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Impact of Pesticides on Soil Fungi and Functions: A Microcosm Approach

GEO 511 Master's Thesis

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Agroscope

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Abstract

Pesticides are important to combat pests and diseases. However, they also influence non-target organisms and may negatively affect soil biodiversity. So far it is still poorly understood whether pesticides influence soil fungi and the ecosystem functions provided by fungi. This master's thesis aimed to understand the impact of pesticides on soil fungi. It is specifically tested whether rare and abundant fungal species that possess different functional traits are differently affected by pesticide exposure. Abundant species are known to play a crucial role in many ecosystem functions and processes. However, rare species, despite their potential significance for certain processes, have often been neglected by research. Therefore, this study provides valuable information on the impact of pesticides on both abundant and rare fungal communities in the fulfilment of various ecosystem functions (i.e., litter decomposition and plant biomass). It was hypothesised that fungal inoculation might improve both parameters, while increased pesticide concentration might negatively affect both abundant and rare fungal taxa, resulting in a reduction of litter decomposition and plant biomass. Finally, a combination of both fungal taxa is hypothesised to potentially decrease the impact of the pesticide. To test this, a microcosm experiment was conducted using different abundant, rare and combined fungal communities with treatments of increasing pesticide concentrations. An additional microplate assay with 95 different carbon sources was performed to gain insights into the metabolic properties of the fungal taxa. Litter decomposition rate and total dry plant biomass were measured to assess differences among pesticide treatments and whether pesticides influence the impact of fungi on plant biomass and litter decomposition. The addition of fungi significantly reduced plant biomass and enhanced litter decomposition. Pesticide treatments did not significantly influence the different fungal communities and their effects upon plant biomass and litter decomposition. Only with the overdosed treatment, plant biomass was reduced, whereas litter decomposition rates were less sensitive. In contrast to abundant communities, which show higher total plant biomass compared to rare communities, decomposition rates were higher for rare species. The combination of abundant and rare communities resulted in the highest total plant biomass and decomposition rates across all pesticide treatments indicating that mixed communities of rare and abundant fungal taxa stabilised ecosystem performance. We did not find significant evidence that rare fungal taxa are stronger affected by

pesticides than abundant fungal taxa. However, the findings of this study provide significant insights into the relationship of various fungal communities under pesticide exposure. Future research could increase their focus by testing an even greater diversity of fungal taxa. An additional emphasis should be placed on different types of pesticides to gain a better understanding of the impacts of pesticide application on soil organisms.

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1. Introduction

1.1. Soil ecosystem functions

The soil ecosystem consists of a complex network of diverse microorganisms, making it one of the most biodiverse singular habitats (Anthony et al., 2023). The soil microbiome plays a crucial role in supporting numerous ecosystem functions, making it the foundation for maximising agricultural production and ensuring food security. Soil microbes provide key roles in many biochemical processes that sustain plant productivity, diversity, and species richness (McGuire et al., 2010). Accordingly, studies suggest that the simultaneous provision of multiple functions (i.e., ecosystem multifunctionality) positively correlates with soil microbial diversity (Wagg et al., 2019). Soil microbes therefore serve as drivers for the soil-nutrient cycle, particularly in nutrient-low soils, where the soil microbes can regulate plant productivity by enhancing the supply of nutrients for the plant (Van Der Heijden et al., 2008). However, our understanding of the complexity of the soil microbiome and its contribution to ecosystem multifunctionality is still in its early stages.

1.2. Fungal diversity and its ecological significance

Microorganisms are the most frequent and diverse organisms of the soil ecosystem, with fungi standing out as one of the most abundant and biodiverse (Anthony et al., 2023; Bardgett Richard D., 2005). Fungi can be categorised into broad categories of mycorrhizal fungi, filamentous fungi and yeast, whereas a suite of traits can frequently co-occur within a group (Treseder et al., 2015). Due to the diverse traits of the fungi, they can contribute to numerous ecosystem processes including plant health, the decomposition of organic carbon, and various other essential processes (Dara, 2019; Treseder et al., 2015). According to the findings of Wagg et al. (2019), approximately 45% of fungal taxa support at least one ecosystem function. Soil fungi play a crucial role in plant health (Figure 1, marked in green), by decomposing organic carbon, a key function that transforms plant litter and exudates into mineral compounds (Purahong et al., 2016). This decomposition process not only facilitates nutrient cycling but also indirectly

enhances the availability of processed nutrients for other organisms and plants, thereby ensuring ecosystem productivity (Bardgett Richard D., 2005; Treseder et al., 2015). Moreover, some fungi directly benefit plants either by acting as pest control agents (e.g., entomopathogenic fungi) (Dara, 2019; Ownley et al., 2004) or by transferring nutrients to plant tissues (e.g., arbuscular mycorrhizal fungi) (Van Der Heijden et al., 2008).

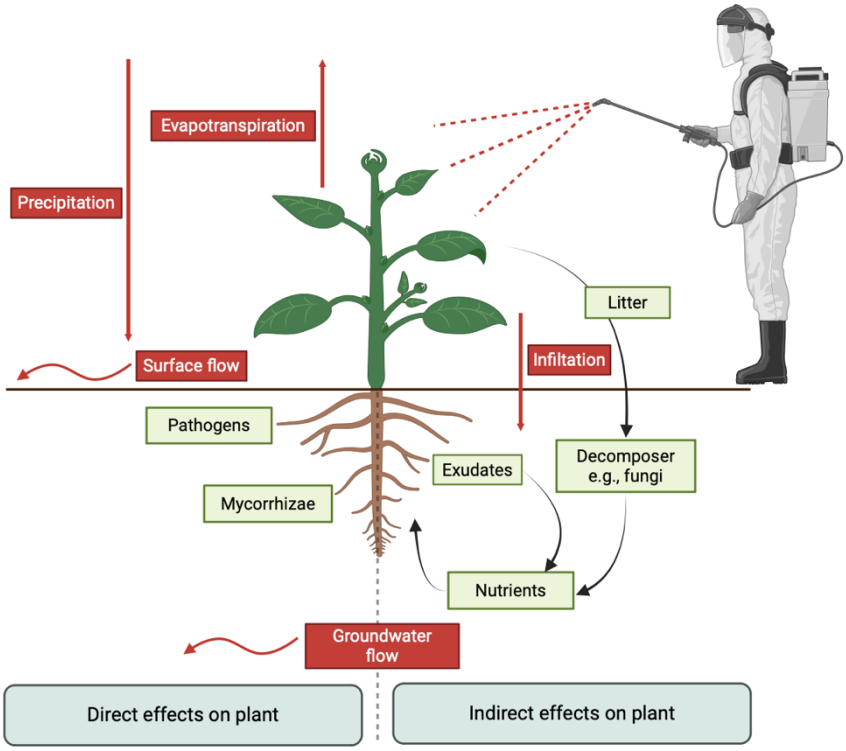


Figure 1: Broad overview of the direct and indirect effects of soil organisms on plant health (green markers). Visualisation of pesticide application and its effects on ecosystems is explained in chapter 1.3 (red markers) (adapted from Bardgett Richard D. 2005: p.87).

The taxa abundance distribution in microbial communities reveals a complex pattern where few species dominate alongside a multitude of rare ones (Jousset et al., 2017; Nemergut et al., 2011). This distinction raises important questions about the relative impact of these diverse fungal groups on ecosystem processes (Anthony et al., 2023; Bahram et al., 2018; Wang et al., 2023), highlighting a significant gap in our understanding of fungal biodiversity and its implications for ecosystem functioning.

Extensive research has focused on abundant microbial taxa and their importance in various ecosystem processes and functions. Specifically, abundant fungal taxa are found more widely distributed across the environment due to being more metabolically flexible and efficient (Wang et al., 2023). According to the results of Wan et al. (2021), abundant fungal taxa also shows a wider range of conditions in which they can respond to environmental factors, allowing them to adapt to changing environmental conditions.

Rare species have often been neglected in research, despite possessing traits that may be significant for certain ecosystem functions. Rare taxa separate from abundant taxa by having unique geographical distributions and biogeographic patterns. The paper of Jousset et al. (2017) summarises the processes and functions which are supported by rare microbes, highlighting the ecological relevance of rare species in biochemical processes, supporting nutrient cycle and plant health. An experiment showed that by reducing the rare species within a soil, plant biomass and nutrient composition increased and suggested a negative effect of rare species. However, when all microbiomes including rare species were present, the plant had higher defence compounds and lower nutrient levels making it less attractive for herbivores and less susceptible to disease (Li et al., 2021; Lynch et al., 2015). Rare taxa are said to play a central role in the fungal co-occurrence network (Xiong et al., 2021). Due to rare species holding a broader functional gene pool, they might be helpful to the functionality of abundant species (Jousset et al., 2017). Overall, future research should prioritise exploring the potential of rare microbial taxa as main indicators for many ecosystem functions and processes (Xue et al., 2020). Moreover, a community assay that combines both rare and abundant taxa, supporting enhanced ecosystem functioning, has been recommended (Li et al., 2021; Xue et al., 2020).

1.3. Pesticides

Since the late 19th and early 20th centuries, humanity has been developing pesticide chemicals to mitigate the impacts of pests and diseases in agriculture (Özkara et al., 2016). Since then, pesticides have been indispensable in modern agriculture and new mixtures are still being developed. Pesticides are grouped by their active ingredients and target. Each group is designed for diverse purposes to control or kill insects (insecticides), weeds (herbicides), fungi (fungicides) or other harmful pests (De Souza et al., 2020; Mahmood et al., 2016). However, the beneficial effects of pesticide usage also

inhibit a potential risk of toxicity to non-target plants, animals, and humans due to incorrect application, excessive concentrations or residues remaining within the ecosystem. Mixtures of different pesticides are often found in the environment, making it hard to evaluate the interaction of the complex substances and possible ecological effects. The increasing global awareness of the impact of pesticides has led to many researchers investigating the environmental pollution caused by pesticides (Diez, 2010; Özkara et al., 2016; Riedo et al., 2023).

The use of pesticide has established them as influential players in many environmental processes, visible in Figure 1 (marked in red). Although pesticides are applied to plants and soil, residues can travel long distances via evapotranspiration and precipitation, but also via surface runoff or infiltration into the groundwater (De Souza et al., 2020). Pesticides are not only getting infiltrated into the water cycle, toxic chemicals are also found in food absorbed by plant roots. Jardim et al. (2012) investigated pesticide residues in fruit and vegetable crops, rice and beans in Brazil. In approximately 50% of the samples, pesticide residues were present, with similar values reported in other countries. Research is also focusing on the effect of various pesticides on the soil environment, especially on being a threat to non-target beneficial microbial activity (Daisley et al., 2022; Srinivasulu et al., 2017). Pesticide application at field application rates is associated with an increase in microbial population, increasing plant productivity and soil organic matter levels (Bünemann et al., 2006; Srinivasulu et al., 2017). However, findings show that increased pesticide concentrations will lead to negative effects and decrease the microbial population. The review of Srinivasulu et al. (2017) suggests that within the analysed studies, soil organisms are less sensitive to herbicides, compared to some insecticides and fungicides.

While current research focuses on the negative impacts of pesticides on the environment, there is a need for future studies to deepen our understanding on how to improve and minimise the effects of pesticides on soil organisms. This effort is essential for maintaining a sustainable agricultural production and healthy soil ecosystem (Mahmood et al., 2016). Furthermore, raising awareness on this topic is essential. It is important to set new regulations, such as the action plan of the Federal Council of Switzerland from 2017, which underscores the urgency of implementing new measures to reduce risks and promote sustainable use of plant protection products, prioritising protection of soil health, non-target organisms, water bodies and human health.

1.4. Aim of thesis

The goal of this study is to understand the contribution of abundant and rare fungal taxa to ecosystem function (i.e., plant growth and litter decomposition). Moreover, this study aims to investigate whether pesticide exposure influences the contribution of abundant and rare taxa to ecosystem function. To test this, a microcosm experiment was performed to assess the impact of fungal inoculation on plant biomass and litter decomposition with and without pesticide exposure. Additionally, individual fungal taxa were screened for their metabolic potential following inoculation into microplates containing 95 different carbon substrates, without the addition of pesticides.

