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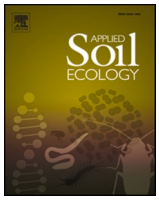

Research paper

Intensive pesticide application history leads to reduced root symbiont functioning but not to long-term adaptation

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ABSTRACT

Frequent pesticide applications have led to their widespread accumulation in agricultural soils. Pesticides can negatively impact plant symbionts such as arbuscular mycorrhizal fungi (AMF) and rhizobia, which are recognized as key plant growth promoters. However, the long-term effects of intensive pesticide use on plant-microbe symbioses and the potential of symbionts to adapt are still poorly understood.

To elucidate this, we collected soil from twelve conventional vineyards with intensive synthetic pesticide use and from twelve organic vineyards without synthetic pesticide use for at least ten years and conducted a greenhouse experiment with red clover. We assessed the impact of management history on plant growth, symbiont performance, and microbial adaptation to fresh application of a fungicide mixture across eight weeks including multiple sampling timepoints. Moreover, we tested the potential of AMF inoculation to mitigate pesticide effects.

Soils with intensive pesticide use history yielded less plant biomass and lower AMF root colonization compared to organic soils. Fresh pesticide application temporarily reduced AMF root colonization and reduced nitrogen fixation at the final harvest. Consequently, plant biomass was also reduced. However, in soils with intensive pesticide use history, neither the AMF nor the rhizobia showed increased tolerance to the fresh application of pesticides, nor was dissipation of the pesticides accelerated. This implies that there was no long-term adaptation of plant symbionts and pesticide degraders to frequent pesticide applications. Inoculation with AMF generally had a positive effect on plant biomass, AMF root colonization, and nitrogen fixation. This was particularly evident in soils with poor performance, such as those with fresh pesticide application. The inoculated *Rhizoglomus irregulare* strain established better in soils with low natural AMF abundance and plant biomass. It mostly replaced other *Rhizoglomus* strains, as revealed by PacBio sequencing of the root AMF communities.

Overall, our study indicates that pesticide use has both short-term and long-term impacts on the functioning of plant symbionts and that their long-term adaptation potential may be limited. While the reduction of pesticide exposure should be prioritized, inoculation of AMF could aid mitigating non-target effects from both historic and fresh pesticide use and restoring soil functioning.

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1. Introduction

Soils are extremely diverse ecosystems, harboring up to 59% of global biodiversity (Anthony et al., 2023). Particularly soil microorganisms provide many relevant ecosystem services, such as nutrient cycling, organic matter degradation, carbon storage, soil aggregation, degradation of contaminants, or the promotion of plant growth (Hartmann and Six, 2023). Amongst plant growth promoters, a key role is played by arbuscular mycorrhizal fungi (AMF) and other mycorrhizal species that form symbioses with around 80% of terrestrial plants (Wang and Qiu, 2006). The best-known function of AMF is the provision of phosphorous to plants but they can also supply nitrogen and micronutrients, as well as improve the plant's water stress tolerance and disease resistance (Ruiz-Lozano, 2003; van der Heijden et al., 2008; Weng et al., 2022). To harness their immense potential in supporting plant growth, AMF inoculation has been promoted by researchers and commercial players, however, a large variability in plant growth responses can be found (Hoeksema et al., 2010; Wu et al., 2024). Next to inoculum quality (Salomon et al., 2022), biotic and abiotic soil properties have a strong influence on AMF inoculation success, with poorly performing soils generally having a higher benefit (Lutz et al., 2023; Rog et al., 2025). Another group of highly important plant symbionts are rhizobia. These bacteria form symbioses with leguminous plants, providing their hosts with nitrogen through atmospheric nitrogen fixation and making them less dependent on external nutrient sources (Ferguson et al., 2010). Legumes comprise many relevant crops for human consumption but also fodder and green manure crops that can be used as soil improvers supplying nitrogen and organic matter (Meena et al., 2018). Soil microorganisms can, therefore, support vigorous plant growth without excessive use of agricultural inputs. However, their functioning might be compromised by soil degradation, e.g. through contamination with pesticides. A growing number of studies have unveiled the ubiquitous occurrence and potential hazards of pesticide residues in agricultural soils as a result of intensive pesticide use over decades (Barmettler et al., 2025; Froger et al., 2023; Geissen et al., 2021; Knuth et al., 2024; Riedo et al., 2021; Silva et al., 2019).

Due to their inherent toxicity, pesticide residues might have unintended non-target effects on soil microorganisms. Various laboratory and greenhouse experiments have documented negative effects from the application of different pesticides on the performance of AMF (e.g. Druille et al., 2013; Okiobe et al., 2022) and rhizobia (e.g. Fox et al., 2007; Paniagua-López et al., 2023). Effects were typically transitory, with the symbiosis recovering after an initial inhibition, but lasting reductions in plant growth and nutrient contents occurred (Abd-Alla et al., 2000; Fox et al., 2007). Pesticides can affect the symbiosis by either acting directly on the microbial symbiont or indirectly by changing root morphology and exudation patterns of the host plant, e.g. through toxic effects from herbicides (Fox et al., 2004; Hage-Ahmed et al., 2019; Zahran, 1999). In the field, effects of pesticide exposure on soil microbes are less well documented, partly because the high complexity of environmental factors that simultaneously affect soil microbiota and pesticide fate makes it challenging to establish causal relationships. However, current screening studies provided strong evidence that pesticide use can strongly affect the soil microbiome and lead to a reduction in soil fungal diversity (Barmettler et al., 2026; Köninger et al., 2026). Previous studies have also documented negative associations between pesticide applications or residues and AMF root colonization (Riedo et al., 2021), phosphorous transfer (Edlinger et al., 2022), and diversity (Vahter et al., 2022).

Experimental studies testing pesticide effects on soil microbes typically used isolated strains or soils that had hardly been exposed to pesticides before (e.g. Abd-Alla et al., 2000; Riedo et al., 2023). Microbiota from intensively managed fields that are regularly exposed to pesticides might adapt over time and react differently to pesticide applications. The sensitivity towards pesticides strongly varies across different soil microbes (Lo, 2010), and also specifically across different species of AMF (Hage-Ahmed et al., 2019; Roshanfekrrad et al., 2025) and rhizobia (Mansotra et al., 2022; Zabaloy and Gómez, 2005). Continuous pesticide application may, therefore, lead to a decline in sensitive taxa and favor robust taxa, making the microbial community overall less sensitive. In line with this, pesticide applications were found to be associated with shifts in microbial (Medo et al., 2021; Schlatter et al., 2017) and AMF community composition (Jin et al., 2013; Rivera-Becerril et al., 2017). Repeated pesticide applications to soil in laboratory settings were also found to increase the pesticide degradation speed and the abundance of pesticide degrading bacteria, as well as the expression of functional genes involved in pesticide degradation (Bælum et al., 2008; Lancaster et al., 2010; Yale et al., 2017). Various rhizobia strains also have pesticide degradation abilities (Liu et al., 1991; Vercellino and Gómez, 2013) and may, therefore, be favored by applications of certain pesticides. Moreover, many rhizobia strains were shown to strongly increase their tolerance to pesticides when cultivated under increasing pesticide concentrations, without compromising their symbiotic potential (Odeyemi and Alexander, 1977; Roslycky, 1985). However, previous studies found no connection between rhizobia strains' pesticide sensitivity and the pesticide exposure of the soil they were isolated from (Drouin et al., 2010; Mårtensson, 1992). While adaptations of AMF towards synthetic pesticides were not studied in detail, specific adaptations to the heavy metal copper have been described, such as avoidance, complexation, or compartmentalization (Ferrol et al., 2009). Overall, numerous studies have demonstrated the adaptation potential of soil microbes towards pesticide exposure in controlled, short-term experiments that typically only included single products. However, it is unclear whether these adaptations also apply to field soils, pesticide mixtures, and over longer periods without pesticide applications.

To address these knowledge gaps, we conducted a greenhouse experiment with soils from conventional and organic vineyards. Conventional vineyards are treated with large amounts of synthetic pesticides, which are forbidden in organic viticulture. Comparing the two farming systems, we could study the impact of increased pesticide exposure under realistic field conditions. Soils were collected in early spring to test the long-term effects of pesticide application after winter break. We studied the establishment of AMF and rhizobia in symbiosis with red clover, a typical cover plant in vineyards. We tested the impact of management history on overall plant and microbial performance, the sensitivity towards a fresh pesticide application, and pesticide degradation speed. Additionally, we assessed the potential of AMF inoculation to mitigate negative non-target effects from pesticide use. We hypothesized that compared to organic vineyards, soil microbes from conventional vineyards would (i) establish less effective symbioses with the host plant, (ii) react less sensitively to fresh pesticide applications, (iii) degrade pesticides faster, and (iv) benefit more from additional AMF inoculation.

