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Department of Geography

Effects of Beneficial Fungi on Wheat Growth

GEO 511 Master's Thesis

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Department of Geography
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I. Abstract

There is growing interest in enhancing the sustainability of agricultural systems and reducing fertiliser and pesticide use. Consequently, microbial inoculation has attracted increasing interest as a sustainable agricultural strategy to enhance nutrient uptake, improve crop resilience, and reduce fertiliser dependence. However, current literature indicates inconsistent results and often tests few plant varieties and microbe species. Very few studies investigated synergistic effects between different fungi on plant growth.

This thesis explores the growth effects of arbuscular mycorrhizal fungi (AMF) and Mortierella fungi inoculation on winter wheat (*Triticum aestivum*). Two greenhouse trials were conducted with the aim to determine factors that shape inoculation efficiency. The first experiment tested the responsiveness of 18 different wheat varieties to inoculation with AMF, specifically examining the mycorrhizal growth response (MGR). The second experiment tested whether AMF and Mortierella fungi complement each other and have synergistic effects on wheat growth compared to single inoculations.

Results revealed high MGR variability across varieties, where *Every* and *Mont-Calme 268* showed positive MGR (5.83% and 0.48% growth increase), while other varieties revealed neutral and even negative values (up to -25.63%), indicating growth suppression by AMF. Biotic (*thrips*) and abiotic stress (heat) in Experiment 1 possibly explain lower root colonisation and suppressed growth benefits. Also, various wheat genotypes have a fine root system, and such genotypes possibly do not rely so much on the symbiosis with AMF. Moreover, the breeding background of varieties emerged as the main driver of MGR and resource allocation strategies, implying genotype-dependent symbiotic efficiency. Older varieties showed trends of higher responsiveness compared to modern ones. Fungal inoculation enhanced the shoot-to-root ratio of most varieties across both experiments despite limited overall biomass gains. A dual inoculation provided growth benefits for the varieties *Montalbano* and *Bonavau*, but they did not consistently exceed the effects of single inoculations on plant growth.

These findings emphasise the contribution of plant genotype in altering fungal associations for growth benefits in wheat. Therefore, future breeding selections should account for microbial inoculum compatibility with crop varieties through contextualised applications in field trials. New technologies such as synthetic microbial communities (SynComs) can help to fully harness microbial benefits for wheat growth. Under future climate scenarios and declining soil quality, contextualised crop-microbe combinations can play a vital role for resilient agricultural systems and food security.

II. Zusammenfassung

Es besteht ein wachsendes Interesse daran, die Nachhaltigkeit landwirtschaftlicher Systeme zu verbessern und den Einsatz von Düngemitteln und Pestiziden zu verringern. Infolgedessen hat die mikrobielle Beimpfung als nachhaltige landwirtschaftliche Strategie zur Verbesserung der Nährstoffaufnahme, zur Verbesserung der Widerstandsfähigkeit der Pflanzen und zur Verringerung der Abhängigkeit von Düngemitteln zunehmendes Interesse gefunden. Die derzeitige Literatur zeigt jedoch widersprüchliche Ergebnisse und testet oft nur wenige Pflanzensorten und Mikrobenarten. Nur sehr wenige Studien untersuchten synergistische Effekte zwischen verschiedenen Pilzen auf das Pflanzenwachstum.

In dieser Arbeit werden die Wachstumseffekte der Inokulation von arbuskulären Mykorrhizapilzen (AMF) und Mortierella-Pilzen auf Winterweizen (*Triticum aestivum*) untersucht. Es wurden zwei Gewächshausversuche mit dem Ziel durchgeführt, die Faktoren zu bestimmen, die die Effizienz der Inokulation beeinflussen. Im ersten Versuch wurde die Reaktion von 18 verschiedenen Weizensorten auf die Inokulation mit AMF getestet, wobei insbesondere die Mykorrhiza-Wachstumsreaktion (MGR) untersucht wurde. Im zweiten Versuch wurde getestet, ob AMF und Mortierella-Pilze sich gegenseitig ergänzen und im Vergleich zu Einzelimpfungen synergistische Effekte auf das Weizenwachstum haben.

Die Ergebnisse zeigten eine hohe Variabilität der MGR zwischen den Sorten, wobei *Every* und *Mont-Calme 268* eine positive MGR (5,83 % und 0,48 % Wachstumssteigerung) aufwiesen, während andere Sorten neutrale und sogar negative Werte (bis zu -25,63 %) zeigten, was auf eine Wachstumsunterdrückung durch AMF hindeutet. Biotischer (*Thripse*) und abiotischer Stress (Hitze) in Versuch 1 erklären möglicherweise eine geringere Wurzelbesiedlung und unterdrückte Wachstumsvorteile. Ausserdem haben verschiedene Weizengenotypen ein feines Wurzelsystem, und solche Genotypen sind möglicherweise nicht so sehr auf die Symbiose mit AMF angewiesen. Darüber hinaus erwies sich der züchterische Hintergrund der Sorten als Hauptfaktor für die MGR und die Strategien der Ressourcenallokation, was auf eine vom Genotyp abhängige Effizienz der Symbiose schliessen lässt. Ältere Sorten zeigten einen Trend zu einer höheren Reaktionsfähigkeit im Vergleich zu modernen Sorten. Die Inokulation mit Pilzen verbesserte das Verhältnis von Spross zu Wurzel bei den meisten Sorten in beiden Versuchen, obwohl der Biomassezuwachs insgesamt begrenzt war. Eine doppelte Inokulation brachte Wachstumsvorteile für die Sorten *Montalbano* und *Bonavau*, übertraf jedoch nicht durchweg die Auswirkungen von Einzelinokulationen auf das Pflanzenwachstum.

Diese Ergebnisse unterstreichen den Beitrag des Pflanzengenotyps bei der Veränderung von Pilzassoziationen für Wachstumsvorteile bei Weizen. Daher sollte bei künftigen Züchtungsmassnahmen die Kompatibilität des mikrobiellen Inokulums mit den Pflanzensorten durch kontextbezogene Anwendungen in Feldversuchen berücksichtigt werden. Neue Technologien wie synthetische mikrobielle Gemeinschaften (SynComs) können dazu beitragen, die mikrobiellen Vorteile für das

Weizenwachstum voll auszuschöpfen. Angesichts künftiger Klimaszenarien und abnehmender Bodenqualität können kontextbezogene Pflanzen-Mikroben-Kombinationen eine entscheidende Rolle für widerstandsfähige landwirtschaftliche Systeme und die Ernährungssicherheit spielen.

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VI. List of Abbreviations

AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
C	Carbon
C3	Plant species using the C3 carbon fixation pathway (cool, moist climates)
C4	Plant species using the C4 carbon fixation pathway (warm, dry climates)
ddH_2O	Double-distilled water
DNA	Deoxyribonucleic acid
DSP	*Delley semences et plantes* SA
FAST	Farming System and Tillage Experiment (Agroscope)
GMO	Genetically Modified Organism
GR	Growth Response (non-AMF specific; Mortierella and dual inoculation)
K	Potassium
LMM	Linear Mixed-Effects Model
MGR	Mycorrhizal Growth Response (AMF-specific)
MHB	Mycorrhiza Helper Bacteria
N	Nitrogen
P	Phosphorus
PERMANOVA	Permutational Multivariate Analysis of Variance
PSB	Phosphate-Solubilising Bacteria
PSF	Phosphate-Solubilising Fungi
qPCR	Quantitative Polymerase Chain Reaction
QTL	Quantitative Trait Locus - genomic region linked to quantitative trait variation
R^2	Coefficient of determination
SAF22	*Rhizoglomus irregulare strain* SAF22
SDGs	Sustainable Development Goals (United Nations)
SOC	Soil organic carbon
S:R ratio	Shoot-to-root ratio
sp.	Unspecified species
SynCom	Synthetic Microbial Community
η^2	Eta Squared – a measure of effect size

1 Introduction

1.1 Wheat Production and Challenges

Almost one-quarter of the global wheat production is traded internationally. Thus, wheat is crucial for global food security, especially in developing countries. Over 2.5 billion people living with less than $2 per day rely on it for their diet (Shiferaw et al., 2013). Approximately 20% of global carbohydrates and protein are provided by wheat, as it accounts for 26% of global cereal production and 30% of total land used for cereal production (Langridge et al., 2022).

Climate change and population growth significantly impact the sustainability of wheat production. Increasing drought and heat stress not only reduce overall yield but also enhance inter-annual wheat production variability by 40%. Langridge et al. (2022) predict an average decrease in wheat yield of 7% for each 1°C temperature increase. Additionally, the available cropping land per capita halved from 1961 to 2018 due to population growth. Models that account for complex climate change effects such as rising night temperatures and changing rainfall patterns are needed for more accurate estimations. Shiferaw et al. (2013) add that by 2050, wheat demand in developing countries is projected to increase by 60%. Per capita demand in developing regions doubled since the 1960s. Furthermore, a large part of these regions is affected by water scarcity, which already accounts for 20 million hectares.

Fertilisers and pesticides usage to ensure more yield for the increasing demand greatly increased, leading to rising financial and environmental costs. Thus, it is essential to focus on research and innovation for sustainable technologies in agriculture. One approach is to replace older wheat variants with newer varieties. These varieties are characterised by higher yield potential, improved disease resistance, and better adaptation to climate stresses, increasing yield stability (Shiferaw et al., 2013). Overall, the main challenge of sustainable wheat production is caused by increased demand and climate change that jeopardise yield.

1.2 Agricultural Intensification

The Green Revolution in the mid-20th century made it possible to meet the growing demand for crops by using new methods and technologies. This involves extensive use of fertilisers and pesticides while incorporating enhanced irrigation processes and modern, high-yield crop varieties (Banerjee et al., 2019). The goal of agricultural intensification is to meet growing demands by increasing productivity while using the same amount of land. However, this comes with higher financial and environmental costs; the consequences are becoming increasingly noticeable. A paper by Richardson et al. (2023) examines the framework of planetary boundaries (**Fig. 1**), which describes nine aspects that are essential

for the Earth's stability and resilience. They argue that six of those nine boundaries have been exceeded. This is linked to human influences, with agricultural intensification playing a major role.

Biosphere integrity is threatened by land conversion, while agricultural intensification impacts genetic diversity through irrigation processes. Land system change is largely caused by agricultural expansion on forest biomes. This can lead to a negative feedback loop and exacerbate climate change, as forest areas are essential for climate regulation. Additionally, over-extraction and pollution of fresh water have pushed this boundary beyond safe limits. Agriculture is also a large emitter of greenhouse gases such as methane and nitrous oxide. Lastly, extensive fertiliser usage has led to excessive phosphorus (P) and nitrogen (N) flows, causing pollution and water eutrophication (Richardson et al., 2023). These problems need to be addressed with increasing urgency to ensure future human well-being.

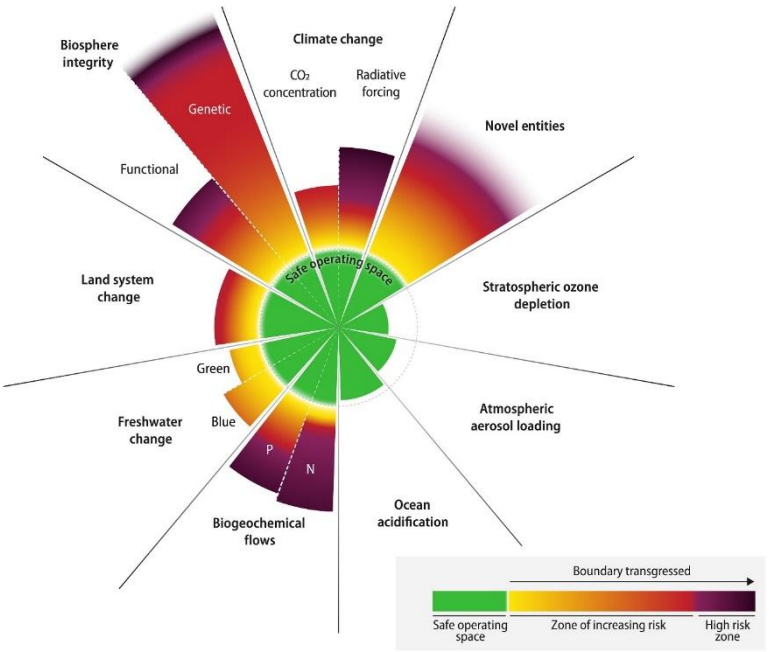

Figure 1: Spider Chart by Richardson et al. (2023), illustrating the framework of planetary boundaries. Biogeochemical flows are in the high-risk zone.

Literature emphasises a growing need for more efficient and sustainable agricultural practices (e.g., Langridge et al., 2022). However, the economic feasibility of large-scale biofertilisation strategies requires more research (Kubiak et al., 2022). Agricultural productivity and maintaining soil health need to be balanced to ensure microbial diversity (Banerjee et al., 2019). Microorganisms play a key role in nutrient cycling, ensuring soil health and mitigating climate change impacts. They are essential for carbon (C) sequestration, carbon storage, and denitrification (Bender, Wagg & van der Heijden, 2016). However, microbial networks are influenced by farming systems. Banerjee et al. (2019) state that organic farming displays higher network complexity, suggesting that sustainable practices need to be promoted to maintain a functional ecosystem.

Macronutrients such as N and P are essential for plant growth and overall health. N is crucial for protein synthesis and chlorophyll production, while P is responsible for energy transfer, root development, and photosynthesis (Dierks et al., 2020). However, P is a finite resource, as it is derived from non-renewable phosphate rock. According to Van Vuuren, Bouwman & Beusen (2010), up to 60% of global P resources could be depleted by the end of the century. Additionally, P is often unavailable in soils because of slow diffusion rates and high fixation (Fabiańska et al., 2020). This further exacerbated the extensive usage

of phosphorus fertilisers to meet rising yield demands. The dependency enhances the vulnerability of the agricultural sector to price fluctuations. Temporal dynamics influencing microbial networks need further research to understand the complex interactions of soil biodiversity and ecosystem functions (Bender, Wagg, & van der Heijden, 2016). Additionally, interactions between different soil organisms and how different agricultural practices affect soil biota communities need investigation (Fabiańska et al., 2020; Bender et al., 2023).

1.3 Plant-Promoting Fungi

Mucoromycota is a phylum of early diverging fungi representing some of the earliest plant-associated terrestrial fungi (Sokołowska et al., 2023). They have versatile ecological potential, which could be promising for the implementation of sustainable agriculture. This master's thesis focuses on two subphyla, *Mortierellomycotina* and *Glomeromycotina*, also known as Mortierella fungi and arbuscular mycorrhizal fungi (AMF), respectively. These two fungi will be incorporated in greenhouse experiments that are part of this master's thesis.

1.3.1 Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi are symbiotic fungi that form associations with the roots of approximately 70–80% of land plant species (Martín-Robles et al., 2018; Cheeke et al., 2019). In the mutualistic relationship between AMF and host plants, the plants provide the fungi with carbohydrates for growth (van der Heijden et al., 2015). The distance from the equator and mean annual precipitation have the strongest effects on fungal abundance and richness, as most fungal groups show the highest diversity in tropical ecosystems (Wardle & Lindahl, 2014). AMF have played a key role in plant-soil interactions for over 400 million years. They penetrate the cortical cells of plant roots, forming arbuscules (**Fig. 2**) (**e**). These tree-like structures extend the plant's root system, increasing the soil volume explored for nutrients (**a**). The molecular mechanisms of nutrient transfer in mycorrhizal networks are complex and require further research (van der Heijden et al., 2015). This extensive hyphal network also improves water uptake, promoting drought resistance (Kozioł & Bever, 2015) (**b**). AMF produce glomalin, a glycoprotein that

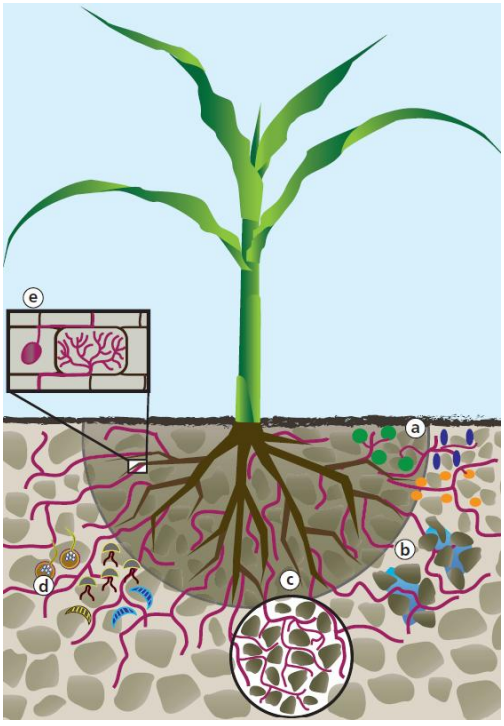

Figure 2: Illustration by Köhl & van der Heijden (2016) showing benefits of AMF for plant growth.

helps bind soil particles together, improving soil structure (Köhl & van der Heijden, 2016) (**c**). Additionally, AMF can improve resistance against certain soil-borne pathogens (Gai et al., 2010) (**d**). This mechanism has the potential to increase plant growth and biomass production, enhance P and N uptake, improve crop yield and quality, and enhance tolerance to abiotic stresses such as salinity and drought (Bender et al., 2019). Berger, F., & Gutjahr, C. (2021) highlight the potential of AMF resistance to heavy metals and their positive influence on plant hormone levels. Furthermore, AMF can influence and contribute to ecosystem diversity by influencing plant composition (Cheeke et al., 2019). However, AMF require a significant amount of N for their metabolic function. In N-limited soils, the fungi might compete for resources with the plant, potentially harming the plant's overall growth (Wang et al., 2018). Moreover, several studies found that AMF nutrient uptake and colonisation are strongly affected by soil P levels; high P availability can suppress the symbiotic interaction, thereby reducing the benefits to the plant, including wheat (e.g., Hetrick, Wilson, & Todd, 1996; Tawaraya, 2003).

1.3.2 Mortierella Fungi

Li et al. (2018) characterise Mortierella fungi as soil-dwelling saprotrophic fungi, often found in deep humus layers. These slow-growing fungi can decompose plant litter and degrade toxic organics. Categorised as R-strategists, they reproduce quickly relative to other fungi and can survive in changing environments. However, “R-strategist” in this context refers more to Mortierella's resource opportunism rather than its fast colonisation of the substrate by establishing long-lasting populations (Li et al., 2018). By synthesising and secreting oxalic acid, Mortierella can dissolve inorganic P. Similar to AMF, Mortierella can also stimulate the production of plant growth hormones (phytohormones). They form biofilms, enhancing soil enzyme activities for C and P degradation (Li et al., 2018). Moreover, Mortierella can produce polyunsaturated fatty acids that mediate plant lipid pathways (Liao, 2021). Ozimek & Hanaka (2021) add that Mortierella decomposes various polymers and thus makes nutrients more available in the soil. Additionally, they produce siderophores, which are iron-chelating compounds, enhancing the bioavailability of iron. Like AMF, Mortierella could be a promising component of biofertilisers, improving plant growth and soil health.

The following table provides an overview of the most important similarities and differences between AMF and Mortierella fungi:

Characteristic	**Arbuscular Mycorrhizal Fungi**	**Mortierella Fungi**
Subphylum	Glomeromycota (Sokołowska et al., 2023)	Mortierellomycotina (Sokołowska et al., 2023)
Habitat	Soil, specifically associated with plant roots (e.g., Kozioł & Bever, 2015)	Soil, not specifically associated with roots (Li et al., 2018)
Relationship with plants	Symbiotic (e.g., Bender et al., 2019)	Generally saprotrophic (Ozimek & Hanaka, 2021)
Primary function	Direct plant growth promotion (e.g., Bender et al., 2019)	Decomposition of organic matter (Li et al., 2018)
Nutrient exchange	Facilitate nutrient exchange with plants (e.g., van der Heijden et al., 2015)	Indirect nutrient cycling in soil (Li et al., 2018)
Plant specificity	Often have specific plant hosts (e.g., Martín-Robles et al., 2018)	Generally, not plant-specific (Li et al., 2018)
Effect on soil	Improve soil structure (e.g., Bender et al., 2019)	Improve soil structure (Ozimek & Hanaka, 2021)
Phosphorus cycling	Enhance phosphorus uptake by plants (e.g., Bender et al., 2019)	Solubilise phosphorus in soil (Ozimek & Hanaka, 2021)
Carbon source	Obtain carbon directly from the plant host (e.g., van der Heijden et al., 2015)	Obtain carbon from soil organic matter (Li et al., 2018)
Growth promotion mechanism	Direct nutrient transfer to plants (Dierks et al., 2020)	Indirectly through improvements in soil (Ozimek & Hanaka, 2021)
Fungal components	Thin, non-septate hyphae; large spores; vesicles (Droh et al., 2023)	Thick, septate hyphae; no vesicles; small spores (Li et al., 2018)

Table 1: Overview of different characteristics and similarities of AMF and Mortierella fungi.

1.3.3 Synergistic Effects

Plant growth response to Mucoromycota has been confirmed in plenty of studies in greenhouse experiments and in the field. Detailed results of multiple studies will be discussed in the next chapter. Synergistic effects between AMF and so-called mycorrhizal helper bacteria (MHB) have been investigated by various studies. MHB promote the functioning and establishment of the symbiosis by AMF and their host plant. They can significantly enhance AMF colonisation of plant roots and improve solubilisation of nutrients like P and N in the soil, making them more accessible for AMF and, subsequently, to plants. (Frey-Klett, Garbaye & Tarkka, 2007). A recent study by Zhang et al. (2024) describes this complex mechanism as "tripartite bacterial-fungal-plant symbiosis" since MHB and AMF symbiotically promote plant growth.

However, only a few studies examined synergistic effects within Mucoromycota, indicating the need for further investigation. Li et al. (2018) point out that Mortierella fungi can help AMF in P acquisition. A study by Zhang et al. (2011) investigated synergistic effects of *Mortierella* sp. (non-identified species) and two strains of AMF on *Kosteletzkya virginica* plants under different salt levels. They conclude that a dual inoculation of *Mortierella* sp. and one AMF strain showed the highest root and shoot biomass. Additionally, *Mortierella* sp. presence enhanced root colonisation of AMF, while *Mortierella* sp. populations were higher with AMF presence. However, a triple inoculation of both AMF strains and *Mortierella* sp. was less effective due to the potential competition of the fungi for resources. Contrary to previous studies (e.g., van der Heijden et al., 1998), results from Zhang et al. (2019) support that a dual inoculation of multiple AMF strains does not provide greater benefits for crop production. Hence, specific fungal combinations need to be explored for optimised results since greater fungal diversity may not always be superior for plant performance.

1.4 Mycorrhizal Growth Response

AMF can significantly promote the yield and quality of various crops, such as wheat, maize, and rice, that make up a major part of global food security. A study by Gai et al. (2010) found a yield enhancement in the previously mentioned crops by 5.5–18.3%. However, mycorrhizal growth response (MGR) heavily depends on environmental factors, soil conditions, and plant species.

