure reduced diversity in conventional soils, this could limit the capacity for adaptive responses. Another factor to consider is the carbon cost of the symbiosis. Arbuscular mycorrhizal fungi rely on carbon provided by the host plant to sustain their functions, and plants might prioritize associations with AMF strains that provide the greatest nutrient return for the least carbon investment (Smith & Smith, 2011). In soils with high pesticide residues, the functional efficiency of AMF may be compromised, causing the plant to allocate fewer resources to the symbiosis, further limiting the potential for adaptation. Our results suggest that the adaptability of AMF to pesticides is complex and influenced by various factors, including species-specific responses, interactions with other soil microorganisms, and the duration of pesticide exposure. These findings highlight the importance of considering multiple ecological and biological factors when assessing the impact of pesticides on AMF communities. Therefore, even though we applied pesticides that are typically used in vineyards, our pesticide mixture, the mode of application, and other environmental factors might have reduced or suppressed the ability of native AMF to adapt to pesticides and therefore show no differences in colonization between our soils from different management practices.

4.5 Different Effects of Rhizoglomus irregulare Inoculation across Management Practices

Inoculation with the *R. irregulare* (SAF22) strain significantly increased total arbuscular mycorrhizal fungi (AMF) community colonization across all management practices (**Figure 6)**. Although root colonization in inoculated plants did not differ significantly across management practices, a trend was observed: the inoculation effect on AMF root colonization followed the contamination gradient, having the highest impact in grassland soils and the lowest in conventional soils. This stronger inoculation effect in grassland soils could be attributed to the lower levels of phosphorus (both bioavailable and non-bioavailable, **Figure 1C**) and the lower levels of pesticide and copper **(Table 2 & Figure 2**) contamination in these soils. Arbuscular mycorrhizal fungi tend to be more effective in colonizing roots in phosphorus-limited soils, and the relatively low pesticide contamination levels in grassland soils may also contribute to this enhanced colonization (Amir et al., 2021; Grant et al., 2005; Riedo et al., 2021b). The high pesticide and copper residue concentrations might explain the weaker inoculation effect in highly contaminated soils, highlighting the possible detrimental effects on the unconditioned inoculated AMF strain reducing the root colonization effect (Pánková et al., 2018). This trend could not be seen in

MGR, which had mostly positive values that were not different depending on management practices or contamination histories **(Figure 7)**. Despite the higher proportional colonization after inoculation in grassland soils (**Figure 6**), MGR didn't vary across management practices (**Figure 7**). The MGR resulted mainly positive, indicating that the AMF inoculation had a beneficial effect on red clover biomass. This result is supported by the meta-analysis conducted by Li et al. (2016), where they reported that MGR was significantly positive across the 410 studies they analyzed. The mean MGR across all the studies was 26%. In our results, the MGR was lower but still positive all over the management practices and pesticide treatment, indicating that the *R. irregulare* SAF22 had a positive effect on the red clover biomass. Several meta-analyses (Hoeksema et al. 2010; Yang et al., 2015) have been conducted studying the effect of inoculation on the plant biomass, and the main results are that MGR is mostly positive but not always the case (Castelli and Casper 2003; Hart et al., 2003) and is dependent on several factors, as for example the host plant's. Supported by Romero et al. (2023), who studied the different dependence on AMF by different plant functional groups, our results that red clover plants profited from AMF are not surprising.

Since the inoculation effect on root colonization was stronger in soils with less contamination, this raises the question of why that isn't also seen in the MGR **(Figures 6 and 7)**. This result is not uncommon, and there are several studies indicating that the AMF root colonization does not always correlate with MGR (Campo et al., 2020; Lutz et al., 2023). One possible reason for this observation in our study could be the overall low P levels in grassland soils. Even though AMF can enhance P uptake, the limited availability of P in the studied grassland soils likely constrained the plants' ability to convert the increased colonization into additional biomass. On the other hand, we could observe that in soils where plants performed poorly without inoculation (**Suppl. Figure 7**), they resulted in having higher MGR, which could be related to the nutrient limitation in those soils and the beneficial part of the symbiosis. Such a result is partly supported by other studies, such as the one conducted by Köhl et al. (2016), where they showed that even in a soil with moderate P availability, clover did not profit from this symbiosis. In the same study, they also found that in soils with relatively low P, the plant profited most from the inoculation; therefore, a clear conclusion can't be made. In a study conducted by Treseder (2013), it has been reported that yes, the root colonization is often associated with increased biomass, but the colonization only explained about 12% of the variability in biomass change. Various other factors (biotic and abiotic) could explain the non-proportional increase in biomass in grassland soils. **Figure 5** shows that even before inoculation, the grassland soils performed less well compared to the other management practices. This suggests that factors not analyzed in this study might have also influenced the plant growth in grassland soils. One factor we haven't examined would be the pathogen pressure. There are studies suggesting that AMF can improve plant health by reducing pathogen load or enhancing plant resistance to diseases (W. Chen et al., 2023; Weng et al., 2022). This could be a significant unstudied factor influencing the observed variability in biomass change, particularly in grassland soils where pathogen pressure might be higher as reflected by the surprisingly low yield. Another plausible explanation could be that *R. irregulare* detection in roots contributed significantly to the pool of DNA detected by the qPCR, but they might have contributed less to the nutrient exchange with clover. Köhl et al. (2016) found similar results, where *R. irregulare* root colonization following inoculation was increased but did not necessarily lead to an increase in red clover biomass. However, *R. irregulare* is known to be efficient in providing nutrients to the plant and often results in a dominant AMF among different species; therefore this might not be a possible explanation for the pattern observed, or only partly. A further factor to consider is the time aspect; if a plant allocates more energy to the initial symbiosis, it could hinder its short-term growth, with the advantages of the symbiosis manifesting only later on. The increased root colonization via inoculation found in grasslands may have led to an elevated MGR during later growth stages. Therefore, our hypothesis that the inoculation effect would be weaker in highly contaminated soils is only partly rejected.