We hypothesised that *(i) plant biomass and litter decomposition are enhanced by the fungal inoculation (abundant or rare)*, *(ii) higher concentrations of pesticides will negatively affect both abundant and rare fungal taxa, leading to a reduction in litter decomposition and plant biomass*. We expect rare taxa to have a higher sensitivity to pesticides than abundant taxa. Ultimately, (iii) *a mixture of abundant and rare fungal taxa will decrease the impact of different pesticide concentrations*, mitigating the impact of the pesticides on litter decomposition and plant biomass.

The outcome of these experiments will provide a fundamental framework for further research to understand the complexity of fungal-plant symbioses, contributing to unravel the contribution of soil biodiversity to ecosystem functioning under pesticide exposure.

2. Material and methods

2.1. Functional traits of fungal taxa

The initial experiments provide insights into a total of 19 fungal taxa, examining metabolic properties of the species. The results of the functional traits assay will provide information about the fungal taxa used in the main experiment, aiding in the interpretation and comprehension of the results.

Abundant and rare fungal taxa selection

The fungal taxa were chosen from a previous study by Labouyrie et al. (2023), which analysed fungal biodiversity across 310 cropland sites across Europe. They grouped the obtained sequences into operational taxonomic units (OTUs) using a 98% similarity threshold. Then, the OTUs were classified using the UNITE 9.1 database and the mean relative abundance was calculated. After narrowing down the selection to OTUs at the species level, the most abundant and rare fungal species were selected. The synthetic fungal taxa were purchased from Westedijk Fungal Biodiversity Institute in Utrecht Netherlands and the Leibniz Institute DMU located in Braunschweig, Germany, and stored in a glycerol stock stored at -80 °C until further use.

Metabolic properties

In this study, FF Microplates™ from *Biolog* were used to determine the ability of the fungal taxa to metabolise different carbon sources. The selected taxa were cultivated and adapted to achieve an optical density (OD) close to the recommended values of 0.9 at 590 nm. In each well, 100 µl of the mycelium suspension was inoculated, and the microplates were incubated for 96 hours at 25± 1 °C at a speed of 130 rpm. Colour developments were recorded 24 hours after inoculation using an absorbance reader with 590 nm and 750 nm wavelengths. Subsequent observations were made at regular 24-hour intervals.

FF Microplate™ results evaluation

A total of 19 fungal species were tested in triplicate using 57 fungi FF Microplates™, ensuring statistical validity. The results were examined to assess the overall colour development by each fungus. For assessing the colour development, the recommendation of Sofo et al. (2019) and Pawlik et al. (2019) was followed. The absorbance reading data was first corrected for turbidity at each measurement timepoint *h* by subtracting both OD values of each well *i*: $i_{(h,corr)} = i_{h,590} - i_{h,790}$. To obtain the blank-corrected OD values, the blank value *b* was subtracted from the corrected OD values of each well *i* at each time point *h*: $i_{h,b_{corr}} = i_{h,corr} - b_h$. The colour development values *c* of each well *i* at each timepoint *h* were calculated by subtracting the blank-corrected values at time 0 from the blank-corrected value at time *h*, setting the negative values to 0: $c_{i,h} = i_{h,b_{corr}} - b_{i,0}$. The obtained *c* values for each well *i* are then used to calculate the average well colour development (AWCD) of each timepoint *h*, where the sum of all c values was divided by the number of carbon sources on the FF Microplate™.

$$AWCD_h = \sum \frac{c_{i,h}}{95}$$

AWCD represents the overall ability of each fungus to utilise the various carbon sources on the FF Microplate™. The carbon sources of each well *i* were then put into substrate categories to allow a more detailed view of the specific ability of each fungus to utilise these sources. All 95 carbon substrates were categorised into carbohydrates, amino acids, amines/amides, carboxylic acid, miscellaneous or polymers (Table A 3). The functional diversity of each fungus was analysed using the substrate richness R_s, which is the number of used substrates c_i higher than a set threshold (Pawlik et al., 2019). In this study, data visualisation was examined to establish an optimal threshold of OD values ≥ 0.01, efficiently filtering out the lower values.

$$R_S = \sum_{i=1}^{95} 1 \ (c_i \geq 0.01)$$

2.2. Microcosm set-up

Magenta™ boxes (GA-7) were used as microcosms to investigate the effects of pesticide application on abundant and rare fungal taxa communities (Figure 2). Accordingly, Magenta™ boxes were filled with 90 g of Sorbix® substrate (type III R). A meshed (36 µm) litter bag, which was filled with 1 g of dried ryegrass (*Lolium multiflorum*) and imbedded in the substrate. Two holes in the lid were created and taped with surgical tape to enable a sterile air exchange. The Magenta™ boxes were individually wrapped in aluminium foil, placed into a large autoclave bag and autoclaved two times at 121 °C for 90 minutes, at an interval of one week between each autoclave cycle. (Zhang, 2023)

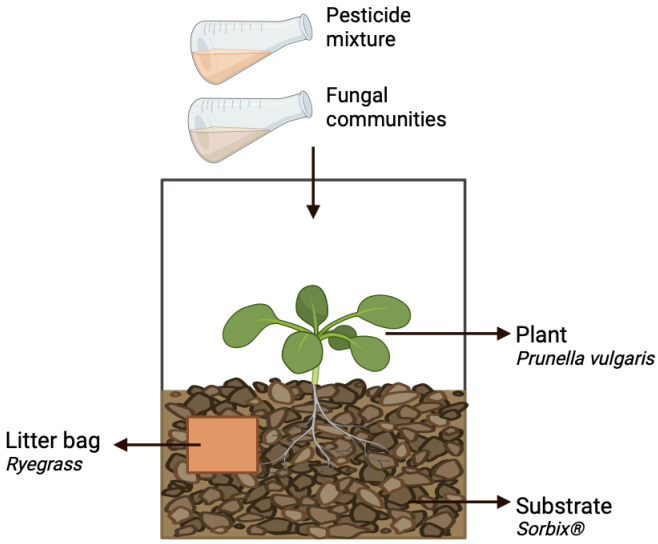


Figure 2: Visualisation of the experimental setup of a Magenta™ box (created in BioRender.com).

2.3. Seed germination

Prunella vulgaris has been widely used in research due to its ecological significance, biological attributes, manageable size, robust growth, and excellent adaptation in small microcosms (Li et al., 2015; Streitwolf-Engel et al., 2001). Firstly, 1 g of *Prunella vulgaris* seeds were surface sterilised for 5 minutes in 70% ethanol and rinsed with distilled water. Thereafter surface sterilised for 5 minutes in 3% NaClO (sodium hypochlorite

solution) and rinsed 3 times with sterilised distilled water. Finally, after washing, the seeds were sown on 10 squared Petri dishes filled with 0.8% agar. 100 seeds were placed on each plate to prevent cross-contamination and provide enough seedlings for the experiment. The plates were then stored in a climate chamber under controlled conditions of 25 °C for 16h and 16 °C for 8h for 10 days. The selected seedlings were visually inspected for contamination before being transplanted into the microcosms. (Zhang, 2023)

2.4. Inocula

The 17 fungal taxa used for this experiment were revived from glycerol stocks stored at -80 °C and cultured on potato-dextrose-agar (PDA). The plates were then incubated at 25 ± 1 °C for 1 to 1.5 weeks. After 10 days of growth, the mycelium of each fungus was transferred to Erlenmeyer flasks containing 300 ml Czapek-Dox Broth to allow them to further grow in a liquid medium. To avoid bacterial contamination, 90 µl of chloramphenicol (100 mg/ml) was added into each. The flasks were then incubated at 25 ± 1 °C at a speed of 150 rpm for 1 to 1.5 weeks. (Zhang, 2023)

To prepare the inoculum, mycelium suspensions from Erlenmeyer flasks were transferred to 50 ml Falcon tubes and then centrifuged at maximum speed (4000 rpm) for 4 minutes. The supernatant was poured out and the mycelium pellet was re-suspended with sterile Phosphate Buffered Saline (PBS) buffer and centrifugated at maximum speed (4000 rpm) for 4 minutes. This step was repeated twice (ATT Bioquest, Inc. 2024). The mycelium cells were then transferred to Falcon tubes and filled up with 15% Hoagland solution to the required volume. Samples were collected from each taxon and plated on PDA to assess the presence of possible external contamination.

To create the different compositions for the fungal communities, 5 ml of the mycelium suspension from the specified fungus were transferred into Falcon tubes and mixed just before inoculation. The communities were allocated randomly, ensuring that each community is distinct and possesses its unique characteristics. There are 5 random compositions of abundant and rare communities, each representing a replicate (Table 1; Table 2).

Table 1: Synthetic abundant fungal taxa and their communities, the numbers represent the community (Abundant community 1-5) and their total occurrence within all communities.

Fungus	Part of Abundant Community	Occurrence in Communities
Mortierella elongata	5	1
Solicoccozyma aeria	1, 2, 4	3
Alternaria peglionii	1, 3	2
Clonostachys rosea	3, 4, 5	3
Solicoccozyma terricola	4	1
Solicoccozyma terrea	1, 2, 3, 4	4
Periconia macrospinosa	2, 3, 5	3
Fusarium redolens	2	1
Mortierella alpina	1, 5	2

Table 2: Synthetic rare fungal taxa and their communities, the numbers represent the community (Rare community 1-5) and their total occurrence within all communities.

Fungus	Part of Rare Community	Occurrence in Communities
Acrostalagmus luteoalbus	1	1
Beauveria bassiana	1, 2, 5	3
Plectosphaerella cucumerina	3, 4, 5	3
Penicillium brevicompactum	1, 2, 3, 4, 5	5
Didymella glomerata	2	1
Gibellulopsis nigrescens	1, 2, 3, 5	4
Trichoderma neokoningii	3, 4	2
Mortierella polycephala	4	1

2.5. Pesticide mixture

The pesticide used in this study were selected from the list of plant protection products of the Federal Office of Agriculture (FOAG) for the application to wheat (Pflanzenschutzmittelverzeichnis, 2024). These were all fungicides namely: Azoxystrobin, Prothioconazole and Bixafen, Difenoconazol and Cyprodinil (Table 3).

The pesticides were used at the following concentrations: the recommended dosage (1x), half of the recommended dosage (0.5x), double dosage (2x), tenfold dosage (10x). The pesticides were diluted in sterile distilled water and applied to the Magenta™ boxes. The final concentration rates of each microcosm were approximately equal in a ratio to the recommendation field application (Table A 1).

Table 3: Information on pesticides used for the mixtures (plant protection product list retrieved from Pflanzenschutzmittelverzeichnis, 2024).

Trade Name	Manufacturer	Active Ingredient	Product Category	Dosing Instructions
Amistar®	Syngenta Agro AG	Azoxystrobin	Fungicide	0.4 l/ha
Aviator Xpro®	Bayer (Schweiz)AG	Prothioconazole, Bixafen	Fungicide	1.25 l/ha
Slick®	Stähler Suisse SA	Difenoconazol	Fungicide	0.5 kg/ha
Unix®	Syngenta Agro AG	Cyprodinil	Fungicide	1 l/ha

2.6. Microcosm assembly

Experimental design and microcosm assembly

The experimental design was a randomised complete block design with 2 treatment factors: factor 1 consisting of 4 fungal community treatments (Non-fungal control, abundant community, rare community and mixture (abundant and rare fungi) and factor 2 consisting of 5 pesticide concentration applications (0x, 1x the recommended rate, 0.5x, 2x and 10x). The treatment combinations were replicated 5 times, resulting in a total of 100 Magenta™ boxes (4 community treatments × 5 pesticide concentrations × 5 replicates).