Soil P content impacts abundance and colonisation success of native AMF on host plants. Root colonisation is negatively correlated with P fertilisation, indicating higher MGR in organic practices (Bender et al., 2019). Greenhouse experiments by Tawaraya (2003) investigated MGR on 13 different plant species with various controlled P levels in sterilised soil. Most species showed high AMF dependency that decreased with increased P levels. Additionally, MGR was higher for dicotyledonous species compared to monocotyledonous species. The latter often have more efficient root hairs, making

them less dependent on AMF for nutrient acquisition. Successional stages of plants largely influence MGR. Late successional plants are more sensitive to AMF than early successional plants, the latter forming more consistent AMF associations (Cheeke et al., 2019). Species that grow more slowly tend to provide more resources for mutualistic relationships, including AMF. Additionally, early successional species have a different root architecture, as there are more root tips per mass, which negatively correlates with mycorrhizal responsiveness (Kozioł & Bever, 2015). A recent study by Lutz et al. (2023) examined MGR on maize across 54 different fields. The large MGR variability, ranging from -12% to +40%, is largely explained through the abundance of certain soil fungal taxa, especially plant pathogens. AMF inoculation reduces plant pathogen abundance, increasing crop productivity. Most experiments in literature focus on short-term experiments, often under controlled conditions, resulting in a large knowledge gap of large-scale field applications over time (Cheeke et al., 2019; Berger & Gutjahr, 2021).

1.4.1 AMF and Mortierella Growth Response on Winter Wheat

Multiple studies have shown a significant MGR on winter wheat (*Triticum aestivum*). A study by Bakonyi & Csitári (2018) tested two winter wheat varieties with AMF inoculation over one growing season in a field in Hungary. Both varieties showed yield increases of 23% and 8%, respectively. This difference could be explained by genetic variability, root architecture, and nutrient uptake efficiency of the varieties. Overall, total yield increased from 7.52 to 8.17 tonnes (t) per hectare (ha). The study also tested the effects of mineral fertilisation, which increased total yields from 7.38 to 8.31 t per ha. However, it is not mentioned what and how much fertilisation was used during the experiment. This indicates that AMF could partially substitute mineral fertilisers for winter wheat production, as there might be a trade-off in the benefits of AMF under intensive fertilisations. The authors highlight that recommendations for farmers regarding different soil types and environmental conditions need to be established.

A similar study by Szentpéteri et al. (2023) supports the findings of the previously mentioned study by Bakonyi & Csitári (2018). In a Hungarian field, the MGR on four different wheat varieties was tested with four different nutrient levels. These nutrient levels differ in N, P, and potassium (K) amounts. The largest MGR of 35% was achieved with medium nutrient content (90 kg/ha N, 30 kg/ha P, 30 kg/ha K). However, the field in which this experiment was conducted (Szeged-Öthalom, Hungary) is classified as a chernozem soil that is characterised by high nutrient content and good water retention properties. It could be plausible that in nutrient-poor soil, higher nutrient levels would be necessary for maximal MGR. This is consistent with the observation that MGR is negatively correlated with P availability in soil, indicating greater AMF benefits in lower P conditions. As winter wheat growing sites are often P-limited, AMF could lead to more stable spring regrowth (Hetrick, Wilson, & Todd, 1996).

According to Ozimek et al. (2018), Mortierella has positive effects on winter wheat seedlings, as both shoot and root fresh weight increased by 40%. Wheat-growing regions with a temperate climate, including cooler spring conditions, can significantly benefit from Mortierella inoculation (Ozimek et al., 2018). However, standardised methods for Mortierella inoculum application and production are lacking (Ozimek & Hanaka, 2021). Apart from fungal inoculum and soil nutrient availability, the host plant itself also plays a significant role in determining MGR, as discussed in the following chapters.

1.4.2 Mycorrhizal Growth Response Differences in Old and Modern Crop Varieties

Not only soil conditions influence MGR but also the crop varieties themselves. Even within the same crop species, MGR differs between modern crops and older varieties. A meta-analysis by Lehmann et al. (2012) concludes that older varieties have an average AMF root colonisation of 40.8%, while newer cultivars showed 32%. This suggests a lower responsiveness or dependence on AMF in modern varieties. However, the study could not find significant differences in P acquisition efficiency or P utilisation efficiency between new and old cultivars.

A study by Martín-Robles et al. (2018) examined MGR in 27 crop species with different P conditions under greenhouse conditions. They found out that domesticated plants did not profit from AMF, while wild progenitors benefitted regardless of P availability. However, AMF symbiosis was only beneficial for domesticated plants in P-limited conditions.

De Leon et al. (2020) performed a study on AMF effects from organic and conventional farms on six different spring wheat varieties released between 1929 and 2016. They used natural soil biota instead of cultured AMF strains to represent field conditions more accurately. Results showed that varieties responded differently to the inoculum. Older varieties showed higher MGR than newer ones. A meta-analysis by Zhang et al. (2019) supports these findings, as older corn and wheat varieties showed higher MGR than newer ones (**Fig. 3**). However, no significant differences in MGR could be detected between organic and conventional AMF strains. Nevertheless, the authors highlight the importance of considering both wheat varieties and AMF strains for developing optimal inoculation strategies. The meta-analysis of MGR by Zhang et al. (2019) also compared C3 and C4 wheat types as well as newer wheat cultivars released after 1950, and older wheat cultivars released

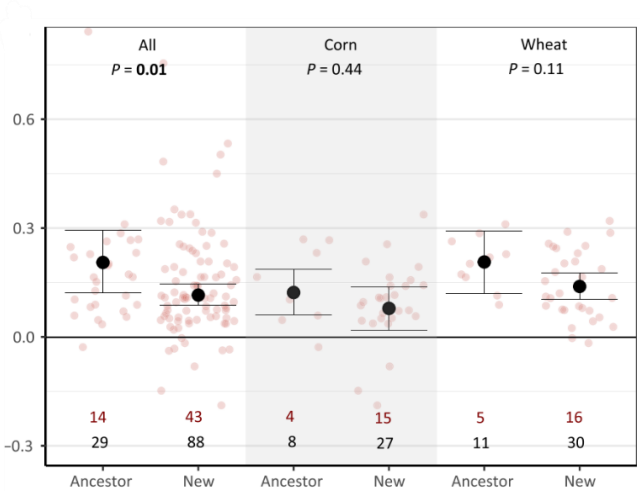

Figure 3: Graph by Zhang et al. (2019), illustrating the differences of MGR between older and newer crop varieties. Older varieties tend to have higher MGR than newer ones. The y-axis describes the standardised yield impact from AMF treatments.

before 1950. They found an overall 17% positive MGR on both C3 and C4 cultivars. Positive effects on newer wheat varieties were less pronounced compared to older ones. However, the study does not provide specifications about the MGR differences nor distinguish between winter and spring wheat varieties.

Although MGR differences require further elucidation, one approach to explaining the differences would be the identification of quantitative trait loci (QTL) to investigate the genetic basis of MGR. Wheat contains 42 chromosomes, including seven homoeologous groups, numbered 1 to 7 with three genomes each, referred to by the letters A, B, and D (Lehnert et al., 2017). The chromosomes 1A, 2A and 5A explain 22% of MGR variation among 94 recombinant inbred wheat lines (Hetrick et al., 1994). Lehnert et al. (2017) identified chromosomes 3A, 4A, and 7A with root colonisation of AMF. For winter wheat, the chromosomes 3D and 7D are associated with AMF response to drought stress conditions (Lehnert et al., 2018). Thirkell et al. (2022) identified chromosomes 2A, 2B, 3B, 4A, 5A, 6A, and 7B for MGR. Overall, these genetic markers can help to better understand and identify MGR mechanisms of different varieties. This in turn could facilitate variety breeding and selection with the highest MGR rates to propose strategies to farmers that maximise efficiency (Lehnert et al., 2017; Thirkell et al., 2022).

1.5 Ecological Engineering

According to Shiferaw et al. (2013), modern wheat varieties come with several advantages compared to older cultivars and have replaced them in most global areas. They generally have higher yield potential, improved pest resistance, and better adaptation to climate stresses. Additionally, they are more responsive to fertiliser inputs and have better water and nutrient use efficiency. Lehmann et al. (2012) argue that newer cultivars may be more dependent on higher P conditions due to their breeding for highly fertilised environments. Thus, the question arises: what advantages can Mucoromycota add compared to modernised crop cultivars in terms of food security and sustainable agriculture? AMF and Mortierella not only have the potential to provide the same benefits; they also offer additional ecological benefits.

AMF benefit plant growth under water-limited conditions, making them more resistant to droughts and heat stress (Hahn et al., 2018). Due to climate change, these events will be more pronounced in the future (Langridge et al., 2022). AMF significantly reduce P fertilisation requirements by up to 80% and even N fertilisation on a smaller basis (Bender et al., 2019). Mortierella could assist AMF in the acquisition of nutrients by plants, potentially enhancing positive effects of AMF (Li et al., 2018). In field trials, AMF could reduce average fertiliser application rates by 21% (Gai et al., 2010). Kozioł & Bever (2015) add that AMF can indirectly promote N use efficiency by improving soil structure and root growth. A study by Dierks et al. (2020) found notable evidence that AMF can transfer nutrients

between species. By connecting different plant species through AMF's mycelia, around one-third of the N that was derived from *Faidherbia albida* trees was transferred to maize plants.

A study by Kozioł, Lubin & Bever (2024) tested 23 AMF inoculants, including commercial products and laboratory-grown fungi. The inoculant was applied while planting crops in pots under organic production standards. They evaluated its effectiveness based on AMF spore concentration, root colonisation and plant growth response. The authors conclude that many commercial products performed worse than lab-grown fungi in terms of root colonisation and growth response. Salomon et al. (2022) found similar results when testing 28 commercial AMF inoculants under greenhouse and field conditions across different continents. Many commercial AMF inoculants lack viable propagules such as spores and hyphae. Large growth response variability in field inoculations stems from inconsistent quality of inoculants and soil properties. The authors of both studies point out the need for improved quality control and standards within the commercial AMF inoculant industry.

Most studies suggest a complementary approach to Mucoromycota inoculation with reduced fertiliser applications. The approach should be used as supplementation rather than a complete replacement (e.g., Cheeke et al., 2019). Factors such as temperature, soil pH, and nutrient content should be considered when deciding on adequate fertilisation application (Tawaraya, 2003). Mortierella can also play an important role in biofertilisation, especially in temperate climates during cool spring conditions (Ozimek et al., 2018). This fertilisation reduction could mitigate the severity of biochemical flows and reduce consequences such as water pollution and nutrient leaching, ensuring human well-being and microbial diversity.

Due to intensive land use and agricultural practices, loss of soil biodiversity communities can occur. This can result in severe impacts on nutrient cycling and thus nutrient availability for agriculture. This was investigated with an experiment by Bender et al. (2023) using lysimeters with different filter sizes that altered soil biota communities. N leaching losses increased by up to 65%, and gaseous emissions of N_2 and N_2O almost doubled. Additionally, N and P uptake by maize was reduced by 20% and 58%, respectively.

Thirkell, Pastok, & Field (2020) found benefits of AMF on wheat cultivars under enhanced CO_2 conditions, indicating improved resistance to future climate change scenarios. Field et al. (2019) conducted a similar experiment with liverworts, AMF, and Mucoromycotina, which is another sub-phylum of Mucoromycota. Interactions were tested under different CO_2 conditions. Mucoromycotina were more effective at transferring P under elevated CO_2 conditions than AMF, making them another interesting candidate for biofertilisation. However, AMF was a more effective C sink than Mucoromycotina fungi. Comparing the nutrient exchange of Mucoromycotina and AMF requires further investigation (Hoysted et al., 2023).

Overall, the effectiveness of AMF and Mortierella fungi in different wheat genotypes regarding the impact of environmental factors needs further analysis. Potential trade-offs between beneficial mycorrhizal associations and breeding modern wheat varieties remain a complex challenge (Zhu et al., 2001).

1.6 Research Question and Hypotheses

This master's thesis investigates MGR in early growth stages (first 60 days) of different winter wheat varieties, including old and new varieties, under controlled conditions. In the second experiment, potential synergistic effects of AMF and Mortierella fungi are explored to see if a dual inoculation provides additional benefits for winter wheat growth. Since studies found that older, less modern wheat varieties showed higher MGR (Zhang et al., 2019; De Leon et al., 2020) and synergistic effects between AMF and Mortierella can be more beneficial than single inoculation (Zhang et al., 2011), the following hypotheses are formulated:

1) There is a significant positive mycorrhizal growth response (MGR) observable in most of the 18 winter wheat varieties.

2) MGR is higher in older and lower-quality varieties than on modern, high-quality varieties.

3) The genetic origin of wheat varieties significantly influences their mycorrhizal growth response (MGR), with varieties from different origins expected to respond differently, possibly reflecting differences in historical input conditions (e.g., high-input, lower MGR).

4) A dual inoculation of AMF and Mortierella is more beneficial for wheat growth than a single inoculation of AMF or Mortierella.

2 Materials and Methods

2.1 Experiment 1

The aim of the first greenhouse experiment is to investigate mycorrhizal growth response (MGR) for various winter wheat varieties recommended to Swiss farmers with different quality classifications and origins in controlled conditions. In addition, older varieties are included to investigate if there is a superior association with AMF. Results test hypotheses 1-3 (Chapter 1.6) and allow variety selection for the second greenhouse experiment (see Chapter 2.2), where Hypothesis 4 is focused.

2.1.1 Winter Wheat Variety Selection

TOP
1. Piznair (Agroscope, CH)
2. Axen (Agroscope, CH)
3. Montalbano (Agroscope, CH)
4. Bonavau (Agroscope, CH)
5. Cadlimo (Agroscope, CH)
I
6. Hanswin (Agroscope, CH)
7. Alpval (Agroscope, CH)
8. Arina (Agroscope, CH)
II
9. Spontan (Secobra, DE)
Feed
10. Campesino (Secobra, DE)
Other (not on the list of recommendations)
11. Blickfang (Secobra, DE)
12. Emblem (DSV, DE)
13. Every (RWA SZ Edelhof, AUT)
14. Kastell (Secobra, DE)
15. LG Mondial (Limagrain, Fr)
Old
16. Plantahof (Plantahof, CH), 1910*
17. Mont-Calme 268 (Mont-Calme, CH), 1926*
18. Probus (Agroscope, CH), 1948*

Each year, Agroscope publishes a list of recommendations for various crops. This experiment incorporates 18 different winter wheat varieties (**Table 2**). Many of them are mentioned in the “List of recommended cereal varieties for the 2024 harvest” (Strebel et al., 2023). These varieties are categorised into different quality classes. The classification is based on several criteria that include yield potential, quality characteristics, and disease tolerance. The list further classifies varieties based on their score in the different categories. The highest-quality varieties are classified as "TOP", followed by classes “I” and "II". Varieties for livestock feeding are in a separate category. Within these categories, varieties are ranked by score (e.g., *Piznair* has the highest quality overall). The experiment incorporates ten varieties from this list, five additional varieties from Germany, Austria, and France, and three older Swiss varieties before the Green Revolution in the 1950s. *Plantahof* originated from the agricultural school and research station Plantahof in Graubünden and *Mont-Calme 268* from the research station at Mont-Calme, near Lausanne (Lehmann, 2003).

Table 2: List of winter wheat varieties that have been used for the first greenhouse experiment. They are categorised by quality and type. The origin and breeders are mentioned in brackets. The year of old varieties stands for the year of inscription.*

2.1.2 Experimental Setup

The 18 different winter wheat varieties were used for the first experiment, grown under controlled greenhouse conditions (Agroscope Reckenholz, Zurich, Switzerland). The experiment consists of eight blocks, each including one control and one AMF treatment for every variety, resulting in 36 pots per block. The wheat plants were grown in autoclaved black, 1.5-litre pots with a plate underneath to prevent soil and water from leaking. Additionally, drainage fleece in each pot provided extra support for drainage. A total of 288 pots and plates were used for the experiment, all of which were carefully indicated with a number and treatment.

2.1.3 Substrate and AMF Inoculum

Soil from a grassland site near Agroscope Reckenholz was sieved with a 5mm mesh before it was mixed with quartz sand (Capito, Landi) at a 1:1 volume ratio with a concrete mixer (see chapter 2.3 for soil parameters). Next, the substrate was autoclaved (121°C, 90 minutes) for sterilisation. Three weeks later, AMF inoculum was added to the substrate. The AMF inoculum used is produced by Inoq GmbH with spores from the *Rhizoglomus irregulare strain SAF22* (a mixture of sand and vermiculite (35:65 v/v)). It was added to the autoclaved soil/sand mixture and homogenised using a cleaned concrete mixer in a 5% v/v ratio. For the control inoculum, we used the same substrate, which was previously autoclaved and then sealed in double-layer plastic bags. In a next step, half of the 1.5L pots were filled with 1.7 kg of control and AMF substrate, respectively, to facilitate the irrigation process during the growth phase.

2.1.4 Seeding and Irrigation

Three samples from the control and AMF substrates were weighed and dried for two days to calculate the water content, which was around 5% for both substrates. Due to the duration of the experiment of 60 days, the wheat plants remain in the vegetative state and will not flower (Duncan et al., 2015). Hence, we decided to focus on the vegetative state of the wheat plants and did not vernalise the seeds prior to sowing. The seeds were surface sterilised using 70% ethanol and 5% bleach-tween solution. On July 4th, 2024, each of the 288 pots received six seeds sown at a depth of 2 cm. During the 60-day growth phase, the plants were irrigated with distilled water three times per week. Once a week, each pot (including the plate) was weighed and irrigated to 20% water content, which corresponds to a weight of 2.0 kg. For the other watering process, three pots from each block were selected and weighed. The average weight was calculated, and the rest of the block was watered to 20% water content based on the average weight of these three pots. Thus, the irrigation process was noticeably faster while still ensuring block-specific watering, as blocks near the greenhouse window usually lost more water.

Water content of 20% per pot was calculated as follows:

Weight of pot and plate = 40g

1700g = 5% (as obtained from drying soil)

Total weight before first irrigation: 1740g

(1.74kg / 1.05) * 1.2 = 1,988kg ≈ 2.0kg

2.1.5 Growth Phase

The greenhouse experiment was conducted in a controlled environment at Agroscope (Reckenholz, Zurich, Switzerland). Plants were grown under long-day conditions (16/8 h photoperiod, additional light from sodium-vapour greenhouse lights) and 22/17°C day/night temperature. Since this experiment was conducted during the summer, the actual temperature mostly exceeded 22°C during the day, which made adequate irrigation even more important. For the first two weeks, the plants were grown and sorted by variety to facilitate further steps. Four days after seeding, plants started to germinate; in particular, older variants grew faster than others in the first few days. After ten days, most of the plants germinated. Two weeks after seeding, the seedlings in each pot were reduced to one. To avoid bias and ensure optimal growth conditions, average-sized seedlings, preferably in the middle of pots, were selected for further growth. For the remaining six weeks, the winter wheat plants were arranged and grown in blocks of 36 pots, each containing a control and AMF treatment of every variety (**Fig. 5**). To avoid bias due to greenhouse gradients such as temperature and light availability, blocks were rotated once a week. Additionally, pots within blocks were randomly rearranged after each irrigation process. After three weeks, wooden sticks and wires were used to stabilise the wheat plants and prevent them from hanging down. While growing, some plants died and were replaced by a corresponding plant from extra pots.

After six weeks, the plants were infested by thrips pests. Thrips feeding leads to leaf tissue damage (**Fig. 4**), which can harm photosynthesis efficiency (Zhichkina, Nosov & Zhichkin, 2023). Some blocks were affected more than others, which could have impacted plant growth and development. However, the differences between blocks were not severe.

Figure 4: Left: Impacts of thrips on winter wheat leaves. Right: Bag of Amblyseius predatory mites.

To combat thrips, *Amblyseius predatory mites* (**Fig. 4**) were used to prevent further pest spread. Additionally, plants were sprayed with distilled water in a spray bottle to combat the pest. During the last two weeks of plant growth, thrips damage did not increase strongly. However, the damage caused might have skewed the results to some extent.

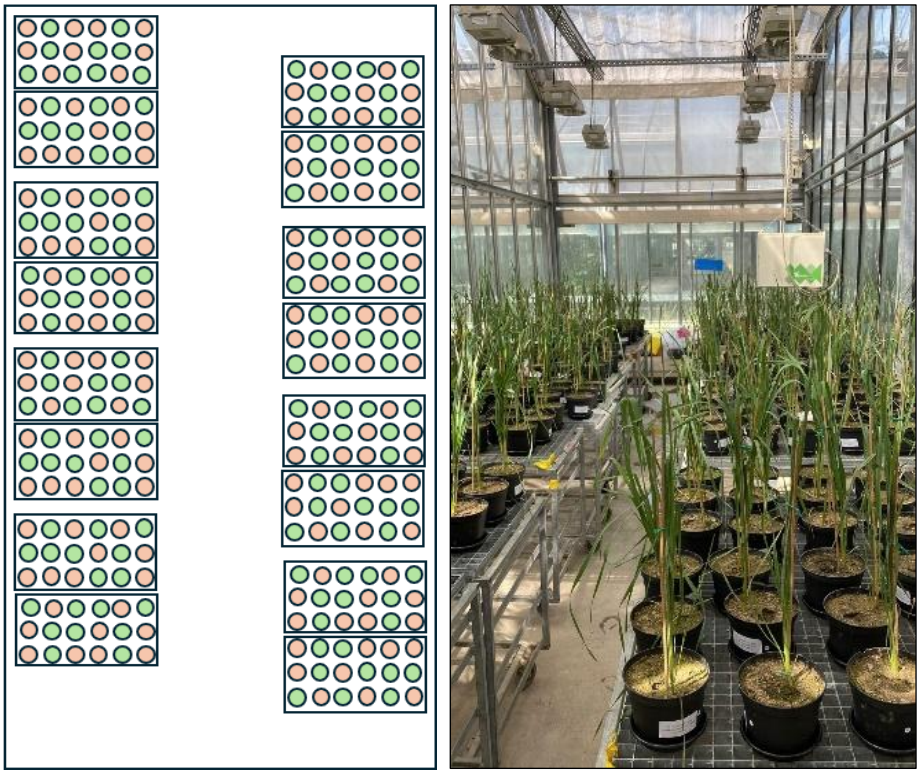

Figure 5: Setup in the greenhouse for Experiment 1. Pots were randomised within blocks (a pair of two tables) and rearranged three times per week. Blocks were rotated every week. Red and green represent control and AMF treatments, respectively.

2.1.6 Harvest and Physiological Parameters

The plants were harvested 60 days after sowing, starting on September 2nd, 2024. Positive, neutral and negative MGR values were observable before harvest (**Fig. 6**). All blocks were harvested within three days. For biomass quantification, each plant was cut with scissors below the lowest node. After that, the fresh shoot weight was determined by a high-precision scale before putting each in a paper bag. Next, the root system and finer roots were collected from the substrate and were then washed clean and dried with household paper. Then, fresh root weight was measured before the roots were cut into 1cm pieces. One part of those root pieces was stored in tubes with 70% ethanol to examine AMF root colonisation under the microscope. Another part was stored in smaller 2ml tubes and freeze-dried for DNA extraction and qPCR analysis (see chapters 2.1.7 & 2.1.8). Remaining roots (often only a small portion around the node) were weighed again before putting them in paper bags as well. Shoots and remaining roots were then dried blockwise at 60°C for two days and weighed again to determine dry shoot and root biomass. The total dry root biomass per sample was calculated by multiplying the dry weight of the small root

subsample by the ratio of total fresh root weight to fresh subsample weight. Every measurement was recorded in detail in an Excel spreadsheet for statistical analysis. Finally, the overall dried biomass was calculated as the sum of dried shoot and dried total root weight for each sample.