The root colonization by the *R. irregulare* strain specifically showed intriguing results (**Figure 6**). *Rhizoglomus irregulare* was present in non-inoculated soils, even though in a very small proportion compared to the total AMF community composition (about 3% of the total AMF community) (**Suppl. Figure 10**), showing that it's present in natural soils and vineyards over the canton of Zürich, as also reported by Köhl et al. (2016), where they observed the presence of this fungus in agricultural soils across Switzerland. Across all management practices, it successfully established itself and contributed to an increase in total AMF root colonization. This is consistent with findings by Alkan et al. (2006) and Janoušková et al. (2013), where they showed that it can successfully establish in a wide range of soil types when native AMF are already present in the natural soils. Werner & Kiers (2015) showed that when *R. irregulare* was simultaneously inoculated with another species, *R. irregulare* exhibited more competitiveness, demonstrating higher abundance than the other species. The establishment of *R. irregulare* did not significantly differ across the management practices (**Suppl. Table 5**). Although no significance was found, an interesting pattern could be observed and is reported in **Figure 6**. In grassland soils, the inoculated strain appeared to act more additively, contributing to an overall increase in AMF root colonization. Conversely, in organic soils, and particularly in conventional soils, it seemed that the potential of the plant to be colonized was almost reached, and the inoculated strain replaced some native fungal species (Hamel et al., 1997). These findings are supported by similar results observed by Bender et al. (2019b). The native AMF in more contaminated soils might be less competitive, as they are already under stress, and therefore *R. irregulare* might be able to outcompete them, and it's also often used for inoculation. These observations might be explained by the *R. irregulare* strain's potential resistance to more contaminated soils, such as those in conventional and organic management systems, where copper contamination and other biological products are present. This can be observed in **Supplementary Figure 10,** where the native *R. irregulare* root colonization increased through pesticide application. This increase is also shown in the **Supplementary Table 4a,** as the interaction between pesticide and inoculation resulted significant on the *R. irregulare* copy numbers. These results indicate that the SAF22 strain (see section 2.2) might be more tolerant to fungicides than other AMF strains. Another hypothesis is that in soils with high bioavailable P, plants only provided limited resources to the AMF, which increased competition among them. This could explain why *R. irregulare* appeared to replace the native AMF in contaminated soils and act more additively in grassland soils. In conclusion, whether *R. irregulare* acts additively or replaces native AMF in our study remains incompletely understood, and it is likely influenced by multiple factors, including soil contamination, nutrient availability, and plant physiological conditions.

4.6 Resilience of Rhizoglomus irregulare to Soil Stressors

Our last hypothesis suggested that the interaction between copper concentration in the soil and additional pesticide application would significantly and negatively impact the establishment of *R. irregularis*. However, the ANOVA results (**Suppl. Table 7**) indicated no significant interaction between copper and pesticide treatments. This finding suggests that *R. irregularis* might maintain its ability to establish and perform well in soils, even under combined stressors such as elevated copper concentrations and a fresh pesticide application. These results align with previous studies showing that *R. irregularis* can tolerate various environmental stressors, including soil contaminants, while continuing to establish and colonize plant roots effectively (Ambrosini et al., 2015; Pozo & Azcón-Aguilar, 2007). This resilience makes *R. irregularis* a robust AMF candidate for use in soils exposed to agricultural chemicals. Additionally, we tested whether copper levels and sensitivity to pesticides were correlated; however, no correlation was found, indicating that copper does not appear to influence AMF resilience or susceptibility to pesticide exposure. This suggests that other factors, such as fungal species composition, environmental conditions, or prior adaptation history, may play a more significant role in determining AMF responses to pesticides.