2. Materials and methods

2.1. Soil sampling and processing

Soils were collected from twelve conventional and twelve organic vineyards in the canton of Zurich in Switzerland and neighboring areas between March 5th and 21st, 2024 (Fig. S1). All conventionally managed vineyards were regularly treated with synthetic pesticides for a minimum of ten years, including the season prior to soil sampling. Contrarily, the organic vineyards were managed according to the guidelines of the Federation of Swiss Organic Farmers that forbids the use of synthetic pesticides for at least ten years (Bio Suisse, 2026). In each vineyard, a 10 × 10 m square was selected and five intact soil cubes of 20x20x20 cm were collected with a spade in five consecutive interrows with a minimal distance of 4 m between soil subsamples. A minimum distance of 10 m from the parcel border was kept. All sampling locations had vegetation cover with varying composition. The soil cubes

were stored at 4 °C until further processing (between one to three weeks). Three weeks prior to the experimental start, the soil cubes were manually broken up and stones and coarse debris were removed. For each vineyard, 5 kg of fresh soil were sampled from each cube and subsamples were pooled to obtain a representative soil sample per vineyard. The pooled soil was air-dried at 8 °C for 72 h to facilitate sieving. Fourteen days before experimental start, the soils were sieved to 5 mm and the water content was measured by drying at 105 °C. Water holding capacity (WHC) was determined by soaking approximately 100 g of soil in water overnight, letting excess water drain and drying the wet soil at 105 °C until constant weight.

2.2. Experimental setup

The greenhouse experiment involved growing red clover (*Trifolium pratense L.*, variety MILVUS) in 1 L pots filled with soils from the various sites specified above. Red clover was selected, as it commonly occurs in Swiss vineyards and it is a suitable model organism to study symbioses with AMF and rhizobia, whereas growing grapevine in a short-term greenhouse experiment is not feasible. The experiment was split into two sub-experiments (Fig. 1). The sub-experiment "continuous harvest" included the treatments management history, pesticide application and multiple harvest timepoints. In the sub-experiment "final harvest", the inoculation of AMF was additionally included, but only the last timepoint was covered (Fig. 1). For each harvest timepoint, separate pots were prepared, resulting in 10 pots per vineyard and 240 pots in total.

A pesticide mix (see section 2.3) was applied to the soil on the first day of the experiment using a spray bottle and thoroughly mixed in by hand after every spray to assure homogeneous distribution. Deionized water was used for the control treatment (pesticide application treatments "control" vs. "applied"). Subsequently, 5% *w*/w (dry soil) AMF inoculum was amended to the soil and mixed in by hand. The inoculum contained spores and mycelium of *Rhizoglomus irregulare* (Błaszkowski et al., 2008; Sieverding et al., 2015) strain SAF22 from the Swiss Collection of AMF. It was produced by growing *Plantago lanceolata* L. in the greenhouse in an autoclaved 65:15:20 (*v*/v/v) sand:soil:oil binder mixture with 5% of previously produced inoculum. Plants were grown for six months and were then left unirrigated until completely dry to stimulate AMF sporulation. As a control we used an inoculum without AMF that was produced under the same conditions, resulting in soils with only native AMF (AMF inoculation treatments "native" vs. "inoculated"). The amount of pesticide treated soil-inoculum mixture that was filled into each pot was adjusted for the soils' varying water contents, targeting 600 g of dry soil per pot. A subsample of this mixture was collected in 50 mL falcon tubes and stored at −20 °C to determine the initial pesticide load.

Twelve red clover seeds were planted in each pot at 1 cm depth using toothpicks. Seeds were surface sterilized, by soaking them in 70% ethanol for 5 min and in 5% bleach (5 drops/L Triton X-100) for 10 min, followed by thorough rinsing with deionized water after both steps. The pots were irrigated to 60% WHC and covered with punctured cling film for three days to avoid drying of the soil surface during seed germination. Soils were split into four blocks, each containing three conventional and organic vineyard soils and the experimental setup was conducted block-wise on four consecutive days (16th to 19th of April 2024) to distribute the workload of setup and harvests across multiple days.

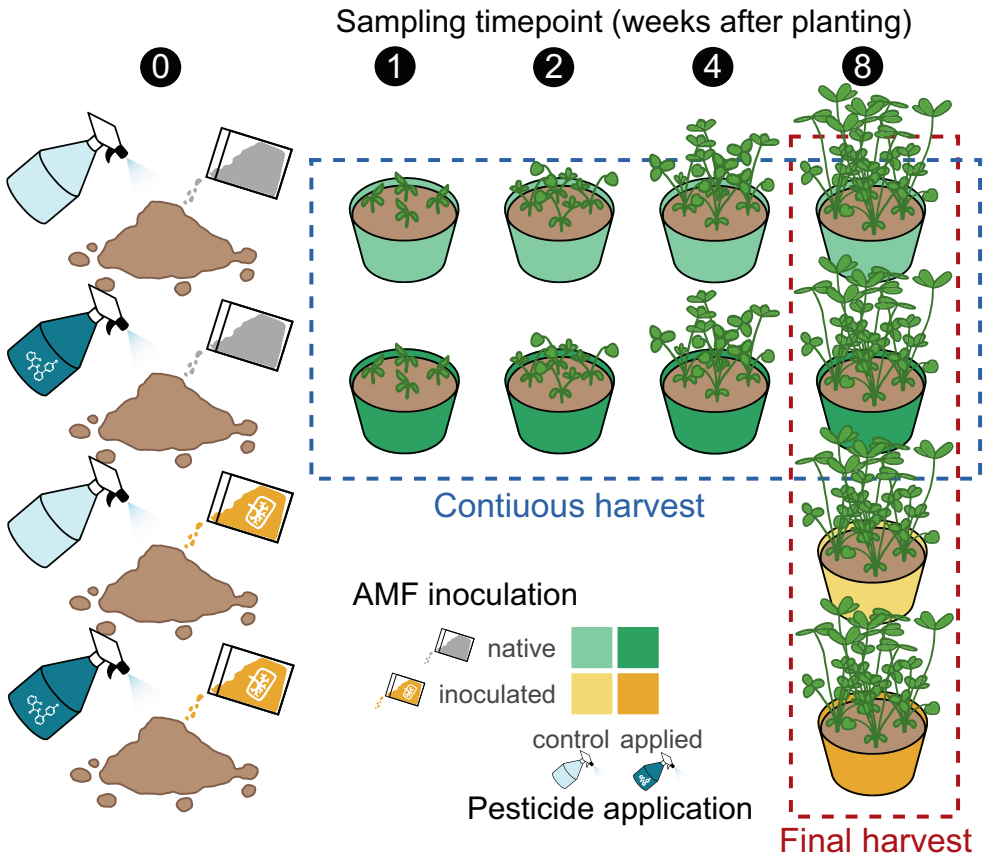

Fig. 1. Experimental design displayed for one of 24 soils showing the experimental treatments "sampling timepoint", "pesticide application", and "AMF inoculation", as well as the two sub-experiments "continuous harvest" and "final harvest".

2.3. Pesticide mixture

A mixture of eight commercial pesticide products containing nine fungicidal active ingredients (AI) was applied in the experiment (Table 1). This was chosen to resemble the application of multiple pesticides across the season, which is typical in viticulture. Pesticides were selected according to the frequency of use, the recommendations in integrated production (IP Suisse, 2024), the target disease, the coverage by our quantification method (Rösch et al., 2023), and their occurrence frequency in vineyard soils according to our previous study (Barmettler et al., 2025). Each product was applied at its minimal recommended single dose (Table 1) to avoid unrealistically high exposure through the combination of multiple pesticides. The applied amount was calculated on the basis of the pots' surface area (13 cm diameter, $1.33*10^{-4}$ m^2 surface area) and diluted in deionized water to reach an applied volume equal to 1270 L/ha.

2.4. Plant growth and harvest

Plants grew in the greenhouse at 22/18 °C and 16/8 h day/night. If the natural light intensity fell below 300 Wm^{-2}, supplementary light of 400 Wm^{-2} was provided. Pots were watered to 60% WHC three to seven times a week, depending on the plant growing stage. After nine days, the plants were thinned out to four plants per pot. Weeds were manually removed regularly. Five weeks after experimental start, an outbreak of powdery mildew was observed that was treated with wettable sulfur (Netzschwefel Stulln, Andermatt Biocontrol, Switzerland). A 0.4% solution of this organic fungicide was applied to all plant shoots using a spray bottle.

Harvest took place 1, 2, 4, and 8 weeks after planting through destructive sampling of whole pots. The plants were separated manually from the soil and remaining soil particles were washed off under the water tab. Plants were patted dry with a paper towel and split into shoot and root. For the first two harvests, the whole root system was collected in a 1.5 mL Eppendorf tube. For the third and fourth harvest, roots were cut into 1 cm pieces, homogenized, and up to 0.4 g of fresh roots were collected in 1.5 mL Eppendorf tubes. These root samples were stored at −20 °C for DNA extraction. The remaining roots and the shoots were collected in paper bags and dried at 60 °C to measure biomass. Fresh roots were weighed before and after sample removal to determine the weight of the whole root system. The soil was homogenized, collected in 50 mL falcon tubes, and stored at −20 °C for pesticide quantification.