The overall dry biomass of all treatments was used to calculate the mycorrhizal growth response (MGR) for each winter wheat variety. This was done by using the following formulas by Köhl, Lukasiewicz & van der Heijden (2016). Every dry biomass AMF treatment was compared to the average dry biomass of the respective control treatments. Depending on the dry biomass weight difference of the AMF treatments compared to the average of the control ones, one of the following formulas was used to calculate the MGR for every AMF treatment.

$$if\ I > C_{mean}, then\ MGR\ (\%) = \left(1 - \left(\frac{C_{mean}}{I}\right)\right) * 100\%$$

$$if\ I < C_{mean}, then\ MGR\ (\%) = \left(-1 + \left(\frac{I}{C_{mean}}\right)\right) * 100\%$$

I = Dry biomass of treatments with mycorrhizal inoculum

C_{mean} = Mean dry biomass of control treatments (average of eight replicates for each variety)

Since each variety has eight AMF replicates, the average was taken to obtain the final MGR for each variety. To check if there is no bias in the result due to unequal conditions across blocks during the experiment, statistical analysis on the mean dry biomass for all blocks was performed (**Fig. S1**; **Fig. S2**, appendix). This included a log-transformed *ANOVA test*, *Shapiro-Wilk Normality test*, and *Levene's Test for Homogeneity of Variance* (**Table S1**, appendix).

Figure 6: Examples of MGR before harvesting. Each picture compares the same winter wheat variety with the control treatment on the left and AMF treatment on the right side. Left: positive effect on Mont-Calme 268, middle: neutral effect on Alpval, right: negative effect on Bonavau.

2.1.7 Root Staining and Microscopy

The wheat roots, conserved in falcon tubes filled with ethanol, were first rinsed with distilled water. (Between each of the following steps, liquids were sucked out with a vacuum, and roots were washed twice with distilled water.) Next, the falcons were filled with potassium hydroxide (KOH, 10% v/v) and were incubated in an 80°C water bath for 20 minutes to clean and prepare them for root staining. An ink-vinegar solution was added to each falcon and incubated in the hot water bath for 20 minutes again. Lastly, 50% glycerine was added to conserve the stained roots.

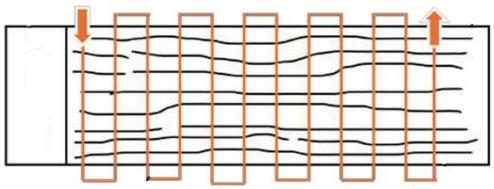

Figure 7: Sketch of a microscopy slide to quantify AMF root colonisation according to McGonigle et al. (1990).

The colonisation rates of AMF are assessed according to a method by McGonigle et al. (1990). Stained wheat roots were aligned parallel on a glass slide with glycerine on it and covered by a cover glass. At 200x magnification, AMF components (arbuscules, hyphae, and vesicles) were quantified for 100 root intersections. The travel direction of the microscope is perpendicular to the roots (**Fig. 7**). The colonisation rates can then be calculated by adding the counted AMF components and then dividing the number of intersections.

2.1.8 DNA Extraction and qPCR Analysis

Winter wheat roots were freeze-dried using a lyophiliser *(VirTis BenchtopK*, USA, New York). Next, roots were ground with glass beads using a FastPrep-24 5G (*MP Biomedicals*, USA, Irvine). From each sample, 20-30mg of root powder was added to a new 2ml for DNA extraction, which was carried out with the NucleoSpin 96 Plant II DNA kit according to a protocol from *Macherey-Nagel* (2024). After extraction, 10 µl of DNA was diluted in 90 µl ddH_2O that was previously added to a PCR plate. DNA concentration was quantified with PicoGreen staining on a Fluorescence Spectrometer (*Cary Eclipse Varian, Agilent Technologies, Inc.*) based on a guide by Valzano-Held (2024). As a last step before qPCR (quantitative Polymerase Chain Reaction), DNA was diluted to 1 ng/µl across all samples using a pipet robot (*Pipetmax 268, Gilson*).

Fungal DNA was quantified using a real-time PCR system (*CFX Opus 384, Bio-Rad Laboratories, Inc.*, USA, Hercules). Triplicates of the samples were pipetted onto a 384-well plate, each consisting of 5 µl of DNA (1 ng/µl) and 5 µl of a mix (2 µl Eva Green 5x HOT FIRE Pol, 2 µl ddH_2O and two primers). Initially, the primers *AMG1F* and *AM1* were used to amplify the *18S rRNA* gene of AMF while a DNA amplification of the host plant was avoided. However, this did not work properly. Therefore, we incorporated strain-specific *SAF22* primers: the forward primer 22KS-F (Bender et al., 2019) and the

reverse primer Alk-R (Alkan et al., 2006), which amplify a 101 base pair DNA fragment. Bodenhausen et al. (2021) validated this method, as results showed strong correlations of root colonisation to quantifications by traditional microscopy.

2.1.9 Statistical Analysis

Statistical analysis of the first experiment was performed with R (4.4.1) and RStudio as the interface. Impacts of AMF inoculation and colonisation were investigated on growth response, shoot-to-root ratio, quality and origin of winter wheat varieties (chapter 3.1). Statistical analysis was performed on 285 samples out of 288. During the experiment, three plants (1x *Alpval* Control, 2x *Plantahof* Control) have died. For every statistical analysis conducted in this study, assumptions were tested, including *Levene's test* for homogeneity of variances and the *Shapiro-Wilk* test for normality of residuals. *Post-hoc* comparisons were performed using *Tukey correction*. Besides one-way and two-way ANOVA models, pairwise *t-tests* with *Bonferroni correction* and *Pearson correlation tests* were performed. Furthermore, *Kolmogorov–Smirnov tests*, PERMANOVA, ANCOVA, and effect size measures (η^2) were applied where applicable. The analysis also includes linear mixed-effects models (LMMs) using the packages "lme4" and "lmerTest" to account for random effects. **Variety** was included as a random factor. ANOVA results showing no significant effect of **Block** (see chapter 3.1.1). Therefore, **Block** was not included as a random factor in the final models to avoid unnecessary model complexity.

As an example, the following LMM was used to evaluate growth response and shoot-to-root ratio change: Growth_response ~ ratio_diff + (1 | Variety_Name)

Detailed statistical tests and results can be found in the appendix (chapter 8).

2.2 Experiment 2

The second greenhouse experiment explores potential synergistic effects of AMF and Mortierella fungi on selected winter wheat varieties from the first experiment (Hypothesis 4). Furthermore, it allows a results comparison from Experiment 1 for selected wheat varieties. Materials and greenhouse conditions are held as similar as possible to Experiment 1.

2.2.1 Winter Wheat Variety Selection

The winter wheat variety selection for the second experiment is based on the results of the first experiment, particularly MGR, origin, and quality. Additionally, recommendations by an internal wheat

expert influenced the decision, as relevant varieties for Swiss agriculture enhance practical applicability. Finally, four varieties were selected for the experiment: *Every*, which had the highest MGR in the first experiment; *Bonavau*, which had the lowest MGR (Chapter 3.1.2); and *Montalbano*, which had a neutral MGR and is highly relevant in Swiss agriculture. It makes up the largest share of wheat areas, with 18.2% of the total area (Strebel, 2024). Lastly, *Campesino* is also included, as it is the largest contributor to feed wheat in Switzerland.

Variety Name	**MGR**	**Quality**	**Origin**
Every	Highest	Other	RWA SZ Edelhof, AUT
Montalbano	Neutral	TOP	Agroscope, CH
Campesino	Low	Feed	Secobra, DE
Bonavau	Lowest	Top	Agroscope, CH

Table 3: Table of winter wheat varieties that have been used for the second greenhouse experiment. These varieties were also used in the first experiment.

2.2.2 Experimental Setup

This experiment contains four different varieties grown with four different treatments. Same as in the first experiment, eight replicates were used, plus an extra replicate as a backup. The same pots were used but cleaned and autoclaved beforehand. However, this time only 144 pots and plates were needed. The experiment included four treatments:

- Control (autoclaved AMF, autoclaved Mortierella)
- AMF (AMF, autoclaved Mortierella)
- Mortierella (autoclaved AMF, Mortierella)
- Dual Inoculation (AMF, Mortierella)

2.2.3 Substrate and AMF Inoculum

The same soil-sand mixture as in the first experiment was used, which was already autoclaved. Again, the same *Rhizoglomus irregulare* strain *SAF22* has been mixed in a 5% v/v ratio, and the same control inoculum (see chapter 2.1.3). However, only 1.6 kg of substrate was used this time to fill the pots to avoid overflooding while irrigating.

2.2.4 Mortierella Inoculum

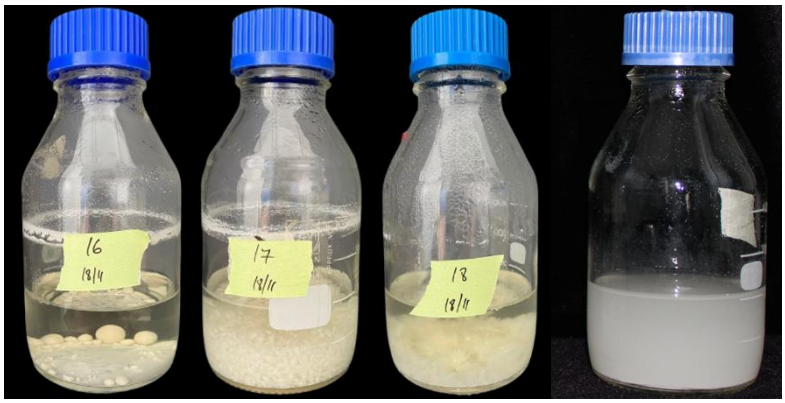

Figure 8: Three Mortierella strains (M. alpina, M. elongata, M. exigua) and a control (right) before mixing them together and inoculation (own caption). Strains could be clearly visually distinguished.

Three Mortierella fungi strains (*M. alpina, M. elongata, M. exigua*) were isolated in November 2023 by the Farming System and Tillage Experiment (FAST) near Agroscope Reckenholz (Zurich, Switzerland). In the laboratory, the Mortierella was cultivated with Pikovskayas Agar[1] in distilled water. Each of the three strains (**Fig. 8**) was grown in eight bottles of 200 ml liquid over 48 hours in a lab shaker (180 rounds per minute). For the control inoculum, four bottles of each strain were autoclaved at 121°C for 20 minutes. Next, both Mortierella treatments have been mixed separately with two kitchen mixers, resulting in 2.4 l of liquid each. On November 29th, exactly two weeks after the wheat seeds had been inoculated, the Mortierella was added to the plants. Some holes were carefully made with a sterilised wooden stick, where each of the 144 pots received 33ml of control or Mortierella liquid.

2.2.5 Seeding and Irrigation

Like the first experiment, we focused on the vegetative state of the plants since they will not flower during a 60-day growth phase. The seeds were prepared according to chapter 2.1.4. However, for this experiment the seeds were not obtained from the Agroscope Reckenholz stock but were instead ordered directly from *Delley semences et plantes* SA (DSP). On November 15th, 2024, each of the 128 received six seeds sown in 2 cm depth. During the 60-day growth phase, the plants were irrigated with distilled water three times per week. Once a week, each pot (including the plate) was weighed and irrigated to 20% water content, which corresponds to a weight of around 1.9 kg. Other watering processes were carried out as in the first experiment. During the second experiment, plants lost significantly less water due to cooler conditions.

[1] https://exodocientifica.com.br/_technical-data/M520.pdf (for further information)

Water content of 20% per pot was calculated as follows:

Weight of pot and plate = 40g

1600g = 5% (as obtained from drying soil)

Total weight before first irrigation: 1640g

(1.64kg / 1.05) * 1.2 = 1,874kg ≈ 1.9kg

2.2.6 Growth Phase

We attempted to keep the conditions in the greenhouse as similar as possible to those in the first experiment (see chapter 2.1.5). This time, the target temperatures were not exceeded. For the first two weeks, the plants were grown sorted by variety to facilitate further steps. Similar to the first experiment, plants started to germinate within 4 days. After a week, most of the plants germinated. We decided to reduce the plant number to three to ensure sufficient root biomass for analysis. To avoid bias and ensure optimal growth conditions, average-sized seedlings, preferably in the middle of pots, were selected for further growth. Two weeks after seeding, holes created by the removal of the plants were used to inoculate Mortierella. For the remaining growth days, the winter wheat plants were arranged and grown in blocks of 16 pots, each containing the four different treatments for every variety (**Fig. 9**). Blocks were rotated once a week, and pots within blocks were randomly rearranged after each irrigation process. Plants grow significantly faster compared to the first experiment. Thus, wooden sticks and wires that were used to stabilise the wheat plants got attached earlier. This time we had no problems with greenhouse pests, and no plant had to be replaced by one of the extra pots, which could be a factor in why the wheat plants grew significantly better within the second experiment.

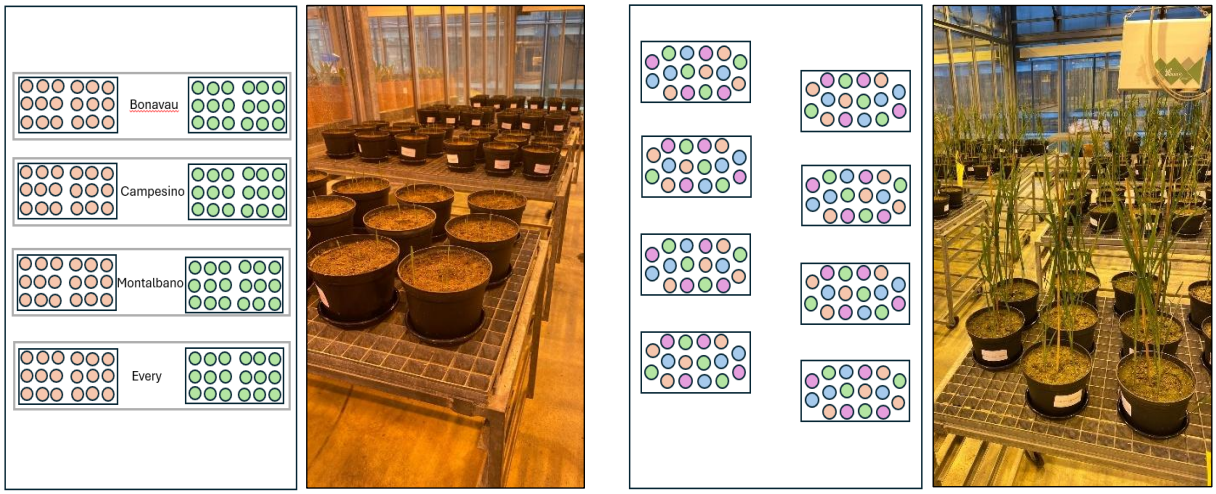

Figure 9: Setup in the greenhouse for Experiment 2. Left: Setup during the first two weeks of growth; right: Setup for the remaining six weeks. Pots were randomised within blocks and rearranged three times per week. Blocks were rotated every week. Red: Control; Green: AMF; Blue: Mortierella; Purple: Dual Inoculation.

2.2.7 Harvest and Physiological Parameters

The plants were harvested on January 14th and 15th, 2025, 60 days after sowing. For biomass quantification, each plant was cut with scissors below the lowest node. The harvest procedure and materials were the same as for Experiment 1 (chapter 2.1.6). However, since we kept three plants per pot, there were significantly bigger root subsamples to dry. Unlike before the harvest of the first experiment, assessing the growth response of treatments by eye was hardly possible (**Fig. 10**). To calculate the total biomass, the dried shoot and root biomass was divided by 3. The overall dry biomass of all treatments was used to calculate the growth response (GR) for the three fungal treatments, using the same formulas below. Each dry biomass fungal treatment for the four varieties was compared to the average dry biomass of the respective control treatments. Depending on their dry biomass weight difference compared to the average of the control ones, one of the following formulas was used to calculate the growth response.

$$if\ I > C_{mean}, then \quad GR\ (\%) = \left(1 - \left(\frac{C_{mean}}{I}\right)\right) * 100\%$$

$$if\ I < C_{mean}, then \quad GR\ (\%) = \left(-1 + \left(\frac{I}{C_{mean}}\right)\right) * 100\%$$

I = Dry biomass of treatments with fungal inoculum

C_{mean} = Mean dry biomass of control treatments (average of eight replicates for each variety)

Since each variety has eight replicates, the average was taken to obtain the final GR of the three fungal treatments for each variety. Again, to check if there is no bias in the result due to unequal conditions across blocks during the experiment, statistical analysis on the mean dry biomass for all blocks was performed (**Fig. S8**; **Fig. S9**, appendix), which included a log-transformed *ANOVA test*, *Shapiro-Wilk Normality test*, and *Levene's Test for Homogeneity of Variance* (**Table S6**, appendix).

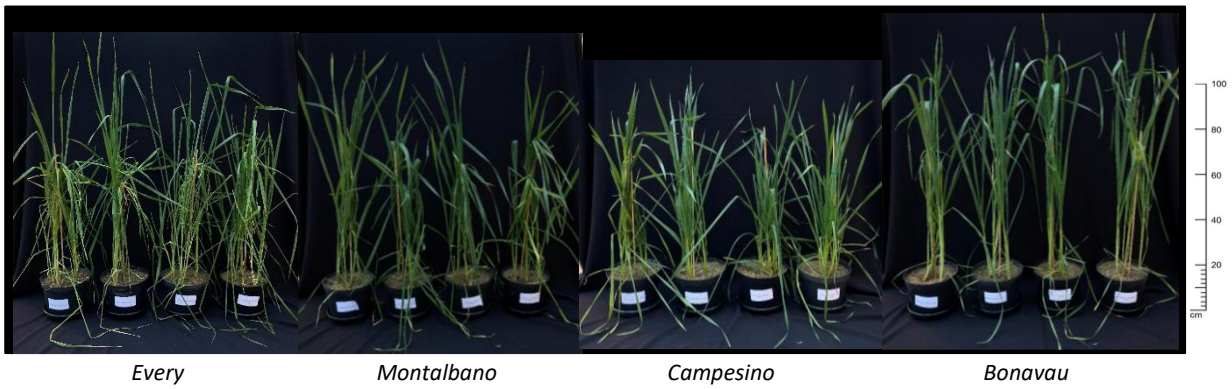

Figure 10: Block 2 before harvest. Each picture compares the same winter wheat variety with the control, AMF, Mortierella, and dual inoculation from left to right.

2.2.8 Root Colonisation and Statistical Analysis

Root colonisation of AMF and Mortierella was investigated via root staining according to chapter 2.1.7. Due to the limited time frame of one year to conduct my master's thesis, qPCR was not performed for the second experiment.

The focus of the second experiment was to compare and verify the result of the first experiment and to investigate synergistic effects between AMF and Mortierella. Thus, the analytical approach was similar to that in chapter 2.1.9, where all statistical tests and packages also used for this experiment can be found. All 128 samples were incorporated into the analysis.

Again, **Block** was treated as a random factor, as ANOVA results showed no significant effects. Additional results can be found in the appendix (chapter 8).

The following LMM was used to model the synergistic effects of AMF and Mortierella: Growth_Response ~ Inoculum + (1 | Variety_Name).

2.3 Soil Analysis

Parameter	Method	Unit	Value	Standard values for arable soils (Switzerland)
Calcium	Soil CEC Cations	cmol+/kg	6.85	5-15
Corg	Soil Organic Carbon (Corg)	Mass-%	3.795	1-3
Humus	Soil Organic Carbon (Corg)	Mass-%	6.545	1.7-5.1
Potassium	Soil CEC Cations	cmol+/kg	0.18	0.2-0.6
Potential CEC	Soil CEC	cmol+/kg	8.0	5-15
Base Saturation	Soil CEC	Mass-%	93.75	70-90
Magnesium	Soil CEC Cations	cmol+/kg	0.42	0.5-2
Sodium	Soil CEC Cations	cmol+/kg	0.05	<0.1
Phosphorus	Soil Total P	mg/kg DS	603.77	30-150
pH Value	Soil pH H_2O	-	7.08	6-7.5
Nitrogen	Soil Total N	Mass-%	–	0.1-0.4 %
Hydrogen	Soil CEC H	cmol+/kg	0.5	Low, the value is reasonable
S-Value	Soil CEC	cmol+/kg	7.5	6 - 16

Table 4: Results from soil analysis that has been mixed with sand as the substrate for both experiments. Standard values for Swiss arable soils were obtained by Richner et al. (2017).

CEC = Cation Exchange Capacity
cmol+ = centimoles of charge per kilogram
DS = Dry Soil

As mentioned in chapter 2.1.3, the substrate for both greenhouse experiments was a 50:50 soil/sand mixture. Soil parameters from a grassland site near Agroscope Reckenholz were analysed (**Table 4**). Since the soil was mixed with sand, mass-dependent parameters such as nutrient contents were halved. Unfortunately, N content could not be quantified.

Standard values for Swiss arable soils were obtained from the book "Basic principles for the fertilisation of agricultural crops in Switzerland" by Richner et al. (2017). While soil organic carbon (SOC) is rather high, potassium, calcium and magnesium are on the lower end, which explains the relatively low sum of the base cations (S-value). However, phosphorus content was very high compared to the standard value.

3 Results

3.1 Results of Experiment 1

Experiment 1 revealed a large variability in biomass and MGR between tested winter wheat varieties. MGR was mostly negative and was significantly influenced by the origin of varieties. Generally, AMF inoculation led to enhanced shoot-to-root ratios for most varieties.

3.1.1 Overall Dry Biomass

The biomass log-transformed *ANOVA test* ($F_{(7,277)} = 1.758$, $P = 0.0957$) confirms that there were no significant differences between blocks, indicating no spatial effects. The *Shapiro-Wilk Normality Test* ($w = 0.9881$, $p = 0.01909$) reveals a slight deviation from a normal distribution for all samples (**Fig. S2**, appendix). However, the large sample size ($n = 285$) mitigates this problem, as ANOVA is generally robust to minor violations of normality. Finally, *Levene's Test for Homogeneity of Variance* ($F_{(7,277)} = 0.9827$, $P = 0.4441$) confirms the homogeneity of variance (**Table S1**, appendix). Although there is a slight deviation from normality, ANOVA assumptions were met. Hence, it is reasonable to continue with the analysis without concern about spatial bias during the experiment.

There were large differences in mean biomass across the 18 different winter wheat varieties (**Fig. 11**). Overall mean dry biomass across all samples was 1.48 grams. *Every* had the highest mean biomass in both treatments with around two grams of dry biomass, while *Cadlimo* had the lowest with around one gram each. One can observe tendencies that the biomass of AMF treatments seems to get lower compared to the control treatment for the respective variety as mean biomass in general decreases. This will be further analysed in MGR correlation results (chapter 3.1.3.1). Control treatments have a higher mean biomass for most varieties. However, *t-tests* for each variety revealed that only *Bonavau*, *Emblem*, and *Arina* had a significantly lower mean biomass in the AMF treatment ($p < 0.05$). The other 15 varieties showed no statistically significant differences between treatments. After applying the *Bonferroni correction*, only *Bonavau* ($p = 0.0232$) has a significant difference for treatments of mean dry biomass (**Table S2**, appendix).