5. Conclusion and Outlook

This thesis showed that pesticides can directly affect the ability of AMF to colonize plants, especially in the early part of growth. We were able to observe that the biomass of the plants was affected by the application of fungicides. This possibly was linked to the observed decrease in early root colonization by AMF and consequent reduced nutrient uptake. This result is crucial as it indicates that in conventional agricultural practices, pesticides can have yield-reducing effects due to negative interference with the symbiosis of plants with fungi. Further studies need to confirm this (e.g., test for reduced nutrient uptake of pesticide-treated plants). From our results, we found that conditioned AMF, which were exposed and conditioned to pesticides for a long time, did not develop a stable and long-lasting tolerance to repeated exposure and treatment with pesticides. This result suggests that in conventional agricultural systems, such as in vineyards, AMF communities may be continually negatively affected by pesticides and have not developed a durable adaptation that can be useful in the long run and be able to react readily to each new pesticide application, without having possible negative effects on the crop as shown above. The results also show that contamination history and pesticide residues (and other heavy metals) have an effect on the ability of AMFs to colonize roots. However, this effect was not as great as we expected, and therefore other factors must be taken into account, such as nutrient levels in the soil, soil structures, and others that may be just as, if not more, crucial to plant growth and the symbiosis between plants and fungi. Therefore, even in soils with high contamination, AMF can play a key role in contributing to plant nutrition and plant fitness and consequently good yields. We noticed that the *R. irregulare* strain SAF22 had different effects depending on the soils where it was inoculated. Firstly, the inoculation worked in all soil types, and the total colonization of AMF was not dependent on soil contamination history. However, the strain mentioned above had different effects depending on the soil characteristics. In nutrient-poor and uncontaminated soils, AMF inoculation increased the overall colonization level much more than in nutrient-rich and more contaminated soils. This result has a significance not to be underestimated on the use of this strain. Inoculation with this strain can be used to enhance colonization potential in nutrient-poor soils, and in highly contaminated soils it can replace native AMF and provide phytoremediation.

Since pesticides reduce the colonization of AMF in the early parts of plant growth, reducing and optimizing the timing of their use would help the native AMF population, which consequently increases the effectiveness of the symbiosis with the plants. The study shows that *R. irregulare* SAF22 can increase the colonization of AMF in nutrient-poor soils and be used in highly contaminated soils to replace the possible present AMF community that may be less resistant to certain active pesticide ingredients. This therefore suggests that target AMF inoculation may be a tool for restoring potentially less performing soils due to high pesticide levels and increasing plant performance and health. Although contamination history resulted less relevant than expected to the success of AMF colonization, there is enough evidence that past and residual contaminants in soils must be taken into account when trying to improve plant-AMF symbiosis.

This study demonstrates that pesticides have an impact on AMF and on plant growth. There are a number of areas that need further attention: First, since our study suggests that AMF do not develop a stable, long-term tolerance to pesticides, it becomes crucial to trace the persistence and long-term effects of pesticide exposure on AMF functionality over several growing seasons. This would make it possible to determine whether AMF communities can gradually adapt under continuous exposure to low doses of pesticides or whether each application causes a new disruption of the symbiosis. Furthermore, several parameters could be observed, including not only colonization but also metabolic activity, glomalin production, nutrient transport efficiency, and the development and length of extraradical hyphae. Moreover, it is possible that different AMF taxa and AMF diversity are influenced by pesticide exposure, and this is an area that needs further attention. Addressing these knowledge gaps will be essential to refine AMF-based soil restoration strategies and develop sustainable agricultural practices that minimize the impact of pesticides while preserving soil microbial health.