Inoculation of the Magenta™ boxes was done under a sterile laminar flow bench. Substrate samples were randomly selected from the Magenta™ boxes, plated on PDA and inoculated at 25 °C for 1 to 1.5 weeks to detect potential external contamination. Before introducing the mixtures, four holes were made in the corners of the Magenta™ box using a sterile rod. The seedlings were transferred from the agar plate using sterile tweezers and subsequently planted into the microcosms. For the control

treatments without fungal taxa, 90 ml 15% Hoagland solution was added. The boxes with either abundant or rare community treatments were inoculated with 20 ml of fungal inoculum (containing 4 species of 5 ml) and 70 ml of 15% Hoagland solution. The microcosms with abundant and rare communities mixed together received 40 ml of fungal inoculum and 50 ml 15% Hoagland solution. The total volume of liquid in each box amounted to 90 ml. The microcosms were incubated in a climate chamber under controlled conditions of 25 °C for 16h and 16 °C for 8h with a humidity of 60% for a total of 9 weeks. Every week, the boxes were randomly rearranged throughout the shelves, to minimise any effect of environmental variability. Plant growth was visually monitored in weeks 6, 7, 8 and 9 by taking pictures and rating the growth from a scale from 0 (100% mortality) to 4 (0% of mortality).

Pesticide addition

5 ml of the pesticide mixture with the concentrations 1x, 2x, 10x or 0.5x (Table A 1) were added one week after inoculation of the synthetic fungal communities and seedlings, to allow the communities and plants to establish within the microcosm before pesticide addition. Pesticide application was conducted in a sterile flow cabinet. The Magenta™ boxes were closed and incubated for 8 more weeks under the same controlled conditions.

2.7. Harvest

The microcosms were harvested 9 weeks after inoculation. After removing the plants, the remaining substrate on the plants was carefully removed with sterile tweezers and washed gently with distilled water. The shoots and the roots were separated with sterile scissors and freeze-dried in paper bags or Petri dishes for 24 hours and weighed. The litterbags were washed gently to remove the remaining substrate, put into Petri dishes, freeze-dried for 24 hours, and weighed. The growth substrate was placed in 50 ml Falcon and stored at -20 °C.

2.8. Biomass collection of roots and shoots

The plant roots and the shoots were dried using a freeze-dryer Beta 1-8K for 24 hours thereafter the dry weights were measured. The total dry biomass was measured by adding up the weight of the dried shoots, the aboveground biomass (AGB), and the dried roots, the belowground biomass (BGB).

$$Total\ dry\ biomass\ (TDB) = BGB + AGB$$

The roots and shoots were then sealed and stored at room temperature 21±2 °C before using for further measurements.

2.9. Assessing litter decomposition

Litter decomposition was quantified by assessing the weight of the litter bag before inserting it in the microcosm and 9 weeks at harvest. The litter decomposition rate was calculated as follows:

$$Decomposition\ rate\ [\%] = \frac{w_{to} - w_{t1}}{w_{to}} * 100$$

The decomposition rate will then be used as an indicator for ecosystem health, by showing the activity of the decomposer organisms, here fungi (Buresova et al., 2019; Zhang, 2023).

2.10. Fungal litter colonisation

DNA extraction

The molecular analysis was done by using the litter from the litterbag samples. DNA was extracted using the NucleoSpin® 96 Plant II Core Kit (Macherey-Nagel GmbH & Co. Ko, Germany), following the protocol from the manufacturer. Ultimately, the extracted DNA was quantified using the VARIAN Cary Eclipse fluorescence spectrophotometer and subsequently standardised at 1 ng DNA per µl.

Fungal biomass determination using qPCR

A quantitative Polymerase Chain Reaction (qPCR) using ITS primers was used to determine the amount of DNA in the samples. To create a master mix for each sample, 2 µl of HOT FIREPol®, 0.5 µl of 10 µl F-primer, 0.5 µl of 10 µl R-primer and 5 µl of ddtH2O were combined. Subsequently, 28 µl of master mix and 7 µl of the DNA of the samples were mixed and added in to a 384-well plate before performing the CFX Opus 384 Real-Time PCR System (Bio-Rad Laboratories, Switzerland). A standardised DNA was utilised to generate the standard dilution, resulting in a linear standard curve with an $R_2 > 0.96$. The mean efficiency of the qPCR ranged between 1.66 and 1.68.

2.11. Statistical analyses

The results were statistically tested and visualised using the R Studio Version 2023.12.1 software. The data was first tested to the requirements for conducting an analysis of variance, here a two-way ANOVA. To verify the assumption for ANOVA, the data for litter decomposition and total dry plant biomass were tested for normality executing the Shapiro-Wilk test. Since both datasets did not follow a normal distribution and attempts to transform the data did not change the outcome, a non-parametric Kruskal-Wallis test was executed to examine potential statistical differences in the median among one or more groups. Pairwise comparison between groups and treatments was conducted using the Dunn's test. The Pearson correlation coefficient was used to test relationship between litter decomposition and substrate richness as well as fungal biomass of the communities. Further, a Tukey HSD test was performed to identify differences in means.

In the study, data visualisation includes bar charts to illustrate the distribution of substrate richness across different fungal taxa. Total dry plant biomass and litter decomposition rate was visualised using boxplots, where the boxes display the distribution of the interquartile range (IQR), the median and the whiskers. Additionally, a cross provides information about the mean of the data. A heatmap was created to visualise the substrate use for all categories across all fungal taxa, displaying the mean of all values within a category with colourization. Additionally, the richness of each species is displayed at the right side of the graph. The visualisations help interpreting the data by showing graphical representations and patterns of the data.

3. Results

3.1. Carbon source utilisation

The optical density (OD) values obtained from the FF microplate™ readings were used to calculate the **average well colour development (AWCD) at 96 hours** of incubation (Figure 3). Higher AWCD values indicate a greater degradation of the carbon substrates within the microplate. *Mortierella alpina* shows the highest AWCD value at around 0.25, but also highest variation for all replicates. *M. alpina* is followed by *Didymella glomerata*, *Exophiala radicis* and *Mortierella elongata*, which are all separating from the 15 other species tested with higher mean AWCD values than 0.05. The 3 yeast species *Solicoccozyma terricola*, *Solicoccozyma terrea* and *Solicoccozyma aeria* have the lowest AWCD values close to 0.

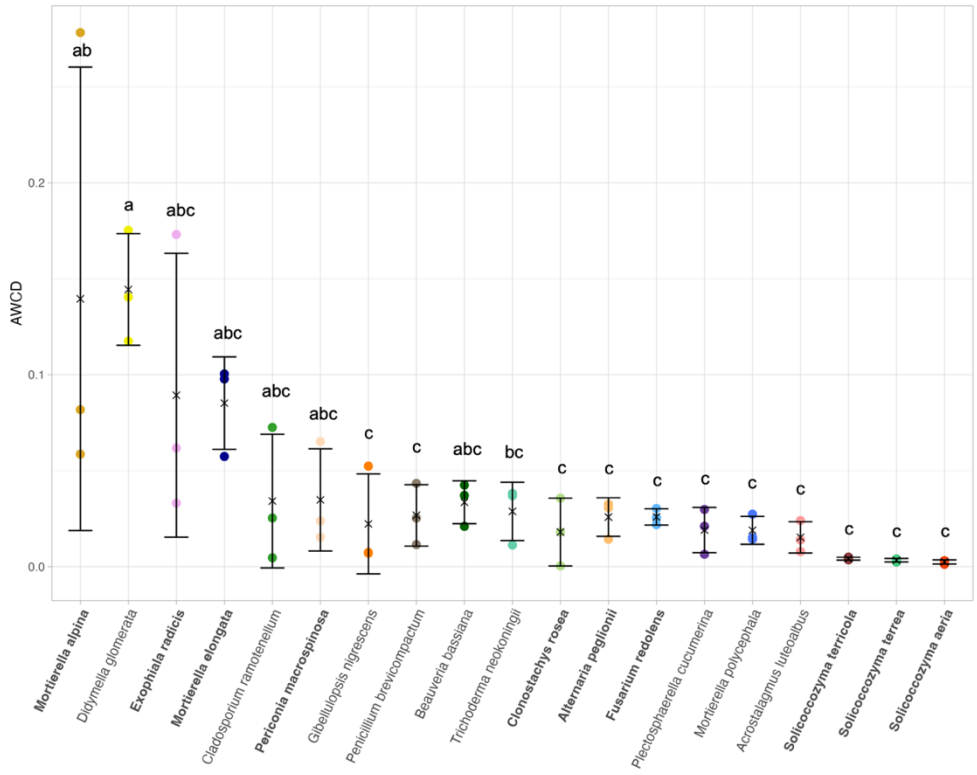


Figure 3: The ability of abundant and rare fungal species to use various carbon sources tested with FF microplates™, average well colour development (AWCD) after 96 hours of incubation for each fungal taxa in decreasing order, abundant species represented in bold. Same letters indicate no significant difference tested by Tukey HSD test, after ANOVA ($p \leq 0.05$). The black cross represents the mean with lines to represent the stand error.

The **temporal development of AWCD** monitored over 97 hours is dependent on the species (Figure 4). The AWCD generally increases for all species within 72 hours. At the timepoint of 96 hours some species already reached their peak, whereas others were slowly reaching a plateau or still increasing in comparison to measurements on 48 hours and 72 hours. *M. elongata* shows the highest increase within the first 72 hours, but a strong decrease at 96 hours. Other species also show a slight decrease for the 96-hour measurements.

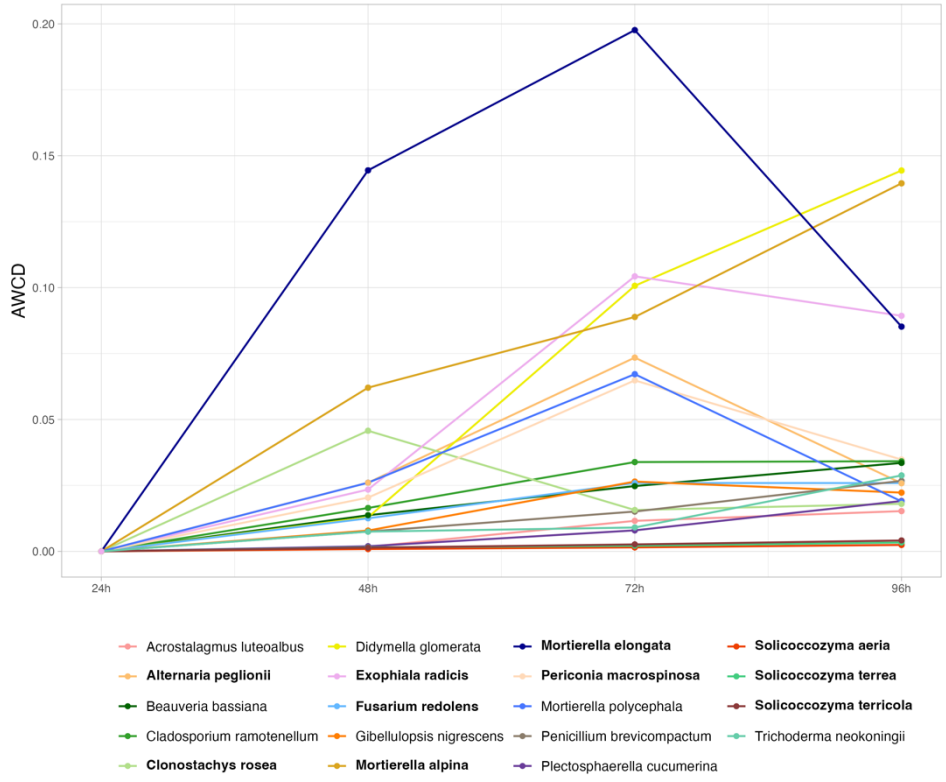


Figure 4: The ability of abundant and rare fungal species to use various carbon sources tested with FF microplates™, with the mean AWCD of 3 replicates at the timepoints 24h, 48h, 72h and 96h. Abundant species represented in bold. See same graph with error bars presented in Figure A 2.

The tested fungal species show different **richness R_S** values ranging from 6 up to 92 of used carbon substrates (Figure 5). Notably, *M. alpina, D. glomerata, E. radicis* and *M. elongata* show a richness of more than 90 substrates with *M. Alpina* having the highest functional diversity with an R_S of 92. Only 4 abundant and 5 rare species indicate R_s values higher than 50, with abundant species notably being more functionally diverse

than rare species. Even if most high substrate users are abundant species, the mean R_S of abundant species of 47 used substrates is lower than the one of rare species with a mean R_S of 52.