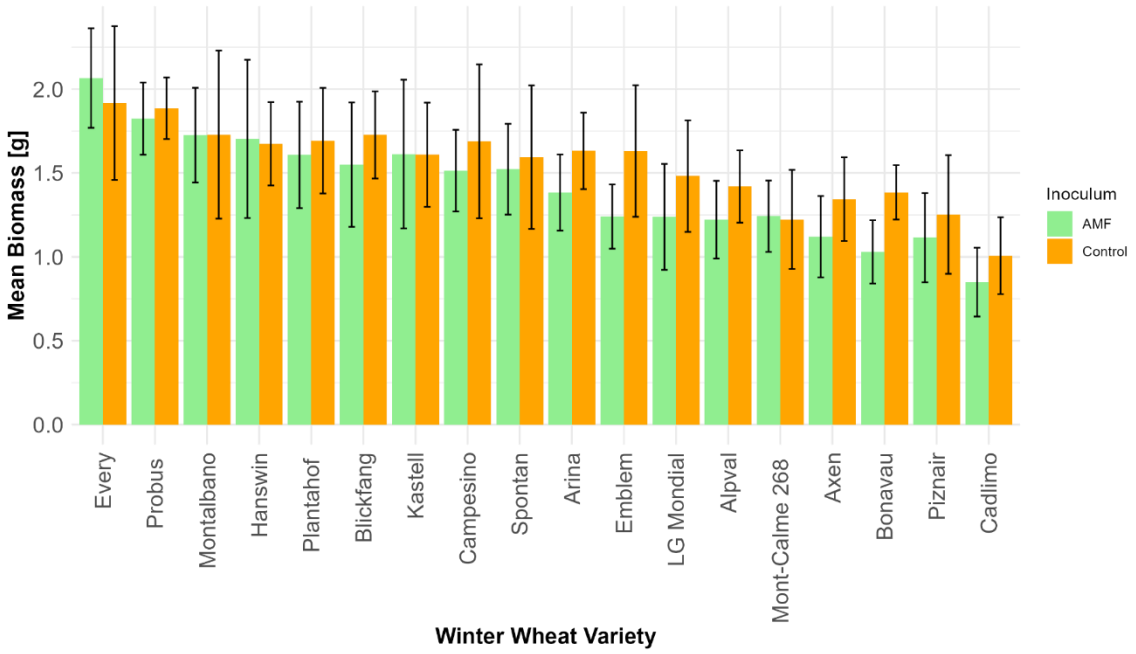

Figure 11: Bar chart of mean dry biomass of different winter wheat varieties sorted by mean biomass, including control and AMF treatments in orange and green, respectively (n = 8). Standard errors (SE) are presented as black bars.

In terms of mean dry biomass for different quality classes, the *TOP* class was notably lower, while other classes are similar. Differences for origins were more pronounced with *RWA SZ Edelhof*, *AUT*, being the highest (**Fig. S3**, appendix). A *two-way ANOVA* (Overall_Biomass ~ Quality * Origin) reveals that both Quality and Origin had a significant impact on dry biomass (Quality, $F_{(5,274)} = 10.20$, $p = 5.62e\text{-}09$; Origin, $F_{(5,274)} = 11.74$, $p = 2.69e\text{-}10$) (**Table S3**, appendix). Origin ($\eta^2 = 0.18$) could explain slightly more variance than Quality ($\eta^2 = 0.16$). Classes in both cases are not represented equally with the number of samples; some classes included only n = 16.

3.1.2 Mycorrhizal Growth Response

Mycorrhizal Growth Response (MGR) was assessed as mentioned in the methodology part (chapter 2.1.6). Contrary to **Hypothesis 1** (chapter 1.6), only *Every* and *Mont-Calme 268* had a positive MGR, while the other 16 winter wheat varieties had a negative one. MGR responses ranged over 30%, from positive 5.83% to -25.63% (**Table S4**, appendix). Mean MGR across all samples was -9.7%. In the following pages, these results will be analysed in terms of quality and origin classification. Furthermore, potential correlations between dry biomass and MGR will be assessed and a potential correlation between AMF colonisation and MGR.

A *two-way ANOVA* (MGR ~ Quality * Origin) was used to determine the impact of both Origin and Quality as factors (**Fig. 12**; **Fig. S4**, appendix). (Quality, $F_{(5,133)} = 1.362$, $p = 0.2428$; Origin, $F_{(5,113)} = 2.780$, $p = 0.0202$) (**Table S3**, appendix). Origin had a significant impact on MGR, while Quality had not, supporting **Hypothesis 3** (chapter 1.6). Quality explained 5% of variance ($\eta^2 = 0.05$), while Origin could explain 9% ($\eta^2 = 0.09$). This trend is illustrated in **Fig. 12**, where different colours tend to be more clustered on the right side.

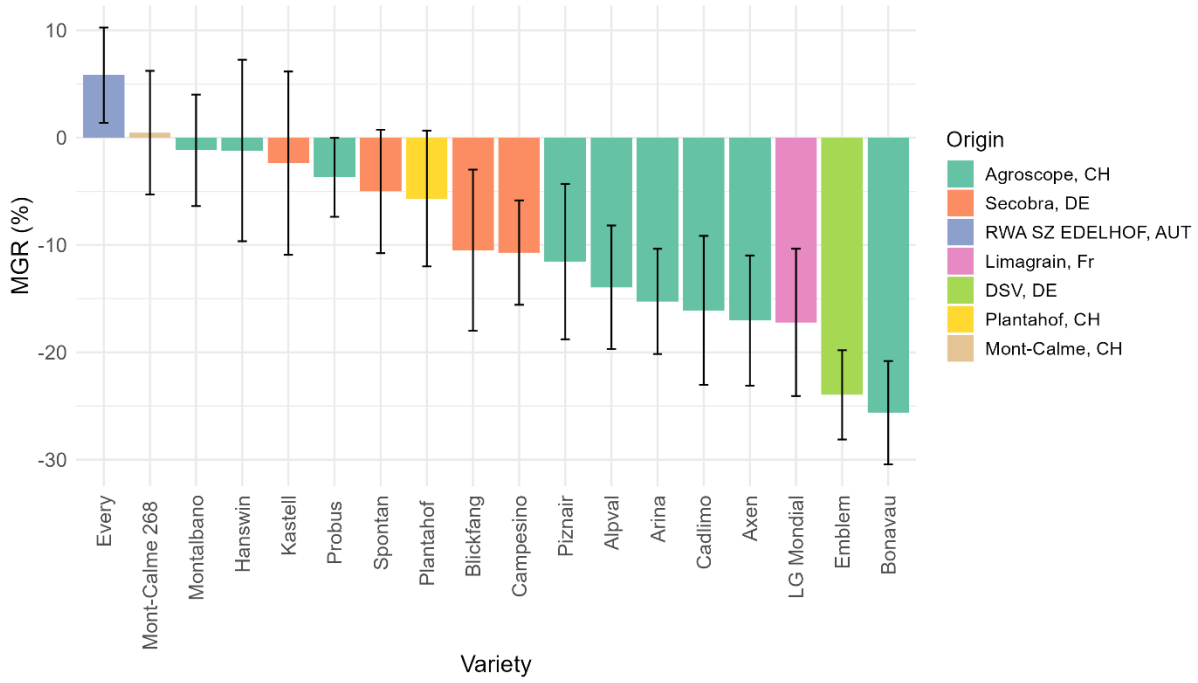

Figure 12: Bar chart of MGR values for winter wheat varieties coloured by origin (n = 8). Two out of 18 varieties show positive MGR values. Standard errors (SE) are displayed as black bars.

Fig. 13 further highlights the larger differences between MGR values for different origins compared to different quality classes. Since Quality classes *TOP, I & II* have Agroscope as a breeder, one can observe an MGR increase independently of the origin, supporting **Hypothesis 2** (chapter 1.6). This hypothesis is further strengthened by relatively high MGR values for class *Old*. The '*Other'* class showed the highest variance, indicating a large potential diversity in terms of quality between varieties (**Fig. 13**).

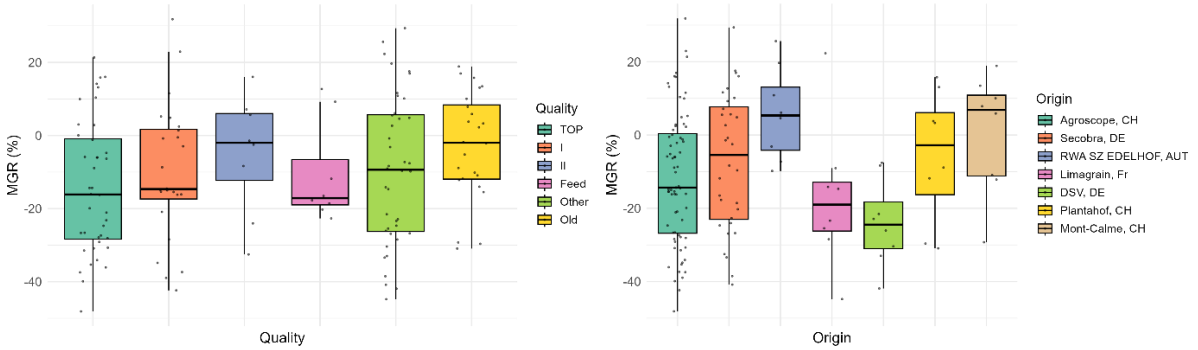

Figure 13: Whisker plots of MGR by quality (left) and origin (right) (n = min. 8). Samples are displayed as black dots. The differences were only significant for origin in the right plot.

3.1.3 Shoot-to-Root Ratio

14 out of 18 varieties had a positive shoot-to-root ratio (S:R ratio) difference, meaning for the same amount of root dry biomass, more shoot dry biomass was produced in most varieties. Thus, AMF tend to enhance the S:R ratio, meaning that the same shoot biomass could grow with fewer roots. The approach, including the formulas, was the same as for MGR (see chapter 2.1.6). Only *Alpval*, *Cadlimo*, *LG Mondial*, and *Probus* had a lower mean S:R ratio of AMF treatments compared to the control average. Overall, differences ranged from +24.4% (*Spontan*) to -7.56% (*Alpval*) (**Fig. 14**), with the mean S:R ratio difference across all samples being +5.11%.

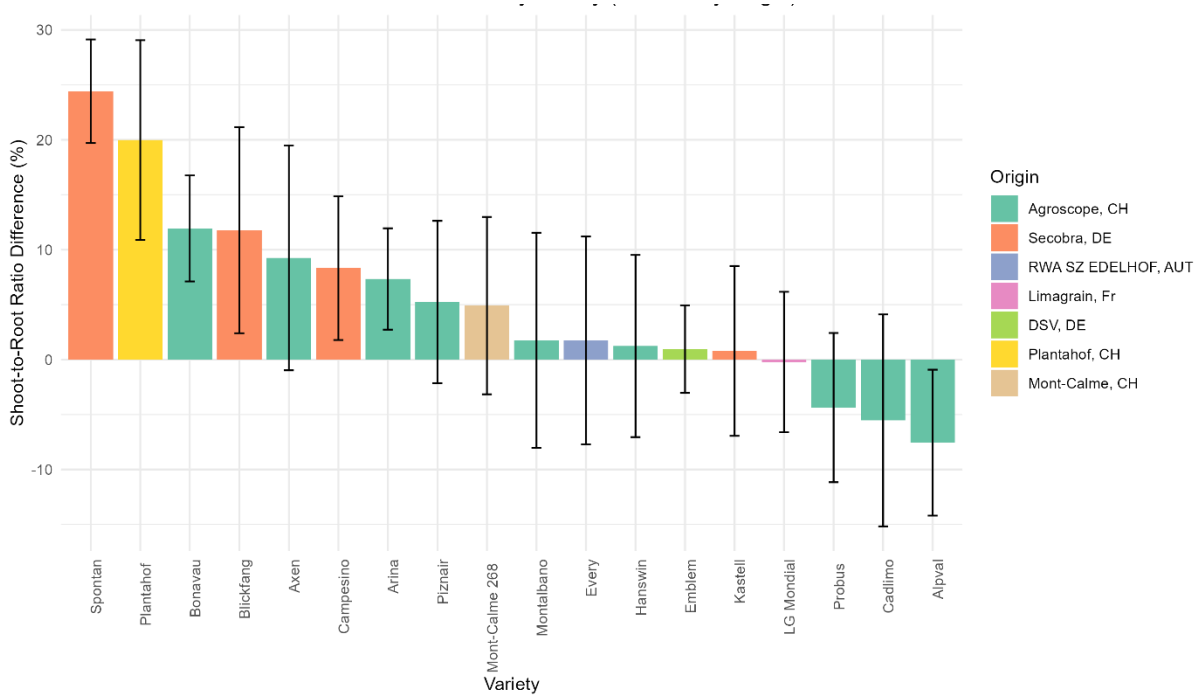

Figure 14: Barplot showing the percentual differences of the mean shoot-to-root ratio of AMF treatments compared to control treatments for origin (n = 8). SE are represented as black bars. Most varieties showed an S:R ratio increase while AMF inoculated.

Unlike for MGR, neither Quality nor Origin had a significant impact on the S:R ratio (Shoot_Root_Ratio ~ Quality * Origin). Quality ($F_{(5,133)} = 1.659$, $p = 0.149$) could explain slightly more variance than Origin ($F_{(5,133)} = 1.164$, $p = 0.330$) (**Table S3**, appendix). Furthermore, no clear statements about differences between older and modern varieties could be made (**Fig. S5**, appendix).

3.1.4 AMF Root Colonisation Rates

3.1.4.1 Root Staining and Microscopy

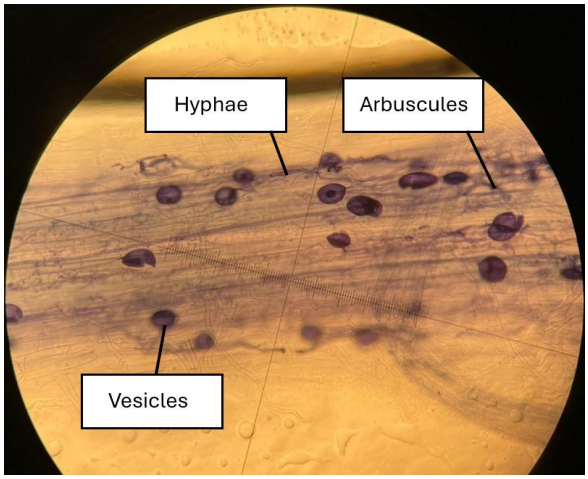

Figure 15: Own caption of Every root under the microscope, colonised by AMF. Switch from 200x to 100x magnification just for the caption.

AMF colonisation was confirmed by the root staining and microscopy (200x magnification) method according to McGonigle et al. (1990). Root colonisation was calculated by dividing the number of intersections showing at least one AMF component (arbuscules, hyphae, vesicles) (**Fig. 15**) by total intersections. AMF colonisation ranged from 10% to 30%. A total of 16 samples were examined, including three AMF samples from *Every*, *Montalbano*, *Campesino* and *Bonavau*. Additionally, one control sample was investigated from each of the four varieties to confirm that there is no AMF colonisation for control samples.

3.1.4.2 qPCR

We used specific *SAF22* primers for qPCR analysis (**Fig. 16**). Three samples were excluded from this analysis due to unreasonably high copy numbers (>250), which deviated strongly from all other values: (Sample 46, *Montalbano*; Sample 94, *Hanswin*; Sample 110, *Alpval*).

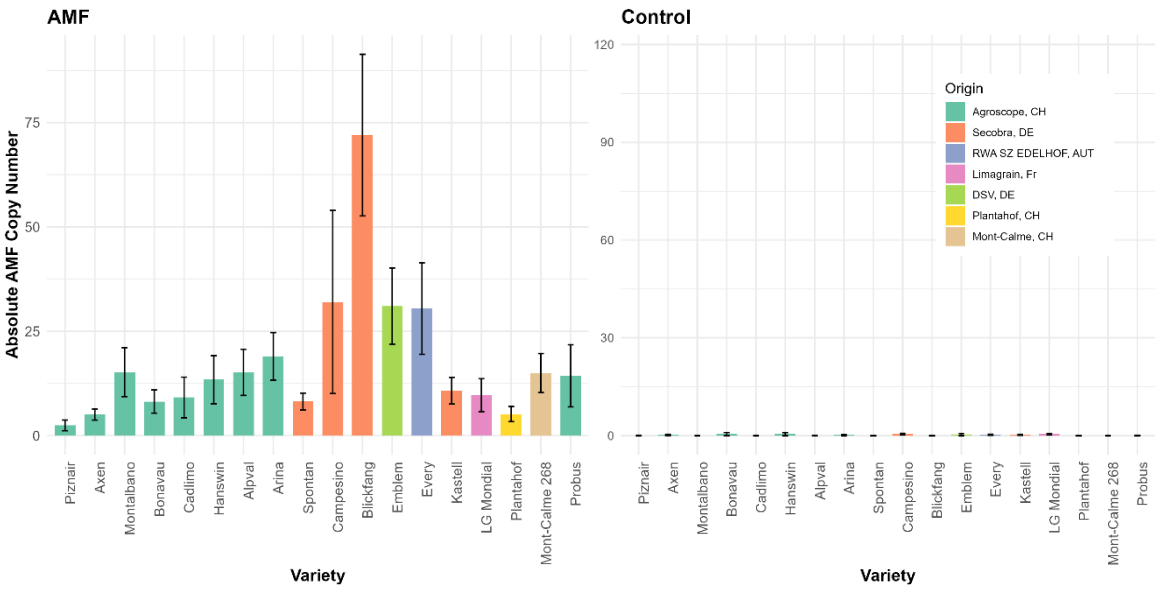

Figure 16: Bar plots of absolute AMF copy number/ng DNA for all varieties (n = 8). Varieties are coloured by origin; SE are included as black lines. All varieties showed visibly higher copy numbers when inoculated.

As expected, absolute *SAF22* copy numbers per ng DNA were visibly higher for inoculated samples compared to control samples. Overall colonisation was low, with values ranging between 5 and 75 copy numbers per ng of DNA. There were no significant differences in copy numbers between varieties (ANOVA: Variety, $F_{(17,114)} = 1.203$, $p = 0.273$). *T-tests* reveal that not all varieties show significant differences compared to respective control groups (**Table S5**, appendix).

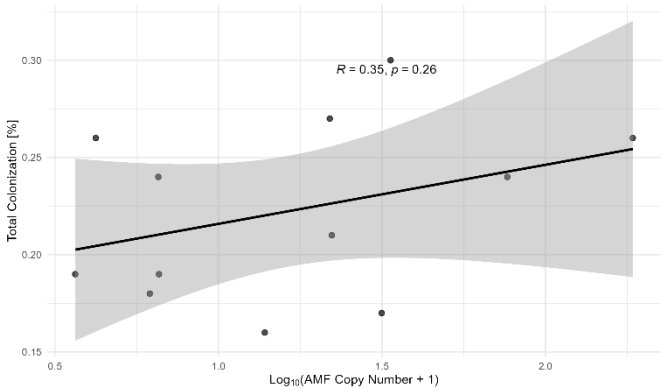

Figure 17: Scatter plot with a linear trend line showing the correlation between microscopy and qPCR results. The x-axis was log_{10} transformed. The +1 is to prevent undefined log (0) values and to visualise samples with a value of 0.

Contrary to the findings of Bodenhausen et al. (2021), no significant correlation ($p = 0.26$, $R = 0.354$) was found between total colonisation rates by microscopy and absolute copy numbers from qPCR (**Fig. 17**).

3.1.5 Mycorrhizal Growth Response Correlations

3.1.5.1 Mycorrhizal Growth Response and Biomass

Fig. 18 illustrates the mean control biomass of each variety on the x-axis and the biomass gain or loss of the respective AMF samples on the y-axis. Although the positive correlation was not statistically significant (**p = 0.0854**), varieties with higher control biomass tended to show higher MGR values (**Fig. 11**). Hence, varieties with an overall higher biomass also potentially had enhanced (or less negative) MGR values.

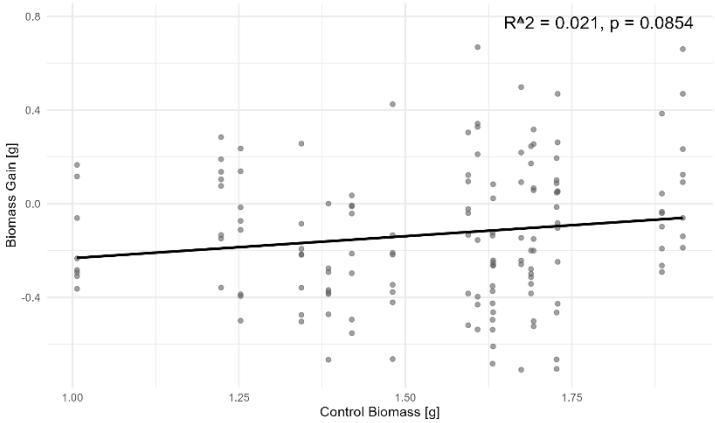

Figure 18: Scatter plot displaying a linear regression of biomass gain respective to the mean control biomass of each variety. Mass gain or loss of inoculated samples of specific varieties are displayed vertically as grey dots.

3.1.5.2 Mycorrhizal Growth Response and Root Colonisation

Root colonisation with microscopy was assessed according to chapter 2.1.7. Only the four winter wheat varieties included in the second experiment were investigated with microscopy. All varieties were examined with qPCR, which is described in the following paragraph. Although there were large MGR differences ranging from -50% to +25%, root colonisation rates were relatively consistent, between 16% and 30% (**Fig. S6**, appendix).

Thus, no clear correlation can be concluded between MGR and AMF colonisation (*Pearson's product-moment correlation*: p = 0.694, R = 0.127). Additionally, three control samples from the four varieties were observed to exclude a potential AMF colonisation for control samples. No AMF components were seen for control samples.

Interestingly, MGR to absolute SAF22 copy numbers revealed a significant positive correlation across all samples assessed by qPCR ($p < 0.01$, $R = 0.29$) (**Fig. 19**). This reflects the slight positive trend from microscopy. However, a correlation test between MGR and absolute copy numbers from qPCR results is more meaningful since the sample size is much larger (n = 131). The same plot coloured by origin can be found in the appendix (**Fig. S7**, appendix).