2.5. Soil analyses

Soil properties were determined according to the reference methods of the Swiss Federal Agricultural Research Station Agroscope (https://www.agroscope.ch/referenzmethoden). Three subsamples of the pre-processed experimental soils were collected one week before the experimental start: One stored at −20 °C for mineral N analyses, one stored at 4 °C to measure microbial biomass, and one dried at 40 °C until constant weight and sieved to 2 mm, which was used for all other soil analyses. Soil texture was fractionated into clay (<2 μm), silt (2–50 μm), and sand (>50 μm) by pipetting. Soil pH was determined in deionized water. Soil organic carbon (SOC) content was quantified via oxidation using dichromate. Total copper and other heavy metals were assessed with a 2 M HNO_3 extraction. Bioavailable P and K were extracted in CO_2 saturated water. Mineral NO_3 and NH_4 were determined by extraction in a 0.01 M $CaCl_2$ solution. Microbial biomass was analyzed in triplicates using the chloroform fumigation extraction method. Prior to analysis the fresh soil was sieved to 2 mm and stored at −20 °C until extraction.

Litter degradation was assessed as a proxy for microbial activity. Three rooibos tea bags (Lipton, United Kingdom) were buried in a 3 L pot using the fresh soil equivalent to 1.8 kg dry soil. Pots were kept in the greenhouse chamber during the experiment for 38 days and were regularly watered to 60% WHC. Tea bags were then removed from the soil and dried at 60 °C until constant weight. The percentage of degradation was calculated using the tea weight before and after burial.

Pesticide concentrations of the untreated field soils were measured following the procedure of Rösch et al. (2023) as described in Barmettler et al. (2025). Briefly, pesticides were extracted with an adapted quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach (Lehotay, 2007) from 5 g soil using acidified acetonitrile (ACN; 2.5% formic acid). Concentrations were quantified using liquid chromatography coupled to triple quadrupole mass spectrometry with electrospray ionization (LC-ESI-MS/MS).

2.6. Pesticide dissipation

To assess the dissipation of the freshly applied pesticides, we quantified the pesticide concentrations in the experimental samples using the same method as described in section 2.5 but with some modifications. Only the applied AI and their transformation products (TP) cyprodinil CGA249287, cyprodinil CGA232449, and trifloxystrobin acid were measured. The soils were lyophilized to avoid potential pesticide dissipation during oven drying. The QuEChERS extracts were diluted 10-fold with acidified ACN to increase the maximum quantifiable concentration. Isotopically labeled internal standards (ILIS) were added after the extraction to limit excessive consumption of ILIS due to strong extract dilution. To verify sufficient extraction efficiency, we measured the absolute pesticide recovery of all field soils by comparing the pesticide peak areas of extracts from pre-extraction spiked soils and post-extraction spiked extracts. All measured pesticides had absolute recoveries close to 100% except for Cyprodinil and its TPs that had mean recoveries between 80 and 90% and a minimal recovery of 69% (Fig. S2). Pesticide concentrations were only compared relatively between samples from different time points of the same soil, which are expected to have the same pesticide recovery. For samples with pesticide concentrations exceeding the linear range of the matrix matched calibration curve, additional 4-fold dilutions were made using acidified ACN.

Table 1
Summary of applied fungicides. Applied dose refers to the amount of product and the expected concentration to the amount of active ingredient. Recommended products (RP) in integrated production (IP Suisse, 2024). Frequent occurrence (FO) in Swiss vineyards according to Barmettler et al. (2025).

Product	Target disease	Applied dose	Active ingredient	Expect. conc. [ug/kg]	Inclusion reason
Cantus® (BASF / Leu + Gygax)	*Botrytis*	1.2 kg/ha	boscalid	1327	FO
Switch® (Syngenta)	*Botrytis*	1.2 kg/ha	cyprodinil	995	RP & FO
			fludioxonil	664	
Flint® (Bayer)	Downy mildew	0.24 kg/ha	trifloxystrobin	265	FO
Pergado® (Syngenta)	Downy mildew	3.2 kg/ha	mandipropamid	354	RP & FO
Ridomil Vino® (Syngenta)	Downy mildew	2.25 kg/ha	metalaxyl-M	241	RP & FO
Slick® (Syngenta / Strähler)	Powdery mildew	0.15 L/ha	difenoconazol	83	RP & FO
Talendo® (Corveta Agriscience)	Powdery mildew	0.4 L/ha	proquinazid	177	RP
Vivando® (Syngenta / BASF)	Powdery mildew	0.32 L/ha	metrafenone	354	RP & FO

2.7. Nitrogen fixation

Nitrogen fixation was quantified for the final harvest using the ^{15}N natural abundance method (Shearer and Kohl, 1986). As a reference plant, we grew perennial ryegrass (*Lolium perenne L.*, variety ARAIAS) under the identical conditions as the clover plants. For each soil, one such reference pot without pesticide application and control inoculum (control-native) was prepared. To increase the ^{15}N signature of the soil, we applied 0.6 mg of ^{15}N (1.67 mg of 98% $^{15}NH_4^{15}NO_3$) to each pot split into three doses and diluted in 50 mL deionized water at 2, 4, and 6 weeks after planting.

Nitrogen contents and ^{15}N isotope ratios of the plants were measured using a vario PYRO cube elemental analyzer coupled to an isoprime precisION isotope ratio mass spectrometer (IRMS; Elementar, Langenselbold, Germany). For clover and reference plants, 1.5 and 2.5 mg of milled shoot material were weighed into tin capsules, respectively. Samples were combusted at 950 °C in the presence of pure O_2 in a continuous He stream. The evolved gases were then converted into N_2 to measure total N and, subsequently, carried to the IRMS to analyze δ15N values, with drift correction and calibration using the USGS-64, USGS-65 and USGS-66 reference materials (U.S. Geological Survey, Reston VA, USA). Blanks and in-house standards were included throughout the runs to ensure analytical accuracy. The data were acquired and processed using the lyticOS and pyrocube software (Elementar).

Percent nitrogen derived from the atmosphere (%Ndfa) was calculated as follows:

$$\%Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{clover}}{\delta^{15}N_{ref} - B} * 100 \quad (1)$$

Where $\delta^{15}N_{ref}$ and $\delta^{15}N_{clover}$ are per mil (‰) excess ^{15}N in the reference and clover plants, respectively, over the ^{15}N abundance in the atmosphere; B is an estimated $\delta^{15}N$ for red clover shoots that are entirely dependent on atmospheric N and was set to −1‰ (Oberson et al., 2013).

2.8. DNA extraction and qPCR

To extract DNA from the roots, the NucleoSpin™ Plant II kit (Macherey-Nagel, Duren, Germany) was used following the manufacturer's instructions. The lyophilized roots were grinded with glass beads using the FastPrep-24™ (MP Biomedicals, Irvine CA, USA) and 20 mg of root powder were used for DNA extraction. If less than 25 mg of root material was available, the whole root system was used. Concentration of DNA was quantified using a Cary Eclipse fluorescence spectrometer (Agilent, Santa Clara CA, USA) and normalized to 1 ng/μL.

We conducted qPCR with specific primers to measure the abundance of the *nifH* gene (PolF_115/PolR_457; Poly et al., 2001), AMF in general (AMG1F/AM1; Helgason et al., 1998; Hewins et al., 2015), and specifically the inoculated strain SAF22 (Rirre_22KS-F/Rirre_Alk-R; Alkan et al., 2006; Bender et al., 2019; see Table S1). Each 10 μL reaction volume contained 2 μL of HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Tartu, Estonia), 500 nM of forward and reverse primer and 2 ng or 5 ng template DNA for *nifH* or AMF, respectively. Amplifications were run on a CFX Opus 384 Real-Time PCR System (Bio-Rad Laboratories, Hercules CA, USA) in triplicates with the specific settings given in Table S2. For the calibration curve, we used two synthetic oligonucleotide standards (Han et al., 2023), one for the *nifH* gene at concentrations between $2 * 10^{-3}$ to $2 * 10^{-7}$ ng/μL (1019 base pairs), and one for both total AMF and SAF22 quantification at concentrations between $5 * 10^{-4}$ to $5 * 10^{-9}$ ng/μL (336 base pairs; see Table S3). Raw data were analyzed using the LinRegPCR program, determining the cycle numbers to reach a common threshold (C_T) and PCR efficiencies. Replicates deviating more than 1 in C_T from the other replicates of the same sample or being more than 0.2 below the mean PCR efficiency were excluded. The C_T of the remaining replicates were averaged per sample. The number of reads per sample were calculated using the calibration curve correcting C_T by mean sample or standard PCR efficiency, respectively. The lowest calibration standard corresponded to 2.7 or 90 copies per ng DNA for AMF and *nifH* qPCR, respectively. Some samples from the first harvest were below these thresholds. However, they passed the quality filtering steps and were retained for the statistical analysis.