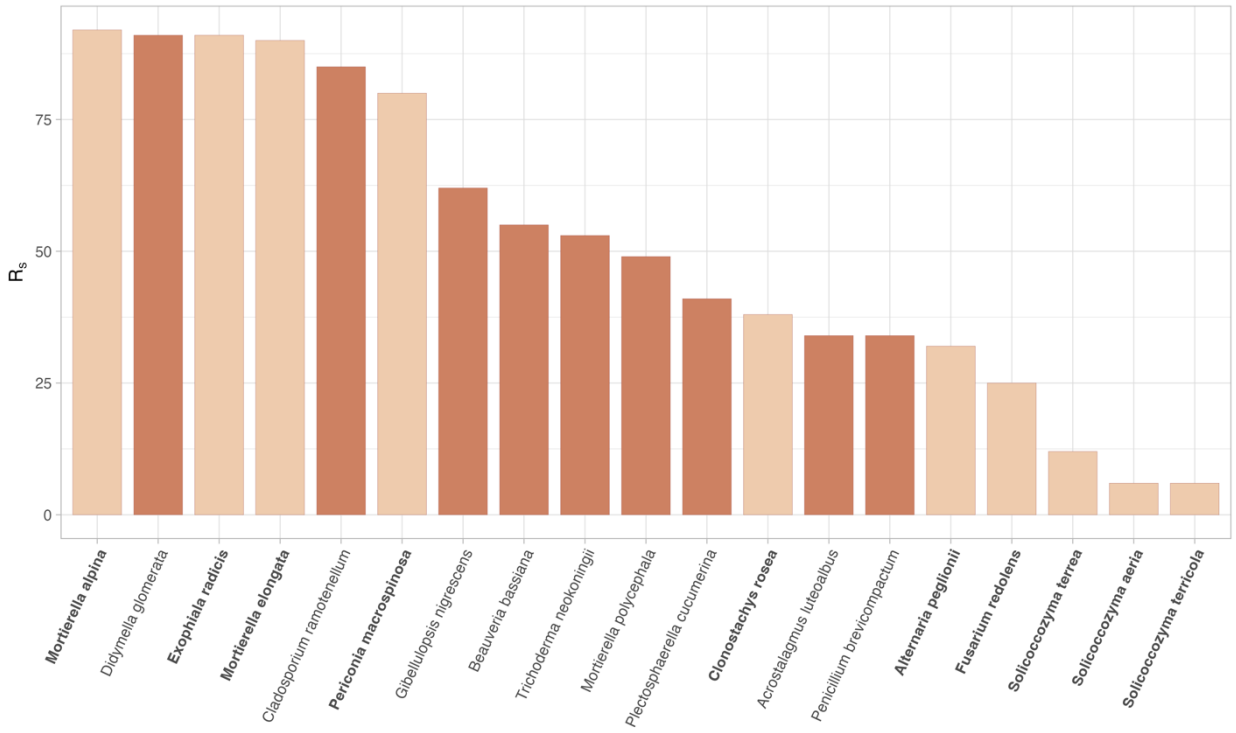


Figure 5: Abundant and rare fungal taxa and their functional diversity using richness R_S (i.e., number of carbon sources used). The number of used substates utilised by each fungus, using all positive OD values with the threshold ≥ 0.01 of all 95 carbon substrates of *Biolog* FF microplate™, abundant species represented in bold.

Despite the richness R_S of the individual fungal taxa, the **utilisation of the various substrates** differs considerably within each substrate category across species (Figure 6). The blank values were 0 for all the values, meaning a successful run of the experiment (Table A 2). Notably, *M. alpina, D. glomerata, E. radicis* and *M. elongata* exhibit highest mean OD values across most substrate categories. Moreover, these 4 species separate by a high substrate use of more than 90% of the categories. *D. glomerata* is the only rare species within the top substrate users mentioned above, with a total richness of 96%. The mean use of a substrate used within a category is dependent on the individual preferences of the fungal species. The 3 different yeast species *S. terricola, S. terrea* and *S. aeria* are lowest in using only 6% to 13% of the total substrates and most mean OD values are situated even lower than the set threshold of 0.01 for most of the categories.

Overall, among the top 10 substrate users, 4 were identified as abundant species and 6 as rare species. Majority of these 6 rare species demonstrate lower substrate utilisation ranging from 52% up to 65% and mean OD for the categories lower than 0.05 in comparison to the top 4 abundant species which show more than 84% substrate utilisation and OD values of around 0.15.

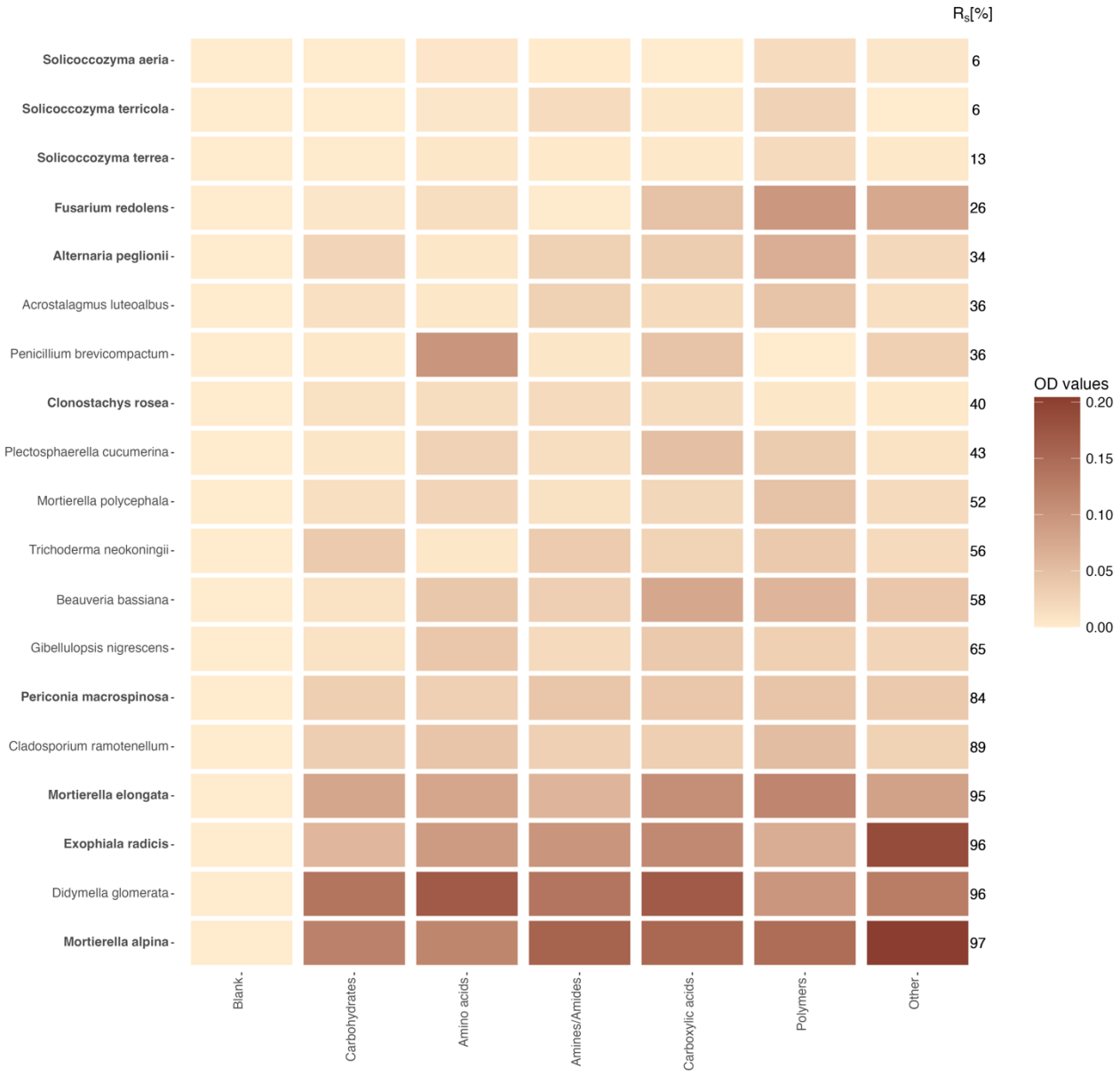


Figure 6: Heatmap representing the utilisation of different carbon substrate of different abundant and rare fungal taxa obtained from Biolog FF Microplates™, abundant species represented in bold. The bars represent the mean OD value of the used substrates of each category (Table A 2); the percentage richness R_s of number of substrates with OD ≥ 0.01 is visible on the right side of the graph. Tested carbon sources are grouped according to Preston-Mafham et al. (2006).

3.2. Plant Biomass

Generally, visual comparison already showed a high variation in plant biomass within a specific treatment and its replicates (Figure 7). Especially the non-fungal group showed relatively high plant biomass compared to fungal groups. Visual comparison between replicates also indicated effects when comparing abundant communities and the mixture of both abundant and rare communities performing better than rare communities on their own (Figure A 1).

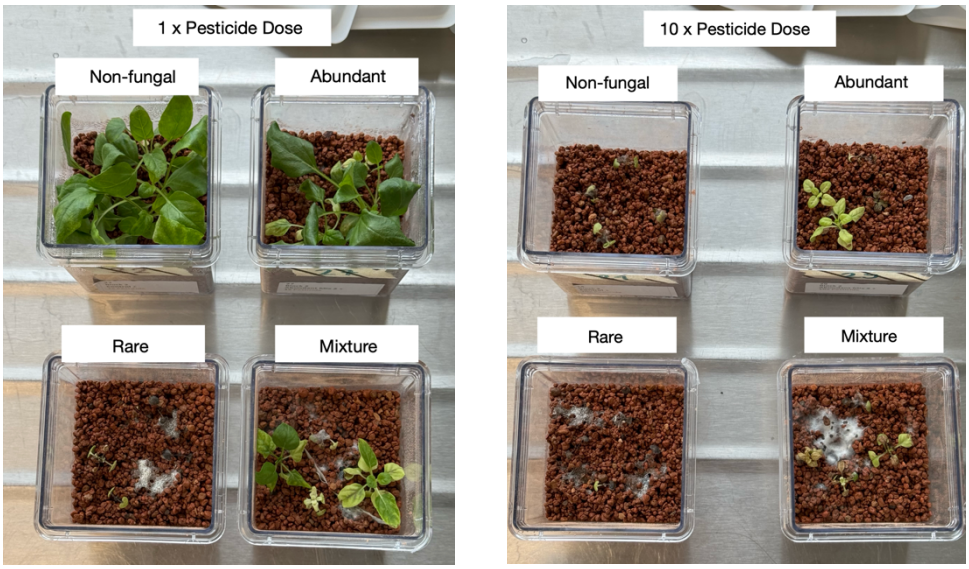


Figure 7: Visual comparison of the 1x and 10x pesticide concentration treatment of replicate 2 with abundant and rare communities.

Results for the non-fungal group show high variation in **total plant biomass** with differences of about 200 mg. For instance, plant biomass in the non-fungal group treated with a 1x pesticide dosage ranges from 21 mg to 239 mg (Figure 8). For the fungal communities, plant biomass was relatively low compared to the non-fungal group. Generally, plant biomass treated with rare fungal communities was lower in comparison to abundant communities and mixtures. However, the mixture of both abundant and rare communities led to higher plant biomass compared to the single communities.

The pesticide treatments did not significantly affect plant biomass in microcosms threated with fungal communities. Negative effects on rare communities were slightly visible at a 2x and 10x concentration. The mixture of both communities shows the most visible negative effect on the 10x treatment with lower plant biomass compared to the single community treatments. The mean plant biomass of microcosms treated with abundant and rare communities was the lowest at the treatment of 10x pesticide dosage. Overall, pesticide concentration showed negative effects on plant biomass in all microcosms treated with the 10x pesticide dosage rate. However, significant differences of the plant biomass for the pesticide treatments were only detected for the non-fungal group.