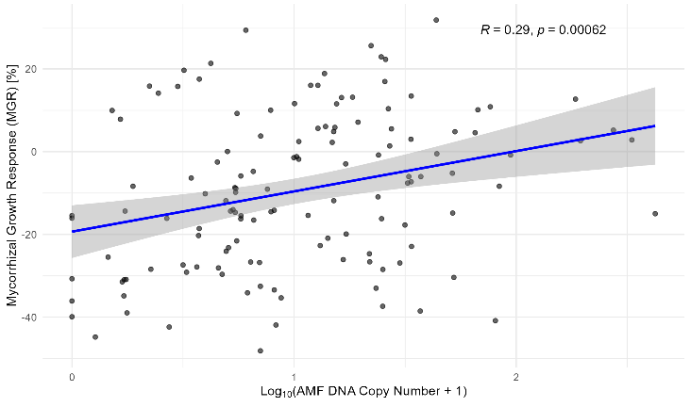

Figure 19: Scatter plot with a linear trend line showing the correlation between SAF22 copy number per ng DNA and MGR. The x-axis was log_{10} transformed. The +1 is to prevent undefined log (0) values and to visualise samples with a value of 0.

3.1.5.3 Mycorrhizal Growth Response and Shoot-to-Root Ratio Difference

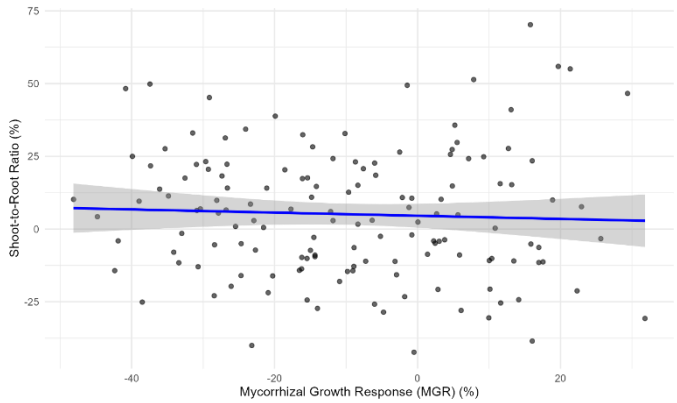

Figure 20: Scatter plot displaying the correlation between MGR and shoot-to-root ratio change of inoculated samples for all varieties.

The analysis for the correlation between MGR and S:R ratio difference of inoculated samples to respective control sample means was performed again with a *Pearson's product-moment correlation.* Since both parameters were conducted with the same method (see chapter 3.1.3), a large sample size of 144 was used. Both parameters compared each AMF sample to the respective control mean of each variety. Results revealed no significant correlation between MGR and S:R ratio changes ($p = 0.5899$, $R = -0.0452$) (**Fig. 20**).

3.2 Results of Experiment 2

Results of the second experiment displayed similar trends in MGR and S:R ratio as observed in the first experiment. Dual inoculation led to inconstant growth benefits. Biomass and S:R ratio were negatively correlated, indicating variety-inherent resource allocation strategies.

3.2.1 Overall Dry Biomass

The biomass log-transformed *ANOVA test* ($F_{(7,120)} = 0.925$, $p = 0.49$) confirmed no significant differences between blocks in terms of mean biomass. The *Shapiro-Wilk Normality Test* ($w = 0.9885$, $p = 0.3627$) revealed that there was no significant deviation from a normal distribution for all samples (**Fig. S9**, appendix). Finally, *Levene's Test for Homogeneity of Variance* ($F_{(7,120)} = 0.6483$, $p = 0.7151$) validated homogeneity of variance (**Table S6**, appendix). Again, potential conditional variations in the greenhouse did not influence plant growth across blocks, which justifies treating **Block** as a random factor for further analysis.

The overall mean dry biomass across all samples was 2.3 grams, notably higher than the previous experiment. Same as Experiment 1, *Every* again had the highest dry biomass. No treatments had statistically significant effects on overall biomass for all varieties (**Fig. 21**). A comparison of whether

the quality or the origin has a greater influence on the biomass was not carried out, as the varieties have the same breeder for each quality and vice versa. For that reason, the focus of the analysis was the effect of origin and inoculum.

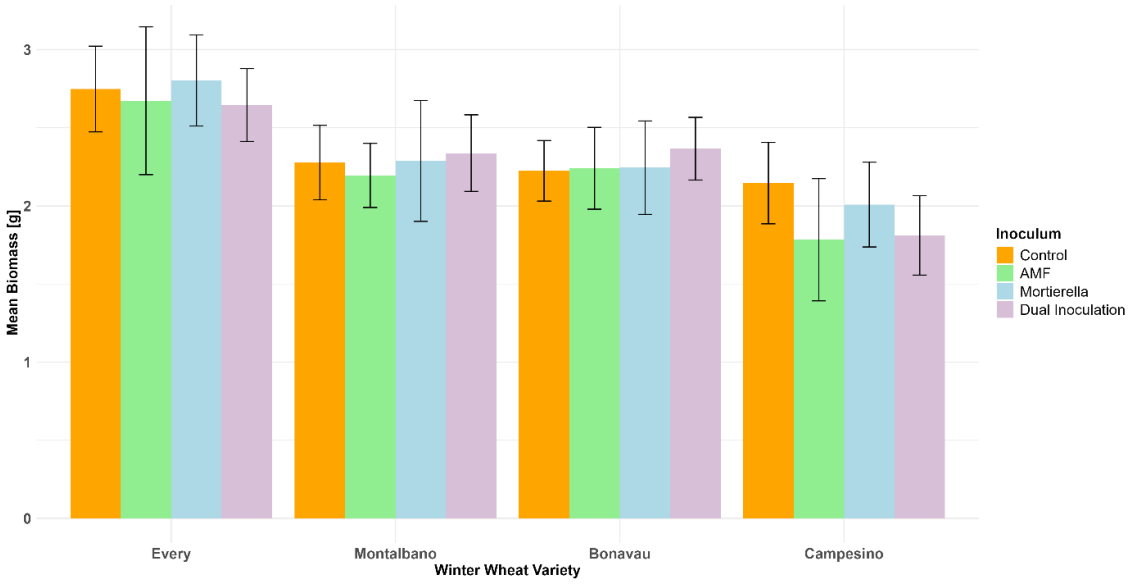

Figure 21: Bar chart of mean dry biomass of different winter wheat varieties sorted by mean biomass including different treatments (n = 8). SE are represented as black bars. No treatment led to significant biomass changes for all varieties.

3.2.2 Growth Response

Growth Response (GR) for all treatments was calculated the same as MGR according to chapter 2.1.6 in the methodology part. GR responses ranged from +5.51% for *Bonavau*, dual inoculation, and -17.07% for *Campesino* (AMF) (**Fig. 22**) (**Table S9**, appendix). Mean GR across all samples was -3.53%. However, *t-tests* show that treatment significantly differed from the control treatment with adjusted p-values for all varieties (**Table S7**, appendix). On the next pages, these results will be further analysed in origin and inoculum classification.

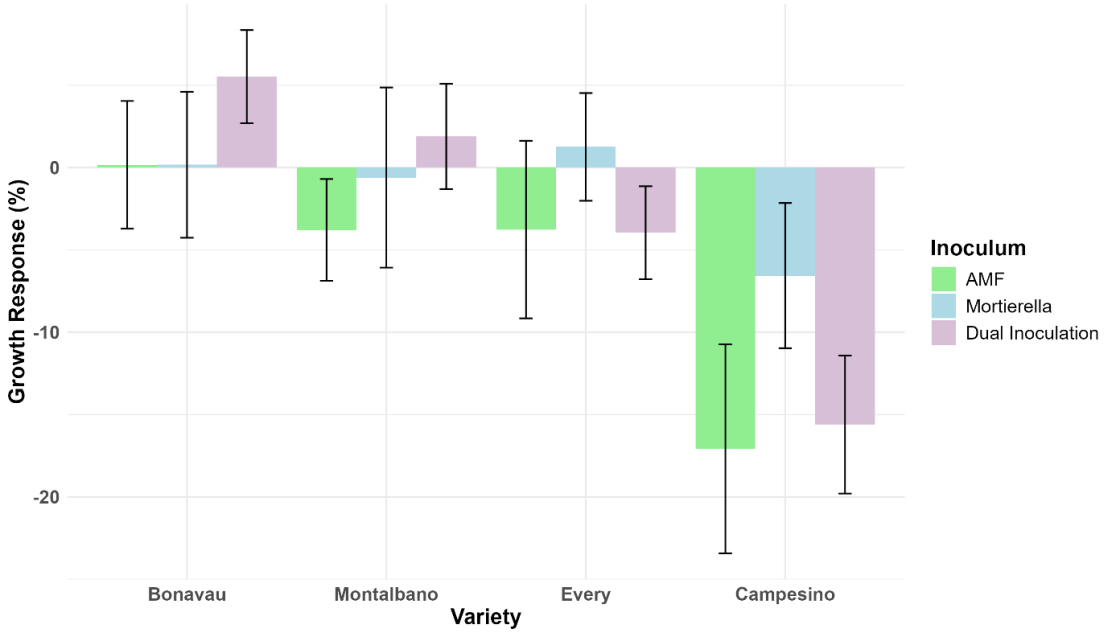

Figure 22: Bar chart of MGR values for winter wheat varieties coloured by treatment (n = 8). Black bars represent the standard error. No treatments showed statistically significant differences from the control mean for any variety.

A *two-way ANOVA* was used to determine the impact of both Origin (Origin, $F_{(2,87)} = 4.6092$, $P = 0.01251$, $\eta^2 = 0.20$) and Inoculum (Inoculum, $F_{(2,87)} = 0.9134$, $P = 0.40498$, $\eta^2 = 0.03$) as factors on growth response (**Fig. 23**). Origin had a strong impact on GR, while Inoculum had not, further supporting **Hypothesis 3**. No strong dependency between the factors was observable (Origin:Inoculum, $F_{(4,87)} = 0.98$, $p = 0.422$). (**Table S8**, appendix). This trend is illustrated in **Fig. 23**, as GR differences are more pronounced in the left graph.

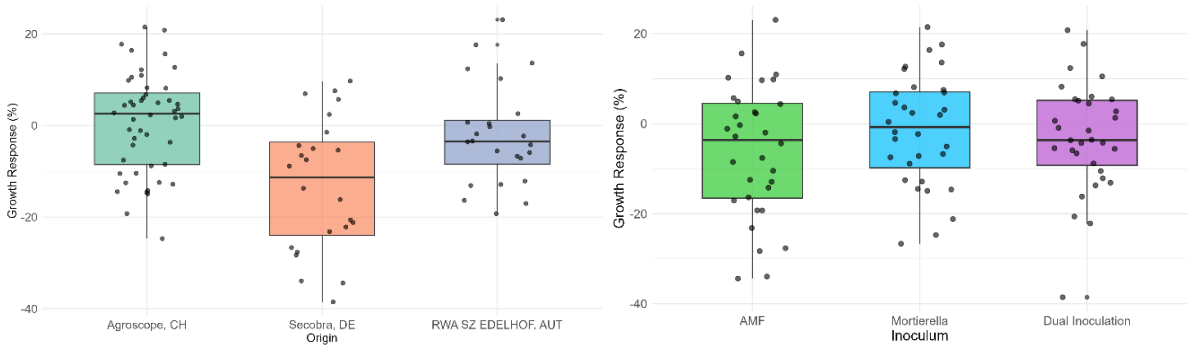

Figure 23: Whisker plots of MGR by origin (left) (n = min. 24) and inoculum (right) (n = 32). Samples are displayed as black dots. The differences were only significant for origin in the right plot.

3.2.3 Shoot-to-Root Ratio

11 out of 14 varieties with different treatments had a positive S:R ratio change compared to their average respective control treatments, meaning for the same amount of root dry biomass, more shoot dry biomass was grown (**Fig. 24**). Notably, AMF especially tend to enhance the S:R ratio.

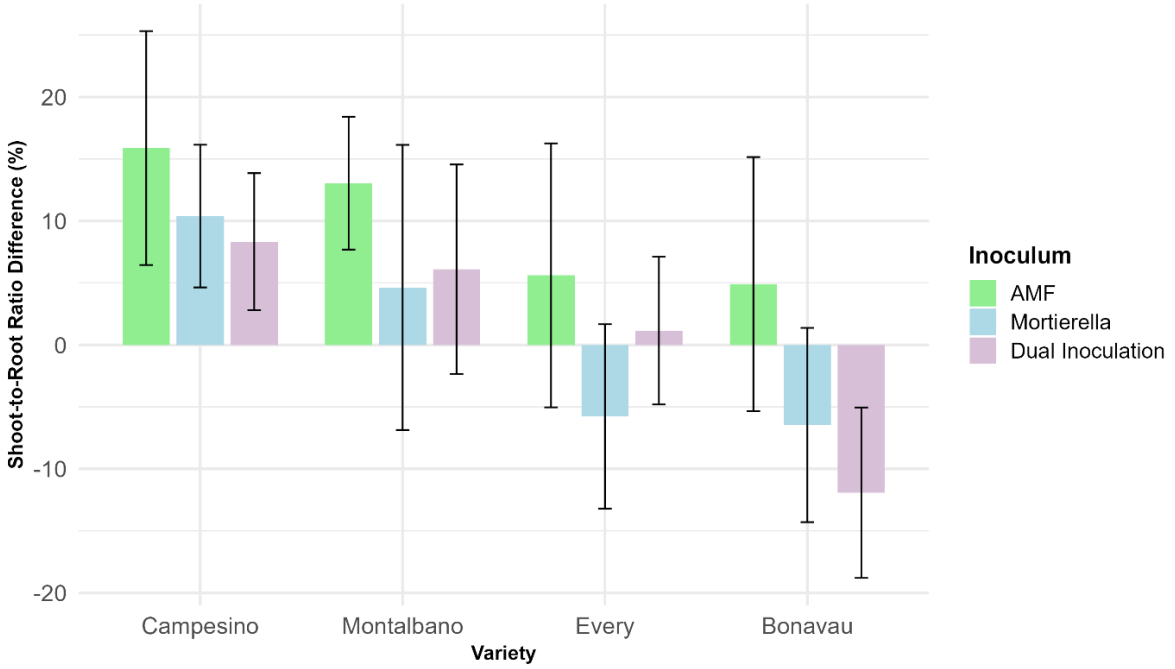

Figure 24: Bar plot showing the percentual differences of mean shoot-to-root ratio of inoculum and varieties compared to the respective control treatment.

A *two-way ANOVA* was used to determine the impact of both origin (Origin, $F_{(2,115)} = 2.0457$, $P = 0.1340$) and inoculum (Inoculum, $F_{(3,115)} = 1.7293$, $P = 0.1649$) on S:R ratio. Although neither factor has a significant impact, the LMM (shoot_root_ratio ~ Inoculum + Origin + (1 | Variety_Name) reveals that AMF had a significant effect on the S:R ratio ($p = 0.00758$) (**Fig. 25**), while every other treatment or origin did not (**Fig. S11**, appendix).

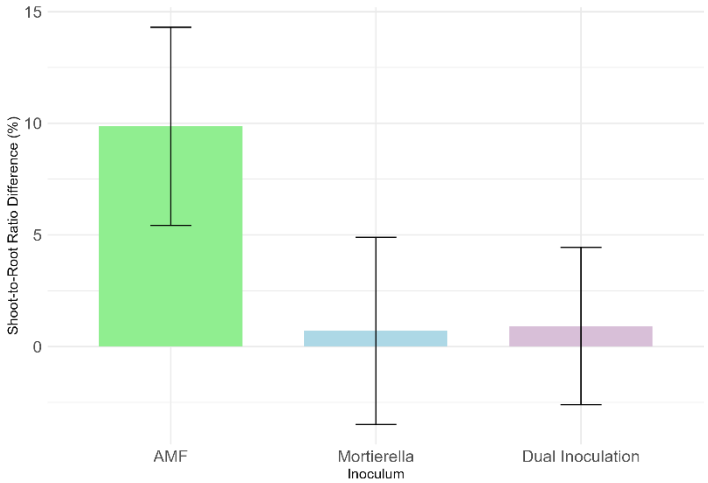

Figure 25: Bar plot showing the percentual differences of the mean shoot-to-root ratio of inoculum for all samples (n = 32). SE are indicated as black bars.

3.2.4 Fungal Colonisation

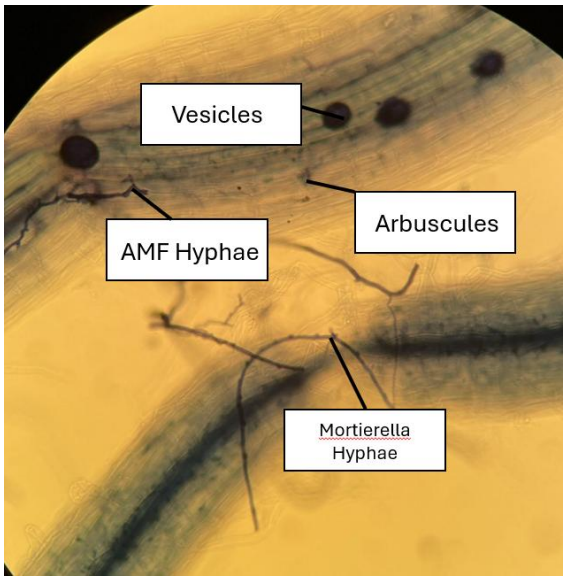

Figure 26: Own caption of Campesino root under the microscope, colonised by AMF and Mortierella (100x magnification).

AMF colonisation was confirmed by root staining and microscopy method as described by McGonigle et al. (1990). Total root for both fungal colonisations was calculated by dividing intersections with at least one AMF component (arbuscules, hyphae, vesicles) or Mortierella hyphae by total intersections. AMF colonisation ranged from 29 to 54%, noticeably higher than in Experiment 1. One block of samples was investigated, which includes each treatment for every variety. Unfortunately, few Mortierella hyphae that are characterised as larger with dark septate were visible under the microscope (**Fig. 26**).

Due to a limited time frame for this master's thesis, qPCR analysis was not performed for the second experiment. However, since AMF colonisation rates are significantly higher, larger copy numbers would be expected in qPCR results.

3.2.5 Growth Response Correlations

3.2.5.1 Growth Response and Biomass

Fig. 27 shows the mean control biomass of the four varieties on the x-axis and the biomass gain or loss for the respective treatment samples. Despite there being no significant positive correlation, a single fungal inoculation of AMF and Mortierella appeared to be more beneficial for wheat varieties with higher control biomass. Growth response for a dual inoculation of both fungi led to even GR unrelated to control biomass.

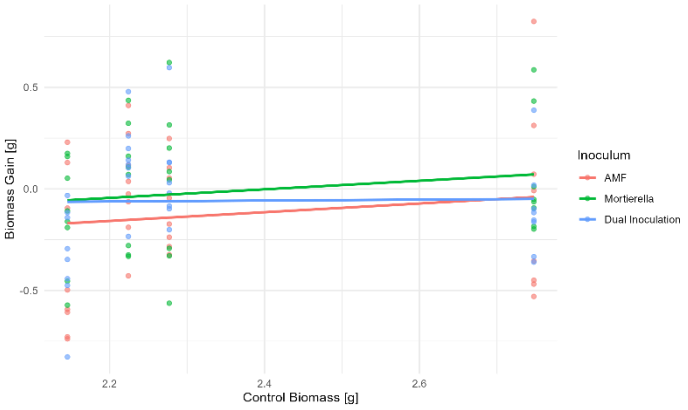

Figure 27: Scatter plot displaying a linear regression of biomass gain respective to the mean control biomass of each variety and treatment.

3.2.5.2 Growth Response and Root Colonisation

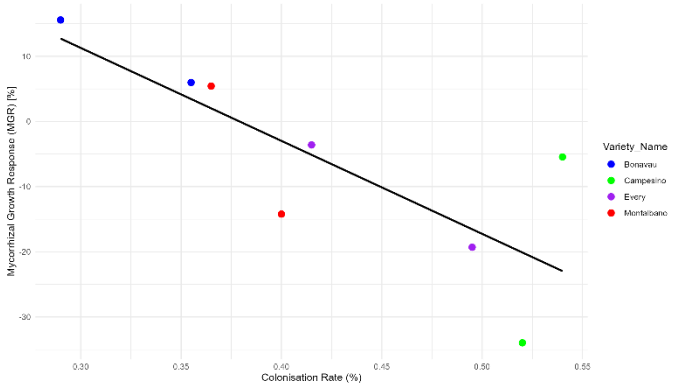

Figure 28: Scatter plot displaying a linear regression of MGR and root colonisation for Bonavau (blue), Campesino (green), Every (purple) and Montalbano (red) (n = 8).

Root colonisation with microscopy was assessed according to chapter 2.1.7. Since Mortierella could not be adequately observed, only one AMF and one dual inoculation sample for each variety were investigated (**Fig. 28**). Contrary to findings from the first experiment, higher root colonisation in this case correlated with lower growth responses (*Pearson's product-moment correlation:* R = -0.792). Again, no fungal components were found in control samples for all varieties.

3.2.5.3 Growth Response and Shoot-to-Root Ratio Difference

Fig. 29 illustrates a strong negative correlation between S:R ratio increases and growth response for all three fungal treatments. The difference between shoot-root ratio and growth response is calculated by comparing the shoot-root ratio or biomass and comparing it with the mean control values for each variety. In total, 96 samples were used for this analysis since 32 are control samples.

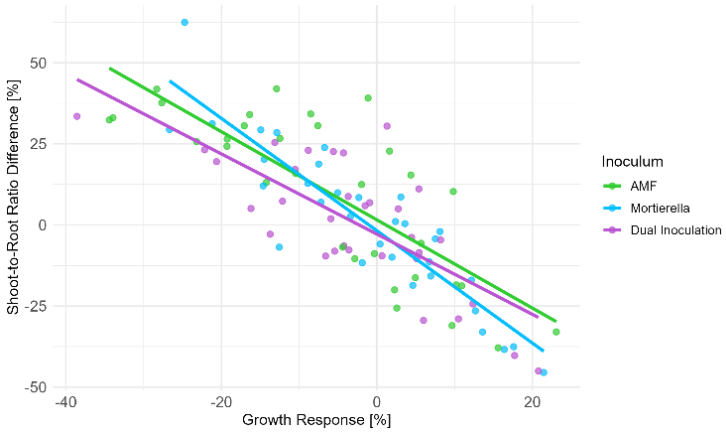

Figure 29: Scatter plot displaying the correlation between growth response and shoot-to-root ratio difference to control average.

An LMM (Growth_Response ~ shoot_root_ratio_diff + (1 | Variety_Name)) reveals highly significant effects of S:R ratio changes on growth response ($p < 2e\text{-}16$) (**Table S10**, appendix). Since the Mortierella treatment showed a steeper slope, the correlation between S:R ratio changes and growth response is even stronger compared to AMF and dual inoculation.