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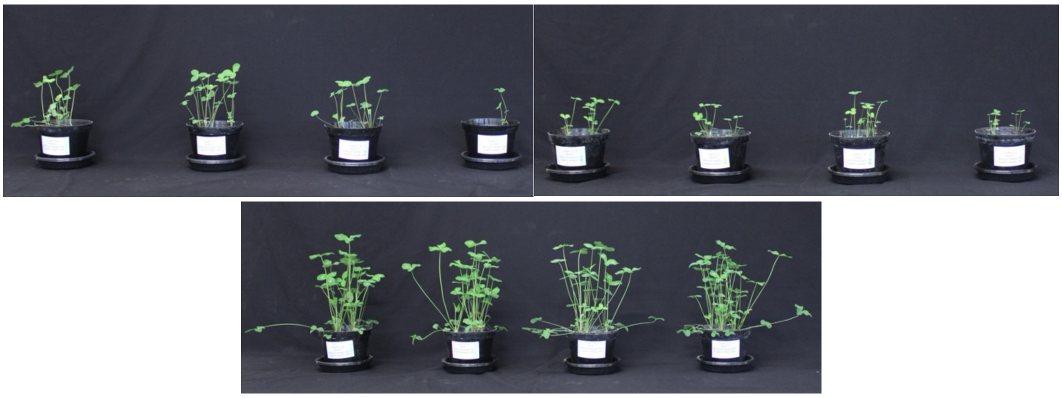
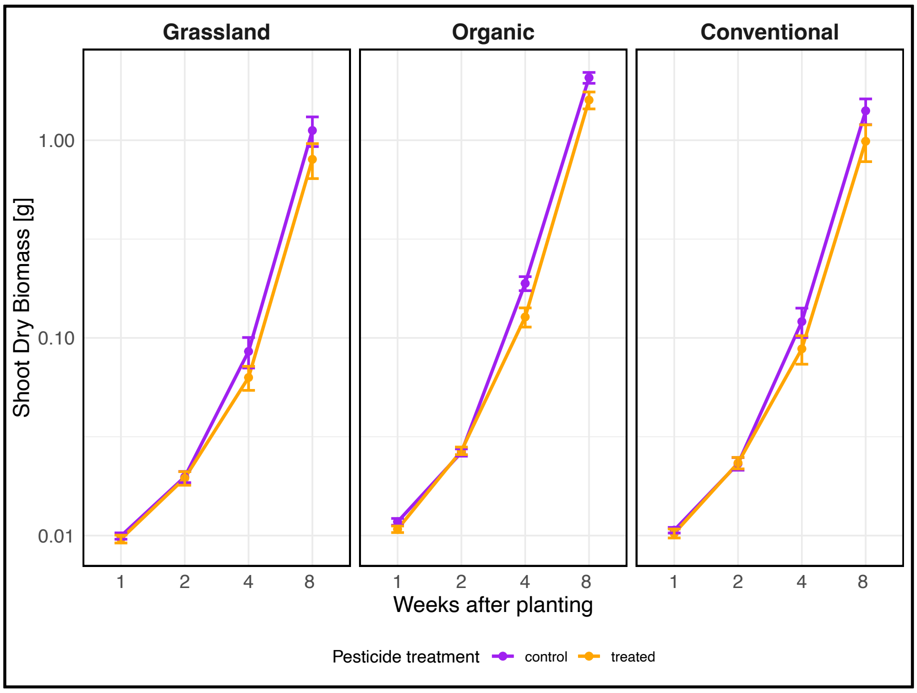
Supplementary Material

Supplementary Figure 1 *Red Clover plants in the greenhouse at Agroscope, Reckenholz, during the experiment.*

Supplementary Figure 2: *Red Clover plant infected with powdery mildew.*



Supplementary Figure 3 *The two excluded grassland soils due to underperformance are shown above, compared to an avarage performing grassland soil shown below.*



Supplementary Figure 4 *Mean shoot dry biomass in g ± SE per sampling timepoint, soil management, and pesticide treatment.*

Supplementary Table 1a: *Anova results for lmer(LOG_copy_nr_dna ~ mngt * Timepoint * Pesticide + (1 | Block) + (1 | soil.id), data = data).*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mngt	2	0.649	0.325	4.2212	0.0249850 *
Timepoint	3	117.104	39.035	507.6164	< 2.2e-16 ***
Pesticide	1	3.673	3.673	47.7594	5.285e-11 ***
mngt:Timepoint	6	0.808	0.135	1.7507	0.1105965
mngt:Pesticide	2	0.089	0.044	0.5757	0.5631798
Timepoint:Pesticide	3	1.688	0.563	7.3161	0.0001075 ***
mngt:Timepoint:Pesticide	6	0.713	0.119	1.5463	0.1643256
Residuals	245	17.218	0.07027755		

Supplementary Table 1b: *Tukey's results for log-10 copy number per ng DNA. The difference between the management practices with the adjusted p-value.*

Comparison	***Estimate***	***p-value***
Conventional - Grassland	*-0.1967*	*0.0704*
Conventional – Organic	*-0.2139*	*0.0342 **
Grassland - Organic	*-0.0171*	*0.9779*

***Supplementary Table 2a**: Anova results for lmer(plant_biomass_dry ~ mngt * Inoculum * Pesticide + (1 | Block) + (1 | soil.id), data = data).*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mngt	2	705024	352512	6.4551	0.004899 **
Inoculum	1	523593	523593	9.5878	0.002597 **
Pesticide	1	5761355	5761355	105.4995	< 2.2e-16 ***
mngt:Inoculum	2	3287	1644	0.0301	0.970363
mngt:Pesticide	2	49359	24680	0.4519	0.637808
Inoculum:Pesticide	1	7254	7254	0.1328	0.716359
mngt:Inoculum:Pesticide	2	14322	7161	0.1311	0.877269
Residuals	120	5108859	42573.83		

***Supplementary Table 2b:** Tukey's results for plant dry biomass The difference between the management practices with the adjusted p-value.*

Comparison	***Estimate***	***p-value***
Conventional - Grassland	*86*	*0.9210*
Conventional – Organic	*-625*	*0.0164 **
Grassland - Organic	*-711*	*0.0092***