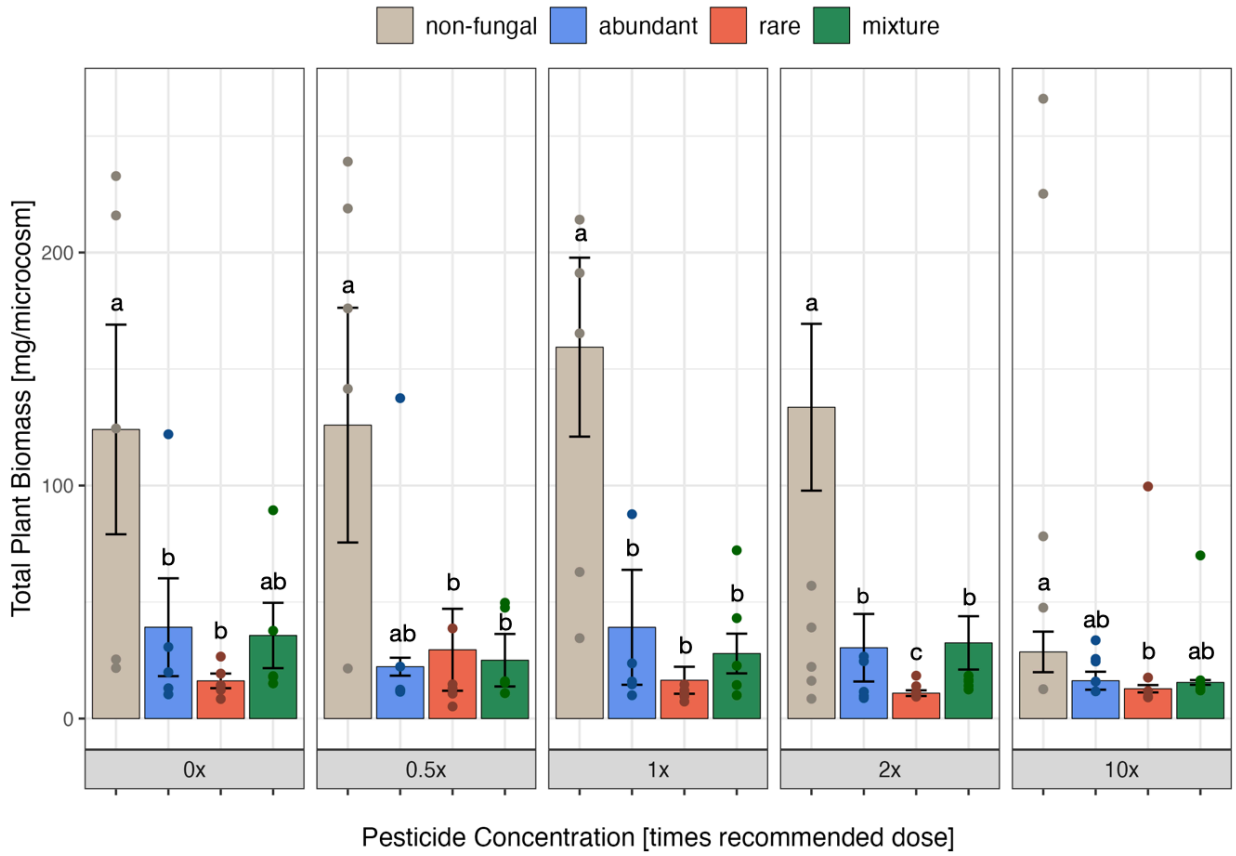


Figure 8: Barplot of total dry plant biomass of non-fungal, abundant, rare and mixed (i.e., abundant and rare) communities at increasing pesticide concentrations of 0x, 0.5x, 1x, 10x (x = recommended dose). Each treatment had 5 unique replicates. The non-parametric Kruskal-Wallis's test and Dunn's test ($p \leq 0.05$) was performed to assess significant differences between pesticide treatments, same letters indicate no significant differences between each group of a treatment, bar height represents mean.

3.3. Litter decomposition

Litter decomposition results depended on the kind of community and pesticide treatment applied (Figure 9). The control group without fungi generally showed around 5% to 10% lower decomposition rates in all pesticide treatments compared to fungal communities. Rare and abundant communities performed similarly with a decomposition rate of 48%. However, both rare and abundant communities show high variation in decomposition rate in their replicates under various treatments. In the 1x pesticide treatment, most abundant communities show lower or the same decomposition rates as the rare communities. The mixture of both abundant and rare fungal taxa led to

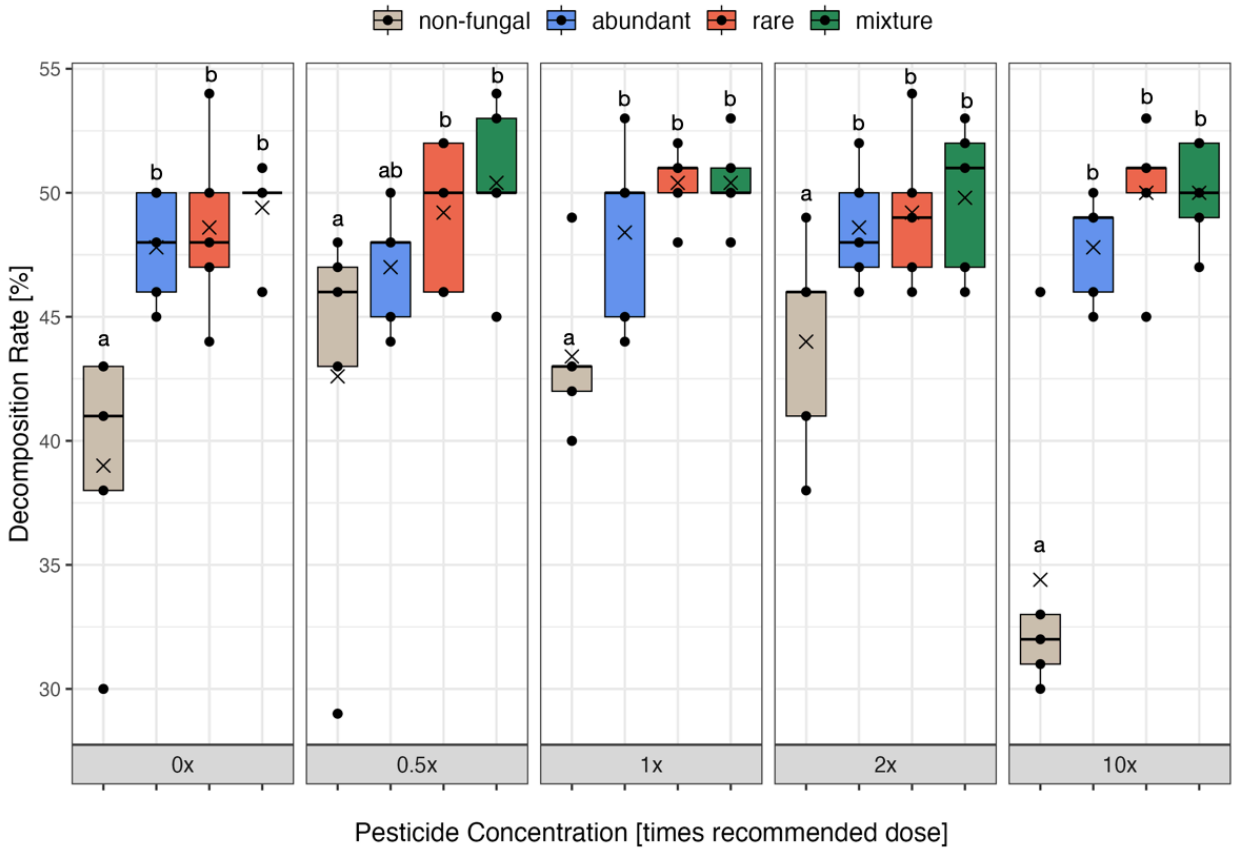


Figure 9: Boxplot of decomposition rate of non-fungal, abundant, rare and mixed (i.e., abundant and rare) communities at increasing pesticide concentrations of 0x, 0.5x, 1x, 10x (x = recommended dose). The non-parametric Kruskal-Wallis's test and Dunn's test ($p \leq 0.05$) was performed to assess significant differences between pesticide treatments, same letters indicate no significant differences between each group of a treatment. Boxplots show the interquartile range (IQR) and the median as a bar, the mean is highlighted with a cross.

the highest decomposition rate, with more than 50% reduction. Generally, no significant differences were detected between the fungal treatments, but the mixture performed similarly or better than rare or abundant on their own, also visible by comparing the mean of the groups.

The pesticide treatments had no significant effect on the ability of the different fungal community groups to decompose litter. Only the non-fungal group showed effects on pesticide concentration at a dose of 10x concentrations with reduced decomposition of about 35%. Generally, pesticide treatments of 0.5x, 1x and 2x had a slight, but not significant positive effect on litter decomposition in comparison to the 0x treatment. This is visible by comparing the mean, which shows an increasing trend for all groups for the treatment 0.5x, 1x and 2x compared to the 0x treatment.

When analysing the combined results of **litter decomposition rate and the functional diversity R_S** of the different fungal communities, only a non-significant negligible correlation of -0.01 was observed. Generally, a high number of substrates used did not necessarily correspond to a high decomposition rate (Figure A 3). 3 out of 5 mixtures (i.e., abundant and rare communities) show high decomposition rates of more than 50%, while all mixtures show richness of 96% or higher. Rare communities overall perform better in decomposition, even if less substrates are used. Mean rare species substrate richness is 90%, which is slightly higher than abundant richness with 84% (Table 4). 3 out of 5 rare communities are higher in decomposition than abundant communities, even though some have higher substrate richness. Only one of the two different communities, abundant mixture 5, used all the substrates, where its decomposition rate is less than 50%. Abundant mixture 4 shows the lowest number of used substrates with only 53%. However, it performs better in the degradation of litter with a rate of about 49% compared to other communities which have a higher substrate richness but lower decomposition rates of 45%.

Table 4: Total number of substrates utilised (OD ≥ 0.01) of individual taxa categorised as rare and abundant. The fungal taxa are assembled according to their mixtures with percentage of used substrates of each mixture, overlapping substrates do not count twice.

community	Total Used Substrates of Community [% (n=95)]	Abundant Species	Number of Used Substrates [n=95]	community	Total Used Substrates of Community [% (n=95)]	Rare Species	Number of Used Substrates [n=95]
Abundant 1	85% (81)	*Alternaria peglionii*	32	Rare 1	86% (82)	*Acrostalagmus luteoalbus*	34
		Mortierella alpina	92			*Beauveria bassiana*	55
		Solicoccozyma aeria	6			*Gibellulopsis nigrescens*	62
		Solicoccozyma terrea	12			*Penicillium brevicompactum*	34
Abundant 2	89% (85)	*Fusarium redolens*	25	Rare 2	98% (93)	*Beauveria bassiana*	55
		Periconia macrospinosa	80			*Didymella glomerata*	91
		Solicoccozyma aeria	6			*Gibellulopsis nigrescens*	62
		Solicoccozyma terrea	12			*Penicillium brevicompactum*	34
Abundant 3	93% (88)	*Alternaria peglionii*	32	Rare 3	91% (86)	*Gibellulopsis nigrescens*	62
		Clonostachys rosea	38			*Penicillium brevicompactum*	34
		Periconia macrospinosa	80			*Plectosphaerella cucumerina*	41
		Solicoccozyma terrea	12			*Trichoderma neokoningii*	53
Abundant 4	53% (50)	*Clonostachys rosea*	38	Rare 4	93% (88)	*Mortierella polycephala*	49
		Solicoccozyma aeria	6			*Penicillium brevicompactum*	34
		Solicoccozyma terrea	12			*Plectosphaerella cucumerina*	41
		Solicoccozyma terricola	6			*Trichoderma neokoningii*	53
Abundant 5	100% (95)	*Clonostachys rosea*	38	Rare 5	83% (79)	*Beauveria bassiana*	55
		Exophiala radicis	91			*Gibellulopsis nigrescens*	62
		Mortierella alpina	92			*Penicillium brevicompactum*	34
		Mortierella elongata	90			*Plectosphaerella cucumerina*	41

3.4. Fungal litter colonisation

The **results of the qPCR** (Figure 10) revealed fungal biomass within the control groups, meaning a potential contamination of some control replicates. The rare communities show higher fungal biomass than the abundant and the mixed communities in all pesticide treatments, meaning that rare taxa colonised the substrate better than abundant taxa. The fungal biomass varies around 8, with highest values for the 10x treatment. The mixed communities are consistent at each pesticide treatment with values around 8. Abundant communities display lowest fungal biomass compared to the other fungal groups. Highest values are observed at around 7, while lowest values are consistently found at the 10x treatment, with fewer than 5 gene copies detected.