3.2.6 Biomass and Shoot-to-Root Ratio

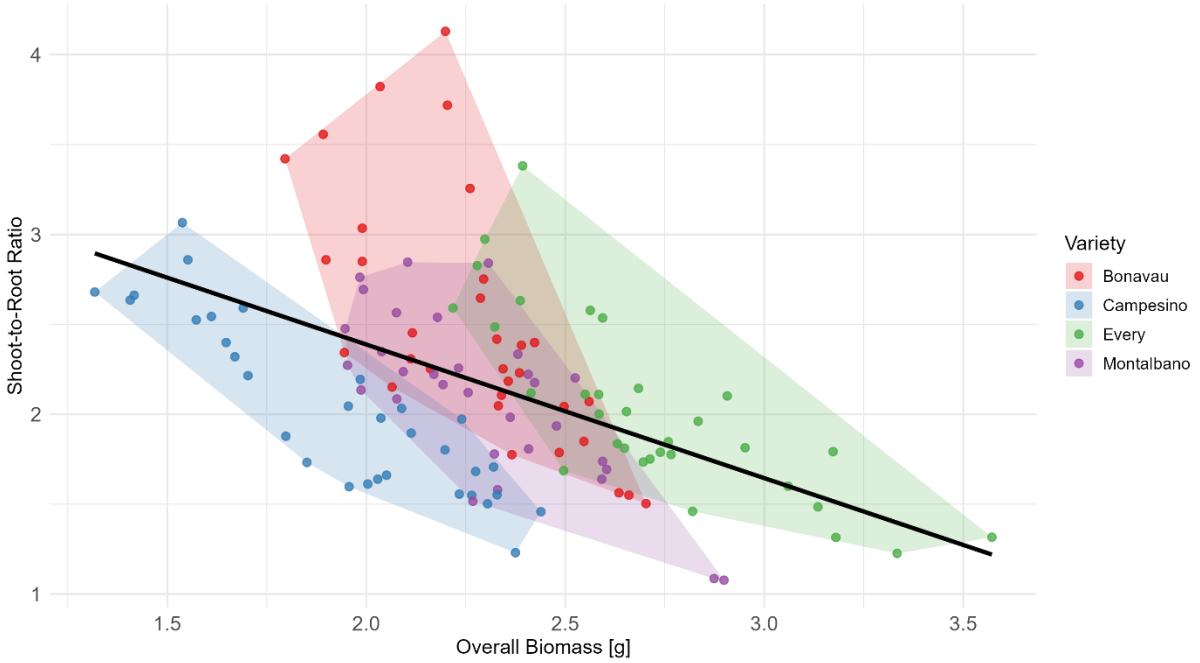

Figure 30: Convex hull plot for wheat varieties on shoot-to-root ratio and overall biomass across all samples (n = 128). Varieties show distinct clusters, indicating variety-specific resource allocations.

PERMANOVA results indicate highly significant clustering of the four winter wheat varieties, based on traits S:R ratio and overall biomass (Variety, $F_{(3,123)} = 13.073$, $P = 0.001$, $R^2 = 0.242$) (**Table S11**, appendix). Since absolute values are used, control samples were also included in this analysis (**Fig. 30**). Consistent with **Fig. 29**, a negative correlation between S:R ratio and total biomass was observed. Varieties that invested more in root biomass tend to have a higher total biomass, indicating distinct strategies in resource allocation linked to overall growth.

4 Discussion

This master's thesis investigated the potential of arbuscular mycorrhizal fungi (AMF) and Mortierella fungi inoculation to promote winter wheat (*Triticum aestivum*) growth. Increasing global food demands and climate change-induced soil quality declines require sustainable alternatives to chemical fertilisers. Here, in two experiments it was tested whether AMF and Mortierella can act as biofertilisation alternatives. The first experiment examined the effect of AMF inoculation on 18 different winter wheat varieties, and the second experiment explored synergistic effects between AMF and Mortierella fungi on four wheat varieties compared to single inoculations. The following discussion interprets the main findings of both experiments individually and compares them to existing literature. A last part combines overall findings and discusses their relevance for future agricultural applications.

4.1 Discussion of Experiment 1

4.1.1 Summary and Overview of Key Results

In the first experiment, the varieties *Every* and *Mont-calme 268* showed positive yet insignificant MGR. This indicates that certain genotypes benefit from AMF inoculation in controlled settings. However, most varieties showed neutral and negative responses (**Fig. 12**). This contradicts **Hypothesis 1**, which suggested a positive MGR for most tested varieties, and thus must be rejected. Although there are trends that lower quality and older varieties have shown higher MGR values, no significant differences to modern and TOP quality varieties can be concluded (**Fig. 13**), providing limiting support for **Hypothesis 2**. However, **Hypothesis 3**, which attributes significant changes in MGR for different origins or breeders, could be confirmed.

Interestingly, AMF inoculation led to enhanced S:R ratios for most varieties, implying a shift in resource allocation to aboveground biomass (**Fig. 14**). However, neither quality nor origin can be attributed to this shift. Furthermore, varieties with higher control biomass showed enhanced MGR, indicating AMF potential in later growth stages (**Fig. 18**).

Overall, these findings are unexpected since many studies primarily found positive MGR for cereals, including crops. However, many factors can impact MGR, such as plant species, substrate composition, environmental conditions and microbial composition. In the following chapters, these findings will be compared to other studies, focusing on genotype-dependent responsiveness, soil nutrient levels and experimental design choices.

4.1.2 MGR Variability Factors

Many studies showed improved plant growth and improved nutrient uptake under AMF influence. Gai et al. (2010) found improved yield results for rice, potatoes and maize by up to 18%, findings that are supported by other meta-studies by Zhang et al. (2019) and Lehmann et al. (2019). However, for wheat in particular, studies also found mixed and negative MGR. Bakonyi & Csitari (2018) tested two winter wheat varieties under farm conditions and found a 15% variability in growth response. Thirkell et al. (2022) conducted a greenhouse experiment where they tested various winter wheat varieties. They found a large variability in shoot growth response of -34% to +89%. However, MGR was calculated using a different approach compared to how this thesis did. Thirkell et al. (2022) defined MGR as the proportional change in mean shoot dry biomass between mycorrhizal and non-mycorrhizal plants for each wheat line. This explains the larger range of MGR values compared to the approach of this thesis (see chapter 2.1.6). Positive MGR values will always be overstated with the formula by Thirkell, compared to the formula used in this thesis. For example, if the biomass of an AMF sample were 3g and the control mean 2g, the formula by Thirkell et al. (2022) would result in a +50% MGR, while the formula used in this thesis by Köhl, Lukasiewicz & van der Heijden (2016) leads to a +33% MGR. Interestingly, the two formulas only differ when the AMF biomass exceeds the control; in the opposite case, both yield the same result. Thus, depending on interpretation, Thirkell's formula may exaggerate positive effects, whereas the method used in this thesis offers a more balanced approach to AMF performance.

Soil nutrient content has a high impact on MGR. Studies found that P content largely influences AMF dependency of plants. Generally, as soil P levels increase, AMF dependency tends to decrease. According to Szentpéteri et al. (2023), this effect also occurs for winter wheat. Tawara (2003) adds that winter wheat revealed low AMF dependency across different P levels in a greenhouse experiment. Soil analysis in chapter 2.3 displayed high P content in the substrate used for both experiments compared to standard values of Swiss arable soils (Richner et al., 2017). This could have inhibited positive impacts of AMF in terms of growth response. Hetrick et al. (1996) found that excess P reduces benefits of AMF-plant symbiosis, resulting in neutral or even negative growth response. Wang et al. (2018) go even further and argue that imbalanced soil conditions, such as high P and low N values, can lead to parasitism by AMF. However, since N content could not be quantified in the soil analysis (see chapter 2.3), this argument should be considered with caution. Furthermore, the performed soil analysis measured total P, which also includes unavailable P for plants. However, most studies only assess plant-available P (e.g., Hetrick et al. 1996; Tawaraya 2003), which reflects plant-accessible P more accurately. Hence, the high-P substrate used in both experiments does not necessarily mean high P availability for wheat plants. Overall, the substrate's high P content, combined with high response variability and general low AMF dependency of winter wheat, explains the mostly neutral to negative growth responses in Experiment 1.

The experimental setting also must be considered. Most studies that highlight positive growth effects on winter wheat were conducted in the field (e.g., Pellegrino et al., 2015; De Leon et al., 2020). Controlled greenhouse settings can hinder positive effects of AMF. Salomon et al. (2022) tested multiple AMF inoculants on various crops such as maize and wheat under greenhouse and field conditions. They conclude inconsistent and weak MGR compared to field trials. Additionally, a sterile environment reduces AMF efficiency as microbial absence limits AMF establishment (Koziol et al., 2024). Thus, this greenhouse experiment may not reflect potential AMF growth benefits in the field.

Compared to maize, another crucial cereal for global food security, wheat typically shows more variable and lower MGR (Zhang et al., 2019). Another master's student conducted a greenhouse experiment with different maize varieties and AMF inoculation (Milanowski, 2024). He used similar materials and the same substrate and inoculum. Most maize varieties yielded negative MGR results ranging from +7.1% to -11.6%. These results strengthen the assumptions that the experimental design combined with high-P substrate led to mostly negative MGR in wheat. Overall, maize showed a more promising response to AMF inoculation compared to wheat. However, plant origin emerged as a key driver for MGR in both crop species.

4.1.3 Quality and Origin Effects on MGR

The effect that older or lower-quality varieties tend to have higher MGR than modern or TOP-quality varieties is visible (**Fig. 13**). Although these effects were not statistically significant, older studies from Hetrick et al. (1992) and Zhu et al. (2001) support these trends. They claim that older cultivars show higher AMF dependency, especially under P-limited conditions. Therefore, differences between these two groups might have been even more pronounced in substrate with lower P content. Newer studies by Lehmann et al. (2012) and Pellegrino et al. (2015) confirm this pattern by reporting that MGR declined in modern crop varieties.

A possible explanation is that breeding methods led to reduced AMF dependence as responsiveness to mineral fertilisers increased (Lehmann et al., 2012; Martín-Robles et al., 2018). De Leon et al. (2020) add that yield prioritisation of conventional farming under high fertilisation practices reduces AMF effectiveness. They also found that cultivars from organic systems show higher MGR than ones bred for conventional agriculture. Li et al. (2024) observed the same effect on rice, as modern cultivars experienced lower MGR.

Results from Experiment 1 indicate that the origin of used wheat varieties significantly impacted MGR ($p = 0.0202$) (**Table S3**, appendix). Studies found that specific genomic regions (QTLs) influence AMF benefits (Lehnert et al., 2017; Thirkell et al., 2022) (see chapter 1.4.2 for detailed information). These QTLs are associated with biomass allocation, P uptake and root colonisation. A genome-wide

association study by Lehnert et al. (2018) states that MGR of genotypes varies under abiotic stresses such as drought. Therefore, controlled greenhouse conditions and regular and sufficient might have further diminished MGR.

Berger & Gutjahr (2021) found that even within the same species, different genotypes (cultivars, lines or varieties) show morphological and genetic variation that can alter AMF effectiveness. Additionally, key plant traits such as the perception of fungal signals, P transporters and control of carbon allocations to fungal symbionts. The authors argue that modern breeding practices that prioritise yield stability and nutrient uptake efficiency unintentionally select against these key traits that enhance AMF benefits. This may explain why origin emerged as a significant factor for MGR in Experiment 1. However, the mechanism of variety-dependent MGR remains highly complex. Interactions between biotic and abiotic aspects and plant and fungal genotypes shape AMF effectiveness for wheat and other plants. To utilise AMF benefits, future breeding programmes need to select for mycorrhizal responsiveness (Martín-Robles et al., 2018; Thirkell et al., 2022).

4.1.4 Shoot-to-Root Ratio Shift

AMF inoculation led to an S:R ratio enhancement of over 5% compared to non-inoculated samples (**Fig. 14**). This indicates a focus on aboveground resource allocation. Smith & Smith (2011) found that plants reduce root growth with higher reliance on fungal nutrient provision. A study on tea plants by Chen et al. (2021) revealed that AMF inoculation altered root morphology and changes in S:R ratio even for non-significant biomass changes. The reason for that is AMF-influenced hormonal signalling, implying a systematic reallocation even in the absence of growth gains. These findings explain overall negative MGR values in the first experiment despite resource allocation shifts towards the shoots, implying altered growth priorities of plants rather than improved productivity. Thirkell et al. (2022) support these findings, as wheat physiological responses to AMF also appear without biomass gains. Smith & Smith (2011) argue that this effect occurs because AMF colonisation demands C from plants. Hence, if nutrient exchange is unnecessary or inefficient, MGR can be neutral or even negative.

Lopez et al. (2023) conducted a meta-analysis on crops under nutrient-deficient conditions. They found that nutrient-deficient conditions generally decrease the S:R ratio as plants alter their allocation strategies for nutrient mobilisation. Regarding experimental results, soil analysis shows rather high nutrient contents. However, since AMF inoculation led to generally increased S:R ratios, AMF probably improved nutrient acquisition of wheat plants.

Interestingly, no correlation between MGR and S:R ratio change was found in Experiment 1 (**Fig. 20**). Additionally, neither origin nor quality played a significant role in S:R ratio shifts of AMF inoculation.

Thirkell et al. (2022) also found no consistent link between growth response, physiological traits and root colonisation, further emphasising the overall complexity of AMF interactions with plants.

4.1.5 Root Colonisation

Root colonisation has been assessed with root staining and qPCR. Both methods displayed rather low colonisation rates (**Fig. S6**, appendix; **Fig. 16**). Furthermore, correlations between MGR and colonisation rates were inconsistent. There was no correlation between colonisation rate by microscopy and MGR (**Fig. S6**, appendix), while there was a strong correlation between absolute AMF copy numbers, obtained by qPCR, and MGR (**Fig. 19**). However, microscopy detects general AMF structures and does not distinguish between strains. For both experiments, only SAF22 was used for inoculation. Thus, a comparison between the two methods offers limited interpretive value. Interestingly, there was no such correlation for the varieties *Every* and *Mont-Calme 268*, which had positive MGR values (**Fig. S7**, appendix). Low colonisation rates of 10-30% could be explained by plant species and environmental conditions. Koziol et al. (2024) noted that sterile substrates and hence microbial absence can significantly limit AMF establishment. Nevertheless, a field study with wheat by Veršulienė et al. (2024) found similar colonisation rates of 10%-59% across 10 wheat varieties. However, such as Thirkell et al. (2022), they found no clear correlations between MGR and root colonisation in wheat. On the other hand, a meta-analysis by Pellegrino (2015) found a positive correlation of AMF root colonisation and grain yield increase. Lutz et al. (2023) also found no correlation for maize. Moreover, the soil fungal microbiome explained significantly higher MGR variation than soil nutrient levels, linking back to the complexity of AMF-plant interactions. For wheat, Hetrick et al. (1992) observed lower colonisation levels in modern cultivars, supporting the argument that such varieties are less dependent on AMF symbiosis. Bakonyi & Csitári (2018) conclude AMF colonisation decreased under fertilisation application. This is a possible explanation for why colonisation results were rather low in Experiment 1 because nutrient contents, especially P, were high.

Both root staining and microscopy and qPCR are valid methods to quantify AMF colonisation on plant roots since studies have shown a high correlation between results of both methods, also for wheat (Bodenhausen et al., 2021; Corona Ramírez et al., 2023). For experiment 1, trends for such correlation were observed but not significant ($R = 0.354$, $p = 0.26$) (**Fig. 17**), likely due to a limited sample size ($n = 12$). Moreover, *t-tests* revealed that not all varieties showed significant differences compared to respective control groups (**Table S2**, appendix). Reasons for that are small numbers of replicates ($n = 8$), large standard errors (SE) for some varieties and adjusted p-values.

Most studies used the root staining and microscopy approach since the qPCR method evolved in the early 2020s. McGonigle et al. (1990) laid the foundation in the microscopy method, which is widely accepted in many studies (e.g., Bakonyi & Csitári, 2018; Szentpéteri et al., 2023). Bodenhausen et al.

2021 compared both methods and discussed advantages and disadvantages. Root staining remains the most used method due to low costs and direct visualisation. It allows a distinction between fungal structures such as arbuscules, hyphae and vesicles. However, microscopy is time-consuming, and observer biased. Additionally, AMF in roots is often unevenly distributed, which could lead to under- or overestimations of root colonisation rates. On the contrary, qPCR provides a more precise and time-efficient approach but is unable to assess fungal structure. Therefore, a combination of both microscopy and qPCR can provide a more dependable view of colonisation dynamics. Hence, future studies may benefit from combining both approaches for root colonisation assessment. (Bodenhausen et al., 2021; Corona Ramírez et al., 2023).

4.1.6 Methodological Limitations

Although experiment 1 was carried out under controlled settings without major complications, various methodological limitations should be considered when interpreting results. Apart from common constraints such as limited sample size and time availability, other experiment-specific factors may also have impacted outcomes.

During the second half of the experiment, Thysanoptera (thrips) infestation of wheat plants was visible. Consequences such as silvery-coloured streaks on leaves and deformation and curling of leaves were clearly visible (**Fig. 4**). Leaf damage reduces photosynthesis efficiency, which significantly weakens growth and development, especially in early growth stages (Zhichkina et al., 2023). Furthermore, thrips infestation was not evenly distributed across pots. Adedayo & Babalola, 2023, point out that this can alter MGR outcomes, as herbivore-induced stress can inhibit the AMF benefits.

The relatively short growth duration of 60 days combined with wheat being monocotyledonous and late successional (see chapter 1.4), MGR tend to be lower and inconsistent (Cheeke et al., 2019). Wheat plants did not reach a flowering state. Therefore, grain yield and quality could not be investigated. Gai et al. (2010) claim that AMF benefits accumulate over time, particularly for late-successional species.

Experiment 1 was conducted during summer with temperatures in the greenhouse regularly exceeding 30°C. In chapter 8 of a book from Wu et al. (2017), they investigated heat stress as an abiotic factor that can impact AMF benefits. They found that heat stress can disrupt AMF functioning, including nutrient exchange. Additionally, the sterile P-rich substrate could have further diminished AMF functioning. However, the book also mentions plenty of studies which state that AMF can improve plant growth under heat stress.

Only the native AMF strain *Rhizoglomus irregulare SAF22* was incorporated in the first experiment. Root colonisation of this strain has been verified by Lutz et al. (2023) in numerous field trials with maize. Moreover, Boussageon et al. (2022) found that *Rhizoglomus irregulare SAF22* facilitated nutrient

acquisition in *sorghum* under varying P and N content, indicating the strain's high efficiency and adaptability. However, one strain does not reflect the natural AMF diversity. Various studies have shown that multiple AMF strains provide complementary and synergistic effects that improve plant responsiveness. Bender et al. (2016) mention that less-diverse soil communities showed lower stress resistance, which supports findings from the last paragraph. Deja-Sikora et al. (2023) highlighted that different AMF species have varying effects on plant growth. Consequently, a different AMF strain could lead to different MGR. Furthermore, inoculant efficiency is highly dependent on strain composition and microbial interactions (Koziol et al., 2024).

The substrate used in both experiments was uniform and sterile, which eliminates microbial communities. Lutz et al. (2023) argue that soil microbiome indicators best explained MGR variability. Moreover, microbes influence AMF signalling dynamics (Koziol et al., 2024; Salomon et al., 2022). Microbial absence likely restricted AMF colonisation and functionality (Compant et al., 2024; Xu et al., 2025). Moreover, using a uniform substrate constrains generalisability to field conditions where microbial diversity and soil structure highly impact AMF dynamics (Lutz et al., 2023; Compant et al., 2024).

Overall, this experiment controlled key variables to investigate genotype effects. However, abiotic and biotic stresses and simplified microbial and soil conditions do not resemble real-world conditions. Thus, more realistic and ecologically complex conditions better mirror AMF functionality for agricultural application.

4.2 Discussion of Experiment 2

Experiment 2 aimed to explore synergistic effects between arbuscular mycorrhizal fungi (AMF) and Mortierella fungi on a selected subset of winter wheat varieties from experiment 1. This follow-up experiment evaluates the potential of combining microbes to further improve biofertilisation compared to single inoculations in controlled settings.

4.2.1 Summary and Overview of Key Results

A dual inoculation of AMF and Mortierella led to positive GR for *Bonavau* and *Montalbano*. However, this trend was not observed for *Every* and *Campesino* (**Fig. 22**). Thus, Hypothesis 4, which states that a dual inoculation of both fungi is beneficial for wheat growth, must be rejected. Overall, experiment 2 revealed a negative fungal growth response (GR) across tested wheat varieties of -3.53%. While *Every*, *Montalbano* and *Bonavau* showed neutral GR, *Campesino* showed clear negative GR (**Fig. 22**).

Interestingly, the variety's background (origin) had a way stronger effect on GR ($p = 0.01251$, $\eta^2 = 0.20$) than inoculum ($p = 0.40498$, $\eta^2 = 0.03$) (**Fig. 23**).

Fungal inoculation increased S:R ratios overall (**Fig. 24**). AMF especially enhanced the S:R ratio, while Mortierella and dual-inoculated treatments and origin showed no significant changes (**Fig. 25**). However, there was a very strong correlation of S:R ratio changes between all fungal and control treatments and MGR ($p < 2e\text{-}16$), indicating a resource allocation shift of fungi towards aboveground biomass that harmed overall growth (**Fig. 29**). Furthermore, PERMANOVA revealed significant clustering ($p = 0.001$, $R^2 = 0.242$) and negative correlation of overall biomass and S:R ratio of tested wheat varieties (**Fig. 30**). This implies that varieties show different allocation strategies that highly impacted their overall growth. Varieties with lower S:R ratios generally had higher biomass, possibly explaining that fungal S:R ratio enhancement hindered wheat growth.

Microscopy revealed AMF colonisation rates of 29-54%. Mortierella detection was very low, yet visible in some samples (**Fig. 26**). Additionally, findings indicate a negative correlation between AMF colonisation and MGR (**Fig. 28**). However, this needs to be interpreted with caution since the sample size is very limited due to the time constraints of this master's thesis.

4.2.2 Individual Effectiveness of Mortierella

Besides AMF, Experiment 2 incorporated three Mortierella strains (*M. alpina, M. elongata and M. exigua*) that were isolated from the FAST trail site in Switzerland. For all varieties, Mortierella inoculation led to the most stable results in terms of growth response and S:R ratio change. Contrary to several studies (Ozimek et al., 2018; Liao, 2021), no significant growth-promoting effects on wheat were observed. Ozimek et al., 2018, conducted a laboratory experiment where they inoculated two Mortierella strains (*M. antarctica* and M. *verticillata*) on the winter wheat variety *Arkadia* under different temperatures of 4°C-28°C. They concluded that the growth response for both Mortierella strains was highest at 15°C and decreased at higher temperatures. Apart from a growth period of only 10 days in treated petri dishes, they applied seed soaking in liquid fungal inoculum, which likely promoted early colonisation. Additionally, they found that Mortierella was able to synthesise phytohormones (e.g., auxin and gibberellins) that promote plant growth under temperate climatic conditions, which makes them a promising biofertiliser in Switzerland. Other studies (Li et al., 2018; Liao, 2021; Ozimek & Hanaka, 2021) also noted that Mortierella enhances plant growth-promoting hormones.

A key mechanism of Mortierella that promotes plant growth is P acquisition, as it can dissolve inorganic P. Since the P content of the experiment's substrate was high (see chapter 3.3), plant dependency on fungal-mediated nutrient mobilisation might be reduced. The absence of microbial communities in the

sterile substrate and limited growth time constrain other growth-promoting effects of Mortierella. These include degradation of toxic organics, enzyme production and recalcitrant substances that contribute to long-term stable soil organic matter (Li et al., 2018; Field et al., 2019; Liao, 2021).