2.9. AMF sequencing and bioinformatics

To characterize the AMF communities, we sequenced a large fragment of rDNA spanning from the SSU to the LSU, including the whole ITS region, as well as other variable regions. We used a modified protocol based on Kolaříková et al. (2021). A two-step PCR was performed, using the two primer pairs NS31/LSUmAr and AML1/LSUmAr for PCR1 and the primer pair NS31_Glo3/LSUmBr for PCR2 (Kolaříková et al., 2021; Krüger et al., 2009; Lee et al., 2008; Simon et al., 1992; see Table S4). In PCR2 we added universal M13 primer tails and a 5AmMC6 modification to avoid ligation of carry-over amplicons from PCR1 to the SMRTbell adapters in subsequent steps. PCR1 was performed separately for both primer pairs and pooled for PCR2, which was done in triplicates. Each 10-μL PCR reaction contained 1× Kapa HiFi HotStart DNA Polymerase ready-mix kit (Roche, Basel, Switzerland), 500 nM of each primer, and 4 ng of template DNA (PCR1) or 4 μL of pooled PCR1 product (PCR2). As a modification from the original protocol, PCR products were not diluted after PCR1 but purified with self-made SPRI Beads (GDC, Zurich, Switzerland) after both PCR steps. Finally, index PCR was performed in a volume of 20 μL with custom-made M13 barcodes as described by Stalder et al. (2025). Per PCR reaction we used 1× Kapa HiFi HotStart DNA Polymerase ready-mix kit (Roche), 300 nM forward and reverse index primer each, as well as 6 μL clean PCR2 product. Specific PCR settings can be found in Table S5. Index PCR products were loaded on a 1% Agarose Gel. The fragments of 1500 to 3000 base pairs were cut out from the gel and purified with the NucleoSpin Gel and PCR Clean up Kit (Macherey-Nagel) according to the manufacturer's instructions. Subsequently, DNA concentration was quantified using the Qubit™ dsDNA Quantification Assay Kit Q32853 (Thermo Fisher, Waltham MA, USA). The samples were pooled using a pipetting robot Liquid Handling Station (BRAND, Wertheim, Germany). The pooled library was diluted to a final concentration of 8 ng/μL and sequenced on the PacBio Revio Sequencer using a SMRT Cell 25 M, 12 h movie (<5 kb libraries; Pacific Biosciences, Menlo Park CA, USA). Library preparation was performed at the Genomic Diversity Centre Zurich (https://gdc.ethz.ch) and sequencing was carried out at the Functional Genomics Centre Zurich (https://fgcz.ch).

Samples were demultiplexed using the PacBio *lima* tool v2.13.0 (https://lima.how) with the specified adapter and primer barcode sequences. Standard parameters were used, with the additional options *–different* (to retain only barcode pairs containing different barcodes) and *–split-names* (to name output files by the resolved barcode-pair identifiers). Primers were trimmed using *cutadapt* v4.8 (Martin, 2011). Sequence quality was assessed with FastQC v0.12.1 (Andrews, 2025). Trimmed sequences were imported into QIIME 2 v2022.11.1–2 (Bolyen et al., 2019) using the SingleEndFastqManifestPhred33V2 format and denoised with the DADA2 (Callahan et al., 2016) *denoise-ccs* plugin (*–-pooling-method pseudo*, *–-p-chimera-method pseudo*, with all other parameters set to defaults). The resulting denoised and filtered amplicon sequence variants were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold.

Taxonomy was assigned to the representative sequences of the 97% OTUs using the feature-classifier *classify-sklearn* method and the EUKARYOME database v1.9.3 (Tedersoo et al., 2024). The OTU table, taxonomy table, and representative sequences were then exported for downstream analyses in R. For OTUs that were not classified at species level, sequences were manually queried against the NCBI nucleotide database using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Assignments with >90% query coverage and > 97% identity were

accepted (Table S6).

2.10. Data transformation

Pesticide dissipation was assessed using the pesticide concentrations of the pesticide-treated soils and subtracting the concentrations of the non-treated soils. The 50% dissipation times (DT50) were calculated using the *kminfit* function from the R package *kmin* v.1.2.10 (Ranke, 2025) assuming single first order dissipation. As dissipation appeared non-linear for fast degrading pesticides (cyprodinil, mandipropamid, metalaxyl, and trifloxystrobin), they were modeled according to double first order (biphasic) dissipation (Fig. S3). The dissipation of trifloxystrobin and its TP trifloxystrobin acid was modeled simultaneously. The DT50 values of trifloxystrobin were not assessed due to its very fast dissipation that could not sufficiently be captured with the selected sampling timepoints. As a comparison, lab and field DT50 values were retrieved from the "Pesticides Properties DataBase" (Lewis et al., 2016; https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm).

Number of reads from qPCR were log-transformed in all statistical tests. The pesticide sensitivity of all response variables was expressed as log-ratios between treatment and control. Mycorrhizal growth response (MGR) was calculated using the following formula (Veiga et al., 2011):

$$If\ M_{inoc} > M_{ctrl}: \quad MGR = \left(1 - \left(\frac{M_{ctrl}}{M_{inoc}}\right)\right)*100 \tag{2}$$

$$If\ M_{inoc} < M_{ctrl}: \quad MGR = \left(-1 + \left(\frac{M_{inoc}}{M_{ctrl}}\right)\right)*100 \tag{3}$$

Where M_{inoc} and M_{ctrl} are the total biomass per plant of inoculated and control treatment, respectively.

2.11. Statistical analysis

Differences in soil characteristics between conventional and organic farming were tested with Welch's *t*-test. If the assumption of normal distribution was violated, transformations were tested (squared, square-root, cube-root, logarithmic, and inverse) in given order until a suitable transformation was found. For pH an exponential transformation was used. If no suitable transformation could be found, the Kruskal-Wallis test was used (only for zinc concentration).

The two sub-experiments (continuous and final harvest) were analyzed separately. Linear mixed models were created using the *aov* function including the vineyard as random factor with the formulas y ~ block + management * week * pesticide + Error(vineyard) and y ~ block + management * pesticide * AMF + Error(vineyard) for continuous and final harvest, respectively. Differences between groups were assessed using the *contrast* function from the *emmeans* package v. 1.11.2 (Lenth, 2025) with MVT *p*-value adjustment for multiple testing (Hothorn et al., 2008).

Using the R package phyloseq v.1.52.0 (McMurdie and Holmes, 2013) ASV and taxonomy tables and the sample data were compiled and rarefied to equal depth (251 reads; Fig. S4). The Shannon index was used to asses AMF diversity (Shannon, 1948) and Bray-Curtis dissimilarities for community composition (Bray and Curtis, 1957). For diversity data the *lmer* function of the *lme4* package v.1.1–37 (Bates et al., 2015) was used for mixed models, due to the unbalanced design resulting from the loss of some samples. To assess treatment effects on AMF community composition, PERMANOVA was used in the *adonis2* function of the *vegan* package v.2.7–1 (Oksanen et al., 2025). Due to the nested design, permutations were restricted using the *how* function in the *permute* package v. 0.9–8 (Simpson, 2025). As the number of samples per vineyard were unbalanced, permutations on vineyard level had to be defined manually. Differential abundance analysis was performed using the *DESeq* function in the *DESeq2* package v.1.48.1 (Love et al., 2014) including the vineyard ID and other experimental variables in the formula before the variable of interest. The options *test* = *"Wald"* and *fitType* = *"parametric"* were used, and contrasts were extracted with the *results* function. All plots were created using the package *ggplot2* v.3.5.2 (Wickham, 2016) and all statistical analyses were performed using R v.4.5.1 (R Core Team, 2025).

3. Results and discussion

3.1. Similar soil properties, but distinct pesticide profiles in conventional and organic systems

Conventionally and organically managed fields did not significantly differ in the basic soil properties and most other characteristics measured, including pH, SOC, texture, macronutrients, microbial biomass, copper, and other heavy metals (Fig. S5). Therefore, we argue that the selected soils are suitable to test the effect of management history on soil microbes with minimal interference of other soil property differences that are known to influence soil microbes (Fierer, 2017). As expected, the number of pesticides and pesticide sum concentrations were significantly higher in conventional vineyards than organic ones ($p < 0.001$; Fig. 2a,b). Conventional and organic vineyards contained median numbers of 16.5 and 5 pesticides and sum concentrations of 60 and 1.4 μg/kg, respectively. Moreover, organic vineyards had a faster degradation of rooibos litter compared to conventional vineyard soils ($p = 0.016$, Fig. 2c).

The nine fungicidal AI that were applied in the experiment, were also commonly found in the vineyards studied and present at higher numbers and concentrations in the conventional vineyards (Fig. S6a). All nine AI and/or their TP were found in at least seven conventional vineyards (Fig. S6b). Fludioxonil and the TP cyprodinil CGA249287 were found in all conventional vineyards and commonly occurred in organic vineyards too. Conventional vineyards contained between four to nine of the nine selected fungicides, whereas organic vineyards contained between zero to four (Fig. S6c). We therefore conclude that conventional vineyards were commonly exposed to the fungicides selected, whereas organic vineyards showed a clearly lower exposure.