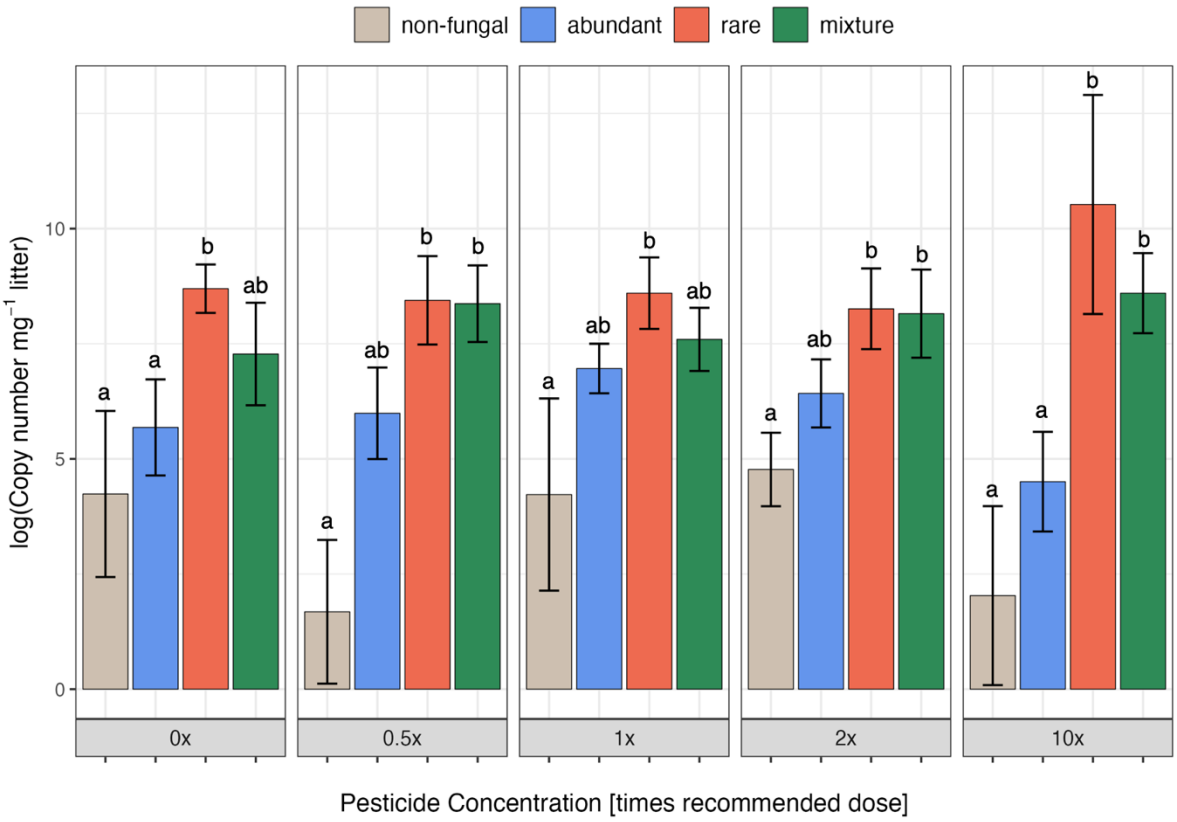


Figure 10: Total fungal biomass (i.e., number of gene copies) of non-fungal, abundant, rare and mixed (i.e., abundant and rare) communities at increasing pesticide concentrations of 0x, 0.5x, 1x, 10x (x = recommended dose). DNA was extracted from the 5 replicates and qPCR determined the number of gene copies. The results are given in log number of copies per mg of litter. The non-parametric Kruskal-Wallis's test and Dunn's test ($p \leq 0.05$) was performed to assess significant differences between pesticide treatments, same letters indicate no significant differences between each group of a treatment.

The pesticide application had no visible effect on the fungal biomass of the mixtures containing both abundant and rare taxa. Pesticide exposure also does not affect rare communities, except at a dosage of 10x, where a positive effect emerges, resulting in an increase in fungal biomass, but also a high variation within the replicates. The effect on abundant communities is most visible. At recommended concentrations the pesticide has a slightly positive effect by increased fungal biomass compared to the control treatment 0x. At the 10x dosage, the abundant communities indicate a decrease in fungal biomass with lowest gene copy numbers compared to each treatment. Overall, fungal biomass of rare and combination of both abundant and rare communities are less affected by especially high pesticide concentrations, showing significant differences compared to the abundant communities.

The Pearson correlation coefficient and linear regression were used to assess the **relationship between litter decomposition rate and fungal biomass** (i.e., number of gene copies) (Figure 11). The analysis revealed a significant positive correlation of 0.62 and an R^2 of 0.39, indicating that communities with a higher fungal biomass tent to degrade higher rates of organic matter.

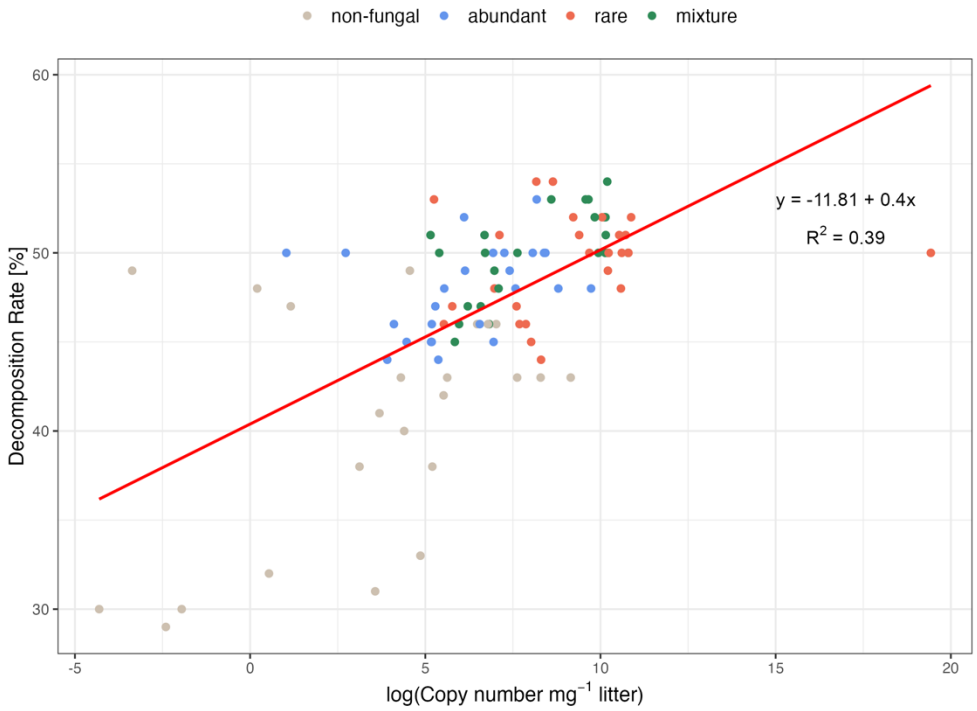


Figure 11: Regression analysis between litter decomposition rate and fungal biomass (i.e., number of gene copies) of non-fungal, abundant, rare and mixed (i.e., abundant and rare) communities, revealing a relationship with a strength by an $R^2 = 0.39$ and a highly statistical significance ($p \leq 0.05$). Pearson correlation coefficient revealed a positive correlation of 0.62 ($p \leq 0.05$).

4. Discussion

In this study, the impact of different pesticide treatments on abundant and rare fungal taxa was investigated. The focus was set on the effects of different pesticide concentrations on two ecosystem functions performed by fungal taxa (i.e., plant growth and litter decomposition). Various experiment outputs were used to assess this effect and to contribute to a better understanding on how pesticide application in agriculture can influence soil microorganisms.

4.1. Pesticide effects on plant growth and litter decomposition

Abundant and rare fungal communities

The results revealed a significant difference in total plant biomass between the non-fungal and fungal communities for all pesticide treatments, with higher plant biomass for the non-fungal group. This suggests that fungal communities potentially acquired nutrients that could otherwise have been utilised by the plants for growth. The non-fungal group separates from the fungal groups with up to 150 mg differences in plant biomass. The variation within the non-group with differences of up to 200 mg could be explained by potential contamination of bacteria or fungi, even if sterile conditions were highly prioritised. Gene quantification results partly support the explanation by showing fungal biomass in the non-fungal group (Figure 10). Alternative explanations may include variation in the substrate used for the experiment, as previous studies were conducted under similar procedures but with a different substrate (Hartman, 2018; Zhang, 2023).

The differences in plant biomass of the fungal communities under various pesticide treatments were not significant. Nevertheless, plant biomass in microcosms treated with rare fungal communities was lower in comparison to abundant communities. Breaking down organic matter is one of the main ecosystem functions of fungi (Purahong et al., 2016). Therefore, the litter decomposition rate provides insights into the ability of the fungal communities to provide nutrients to the plants to promote plant health and growth. The decomposition rate of the control, non-fungal group, was as expected lower than fungal communities under each pesticide treatment. However, the detected fungal

biomass in the control non-fungal group might have even enhanced the decomposition of organic matter and therefore indicating potential higher results. Nevertheless, the non-fungal group shows a pattern of an increase in decomposition for lower pesticide treatments, except for the 10x dosage the rate reduced significantly. Comparing this to the fungal communities, rare and abundant fungal communities and their mixture performed better in decomposition rate under each treatment. These findings support the fact that fungi play a crucial role in the decomposition of organic matter and plant health (Dara, 2019; Treseder et al., 2015).

Pesticide application treatments did not significantly influence the tested ecosystem functions provided by the fungi, but visible effects in total plant biomass and decomposition rate for both abundant and rare communities were found with higher dosages of pesticides, thereby reinforcing findings from previous studies (Shaheed et al., 2006; Shakir et al., 2016). The increased concentrations of pesticides can have impacts on various physiological and biochemical processes, influencing the growth and biomass of plants (Shaheed et al., 2006). Additionally, present fungi are affected by pesticide application and concentration while fulfilling ecosystem functions like providing nutrients to plants, leading to limitations in plant growth. Abundant communities were affected more by the 10x pesticide treatment, resulting in a decline of fungal populations under overdosed pesticide exposure. Conversely, rare communities exhibited contrasting behavior, with higher population levels observed under the same conditions of pesticide overdose.

Plant biomass of microcosms treated with abundant communities was higher for all pesticide treatments than the rare communities, supporting the fact that abundant taxa have potentially broader response thresholds to environmental changes than rare taxa (Jiao et al., 2020). Rare species show relatively low plant biomass when exposed to pesticides, especially to high dosages of chemicals, whereas abundant taxa show a less visible decrease. The lower plant biomass in microcosms treated with rare communities could also be attributed to the traits of rare taxa. This effect of rare taxa on plant biomass was also found by Hol et al. (2010), who observed that a decrease of rare taxa within the soil resulted in higher plant biomass. The results of the gene quantification disagree with these results, by showing more rare fungal biomass for each treatment than abundant biomass. Thus, the lower plant biomass outcomes observed in rare communities cannot solely be explained by fungal population size (Hol et al., 2010). Rather, they may be

caused by the impacts of pesticides on the traits of rare fungal taxa. However, the decomposition rate of rare communities seems to be less sensitive to pesticide exposure. The positive correlation between fungal biomass and litter decomposition rate also emphasises that rare species are performing better in decomposing than abundant species. At a dosage of 10x, rare communities not only exhibit a mean decomposition rate approximately 5% higher than that of the abundant communities but also demonstrate double the amount of fungal biomass. This underscores the fact that rare taxa could potentially better adapt to stresses introduced by toxic pesticides. Additionally, the elevated decomposition rate of rare communities might support the fact that rare species and their taxonomically and functionally diverse biosphere are potentially even more important for ecosystem functioning than assumed (Liang et al., 2020; Logares et al., 2015; Xue et al., 2020). The higher decomposition rate also indicated that rare species decompose pollutants and contribute to ecosystem resilience to external pollution (Jousset et al., 2017). The higher rates in decomposition for rare communities in comparison to abundant and non-fungal could therefore mean that rare species are more resilient to high concentrations of pesticide. It is necessary to further understand how pesticides influence the ecosystem functions and processes provided by abundant and rare taxa, potentially even going further to unveil the complexity of the dynamic between the chemicals and individual fungal taxa.