A further explanation for the lack of significant growth promotion could be that tested wheat genotypes show low root exudates such as sugars and organic acids. Unlike AMF, Mortierella is a non-symbiotic fungus and might rely more on root exudates to activate growth-promoting traits (Ozimek & Hanaka, 2021). Low AMF responsiveness in both experiments could be linked to low root exudates and thus fewer resources and signals for Mucoromycota, including Mortierella (Berger & Gutjahr, 2021). This would align with the fact that modern breeding unintentionally selected against key traits that enhance fungal responsiveness (see chapter 4.1.3). A study by Iannucci et al. (2017) investigated different factors that alter wheat root exudates. They found that root exudate profiles significantly varied among genotypes. Additionally, soil composition further affected root exudates, indicating interactions between genetic and environmental factors. Moreover, they observed that different genotypes promote different soil compositions and that exudation profiles could have an adaptive value. These findings should further encourage breeders to select for microbial responsiveness.

4.2.3 Dual Inoculation - Potential and Limitations

In theory, complementary benefits of AMF and Mortierella can lead to synergistic effects that promote plant growth. While root symbiosis with AMF improves nutrient uptake, particularly P (e.g., Bender et al., 2019), Mortierella complements by P solubilisation and phytohormone production (Li et al., 2018). However, Experiment 2 revealed only limited and no consistent synergistic effects on wheat. Interestingly, *Bonavau* and *Montalbano*, both top-quality varieties bred by Agroscope, showed positive yet not significant results with dual inoculation, strengthening findings of origin being the main driver behind GR.

Multiple studies indicated a complementary effect of Mortierella to AMF. Li et al. (2018) state that *Mortierella elongata* enhances nutrient availability, assisting AMF benefits. Zhang et al. (2011) observed improved enzyme activity and plant growth in co-inoculated treatments. Lutz et al. (2023) support these findings, as they observed higher MGR and AMF colonisation rates in fields with high Mortierella abundance. Tamayo-Velez & Osorio (2017) investigated synergistic effects between *Rhizoglomus fasciculatum* and *Mortierella sp.* on avocado plantlets. They found significant growth benefits of a dual inoculation compared to single inoculations. Interestingly, root colonisation of both fungi almost halved when dual inoculated, which suggests competition over resources between fungi. Despite that, additive effects were still significant.

However, effects of a dual inoculation with AMF and Mortierella are context dependent. Zhang et al. (2011) tested two AMF strains (*Aggregatum* and *Mosseae*) and *Mortierella sp.* on *Kostelelzkya virginica* plants. They found that a dual inoculation with one AMF strain and Mortierella was more beneficial than inoculating all three strains. Like Tamayo-Velez & Osorio (2017), they claim a potential competition of fungi over root exudates. Additionally, soils with AMF and Mortierella had higher available P concentrations than soils with a single inoculation of either fungus.

Although no studies have yet investigated synergistic effects of AMF and Mortierella on wheat, some older studies used other phosphate-solubilising fungi (PSF) which provide growth benefits such as those through comparable mechanisms. Tarafdar & Marschner (1995) conducted an experiment with wheat *'Star'* under controlled conditions and a 50-day growth time. Results showed that a dual inoculation of *Glomus mosseae* (AMF) and *Aspergillus fumigatus* (PSF) significantly enhanced wheat shoot and root biomass compared to single inoculations. They supplemented low P, sterilised soil with organic P (Na-phytate), which the PSF was able to mineralise, and AMF improved P uptake.

Singh & Kapoor (1999) also conducted a controlled experiment with wheat (*Triticum aestivum*) with an extended growth time of almost half a year. The substrate consisted of sandy, nutrient-deficient soil and partially added rock phosphate. AMF (*Glomus sp.*), PSF (*Cladosporium herbarum*), and phosphate solubilising bacteria (PSB) (*Bacillus circulans*) were inoculated. They also concluded improved plant growth and nutrient uptake for dual inoculations, highlighting complementary effects.

In the context of Experiment 2, genotype-determined root exudates likely played a big role in dual inoculation efficiency, which would explain variable GR for varieties (Iannucci et al. 2017). Competition over C and limited root space can negate benefits, as we used 1.5L pots and three plants per pot. As mentioned in previous paragraphs, the sterile and high-P substrate lowered plant dependency on additional fungal nutrient supply. In the two studies above, plant dependency of microbial P mineralisation was likely higher, which explains significant growth improvements and complementary effects.

4.2.4 Root Colonisation and its Limitations

Despite higher root colonisation rates of 29-54%, MGR values remained neutral or negative. This strengthens findings from Experiment 1, which showed that root colonisation alone does not predict MGR reliably. Nevertheless, there appears to be a negative correlation between root colonisation and MGR, meaning that higher colonised samples displayed negative MGR (**Fig. 28**). This contradicts several studies that could not conclude consistent correlations for wheat (Thirkell et al., 2022; Veršulienė et al., 2024). However, this should be interpreted with caution because the sample size is very limited.

Moreover, AMF colonisation of dual inoculated samples showed no significant differences compared to single inoculated samples, contrary to findings from Tamayo-Velez & Osorio (2017).

Mortierella was almost non-detectable via microscopy, with only a few structures, which cannot definitively be assigned. Contrary to AMF, Mortierella does not form intraradical structures and primarily colonises the rhizosphere and not root tissue (Ozimek & Hanaka, 2021). Moreover, it can be difficult to distinguish structures from other fungi (Li et al., 2018). However, this contradicts findings from Liao et al. (2019), who argue that Mortierella can be visualised under the microscope since it is frequently isolated from plant roots. Nonetheless, their study does not mention a specific protocol for microscopic detection.

Thus, qPCR with soil DNA might be more suitable for Mortierella quantification. However, this was not part of this master's thesis due to time constraints and unclear results of qPCR from Experiment 1. A recent master's thesis (Wroblewski, 2024) successfully detected several Mortierella species from maize roots and specific primers that have been tested and validated by the plant-soil interaction group at Agroscope Zürich. Despite this, it is unclear if this would work for wheat plants too.

4.2.5 Variety-Specific Responses

Many aspects of Experiment 1 that have been discussed in chapters 4.1.2 – 4.1.4 can be applied to the second experiment. Distinct differences and similarities are discussed in the following chapter 4.3. This section solely focuses on results of Experiment 2 that differed from Experiment 1.

Overall, GR across all treatments was higher, yet still negative. Reasons for that could be absent biotic and abiotic stressors (*thrips* and heat stress) that can disrupt AMF functioning and establishment (Wu et al., 2017). Additionally, freshly ordered seeds from DSP potentially increase fungal responsiveness and lead to enhanced plant growth overall. However, AMF still showed the lowest GR compared to other treatments, underlining the general low dependency and even harmful relationship of AMF and modern wheat varieties under high P conditions (Hetrick et al., 1996; Wang et al., 2018; Szentpéteri et al., 2023). Also, origin was the driving factor, as it explains 20% of GR variability, confirming that morphological and genetic variation of genotypes, even within the same species, highly impacts GR of Mucoromycota (Martín-Robles et al., 2018; Berger & Gutjahr, 2021; Thirkell et al., 2022).

AMF inoculation led to an S:R ratio enhancement of almost 10%, while Mortierella and dual-inoculated samples showed neutral effects (**Fig. 25**). Interestingly, results from Experiment 2 show a very strong negative correlation between S:R ratio increase and growth response (**Fig. 29**). In other words, inoculum-related changes in S:R ratio significantly determined growth response. Against expectations, this correlation was strongest for Mortierella inoculation. Resource allocation shifts due to fungal inoculation stem from altered root morphology and hormonal signalling (Chen et al., 2021; Thirkell et

al., 2022). These results support findings from Smith & Smith (2011) which state that demands from fungi can outweigh their contribution to nutrient acquisition by plants in nutrient-rich environments. However, it is not clear why this effect was not present in the first experiment. One potential reason could be the higher colonisation rate due to seed quality and three plants per pot that provided more root exudates that nourished fungi and thus promoted symbioses.

Interestingly, the four tested varieties showed distinct clustering based on overall biomass and S:R ratio (**Fig. 30**). This implies strong genotype-based allocation strategies of wheat varieties and should be highly considered in future breeding programmes to maximise benefits on Mucoromycota in agricultural applications, as Martín-Robles et al. (2018) and Thirkell et al. (2022) request.

4.2.6 Practical Implications and Limitations

Findings from Experiment 2 yield relevant insights for fungal application in agriculture. Although growth response was mainly negative, consistent physiological responses could be observed. Especially AMF altered the S:R ratio of wheat plants and thus influenced plant allocation strategies in nutrient-rich conditions. This factor should be considered in future breeding strategies, as this might lead to a higher growth response in field conditions where microbial composition and nutrient-scarce areas potentially enhance fungal benefits. Extended growth times in the field allow late-stage effects, as AMF benefits for wheat can accumulate over time (Pellegrino et al., 2015; Thirkell et al., 2022).

Translating experimental findings to field conditions is challenging since the composition of soil microbial communities is complex, which strongly influences nutrient cycling and plant–microbe interactions (Lutz et al., 2023; Zhang et al., 2024). Beneficial effects of Mortierella may rely on microbial synergies and complex rhizosphere interactions, which were lacking in sterilised conditions (Li et al., 2018).

Furthermore, many commercial AMF products do not provide consistent growth benefits, which are highly context dependent. For AMF, Salomon et al. (2022) point out field inconsistencies, strain specificity and worse establishment under high fertilised conditions. Koziol et al. (2024) add that many products contain contaminated, low-quality spores that fail to colonise plant roots effectively. On the other hand, commercial inoculants for Mortierella are not available yet. Although they are promising candidates for biofertilisation, particularly in temperate, low-phosphorus soils, commercial standardisation is lacking (Ozimek et al., 2018; Ozimek & Hanaka, 2021).

Overall, enhanced manual work for inoculum application, combined with poor quality standards and inconsistent growth responses, makes current commercial products unprofitable. Thus, standardisation practices and improved quality control within the AMF inoculant industry need to be established

(Salomon et al., 2022; Koziol et al., 2024). However, since many complex interactions influence fungal inoculation in agriculture, doing so remains challenging.

4.3 Integrative Discussion

4.3.1 Key Takeaways

The results of both greenhouse experiments revealed various morphological similarities of winter wheat with fungal inoculation. Although AMF and Mortierella application was expected to generally promote wheat growth, only a minority of varieties showed positive growth responses. While *Every* emerged as a promising candidate for fungal inoculation, displaying the highest biomass and MGR values in the first experiment, growth responses in the second experiment were neutral. However, *Every* showed consistently high overall biomass, making it worth considering for field trials. *Montalbano* showed a neutral growth response across both experiments, while *Campesino* showed clear negative growth responses under both fungal inoculations. Despite these findings, integrating AMF and Mortierella as biofertilisers may be more effective under field conditions with the presence of microbial communities and environmental stresses. In microbic complexes, P-deficient soils with biotic and abiotic stresses being present, fungal inoculation was shown to be more beneficial for plant growth, including wheat (e.g., Pellegrino et al., 2015; Bakonyi & Csitári, 2018; Zhang et al., 2019). This statement is further supported by a recent study by Rog et al. (2025), which concludes that growth response negatively correlated with soil health.

Most notably, the genetic origin of varieties emerged as a significant growth response driver in both experiments. Interestingly, neither quality classification (e.g., TOP, older varieties) nor different treatments in Experiment 2 could significantly explain growth response variation. This highlights that the genetic background linked to breeding and lineage highly impacts the symbiotic compatibilities with AMF and Mortierella. However, there were still some trends that older and lower-quality varieties displayed better GR. Previous studies argued that intensive, modern breeding practices might have unintentionally selected against traits that enhance fungal inoculation benefits (Hetrick et al., 1992; De Leon et al., 2020; Li Y. et al., 2024). Varieties such as *Campesino,* which has been bred to maximise yield for livestock feeding, show lower fungal dependence and potentially contain unfavourable characteristics for symbiosis, such as root morphology or root exudate characteristics (Lehmann et al., 2012; Berger & Gutjahr, 2021). Additionally, *Campesino* displayed relatively high colonisation rates compared to other variants (**Fig. 28**). Overall, the genetic origin should be highly taken into consideration for wheat variety selection when incorporating biofertilisation.

Fungal inoculation led to enhanced S:R ratios for most varieties, especially with AMF inoculation. This resource allocation shift to aboveground biomass suggests enhanced growth efficiency (Chen et al.,

2021). In most cases, however, total biomass decreased potentially due to high fungal C demand, a phenomenon that has been observed in other studies (Smith & Smith, 2011; Thirkell et al., 2022). While the S:R ratio enhancement had no negative correlation with MGR in the first experiment, there were very strong negative correlations in Experiment 2 (**Fig. 20**; **Fig. 29**). A reason for that could be the higher colonisation rates, induced by favourable conditions and higher root density since there were three plants per pot instead of one. Despite this, the fungal-induced allocation shift could be beneficial under harsher field conditions and longer growth periods (Berger & Gutjahr, 2021; Lopez et al., 2023).

Contrary to findings by Thirkell et al. (2022), varieties that displayed higher control biomass showed higher growth response in both experiments (**Fig. 18**; **Fig. 27**). However, in this study, different genotypes were used as in the study by Thirkell et al. (2022), perhaps explaining this difference. Although these trends were non-significant, such contradictions emphasise complex interactions between genotype-specific traits and physiological factors that alter plant-fungi relationships. Interestingly, this trend occurred across both of our experiments, despite environmental differences in biotic and abiotic stressors, indicating that substrate composition might play a key role.

The correlation between fungal root colonisation in wheat and growth response was inconsistent across both experiments. While qPCR results of Experiment 1 showed a positive correlation between AMF colonisation and MGR, microscopy with Experiment 2 samples showed negative correlation trends of these two factors. However, microscopy sample sizes for both experiments were quite low (n = 12; n = 8). Despite that, these findings reflect differences and inconsistencies across several studies. Whereas some studies found no correlation between MGR and root colonisation (Thirkell et al., 2022; Veršulienė et al., 2024), other studies did (e.g., Pellegrino et al., 2015). This circumstance emphasises the statement from Lutz et al. (2023) that root colonisation is insufficient to explain growth response.

One key difference between the experiments was biotic and abiotic stressors. In the first experiments, winter wheat plants were exposed to thrips and heat stress, which can significantly impact colonisation rates and growth response. Wu et al. (2017) point out that stress during early growth stages can alter plant signalling pathways and hence symbiotic establishments. Experiment 2 was absent from such stress factors, which likely explains significantly higher root colonisation and, overall, less negative growth responses under fungal inoculations. This emphasises the environmental sensitivity of AMF establishment (Hahn et al., 2018).

Interestingly, *Bonavau* showed very different growth responses across both experiments. While it had the lowest growth response in Experiment 1, it revealed the highest in Experiment 2, especially when dual inoculated. The most logical explanation for this is stress factors that were present during the first and microbial factors that shape the inoculation efficiency of AMF and Mortierella. Bakonyi & Csitari (2023) found that under drier conditions, AMF benefits were highly variety dependent and yields overall were reduced. This was likely the case for *Bonavau* as well.

4.3.2 Future Perspectives

Results from both experiments emphasise the importance of a context-dependent approach to microbial inoculation. Biotic and abiotic stressors, environmental conditions and wheat genotype selection led to large growth response variation of AMF and Mortierella inoculation. These interactions suggest that there is no universal inoculation strategy that consistently succeeds in diverse agricultural settings. Thus, future investigations should focus on pre-application assessment tools that maximise benefits of fungal inoculants to specific crop genotypes under certain conditions (Compant et al., 2024). However, field trials will provide a more sophisticated understanding of complex physiological and ecological dynamics (e.g., Lutz et al., 2023) than controlled greenhouse experiments (Thirkell et al., 2022).

One recent, promising approach towards improving scalability and reliability of context-dependent microbial applications is synthetic microbial communities (SynComs). SynComs describe tailored microbial associations that show improved ecological resilience compared to single inoculations (Xu et al., 2025). Synergistic and complementary traits such as improved nutrient uptake from AMF and enhanced P-solubilisation from Mortierella characterise SynComs. To promote this technology, Compant et al. (2024) and Xu et al. (2025) highlight the need for considering standardisation protocols, region-specific soil microbiomes, crop genotypes and public strain libraries. Northen et al. (2024) agree that SynComs have the potential to enhance reliable plant benefits in real-world applications. Although SynComs show great potential, they are still in the early stages of development and have not been widely implemented in large-scale agricultural practices yet. Due to obstacles mentioned in the last paragraph, achieving field-level consistency is challenging (Compant et al., 2024; Xu et al., 2025). Nevertheless, SynComs present a viable application towards sustainable agriculture and closing the gap to fulfilling the sustainable development goals (SDGs), particularly SDG2 (zero hunger), SDG15 (life on land), and SDG3 (good health and well-being) (Compant et al., 2024).

As plant biotechnology advances, one emerging, yet controversial, branch is using genetically modified (GMO) plants to meet increasing food demands. A recent study by Cook et al. (2025) discovered a mutated plant gene (CNGC15) that improves plant signalling to microbes, such as AMF, through small spontaneous pulses of calcium in root cells. They were able to implement this gene in wheat and found significantly improved AMF root colonisation and nutrient uptake in wheat plants. This was also the case in highly fertilised soils that usually suppress AMF benefits (Bakonyi & Csitari, 2023). GMOs like this could further reduce dependency on chemical fertiliser in the future, providing a vital part in achieving sustainable agriculture.

A recent study by Raza et al. (2025) highlights the need for considering soil parameters, as only 10% of plant breeding currently do so. Climate change accelerates nutrient loss and soil degradation in croplands. This is problematic since many breeding programmes favour high-yield performance in nutrient-rich conditions and neglect heterogeneous conditions in soils. This can lead to overestimations

of selected cultivars as soil quality declines. Additionally, Raza et al. (2025) advocate future breeding programmes to emphasise root system architecture traits such as root depth and exudation profiles. Achieving this requires high-resolution soil imaging and improved collaboration between soil scientists and plant geneticists. Incorporating biofertiliser strategies such as AMF and Mortierella inoculation could further improve cultivar resilience for future agricultural practices.

Overall, AMF and Mortierella inoculation can help to reduce agriculture's large environmental footprint by reducing fertiliser application while maintaining yield (Gai et al., 2010). Thirkell et al. (2020) found that microbial-induced nutrient uptake benefits remain for elevated CO_2 levels in the future, underlining their potential for sustainability. Therefore, we suggest the inclusion of AMF responsiveness as a factor in official cultivar evaluation protocols in the future. Furthermore, this encourages farmers and breeders to utilise varieties that are responsive to microbial benefits under certain soil conditions.

5 Conclusion

This master's thesis examined the biofertilisation potential of arbuscular mycorrhizal fungi (AMF) and Mortierella fungi on early winter wheat (*Triticum aestivum*) growth. The focus of this work lay on growth response variability, morphological and ecological traits investigation and inoculation strategies. Considering resource limitations, climate change and global food demand increases, this work aims to address sustainable fertilisation alternatives to chemical fertilisation while maintaining yield. Therefore, two greenhouse experiments, using sterilised soil/sand mix as a substrate, were conducted. Experiment 1 assessed the mycorrhizal growth response (MGR) of 18 different winter wheat varieties from seven European breeders and different quality classifications, including three older varieties. Experiment 2 focused on the synergistic effects of AMF and Mortierella on a subset of four varieties based on results from the experiment prior.

Both experiments mainly yielded negative and neutral growth responses to fungal inoculation, suggesting limited benefits under high-phosphorus and microbial-absent environments. Varieties of different breeders showed great growth response and resource allocation variability. The origin of varieties significantly altered growth response and resource allocation, contrary to quality classifications and different inoculants. This implies genotype-dependent symbiotic compatibilities with microbes, such as AMF and Mortierella, which is potentially linked to breeding practices. Fungal inoculation generally enhanced the shoot-to-root (S:R) ratios of wheat plants, indicating biomass allocation shifts even in the absence of total biomass gains. Varieties with higher control biomass tend to benefit more from fungal inoculation across both experiments.

Fungal root colonisation varied significantly between experiments, likely due to biotic and abiotic stressors and lower root density in Experiment 1, which can suppress symbiotic relationships between fungi and plants. The consistency of growth responses among the wheat varieties varied between the two experiments. *Every* did not replicate the growth response from the first experiment, as values in the second experiment were neutral. *Montalbano* and *Campesino* showed consistent growth response with neutral and negative values, respectively. Interestingly, *Bonavau* displayed the lowest MGR in Experiment 1 but the highest in Experiment 2. While a dual inoculation benefitted *Bonavau* and *Montalbano*, synergistic benefits were non-significant overall. These inconsistencies emphasise the complex morphological and ecological dynamics that alter inoculation efficiency.

In summary, this master's thesis reveals that despite potential microbial benefits towards sustainable agriculture, their efficiency is highly context-dependent, varying with environmental conditions, genotype, and management practices. Thus, research and future breeding programmes should implement microbial responsiveness and soil dynamics as traits for variety selection to maximise their potential and enhance resilience and food security in modern agriculture.

6 Outlook

Beyond the scope of this master's thesis, we decided to conduct a field experiment including the four winter wheat varieties that were selected for Experiment 2. The goal of this field experiment is to investigate the potential of AMF inoculation on winter wheat under real-world conditions. On November 14th, 2024, we sow the wheat seeds on one of my grandfather's fields in Dietwil (6042, AG, Switzerland; Coordinates: 47.15259° N, 8.40597° E), which he uses conventionally for growing maize and *Campesino* wheat for livestock feeding.

Fig. 31 provides an overview of the experimental setup. Six blocks, including all four varieties under both treatments, were randomly sown in 15cm wide and 1 metre long lines. We incorporated two sorts of granules that we inoculated with the wheat seeds: one inoculated with AMF propagules for treatment groups and one untreated for controls. Between each line, we left a spacing of one metre to avoid contamination of control samples. Based on recommendations (Strebel, 2024), we calculated the seed density and granule amount based on 350 seeds/m² and 200 kg/ha, respectively. Thus, we used 50 seeds and 3 g of granulate for all 48 rows.