3.2. No adaptation for pesticide degradation

To better understand the general trend of pesticide exposure during the experiment and assess the effect of management history on pesticide degradation, we calculated DT50 values. Multiple AI had DT50 values between a few days to few weeks (Fig. S7), indicating that overall pesticide exposure was clearly lower by the end of the experiment compared to the beginning. Except for fludioxonil and metrafenone, the DT50 values were clearly lower compared to lab and field DT50 values from literature (Fig. S7). The greenhouse conditions could have accelerated pesticide dissipation, e.g. higher temperatures and more stable soil moisture compared to the field. Moreover, plant exudates could increase pesticide desorption (Luo et al., 2006) and boost microbial activity (Piutti et al., 2002), compared to laboratory essays with pure bulk soil.

There was no difference in dissipation speed between organic and conventional farming for any of the nine applied AI (Fig. S7). Also, when comparing the soils where the pesticide was detected in the field to those without detection, no significant difference was found. This indicates that there was no adaptation effect for pesticide degradation from previous pesticide exposure in the field, in contrast to previous studies. It has been shown that repeated pesticide applications can accelerate pesticide degradation through microbial adaptation in short-term lab experiments (Bælum et al., 2008; Lancaster et al., 2010), as well as long-term field trials (Harvey, 1987; Paporisch et al., 2020). However, adaptation to pesticide degradation is also highly substance specific (Harvey, 1987; Pose-Juan et al., 2018). The long period of approximately six months between the last pesticide application and sample collection could also partly explain the lack of adaptation, despite

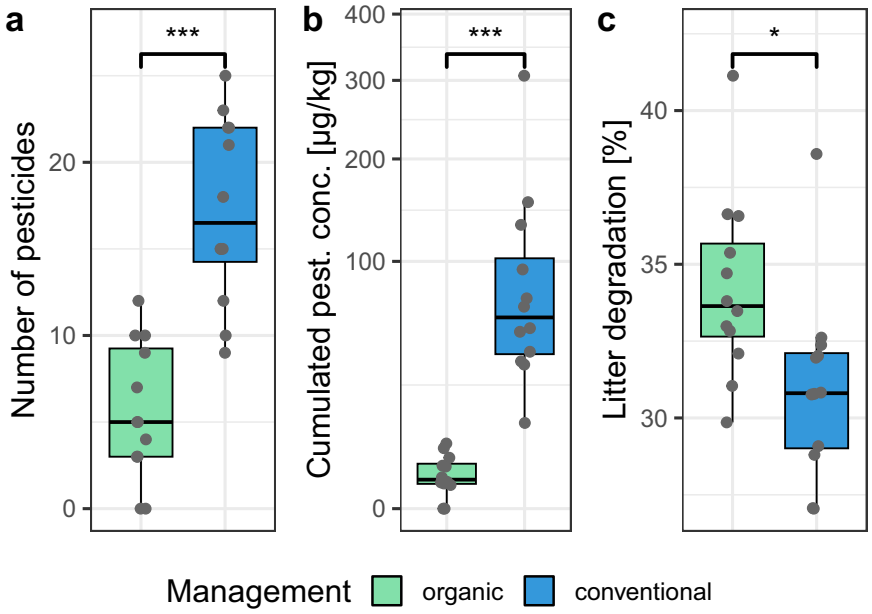

Fig. 2. Soil characteristics that significantly differed between conventional and organic vineyard soils. a) Number of detected pesticides, b) cumulated pesticide concentrations, and c) percent of rooibos litter degradation. Significant differences are displayed using asterisks ($p < 0.001$ (***), $p < 0.01$ (**), and $p < 0.05$ (*)).

pesticides still being present in the soils (Fig. S6).

Although pesticide dissipation is to a large extent mediated by microbial degradation, DT50 values did not correlate with microbial biomass nor litter degradation. Such general microbial markers might be limited in explaining pesticide dissipation, as pesticide degraders are specialized microbes typically constituting a small fraction of the microbiome (Malla et al., 2022). Moreover, AMF inoculation did not affect pesticide dissipation. Contrarily, previous studies have documented an increased dissipation of pesticides and other pollutants through AMF inoculation, which was attributed to increased plant uptake and soil biological activity (Shi et al., 2022; Wang et al., 2011).

3.3. Lower plant biomass and AMF root colonization in conventional systems

Plant biomass continuously increased over the four sampling timepoints (Fig. 3a). Plants grown in pots with organic soils yielded more biomass than those grown in pots with conventional soils ($p = 0.012$). This difference was not constant over the whole experiment, as indicated by a strong management-week interaction ($p < 0.001$). While plant biomass did not differ between management systems one and two weeks after planting, biomass from organic soils was significantly higher compared to conventional in week four and eight. At the final harvest, the mean biomass in organic soils was 47% higher than in conventional soils (Fig. 3a). Similar patterns were found for AMF root colonization. There was a sharp increase of AMF root colonization until week four and a slight decrease towards the final harvest (Fig. 3b). As with biomass, AMF root colonization differed across management systems ($p = 0.037$) with organic soils having higher colonization rates. This difference tended to be strongest two weeks after planting (Fig. 3b).

The better plant performance in organic soils compared to conventional soils could be due to biological activity, as abiotic soil properties did not significantly differ between management systems (Fig. S5). The overall higher and faster root colonization of AMF (Fig. 3b) could have led to an improved nutrient supply to the plants and, therefore, a higher biomass towards the end of the experiment. Similarly to our results, Heuck et al. (2025) found a higher AMF root colonization and plant biomass from AMF originating from organic vegetable fields compared to conventional fields. Moreover, organic soils exhibited a faster litter degradation (Fig. 2c) which could point towards higher microbial activity in general and increased nutrient provision through mineralization. Organic farming was generally found to positively affect microbial activity, typically in combination with higher SOC contents and microbial biomass (Lori et al., 2017). In the current study, we only found a slight trend for higher microbial carbon (74 vs. 59 mg/kg, $p = 0.14$) and microbial nitrogen (12 vs. 9 mg/kg, $p = 0.11$) in organic compared to conventional vineyards, and no difference was found for SOC (SOC: 2.4% vs. 2.3%, $p = 0.53$; Fig. S5).

3.4. Pesticide application affects biomass and AMF differently over time

Pesticide application led to a pronounced decrease in plant biomass ($p < 0.001$), however, this effect was not constant over time, as visible in a highly significant pesticide-week interaction ($p < 0.001$; Fig. 3c). Plants appeared unaffected by pesticide application the first two weeks after planting, whereas four and eight weeks after planting there was a strong reduction in plant biomass, namely a median of −27% at the final harvest (Fig. 3c). Root colonization by AMF was also strongly reduced by pesticide application ($p < 0.001$), and, as with biomass, this reduction depended on the timepoint, as indicated by a highly significant pesticide-week interaction ($p < 0.001$; Fig. 3d). However, in contrast to plant biomass, the reduction in root colonization was strongest at the early harvest timepoints and by the end of the experiment, no significant reduction was found anymore (Fig. 3d). This might be explained by the plateauing of AMF root colonization (Fig. 3b), allowing the freshly pesticide-treated AMF to catch up. Additionally, AMF might have adapted to pesticides and have been better protected once entered the plant root but also suffered from lower pesticide toxicity at later harvest timepoints due to dissipation, allowing them to recover. Similaryl, Riedo et al. (2025), reported transient effects on soil fungal communities from abrupt pesticide applications, whereas gradual applications led to persistent shifts.

The reduction of plant biomass at later growth stages due to pesticide application could be explained by the initial reduction in AMF root colonization (Fig. 3c,d). The delayed establishment of the plant-AMF symbiosis could have led to reduced nutrient acquisition, which finally resulted in lower biomass. We consider a direct effect of the applied fungicides on the plants unlikely, as plant biomass was not