Combination of abundant and rare fungal communities

The mixture of abundant and rare species not only leads to higher plant biomass and decomposition rates but also reveals slightly protective effects to pesticide exposure. Additionally, fungal population was found to be relatively stable for all pesticide treatments. The positive effects of combining the communities support that rare species help soil stability (Lynch et al., 2015) by potentially having positive effects on abundant species. The protective effects of rare microbes on plants against some pesticides are also found to be higher when rare taxa are present (Hol et al., 2010). According to Jousset et al. (2017), rare species could induce metabolic responses in abundant species and therefore have indirect effects on ecosystem functions. All these findings suggest that within community combinations, the presence of rare species might help to reduce the effects of pesticides on both the fungal community and the plant itself. This phenomenon potentially contributes to the observed stability in fungal populations as identified in this study.

Another explanation for the mixture performing better is the difference in sensitivity of the microbial makers (Riedo et al., 2023). Pesticide application can be harmful to some fungal species due to toxic chemicals. However, studies have shown that some fungal species might have better responses and get stimulated by the presence of some pesticides and are degrading or using them as an energy source (Pinto et al., 2012; Riedo et al., 2023). The study of Pinto et al. (2012) investigated the potential degradation of Difenoconazole by *P. Brevicompactum* and other fungal species. The fungus was able to successfully degrade about 90% of the pesticide after 10 days. Therefore, by combining communities from both rare and abundant pools, a broader range of microbial responses is created, potentially reducing the sensitivity to pesticide exposure. This explanation also highlights the possibility of individual fungal taxa showing species-specific responses to pesticide exposure. In other words, certain rare species might demonstrate better resilience or tolerance to pesticide exposure, while abundant species may be more susceptible to its effects, and vice versa. The combination of rare and abundant fungal taxa created a microbial community that is less sensitive to pesticide exposure and weakens the effect of even high dosages of toxic chemicals (Shakir et al., 2016). This resulting resilience explains the higher decomposition rate and total plant biomass for the mixture of both abundant and rare communities, even for higher dosages of pesticides.

4.2. Metabolic diversity of fungal taxa influences litter decomposition

As the results emphasise, the mixture of abundant and rare species promotes ecosystem functions under various pesticide applications. Nevertheless, only a weak negligible correlation between decomposition and substrate richness is detected. Richness tends to increase when mixing the communities, a logical outcome given the combination of diverse species utilising a variety of substrates. However, the decomposition rate is not consistently highest in mixtures, as some individual abundant or rare communities exhibit higher decomposition rates. Therefore, a higher usage of substrates does not necessarily follow a higher decomposition rate. This could be explained by potential antagonistic interactions between fungi species within the community (Fukami et al., 2010; Purahong et al., 2016). The species invest more energy into fighting other species than producing enzymes for decomposition. Another reason could be that some species

produce high quantities of these enzymes to be more competitive and exclude other fungi from fulfilling their functions (Tsujiyama et al., 2005).

Different fungal taxa are found to have different abilities to decompose specific carbon sources, with some displaying more specialisation and more effective substrate utilisation (Leifheit et al., 2024; Treseder et al., 2015). Yeast is observed to play a less important role in decomposition compared to its significance in stress tolerance. In contrast, free-living filamentous fungi possess traits related to decomposition processes (Treseder et al., 2015). These results also support our findings, by yeast species showing the lowest richness values. The low importance of the decomposition of yeast can partly explain the lower decomposition rate of the abundant communities. For instance, by focusing on the abundant community 4, where 3 out of 4 species are yeast, one can find the lowest richness of all communities as well as a decomposition rate under 50%. Other abundant communities also include yeast species, which also might influence the decomposition rate. Additionally, Mucoromycota fungi, which are discovered to prefer simple glucose are performing worse in decomposition (Leifheit et al., 2024). In this study, abundant *Mortierella* species tend to perform well in substrate utilisation, with the highest number of substrate utilisation. Nevertheless, abundant communities containing *M. alpina* and *M. elongata* perform well in decomposition but do not rank as the best performers compared to the decomposition rate of other communities. Differences in the outcomes of this study and Leifheit et al. (2024) could be explained by differences in experimental designs, with different approaches and potentially different fungal isolates.

4.3. Limitations of study

While this study provides valuable insights into the impact of pesticide exposure on various fungal communities and the potential influence of different concentrations, it is essential to acknowledge its limitations. Firstly, the experimental setup itself may have introduced constraints. The substrate used for this experiment and the timing of pesticide application could have led to restricted plant growth. Secondly, the focus on rare and abundant communities involved replicates with different fungal taxa compositions. This may have led to species-specific effects, with certain species asserting greater dominance over others. Therefore, additional DNA sequencing could add more information and clarity on community composition, highlighting dominant species and potential competitive dynamics among taxa.

5. Conclusion

This study aimed to understand the dynamics of the plant-fungal symbiosis when exposed to various pesticide concentrations. Visible effects have been detected on the dependency of fungal inoculation on plant biomass and litter decomposition. Abundant communities showed higher total plant biomass values, whereas the decomposition rates were detected higher for rare species. The exposure to pesticides at recommended and slightly increased dosages did not affect the different fungal communities. Only with the 10x dosage treatment, fungal communities showed negative effects in total plant biomass, whereas litter decomposition rates were less sensitive. Overdosed pesticide concentrations had less effect on the decomposition rate of rare species than abundant species. In both assessed ecosystem parameters, the combination of abundant and rare communities yielded to highest total plant biomass and decomposition rates across all pesticide treatments. Notably, the application of an overdose of pesticide at a 10x concentration affected the combined communities across both parameters.

Future research could increase their focus by testing an even greater diversity of fungal taxa. Additionally, emphasis should be set on different types of pesticides to gain a better understanding of the impacts of pesticide application on soil organisms.

Appendices

A.1. Pesticides

Amistar®

It is a fungicide with concentrated suspension containing 250 ml/l azoxystrobin from Syngenta Agro AG to control diseases in field crops, fruit growing, vegetable growing, berries and ornamental plants (Syngenta Agro AG, 2016).

Aviator Xpro®

It is a fungicide with concentrated emulsion containing 150 g/l prothioconazol and 75 g/l bixafen from Bayer (Schweiz) AG to control diseases in crops (Bayer (Schweiz) AG, 2023).

Slick®

It is a fungicide with concentrated emulsion containing 250g/l difenoconazol from Stähler Suisse SA for infection stopping and preventive effect for a variety of crops and diseases (Stähler Suisse SA, 2023).

Unix®

It is a fungicide with a water dispersible granule containing 750g/kg cyprodinil from Syngenta Agro AG to control of diseases in crops (Syngenta Agro AG, 2023).

A.2. Tables

Table A 1: Amount of pesticide for the different concentrations 1x, 2x, 10x and 0.5x.

Trade Name	Dosing per Box with Pesticide Concentration			
	1 x	**2 x**	**10 x**	**0.5 x**
Amistar®	0.23 µl	0.46 µl	2.32 µl	0.12 µl
Aviator Xpro®	0.73 µl	1.45 µl	7.26 µl	0.36 µl
Slick®	0.26 µl	0.58 µl	2.90 µl	0.15 µl
Unix®	0.58 mg	1.16 mg	5.81 mg	0.29 mg

Table A 2: Mean OD values of different categories for the different fungal taxa. The values show the mean of all values within a category for 3 replicates.

Species	Blank	Carbohydrate	Amino Acid	Amines/ Amides	Carboxylic Acid	Polymer	Other
A.Luteoalbus	0	0.012	0.005	0.029	0.020	0.045	0.013
A.Peglionii	0	0.024	0.005	0.030	0.034	0.069	0.022
B.Bassiana	0	0.010	0.041	0.031	0.077	0.061	0.040
C.Ramotenellum	0	0.032	0.044	0.030	0.031	0.052	0.028
C.Rosea	0	0.011	0.016	0.019	0.017	0.004	0.004
D.Glomerata	0	0.138	0.169	0.137	0.170	0.097	0.127
E.Radicis	0	0.058	0.091	0.099	0.112	0.070	0.187
F.Redolens	0	0.005	0.015	0.002	0.047	0.096	0.076
G.Nigrescens	0	0.010	0.040	0.019	0.039	0.030	0.025
M.Alpina	0	0.123	0.117	0.159	0.153	0.147	0.204
M.Elongata	0	0.079	0.078	0.062	0.107	0.117	0.084
M.Macrospinosa	0	0.032	0.030	0.041	0.040	0.043	0.038
M.Polycephala	0	0.014	0.025	0.011	0.022	0.047	0.019
P.Brevicompactum	0	0.003	0.099	0.006	0.045	0.000	0.031
P.Cucumerina	0	0.006	0.027	0.015	0.050	0.035	0.010
S.Aeria	0	0.000	0.006	0.001	0.000	0.018	0.005
S.Terrea	0	0.001	0.004	0.003	0.003	0.019	0.003
S.Terricola	0	0.000	0.005	0.018	0.004	0.028	0.000
T.Neokoningii	0	0.037	0.004	0.036	0.027	0.038	0.019

Table A 3: Allocation of different carbon substrates to broader categories, substrates are grouped according to Preston-Mafham et al. (2006).

Category	Substrate
Blank	*Water*
Carbohydrates	*Adonitol* *Arbutin* *D-Arabinose* *D-Arabitol* *D-Cellobiose* *D-Fructose* *D-Galactose* *D-Mannitol* *D-Mannose* *D-Melezitose* *D-Melibiose* *D-Psicose D-Fructose* *D-Raffinose* *D-Ribose* *D-Sorbitol* *D-Tagatose* *D-Trehalose* *D-Xylose* *Gentiobiose* *i-Erythritol* *L-Arabinose* *L-Fucose* *L-Rhamnose* *L-Sorbose* *Lactulose* *m-Inositol* *Maltitol* *Maltose* *Maltotriose* *N-Acetyl-DGalactosamine* *N-Acetyl-DGlucosamine* *N-Acetyl-DMannosamine* *Palatinose* *Sedoheptulosan* *Stachyose* *Sucrose* *Turanose* *Xylitol* *α-D-Glucose* *α-D-Lactose* *α-Methyl-D-Galactoside* *α-Methyl-D-Glucoside* *β-Methyl-D-Galactoside* *β-Methyl-D-Glucoside*
Amino acids	*Glycyl-L-Glutamic Acid* *L-Alanine* *L-Alanyl-Glycine* *L-Asparagine* *L-Aspartic Acid* *L-Glutamic Acid* *L-Ornithine* *L-Phenylalanine* *L-Proline* *L-Pyroglutamic Acid* *L-Serine* *L-Threonine* *γ-Amino-butyric Acid*
Amines/amides	*2-Amino Ethanol* *Alaninamide* *D-Glucosamine* *Glucuronamide* *Putrescine* *Succinamic Acid*
Carboxylic acids	*2-Keto-D-Gluconix Acid* *D-Galacturonic Acid* *D-Gluconic Acid* *D-Glucuronic Acid* *D-Malic Acid* *D-Saccharic Acid* *Fumaric Acid* *L-Lactic Acid* *L-Malic Acid* *N-Acetly-L-Glutamic Acid* *p-Hydroxyphe-nyl-acetic Acid* *Quinic Acid* *Sebacic Acid* *Succinic Acid* *α-Keto-glutaric Acid* *β-Hydroxy-butyric Acid* *γ-Hydroxy-butyric Acid*
Miscellaneous	*Adenos-ine-5′-Mono-phosphate* *Adenosine* *Amygdalin* *Bromosuccinic Acid* *D-Lactic Acid Methyl Ester* *Glucose-1-Phosphate* *Glycerol* *Salicin* *Succinic Acid Mono-Methyl Ester* *Uridine*
Polymers	*Dextrin* *Glycogen* *Tween 80* *α-Cyclodextrin* *β-Cyclodextrin*

A.3. Figures

Figure A 1: Visual assessment of the observed growth rates [%] during the experiments grouped into pesticide treatments (0x, 0.5x, 1x, 2x and 10x of recommended dose). Growth was described after 5 weeks up to harvest, each box was inspected and checked for growth by comparing with photos. The visual inspection was then transformed into numbers from 0 (100% mortality) to 4 (0% mortality) to assess visual growth.