***Supplementary Table 3a**: Anova results for lmer(LOG.copy.nr.per.ng.dna. ~ mngt * Inoculum * Pesticide + (1 | Block) + (1 | soil.id), data = data).*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mngt	2	0.52394	0.26197	5.7660	0.008108 **
Inoculum	1	0.95637	0.95637	21.0501	1.416e-05 ***
Pesticide	1	0.01681	0.01681	0.3700	0.544521
mngt:Inoculum	2	0.24974	0.12487	2.7484	0.069310 .
mngt:Pesticide	2	0.00196	0.00098	0.0215	0.978703
Inoculum:Pesticide	1	0.00850	0.00850	0.1870	0.666430
mngt:Inoculum:Pesticide	2	0.01504	0.00752	0.1656	0.847676
Residuals	120	4.56231	0.03801925		

***Supplementary Table 3b:** Tukey's results for LOG.copy.nr.per.ng.dna. The difference between the management practices with the adjusted p-value.*

Comparison	***Estimate***	***p-value***
Conventional - Grassland	*-0.289*	*0.0052 ***
Conventional – Organic	*-0.118*	*0.3170*
Grassland - Organic	*-0.171*	*0.1242*

Supplementary Table 4a: *Anova results for lmer(LOG_SAF_DNA ~ mngt * Inoculum * Pesticide + (1 | Block) + (1 | soil.id), data = filtered_data).*

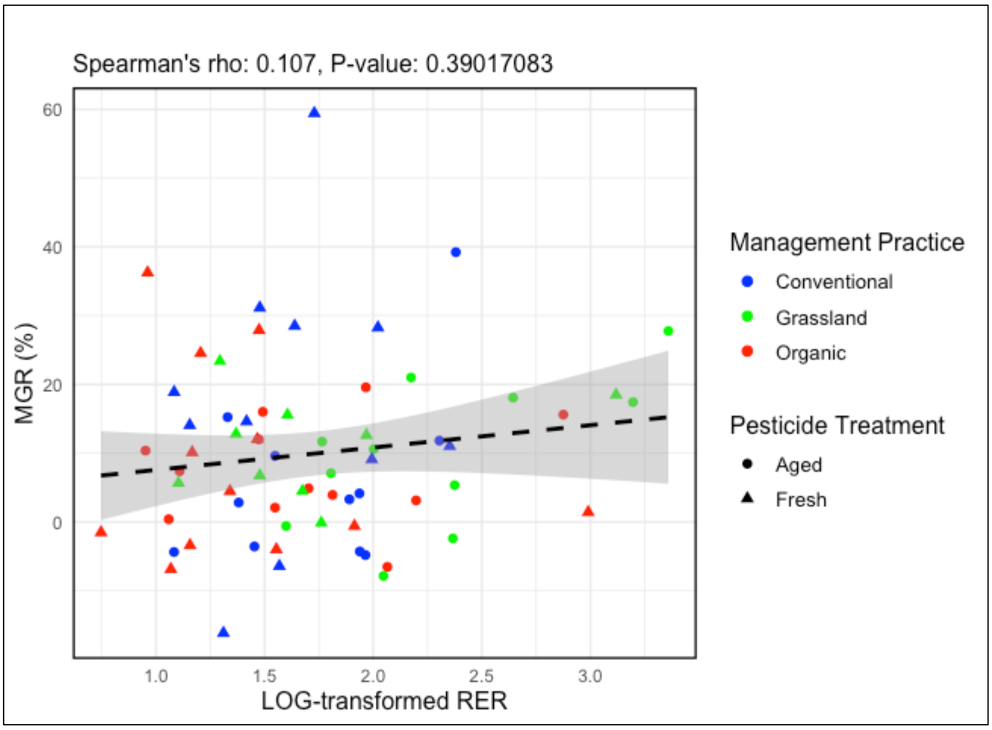
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
mngt	2	0.526	0.263	3.4072	0.0472785 *
Inoculum	1	83.964	83.964	1088.5071	< 2.2e-16 ***
Pesticide	1	0.171	0.171	2.2212	0.1395361
mngt:Inoculum	2	1.541	0.771	9.9908	0.0001179 ***
mngt:Pesticide	2	0.049	0.025	0.3188	0.7278122
Inoculum:Pesticide	1	0.627	0.627	8.1252	0.0053831 **
mngt:Inoculum:Pesticide	2	0.161	0.080	1.0429	0.3565504
Residuals	120	8.148559	0.06790466		

Supplementary Table 4b *Tukey's results for LOG_SAF_DNA. The difference between the management practices with the adjusted p-value.*

Comparison	***Estimate***	***p-value***
Conventional - Grassland	*-0.1667*	*0.1484*
Conventional – Organic	*-0.2089*	*0.0418 **
Grassland - Organic	*-0.0422*	*0.8769*

Supplementary Table 4c *Tukey's results for LOG_SAF_DNA. The difference between the management practices & inoculation with the adjusted p-value. (Inoculated)*

Comparison	***Estimate***	***p-value***
Conventional - Grassland	*-0.3771*	*0.0021 ***
Conventional – Organic	*-0.1690*	*0.2141*
Grassland - Organic	*0.2081*	*0.1287*