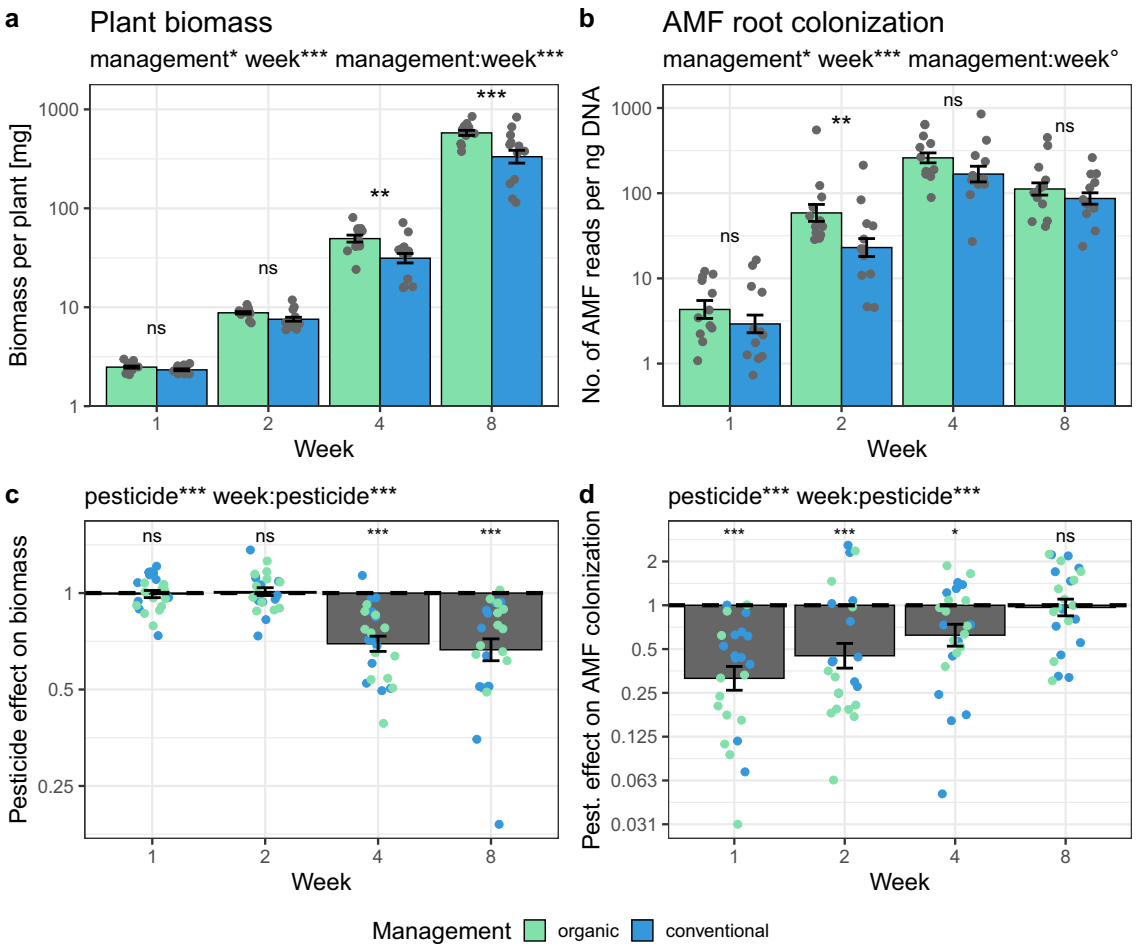

Fig. 3. Temporal development of plant biomass and arbuscular mycorrhizal fungi (AMF) root colonization, and effect of pesticide application across time. a & b) Plant biomass & AMF root colonization grouped by harvest timepoint and management, and averaged across pesticide treatments. c & d) Effect of pesticide application on plant biomass & AMF root colonization grouped by harvest timepoint. Pesticide effect expressed as ratio between pesticide applied and control treatment. Dashed line indicates ratio of 1, i.e. no pesticide effect. Means ± SE and individual data points are shown. Asterisks above panels indicate significance of variables from linear mixed models. Asterisks within plots indicate significant difference between organic and conventional (a & b) or significant difference from 1 (c & d) using MVT correction for multiple testing ($p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (°), and $p > 0.1$ (ns)).

affected at the first two harvest timepoints (Fig. 3c), where pesticide concentrations were highest. Also, a meta-study including 369 experiments overall found no effects of fungicides on plant biomass (Song et al., 2025). Studying 24 distinct natural soils, our results confirm the generally negative effect of fungicides on native AMF, which has been documented in individual fields in previous studies (Jin et al., 2013; Rivera-Becerril et al., 2017).

As we applied a pesticide mixture, it is not possible to assign the observed pesticide effects to a specific AI. We chose to apply a mixture because it is common practice in viticulture and other intensive agricultural systems, to apply two to three pesticides at once and over the growing season a broad range of pesticides is typically used. However, avoiding pesticides that are particularly toxic to AMF could support overall AMF performance. Roshanfekrrad et al. (2025) found very strong inhibitory effects of fludioxonil on AMF spore germination, even at 0.01-fold field dose. Negative effects of fludioxonil on AMF symbiosis were also documented in other studies (Brieuc et al., 2025; Papadopoulos et al., 2025). Some negative effects on AMF were also reported for boscalid (Okiobe et al., 2022; Riedo et al., 2023), metalaxyl (Hilbig and Allen, 2019; Jin et al., 2013), and difenoconazole (Brieuc et al., 2025), however, evidence is relatively scarce or even missing for the remaining applied fungicides. While further studies on individual pesticides would be necessary to assess their specific impact on AMF, substituting the botryticide fludioxonil in viticulture, e.g. with cultural techniques, may be favorable for AMF.

There was no significant management-pesticide interaction, neither for biomass nor AMF root colonization (Fig. 3b,d), which indicates that there was no difference in pesticide sensitivity between organic and conventional soils. A trend for a management-pesticide-week interaction for AMF root colonization ($p = 0.074$) points towards a stronger reduction of AMF in organic farming at early harvest timepoints, followed by a faster recovery compared to conventional farming (Fig. S8). However, these findings overall do not suggest an adaptation towards pesticide application in conventional farming due to regular pesticide exposure. While it has been shown that different AMF strains differ in pesticide sensitivity (Roshanfekrrad et al., 2025) and pesticide applications lead to shifts in AMF community composition (Jin et al., 2013; Rivera-Becerril et al., 2017), these changes might not lead to a long-term adaptation of AMF communities. In addition, survival mechanisms of AMF against pesticides (Ferrol et al., 2009) might not endure over longer periods.

3.5. *AMF inoculation success depends on native AMF community*

For the final harvest after eight weeks, AMF inoculation was included as an additional treatment factor. The inoculated strain SAF22 established successfully in all treated pots (Fig. S9a). Inoculation significantly increased total AMF root colonization and plant biomass (Fig. S9b,c).

Mycorrhizal growth response was overall positive, with a median of +10% (Fig. 4a). Although not significant, MGR tended to be higher in pesticide treated soils ($p = 0.066$) and conventional soils compared to organic ($p = 0.101$). For instance, the median MGR was +19.7% in conventional-pesticide treated and + 4.8% in organic-untreated soils (Fig. 4a). There was a strong negative correlation between MGR and biomass of the non-inoculated plants ($R^2 = 0.59$, $p < 0.001$; Fig. S10a), indicating that plants in poorly performing soils benefited more from AMF inoculation compared to well performing ones. Similar findings have been described previously (Qin et al., 2020; Rog et al., 2025). Additionally, there was a negative trend between MGR and AMF root colonization in the non-inoculated treatment ($p = 0.063$; Fig. S10b). Altogether, this could imply that a malfunctioning of AMF symbiosis due to pesticide application or intensive management could partly be compensated for by AMF inoculation, whereas well-functioning AMF communities hardly benefit.

Moreover, there was a negative relationship between the relative abundance of SAF22 in the inoculated treatment and AMF root colonization in the non-inoculated treatment ($R^2 = 0.34$, $p < 0.001$; Fig. 4b). In other words, the better the native AMF community colonized the roots, the less well the inoculated SAF22 strain could establish. This suggests that well-performing AMF communities are more competitive towards an inoculated strain, whereas poorly performing AMF communities are easily outcompeted. A similar observation was made by Bender et al. (2019).

3.6. AMF strains differ in competitiveness and pesticide sensitivity

We sequenced root AMF to assess the effects of experimental treatments on community structure. The relative abundances of AMF species and genera are presented in Fig. 5, suggesting differences between organic and conventional vineyards, as well as shifts over time and due to pesticide application and AMF inoculation. Diversity was slightly higher in organic fields compared to conventional ones in the continuous harvest, although not significant ($p = 0.051$; Fig. S11b). This trend is in line with the study of Heuck et al. (2025) that documented higher diversity in organic vegetable fields compared to conventional ones. In addition, community composition was affected by management ($p = 0.041$; Fig. S12a). Over time, AMF diversity decreased ($p = 0.001$; Fig. S11a,b) and there was a strong shift in community composition ($p < 0.001$; Fig. S12a,b), which might be explained by competition amongst AMF. Less competitive AMF might decline or even disappear over time (Engelmoer et al., 2014), resulting in lower diversity at later growth stages. A shift in AMF community composition was also observed in the field trial of Gao et al. (2019), however, they found an increase of AMF diversity over time. Interactions between AMF can be highly complex and range from competition to facilitation depending on combination of strains and external factors (Thonar et al., 2014). Moreover, AMF inoculation led to a strong reduction in AMF diversity and a pronounced shift in AMF community composition (both $p < 0.001$; Fig. S11a,c; Fig. S12c,d). Alteration in community composition was consistent with the study of Lutz et al. (2023) but averaged over all fields no effect of AMF inoculation was found on AMF diversity in that study. Fresh pesticide application had no effect on AMF diversity but affected community composition in the continuous harvest (Fig. S12a). This shift could be explained by varying pesticide sensitivities of different AMF strains (Roshanfekrrad et al., 2025), with pesticide tolerant AMF having a relative advantage over more sensitive ones.