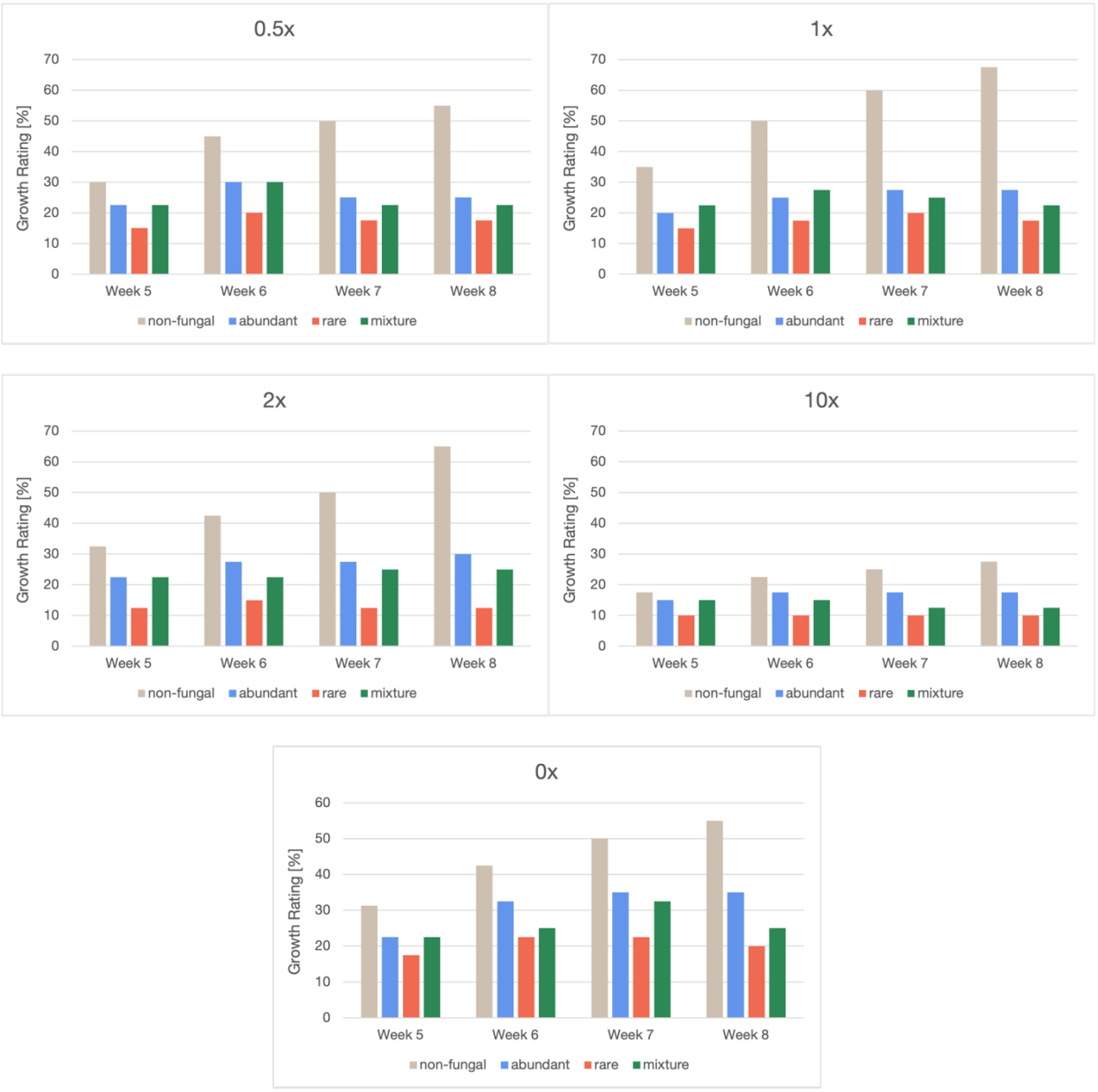


Figure A 2: The ability of abundant and rare fungal species to use various carbon sources tested with FF microplates™, with the mean AWCD and standard error of the 3 replicates at the timepoints 24h, 48h, 72h and 96h. Abundant species represented in bold.

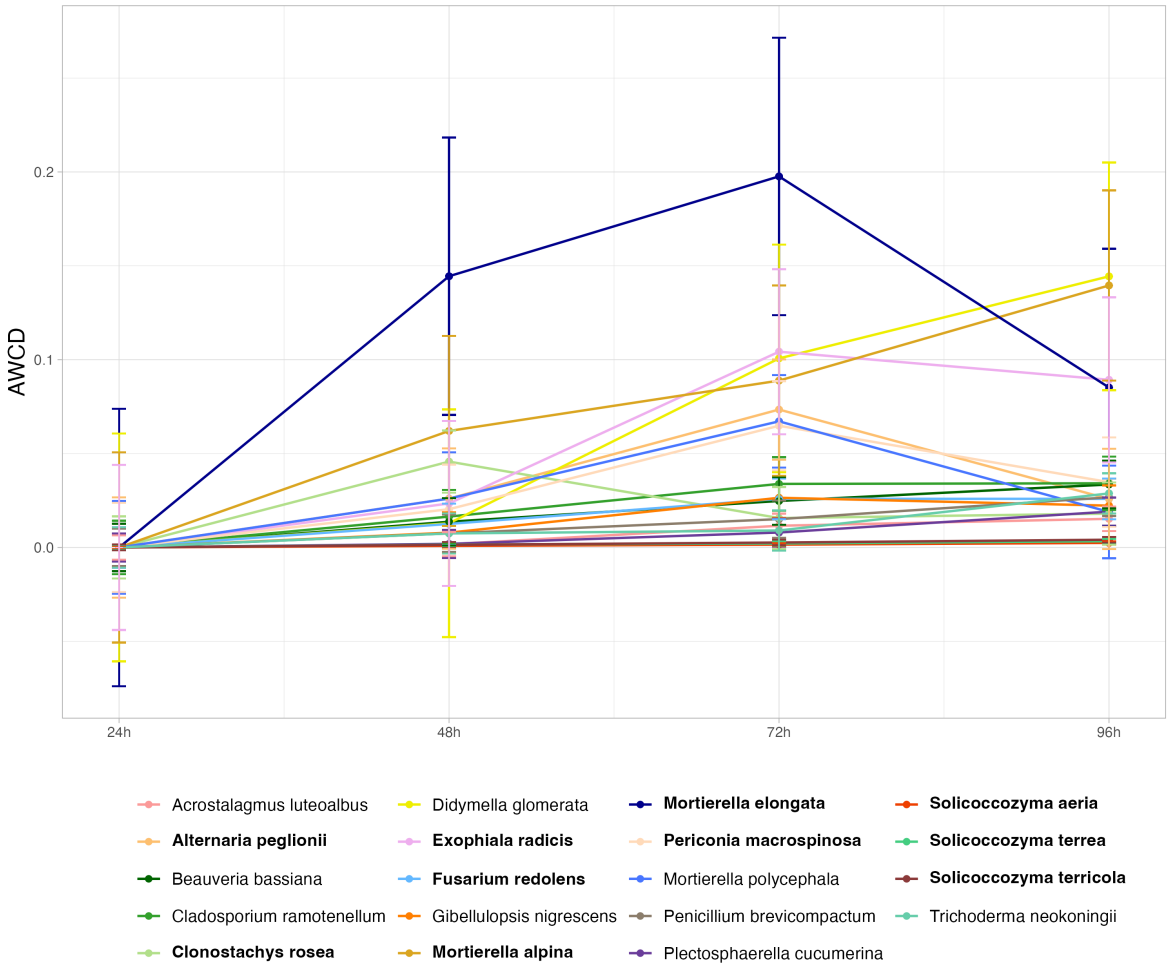
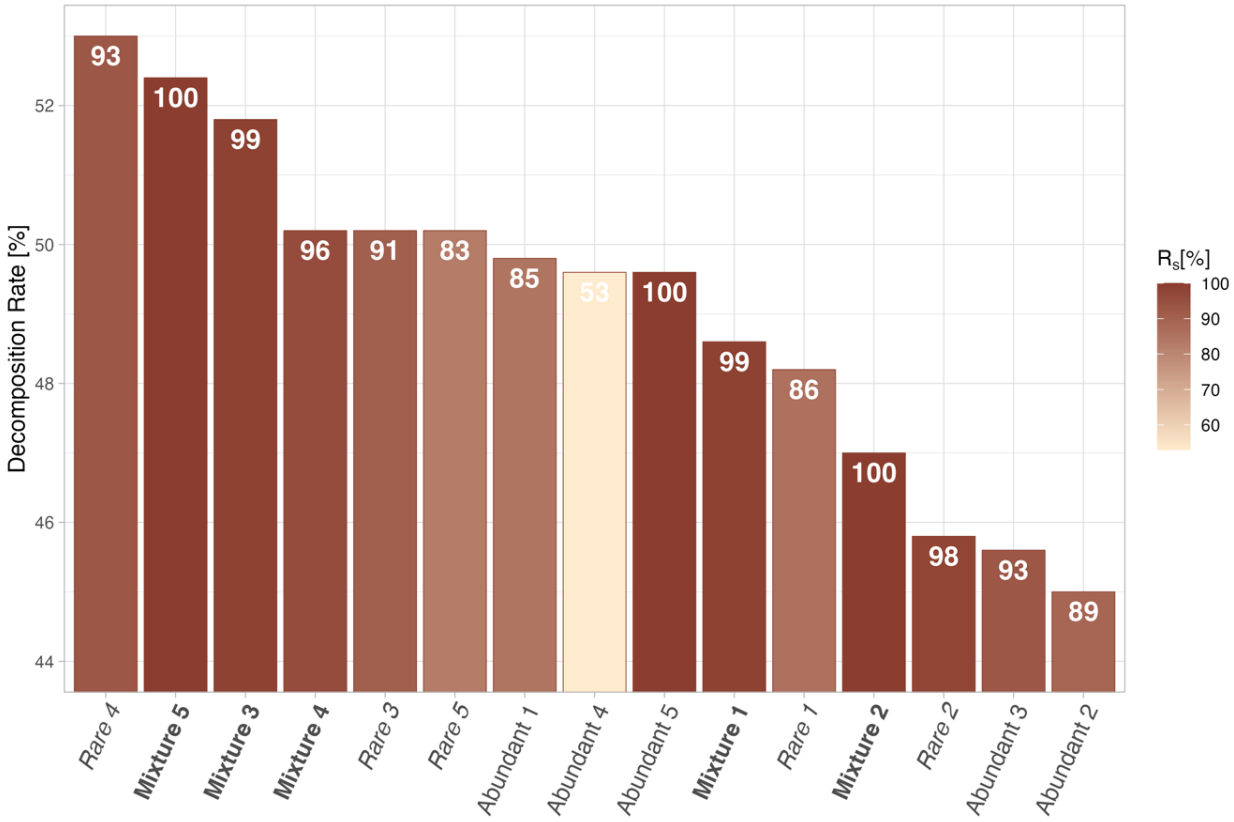


Figure A 3: Decomposition rate of all abundant, rare and mixed (i.e., abundant and rare) communities and their functional diversity using percentage richness R_S with number of used substrates with an OD ≥ 0.01. Each group is a unique and random replicate. Pearson correlation coefficient was -0.01 indicating a non-significant and very weak or negligible linear relationship (p=0.95).



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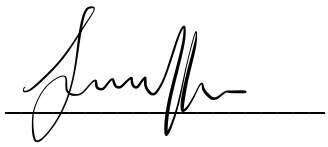
Personal Declaration

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

Zurich, 30 April 2024



Summermatter Jenny