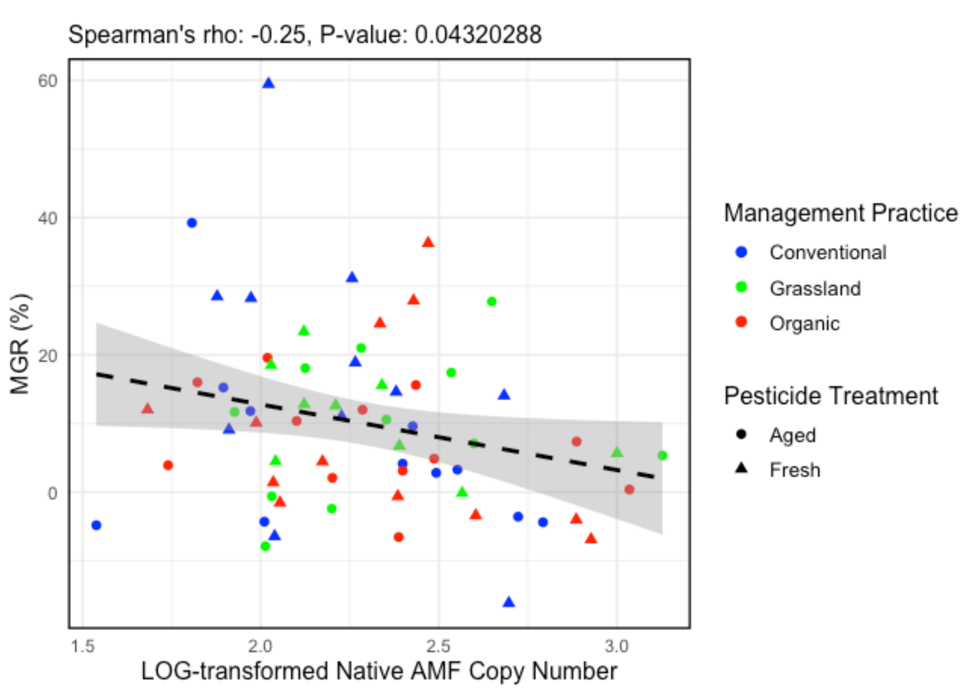
Supplementary Figure 5 *Relationship between RER and MGR. The log10-transformed relative establishment ratio of R. irregulate on the x-axis and the mycorrhizal growth response in % on the y-axis. The 95% confidence interval in grey. Pesticide treatment "Aged" as control (no application) and "Fresh" treated with pesticides. Spearman's rho: 0.107 and p = 0.390*

Supplementary Table 5: *Anova for lmer(RER ~ mngt * Pesticide + (1 | soil.id), data = AMF).*

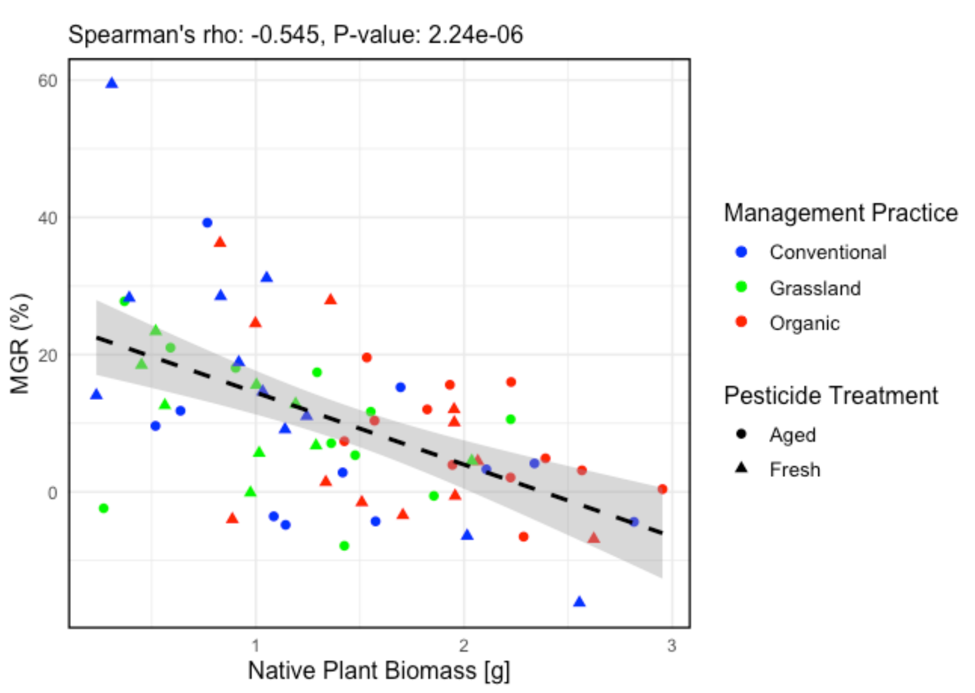
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mngt	2	0.042607	0.021303	1.0133	0.37499
Pesticide	1	0.081550	0.081550	3.8788	0.05777 .
mngt:Pesticide	2	0.054836	0.027418	1.3041	0.28573
Residuals	31				