Differential abundance analysis indicated that at the final harvest, multiple OTUs from the genus *Rhizoglomus* were increased compared to the first week of the experiment, whereas many other OTUs were reduced (Fig. S13a). Fresh pesticide application showed only weak effects at OTU level (Fig. S13b). In contrast, inoculation of AMF led to a strong increase of the inoculated strain SAF22 whereas many other OTUs from the genus *Rhizoglomus* were significantly reduced (Fig. S13c). Overall, 14.5% of *Rhizoglomus* OTUs were significantly reduced due to AMF inoculation, whereas for other genera it was clearly less (*Septoglomus* 7.1%, *Funneliformis* 4.2%, *Dominikia* 1.5%, none for other genera at $p < 0.05$; Fig. S13c). The cumulated relative abundance of native OTUs from the genus *Rhizoglomus* increased over time and was strongly reduced by AMF inoculation (Fig. S14a,c), in line with the differential abundance analysis. Additionally, fresh pesticide application led to a significant increase of *Rhizoglomus* relative abundance, especially at the final harvest ($p < 0.001$; Fig. S14). Overall, this suggests that

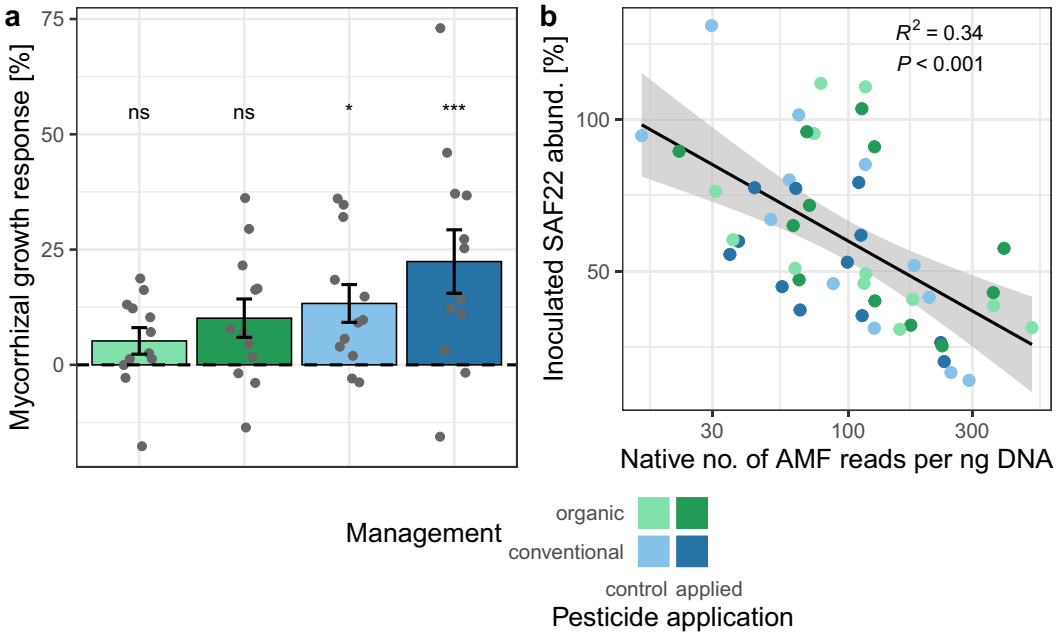

Fig. 4. Arbuscular mycorrhizal fungi (AMF) inoculation effect on plant biomass and AMF root colonization. a) Mycorrhizal growth response (MGR) grouped by management and pesticide application. Means ± SE and individual data points are shown. Dashed line indicates MGR of 0%, i.e. no inoculation effect. Asterisks within the plot indicate significant difference from 0 using MVT correction for multiple testing ($p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (°), and $p > 0.1$ (ns)). b) Correlation between percentage of the inoculated AMF strain SAF22 in the inoculated treatment and total AMF root colonization in the native treatment.

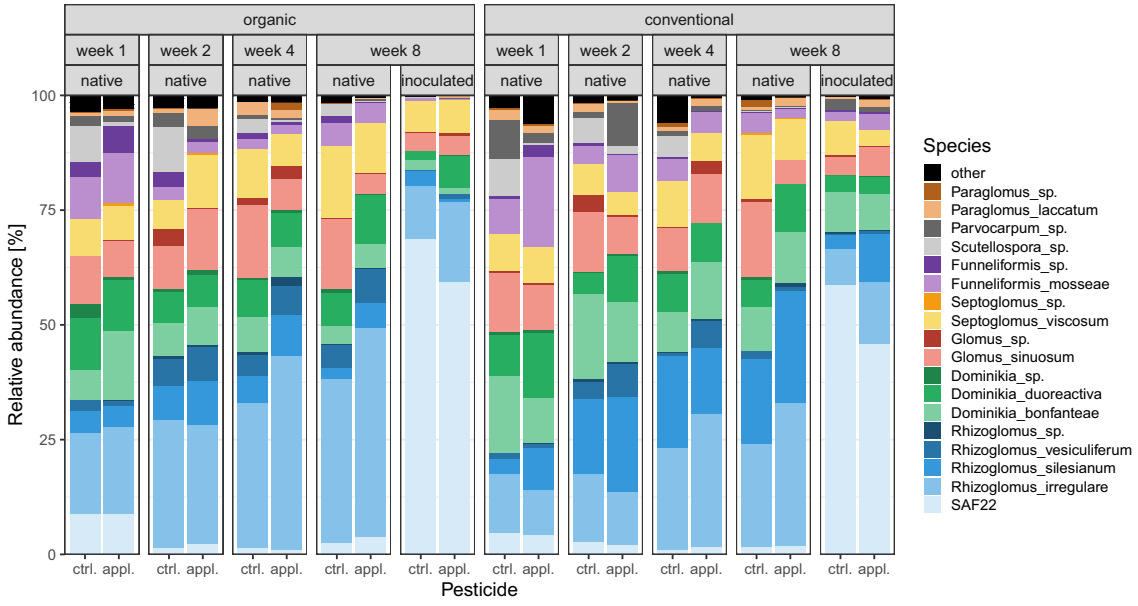

Fig. 5. Species composition of arbuscular mycorrhizal fungi (AMF) in plant roots grouped by management (organic vs. conventional), harvest timepoint (weeks 1–8), AMF inoculation (native vs. inoculated), and pesticide application (control vs. applied). Genera are shown in colors, and species are displayed as different shades. SAF22 was the inoculated *Rhizoglomus irregulare* strain.

Rhizoglomus species are especially competitive against other AMF and might have a higher tolerance against pesticides. Numerous studies have shown that compared to other genera, *Rhizoglomus* strains were particularly efficient at colonizing roots (e.g. Caccia et al., 2024; Köhl and van der Heijden, 2016; Wen et al., 2025), which might give them a competitive advantage. Also, many commercial AMF inoculation products are based on *Rhizoglomus* strains. Fast root colonizers could also be better protected from pesticides, as a larger share of mycelium is inside the plant root compared to AMF forming extensive extraradical hyphal networks, such as the family Gigasporaceae. Due to the low abundance of AMF outside the Glomeraceae family, we could not test this hypothesis. Regarding literature about pesticide tolerance, we found no systematic comparisons of pesticide sensitivity across different genera or families. Studies comparing single strains cannot be used for general comparisons, as different species within the same genus can strongly vary in pesticide sensitivity (Roshanfekrrad et al., 2025). Also, the sensitivity towards different pesticides can vary across AMF strains (Rodriguez-Morelos et al., 2021).

The strong reduction of native *Rhizoglomus* strains due to inoculation might be due to competition for the same niches of closely related AMF strains, whereas distantly related strains exhibit different root colonization strategies (Hart and Reader, 2002). A similar trend was found in field inoculations with *R. irregulare* (Lutz et al., 2023). This is in line with the study of Maherali and Klironomos (2007) showing that inoculated AMF strains from different families could better co-exist in plant roots than the same number of strains from only one family. Contrarily, Roger et al. (2013) observed that closely related *R. irregulare* strains co-occurred, whereas distantly related strains of *R. irregulare* often outcompeted each other. Van't Padje et al. (2022) documented a stronger fungal growth when distantly related AMF strains were inoculated compared to closely related ones, with no effect on plant biomass.

3.7. Rhizobia symbiosis affected by pesticides and AMF inoculation

Root colonization with rhizobia was assessed through the *nifH* gene. Rhizobia colonization slowly increased from week one to week four and showed a sharp increase towards the final harvest (Fig. S15a). Pesticide application had a negative effect on rhizobia root colonization ($p = 0.007$; Fig. S15a). There was a trend for a pesticide-week interaction ($p = 0.083$) with pesticide effects being strongest in week four and less clear at the other timepoints. Visually, first nodules were observed from week 4 and only at the final harvest were nodules frequently found. Nodules could also be a source of variation in the *nifH* analysis, as it was a challenge to representatively sample them for DNA extraction.