Supplementary Table 6: *Anova for lmer(MGR ~ mngt * Pesticide + (1 | soil.id), data = AMF).*

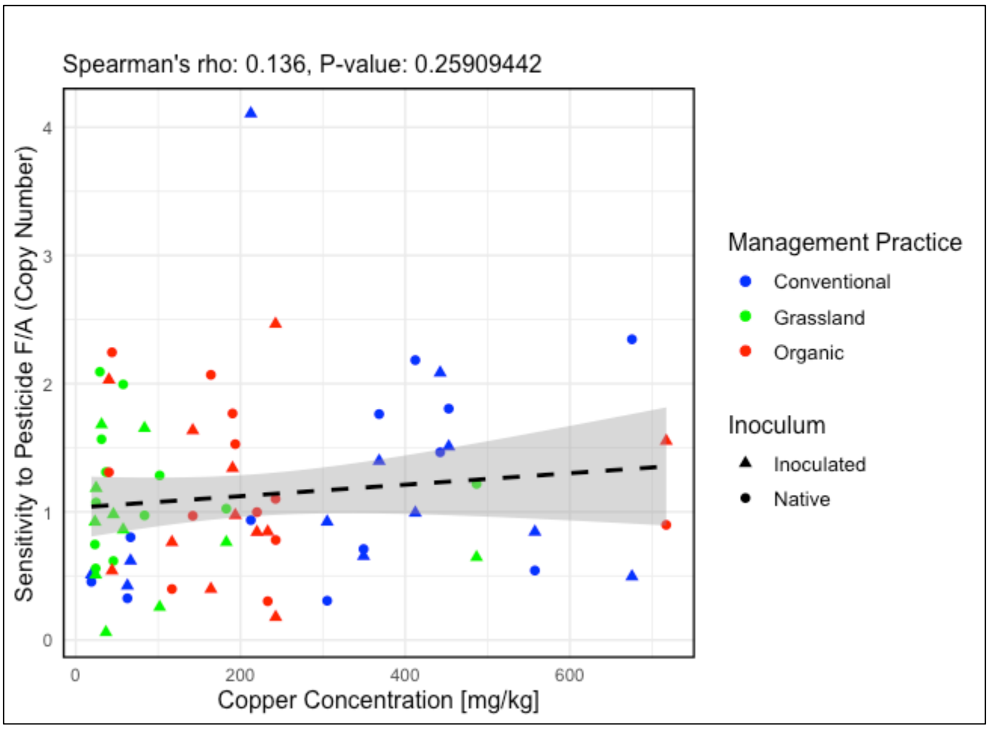
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mngt	2	143.46	71.728	0.5631	0.5764
Pesticide	1	290.07	290.075	2.2772	0.1442
mngt:Pesticide	2	303.63	151.816	1.1918	0.3208
Residuals	58	6062.099	104.5189		



Supplementary Figure 6 *Relationship between native AMF colonization and MGR. The log10-transformed native AMF copy number per ng DNA on the x-axis and the mycorrhizal growth response in % on the y-axis. The 95% confidential interval in grey. Pesticide treatment "Aged" as control (no application) and "Fresh" treated with pesticides. Spearman's rho: -0.25 and p = 0.042.*



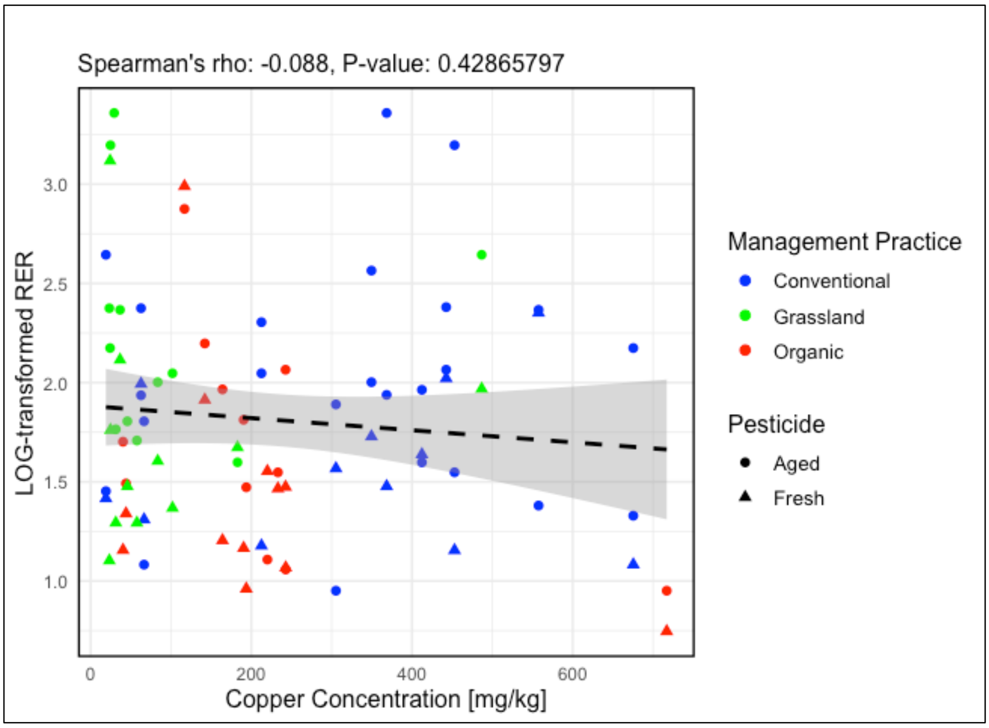
Supplementary Figure 7 *Relationship between native plant biomass and MGR. The plant dry biomass of non-inoculated pots on the x-axis and the mycorrhizal growth response in % on the y-axis. The 95% confidential interval in grey. Pesticide treatment "Aged" as control (no application) and "Fresh" treated with pesticides. Spearman's rho: -0.545 and p < 0.001.*