To evaluate the performance of the plant-rhizobia symbiosis, we assessed atmospheric nitrogen fixation at the final harvest. On average, 58% of plant nitrogen was derived through nitrogen fixation (Fig. 6). Nitrogen fixation rate was strongly negatively affected by pesticide application ($p < 0.001$) leading to a reduction of −11.4% at median,

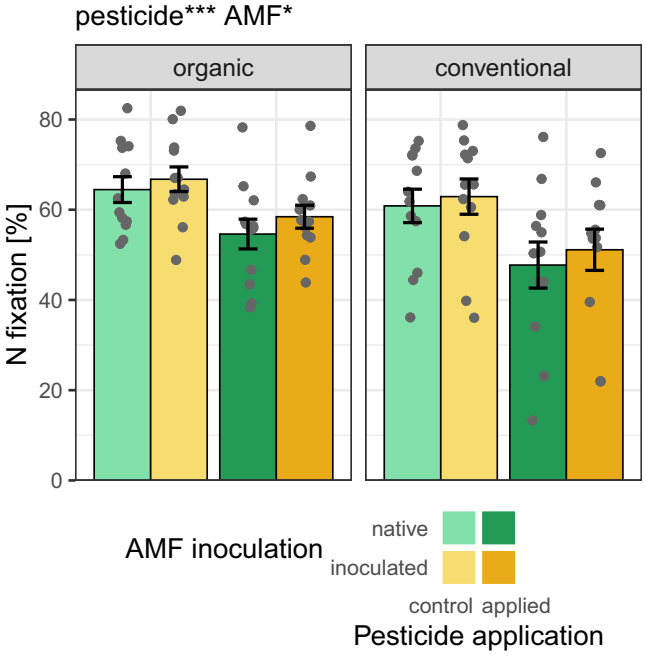

Fig. 6. Percent fixed nitrogen grouped by management (organic vs. conventional), pesticide application, and AMF inoculation. Means ± SE and individual data points are shown. Asterisks above panels indicate significance of variables from linear mixed models ($p < 0.001$ (***), $p < 0.01$ (**), and $p < 0.05$ (*)).

whereas inoculation of AMF had a positive effect on nitrogen fixation rate ($p = 0.036$) with a median increase of +3.4% (Fig. 6). Moreover, there was a strong positive correlation between nitrogen fixation rate and plant biomass ($R^2 = 0.4$, $p < 0.001$; Fig. S16), indicating that better performing plants derived more nitrogen through the rhizobial symbiosis. This could be due to the higher nitrogen demand of bigger plants, which might not be met by uptake from the soil and could lead to an increased allocation of resources into the plant-rhizobia symbiosis. Indeed, especially for conventional soils, there was a clear plateauing effect of nitrogen uptake from soil with increasing plant biomass, whereas nitrogen derived from atmospheric fixation disproportionally increased (Fig. S17). The effects of pesticides and AMF inoculation on nitrogen fixation could, therefore, also be indirectly explained by their effects on plant biomass. It has also been shown that phosphorous limitation reduces plant biomass and simultaneously negatively affects plant-rhizobia symbiosis (Christiansen and Graham, 2002; McCulloch and Porder, 2020). Impacts on the AMF symbiosis, which is highly relevant for phosphorous supply (van der Heijden et al., 2008), could, therefore, indirectly affect the rhizobia symbiosis. A direct reduction of rhizobia due to pesticide application from the beginning of the experiment is not clearly supported by the data presented (Fig. S15a). This is in line with a relatively high pesticide tolerance and adaptability of rhizobia reported in previous studies (Odeyemi and Alexander, 1977; Roslycky, 1985).

Neither rhizobia abundance nor nitrogen fixation rate was significantly different between organic and conventional management and there was no interaction with pesticide application or AMF inoculation (Fig. 6, Fig. S15). This suggests that the plant-rhizobia symbiosis is overall less responsive to management history compared to AMF. Similarly, studies on rhizobia isolates found no effect of pesticide exposure from the soil they originated from on the strains' pesticide sensitivity (Drouin et al., 2010; Mårtensson, 1992).

3.8. Variable effects of copper contamination

In addition to pesticide applications, copper application history and its accumulation over time might also impact the performance of soil microbes. Copper displayed a negative correlation with microbial biomass ($R^2 = 0.38$, $p = 0.001$), but only a slight negative trend with litter degradation ($R^2 = 0.14$, $p = 0.071$; Fig. S18). This is in line with previous studies documenting decreases in microbial biomass and activity with increasing copper contamination (Shaw et al., 2020; Wang et al., 2007). Therefore, copper would also be expected to hamper pesticide degradation. However, we found negative correlations between copper concentrations and the dissipation of metrafenone ($R^2 = 0.26$, $p = 0.01$) as well as cyprodinil ($R^2 = 0.19$, $p = 0.033$; Fig. S19). In other words, pesticide dissipation tended to be faster, when high copper concentrations were present in the soil. In any case, previous studies commonly found no strong association between copper levels and pesticide dissipation, although the mobility of some pesticides might be altered, e.g. through the formation of copper-pesticide complexes (Dewey et al., 2012; Dousset et al., 2007; Jacobson et al., 2005).

Moreover, we found negative correlations between copper concentrations and AMF root colonization at the first two harvest timepoints, whereas at later timepoints the negative relationship became insignificant (only treatment without pesticide application considered; Fig. S20a). In line with this, AMF were found to be especially sensitive at the pre-symbiotic phase (Hage-Ahmed et al., 2019) and high copper concentrations could, therefore, delay the establishment of the symbiosis with the plant. Once established, AMF are expected to be more resistant and better protected by the plant. Elevated copper concentrations could also lead to amplified pesticide effects, due to exposure to multiple stressors (Wang et al., 2015). However, higher copper concentrations were not associated with a higher sensitivity to pesticides in AMF (Fig. S20b).

3.9. Reduced performance of contaminated soils but no adaptation to pesticides

Soils from organic vineyards produced more plant biomass and had a higher AMF root colonization compared to conventional vineyards (Fig. 3a,b). This supports our first hypothesis that a management history with intensive pesticide application reduces the effectiveness of plant-symbiont interactions and leads to lower plant performance. Contrary to AMF, no management effect was found for rhizobia (Fig. 6, Fig. S15). Indeed, AMF are thought to be especially sensitive to pesticide applications, particularly to fungicides (Roshanfekrrad et al., 2025) that are used in large quantities in viticulture (FOAG Federal Office for Agriculture, 2024). The higher plant performance in organic soils could also be related to the overall higher microbial activity (Fig. 2c), leading to increased nutrient provision through mineralization. In line with this, we found that the nitrogen uptake from soil clearly plateaued in conventional soils with increasing plant biomass, whereas in organic soils no such limitation was observed (Fig. S17a). The performance differences between organic and conventional could be explained by pesticide application history but may also be linked to other contrasting management practices. However, in our previous study we found that management practices that can impact soil microbes, such as soil disturbance or organic fertilizers, were very similar between conventional and organic vineyards in Switzerland (Barmettler et al., 2026).

Our second and third hypotheses were not supported by our findings. In conventional vineyards, plant symbionts did not display a reduced sensitivity towards fresh pesticide applications (Fig. 3d, Fig. 6, Fig. S15) and pesticide degradation was not accelerated (Fig. S7). Within the long period of six months between the last pesticide application in the field and sample collection, microbial adaptation might have been reduced. It could also be that the use of a fungicide mix with nine AI masked symbionts' adaptation to individual pesticides, i.e. increased tolerance to one pesticide might not be visible due to harm from another pesticide. However, a combination of pesticides and a changing selection across years is common practice in viticulture and the fungicides we applied are commonly used products that were also found in many of the studied vineyards (Fig. S6). Our findings suggest that at the beginning of the growing season, the soil microbiome in conventional vineyards might not be prepared for the first pesticide applications. Whether microbes exhibit short-term adaptations during the growing season would have to be tested by collecting soil in the summer months or experimentally expose the soil to repeated applications.

Inoculation of AMF increased plant growth, especially in poorly performing soils, such as conventional vineyard soils. This supports our fourth hypothesis that soils with intensive pesticide application history benefit more from AMF inoculation. Long-term exposure to pesticides could reduce native AMF's ability to support plant growth, which could partly be mitigated by inoculated AMF strains. Also, soils freshly treated with pesticides tended to have a higher MGR compared to non-treated soils. This could indicate that the inoculated strain was relatively pesticide tolerant compared to native AMF. Accordingly, pesticide reduction led to an increased abundance of *Rhizoglomus* (Fig. S14), pointing towards a higher pesticide tolerance of this genus than other genera.

Our study suggests that intensive pesticide application history negatively affects plant-AMF symbiosis without leading to long-term adaptation of soil microbes. Inoculation of AMF could be a measure to partly compensate for a reduced performance of native AMF but cannot substitute a sustainable management of the soil. Our results indicate that, next to implementing already known soil conservation practices, reducing pesticide use would be favorable to sustain soil functioning in the long term.

CRediT authorship contribution statement

Elias Barmettler: Writing – review & editing, Writing – original

draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matteo Torino:** Investigation. **Alain Y. Valzano-Held:** Writing – review & editing, Methodology. **Kathleen A. Mackie-Haas:** Writing – review & editing, Supervision, Conceptualization. **Thomas D. Bucheli:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Marcel G.A. van der Heijden:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Stefanie Lutz:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107200.

Data availability

All data is available as part of this manuscript and the supplementary material. The sequencing data has been deposited in the Sequence Read Archive database under the accession number PRJNA1423224.

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