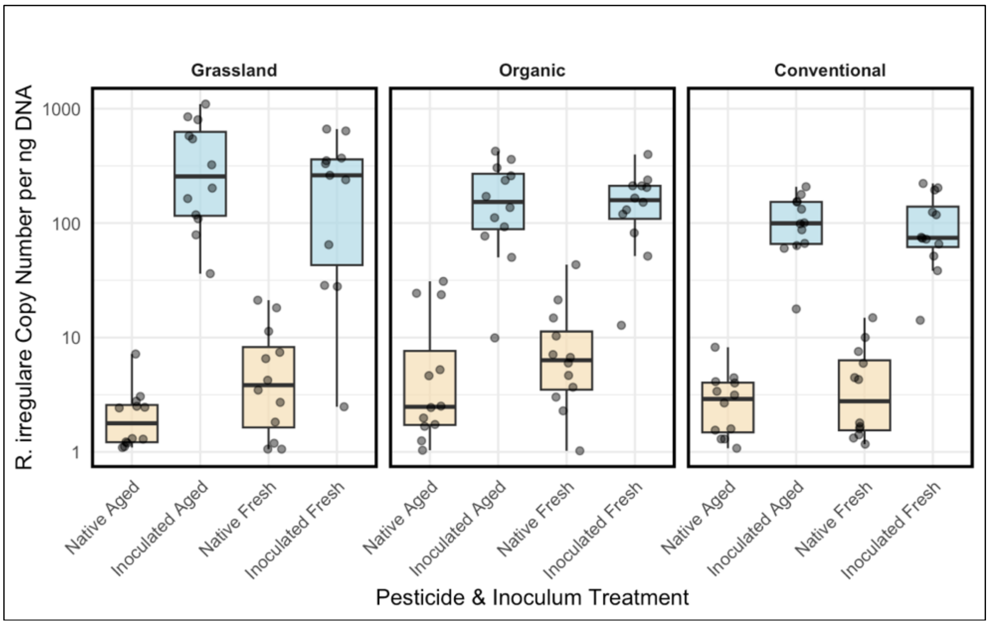
Supplementary Figure 8 *Relationship between the copper concentration and the sensitivity towards pesticide. Sensitivity, calculated as the ratio of copy number of pesticide-treated pots to non-treated pots. The 95% confidential interval in grey. Pesticide treatment "Aged" as control (no application) and "Fresh" treated with pesticides. Spearman's rho = 0.136 and p = 0.258.*

Supplementary Table 7: *Anova for lmer(RER ~ Coppert * Pesticide + (1 | soil.id), data = AMF).*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Copper	1	0.054819	0.054819	2.5903	0.1174
Pesticide	1	0.046810	0.046810	2.2119	0.1462
Copper:Pesticide	2	0.000271	0.00271	0.0128	0.9106
Residuals	34				



Supplementary Figure 9 *Relationship between the copper concentration and RER. Copper concentration on the x-axis and log10-transformed relative establishment of R. irregulare on the y-axis. The 95% confidential interval in grey. Pesticide treatment "Aged" as control (no application) and "Fresh" treated with pesticides. Spearman's rho = -0.088 and p = 0.428.*



Supplementary Figure 10 *Rhizoglomus irregulare copy numbers per ng DNA in native and non-pesticide-treated pots (Native Aged); in inoculated and non-pesticide-treated pots (Inoculated Aged); in native and pesticide-treated pots (Native Fresh); and inoculated and pesticide-treated pots (Inoculated-Fresh). The colors indicate the inoculated samples in lightblue and the non-inoculated in yellow.*

Personal declaration

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

Schlieren, January 31, 2025

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