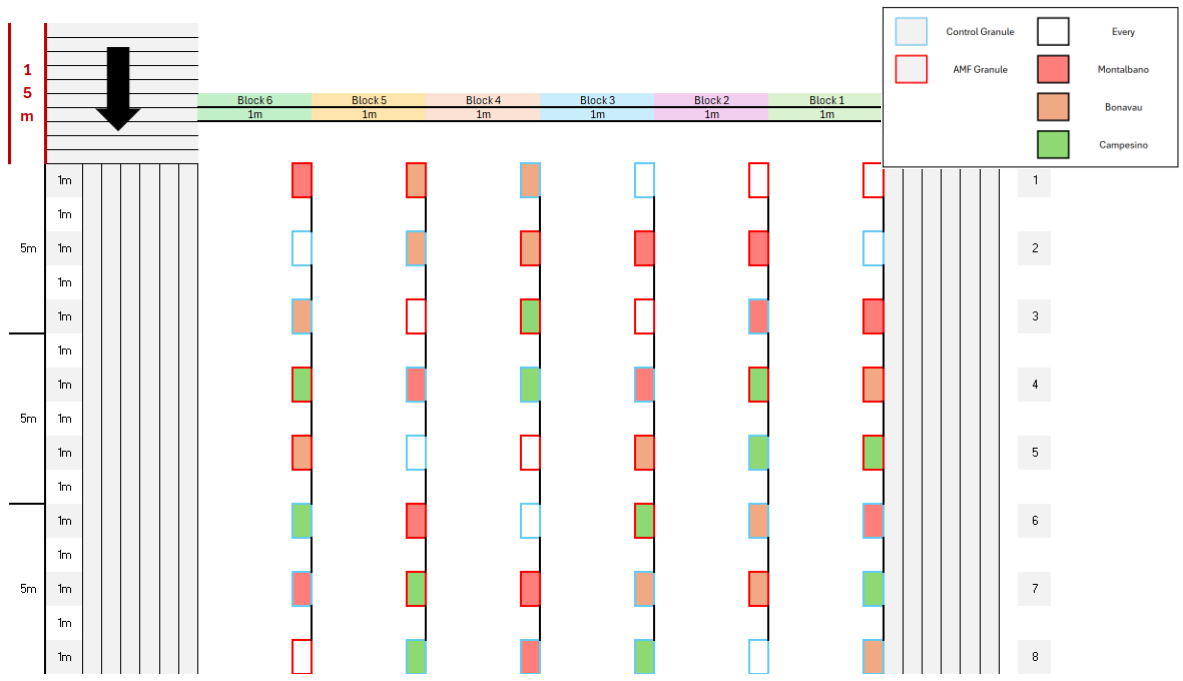

Figure 31: Schematic setup of the field experiment. The same varieties as for experiment 2 were used (Every, Montalbano, Campesino, Bonavau). Six blocks made of 15-metre lines each randomly include all 4 varieties inoculated with control and AMF granulate.

We are planning to harvest the full-grown winter wheat at the beginning/mid of July 2025. Expected outcomes are difficult to predict, as many factors impact results. While meta-studies generally found positive effects of AMF inoculations in field trials with wheat (Pellegrino et al. 2015; Zhang et al. 2019), weather conditions and wheat varieties could alter MGR. A follow-up study from Bakonyi & Csitari

(2023) of their study five years prior tested the same wheat varieties in an intensive crop-producing farm. They found that high N and P content suppressed AMF benefits, but they were more pronounced in the absence of mineral fertilisers. Additionally, they found that under drier conditions, AMF benefits were highly variety dependent, and yields overall were reduced. My grandfather focuses on high N input, incorporating ammonium nitrate fertiliser and slurry, leading to likely high N conditions. Nevertheless, we expect less negative MGR results compared to our greenhouse experiments but similar patterns for tested varieties, mainly since microbial absence likely restricted AMF colonisation and functionality (e.g., Company et al., 2024).

7 Literature

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8 Appendix

8.1 Experiment 1

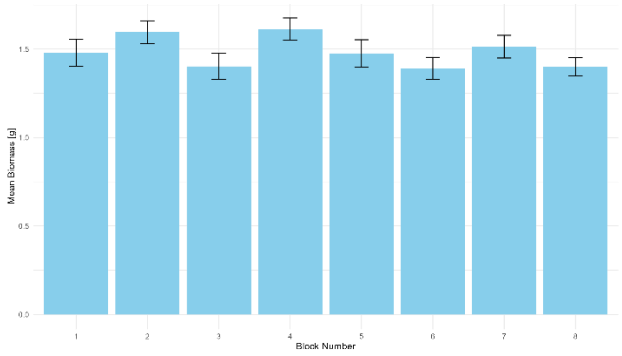


Figure S1: Diagram of mean dry biomass of winter wheat plants for all eight blocks including error bars.

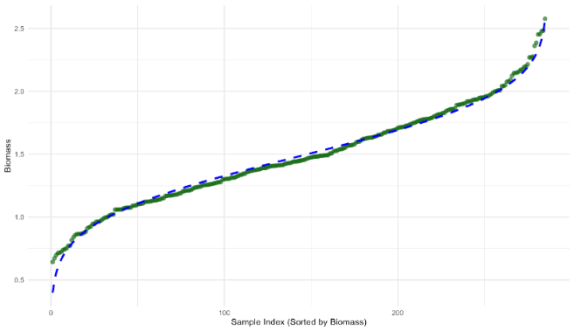


Figure S2: Samples (green dots) sorted by dry biomass weight overlayed on a normal distribution (blue dashed line).

```
# Print ANOVA summary
> print(summary(anova_result_log))
               Df Sum Sq Mean Sq F value Pr(>F)
factor(Block)   7  0.971 0.13868   1.758 0.0957 .
Residuals     277 21.848 0.07888
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
> shapiro_test_result <- shapiro.test(residuals(anova_result_log))
> print(shapiro_test_result)

        Shapiro-Wilk normality test

data:  residuals(anova_result_log)
W = 0.9881, p-value = 0.01909

> levene_test_result <- leveneTest(Log_Biomass ~ factor(Block), data = data_clean)
> print(levene_test_result)
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group   7  0.9827 0.4441
      277
```

Table S1: Statistical tests to verify Block as a random factor. Biomass across all samples was log-transformed and tested with ANOVA which was unsignificant. Shapiro-Wilk normality test revealed significant results; Levene's Test for Homogeneity showed insignificant results.

```
# Perform t-test for each variety
> results <- data %>%
+   group_by(`Variety_Name`) %>%
+   t_test(`Overall_Biomass` ~ Inoculum) %>%
+   adjust_pvalue(method = "bonferroni") %>%
+   add_significance()
>
> # Print results
> print(results)
# A tibble: 18 x 11
   Variety_Name      .y.    group1 group2    n1    n2 statistic    df       p  p.adj p.adj.sig
nif
   <chr>             <chr>  <chr>  <chr>  <int> <int>     <dbl> <dbl>   <dbl>  <dbl> <chr>
 1 "Alpval"          Overa… AMF    Contr…     8     7   -1.71    12.9 0.11    1      ns
 2 "Arina"           Overa… AMF    Contr…     8     8   -2.18    14.0 0.0467  0.841  ns
 3 "Axen"            Overa… AMF    Contr…     8     8   -1.82    14.0 0.0902  1      ns
 4 "Blickfang"       Overa… AMF    Contr…     8     8   -1.11    12.5 0.289   1      ns
 5 "Bonavau"         Overa… AMF    Contr…     8     8   -4.03    13.7 0.00129 0.0232 *
 6 "Cadlimo"         Overa… AMF    Contr…     8     8   -1.45    13.8 0.17    1      ns
 7 "Campesino"       Overa… AMF    Contr…     8     8   -0.953   10.7 0.362   1      ns
 8 "Emblem"          Overa… AMF    Contr…     8     8   -2.53    10.2 0.0294  0.529  ns
 9 "Every"           Overa… AMF    Contr…     8     8    0.773   12.0 0.455   1      ns
10 "Hanswin"         Overa… AMF    Contr…     8     8    0.156   10.6 0.879   1      ns
11 "Kastell"         Overa… AMF    Contr…     8     8    0.0212  12.5 0.983   1      ns
12 "LG Mondial"      Overa… AMF    Contr…     8     8   -1.50    14.0 0.156   1      ns
13 "Mont-Calme 268 " Overa… AMF    Contr…     8     8    0.148   12.7 0.885   1      ns
14 "Montalbano"      Overa… AMF    Contr…     8     8   -0.0149  11.0 0.988   1      ns
15 "Piznair"         Overa… AMF    Contr…     8     8   -0.883   13.0 0.393   1      ns
16 "Plantahof "      Overa… AMF    Contr…     8     6   -0.498   11.0 0.628   1      ns
17 "Probus "         Overa… AMF    Contr…     8     8   -0.612   13.7 0.55    1      ns
18 "Spontan"         Overa… AMF    Contr…     8     8   -0.402   11.8 0.695   1      ns
>
```

Table S2: T-tests of all varieties to determine whether biomass varied significantly between AMF and control samples. Only Bonavau showed significant changes with adjusted p values with the Bonferroni correction.

```
> # Perform Two-Way ANOVA
> anova_two_way <- aov(Overall_Biomass ~ Quality * Origin)

summary(anova_two_way)
             Df Sum Sq Mean Sq F value   Pr(>F)
Quality       5   6.10  1.2193   10.20 5.62e-09 ***
Origin        5   7.02  1.4033   11.74 2.69e-10 ***
Residuals   274  32.75  0.1195
---

# Perform Two-Way ANOVA
> anova_two_way <- aov(MGR ~ Quality * Origin, data = data_clean)
>
> # Show ANOVA table
> summary(anova_two_way)
             Df Sum Sq Mean Sq F value Pr(>F)
Quality       5   2124   424.8   1.362 0.2428
Origin        5   4337   867.3   2.780 0.0202 *
Residuals   133  41488   311.9
---

> # Perform Two-Way ANOVA
> anova_two_way <- aov(Shoot_Root_Ratio ~ Quality * Origin)
>
> summary(anova_two_way)
             Df Sum Sq Mean Sq F value Pr(>F)
Quality       5   3875   775.1   1.659  0.149
Origin        5   2718   543.5   1.164  0.330
Residuals   133  62123   467.1
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S3: Two-Way ANOVA tests to show in where the factors origin and quality have significant impacts. Both factors were highly significant on overall biomass; only Origin was significant on MGR; no factor was significant on S:R ratio.

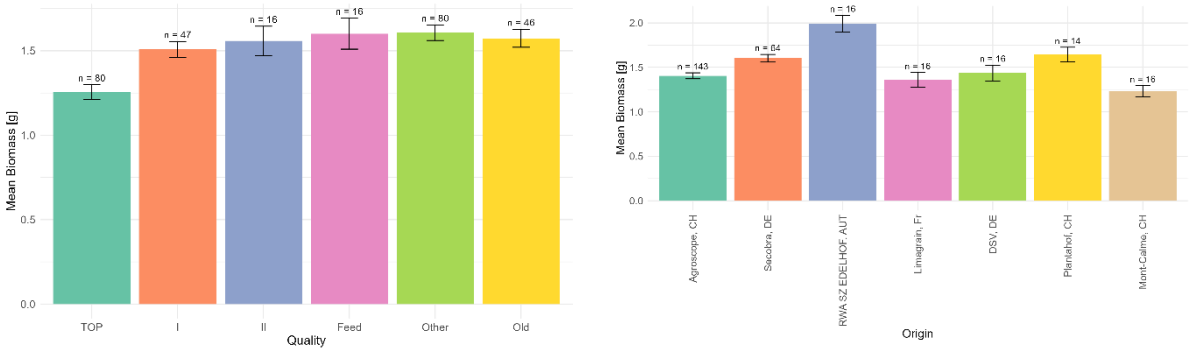


Figure S3: Bar chart of mean dry biomass of different winter wheat varieties by quality (left) and origin (right).

	Variety	MGR	SE	Quality	Origin
1	Every	5.83	4.45	Other	RWA SZ EDELHOF, AUT
2	Mont-Calme 268	0.48	5.76	Old	Mont-Calme, CH
3	Montalbano	-1.17	5.19	TOP	Agroscope, CH
4	Hanswin	-1.18	8.46	I	Agroscope, CH
5	Kastell	-2.37	8.54	Other	Secobra, DE
6	Probus	-3.69	3.68	Old	Agroscope, CH
7	Spontan	-5.00	5.76	II	Secobra, DE
8	Plantahof	-5.66	6.33	Old	Plantahof, CH
9	Blickfang	-10.48	7.51	Other	Secobra, DE
10	Campesino	-10.70	4.87	Feed	Secobra, DE
11	Piznair	-11.54	7.24	TOP	Agroscope, CH
12	Alpval	-13.93	5.76	I	Agroscope, CH
13	Arina	-15.25	4.90	I	Agroscope, CH
14	Cadlimo	-16.08	6.94	TOP	Agroscope, CH
15	Axen	-17.04	6.07	TOP	Agroscope, CH
16	LG Mondial	-17.20	6.86	Other	Limagrain, Fr
17	Emblem	-23.96	4.16	Other	DSV, DE
18	Bonavau	-25.63	4.82	TOP	Agroscope, CH

Table S4: Overview of MGR values of different winter wheat varieties used.

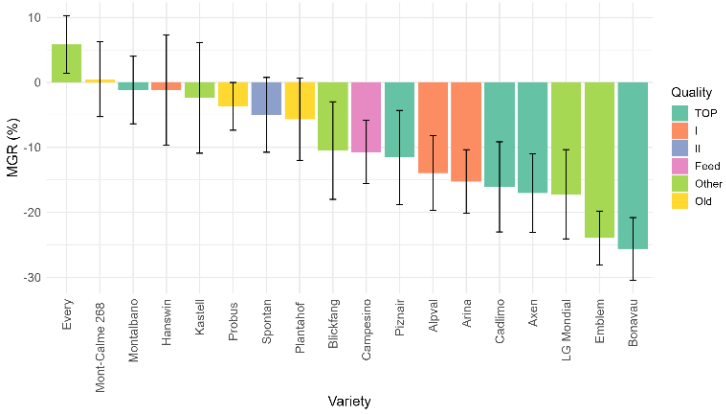


Figure S4: Bar chart of MGR values for winter wheat varieties coloured by Quality.

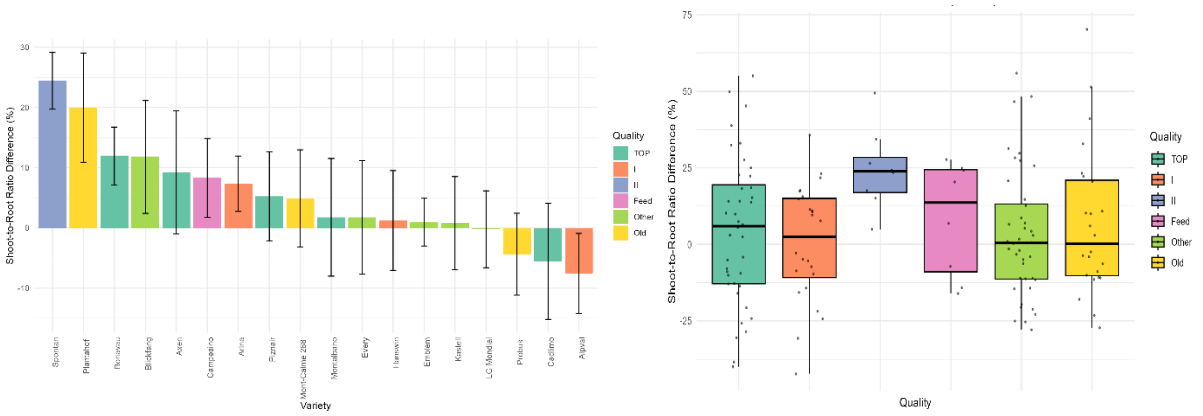


Figure S5: Bar and whisker plots showing the percentual differences of mean shoot-to-root ratio of AMF treatments compared control treatments for quality.

	Variety_Name	p	p.adj	p.adj.signif
	<fct>	<dbl>	<dbl>	<chr>
1	Piznair	0.111	0.145	ns
2	Axen	0.0107	0.0468	*
3	Montalbano	0.232	0.237	ns
4	Bonavau	0.0326	0.0596	ns
5	Cadlimo	0.104	0.145	ns
6	Hanswin	0.209	0.235	ns
7	Alpval	0.237	0.237	ns
8	Arina	0.013	0.0468	*
9	Spontan	0.00492	0.0468	*
10	Campesino	0.194	0.233	ns
11	Blickfang	0.00745	0.0468	*
12	Emblem	0.0121	0.0468	*
13	Every	0.0331	0.0596	ns
14	Kastell	0.0164	0.0492	*
15	LG Mondial	0.0579	0.0947	ns
16	Plantahof	0.029	0.0596	ns
17	Mont-Calme 268	0.0235	0.0596	ns
18	Probus	0.113	0.145	ns

Table S5: T-test results of SAF22 colonisation rates between inoculated and control treatments for each variety. Only 6 of 18 varieties showed significant differences.

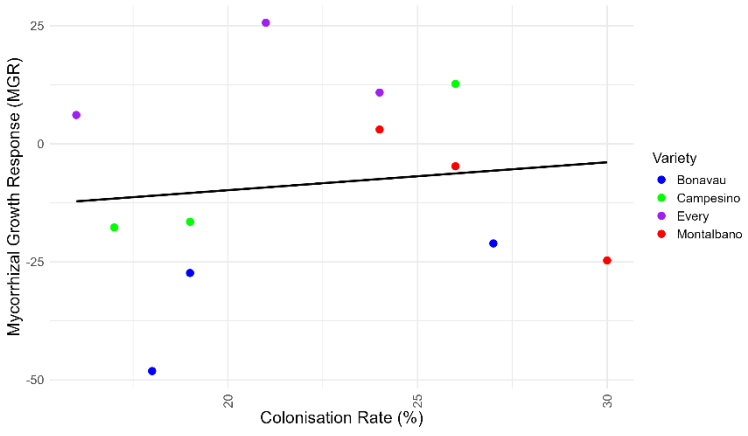
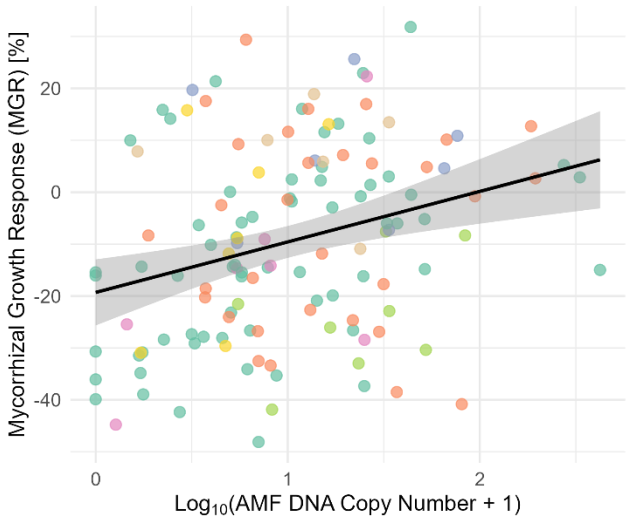


Figure S6: Scatter plot displaying a linear regression of MGR and root colonisation for Bonavau (blue), Campesino (green), Every (purple) and Montalbano (red) (n = 12). Three samples from each variety were investigated.



Origin	n	R	p_value
<chr>	<int>	<dbl>	<dbl>
1 Agroscope, CH	66	0.406	0.000722
2 DSV, DE	8	0.485	0.223
3 Limagrain, Fr	7	0.653	0.112
4 Mont-Calme, CH	6	-0.132	0.803
5 Plantahof, CH	7	0.535	0.216
6 RWA SZ EDELHOF, AUT	7	-0.045	0.924
7 Secobra, DE	31	0.073	0.698

Figure S7: A scatter plot shows a straight-line relationship between MGR and SAF22 copy numbers, indicating a strong positive connection ($p < 0.01$, $R = 0.29$) for all samples. Samples are coloured by Origin. Varieties (Every and Mont-Calme 268) from origins with slight negative correlations displayed overall positive MGR.

8.2 Experiment 2

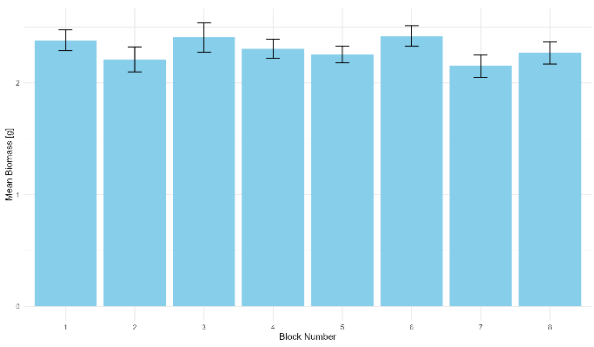


Figure S8: Diagram of mean dry biomass of winter wheat plants for all eight blocks including error bars.

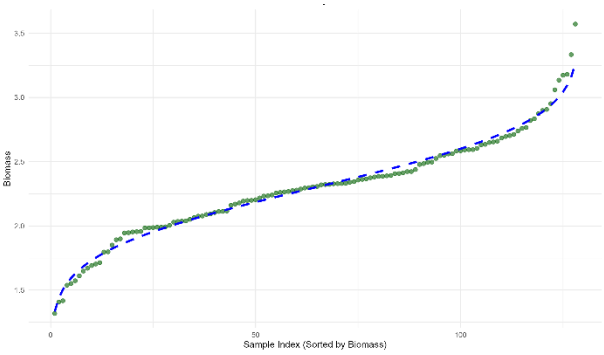


Figure S9: Samples (green dots) sorted by dry biomass weight overlayed on a normal distribution (blue dashed line).

```
Perform one-way ANOVA on log-transformed data
> anova_result_log <- aov(Log_Biomass ~ factor(Block), data = data_clean)
>
> # Print ANOVA summary
> print(summary(anova_result_log))
              Df Sum Sq Mean Sq F value Pr(>F)
factor(Block)   7  0.207 0.02956   0.925   0.49
Residuals     120  3.834 0.03195
>
> shapiro_test_result <- shapiro.test(residuals(anova_result))
> print(shapiro_test_result)

        Shapiro-Wilk normality test

data:  residuals(anova_result)
W = 0.9885, p-value = 0.3627

> levene_test_result <- leveneTest(Overall_Biomass ~ factor(Block), data = data_cle
an)
> print(levene_test_result)
Levene's Test for Homogeneity of Variance (center = median)
       Df F value Pr(>F)
group   7  0.6483 0.7151
      120
```

Table S6: Statistical tests to verify Block as a random factor. Biomass across all samples was log-transformed and tested with ANOVA which was unsignificant. Shapiro-Wilk normality test revealed no significant results; Levene's Test for Homogeneity also showed insignificant results.

```
> # Statistical analysis: Perform t-tests for each variety comparing each inoculum with contro
l
> test_results <- data_mgr %>%
+   group_by(Variety_Name) %>%
+   t_test(Overall_Biomass ~ Inoculum, ref.group = "Control") %>%
+   adjust_pvalue(method = "bonferroni") %>%
+   add_significance()
>
> print(test_results)
# A tibble: 12 × 11
   Variety_Name .y.            group1 group2    n1    n2 statistic    df     p p.adj p.adj.sig
nif
   <fct>        <chr>          <chr>  <chr>  <int> <int>     <dbl> <dbl> <dbl> <dbl> <chr>
 1 Every        Overall_Bioma… Contr… AMF        8     8     0.390   11.2 0.704 1     ns
 2 Every        Overall_Bioma… Contr… Morti…     8     8    -0.387   13.9 0.705 1     ns
 3 Every        Overall_Bioma… Contr… Dual …     8     8     0.806   13.6 0.434 1     ns
 4 Montalbano   Overall_Bioma… Contr… AMF        8     8     0.740   13.7 0.472 1     ns
 5 Montalbano   Overall_Bioma… Contr… Morti…     8     8    -0.0638  11.7 0.95  1     ns
 6 Montalbano   Overall_Bioma… Contr… Dual …     8     8    -0.501   14.0 0.624 1     ns
 7 Campesino    Overall_Bioma… Contr… AMF        8     8     2.18    12.2 0.049 0.588 ns
 8 Campesino    Overall_Bioma… Contr… Morti…     8     8     1.03    14.0 0.319 1     ns
 9 Campesino    Overall_Bioma… Contr… Dual …     8     8     2.60    14.0 0.021 0.252 ns
10 Bonavau      Overall_Bioma… Contr… AMF        8     8    -0.141   12.9 0.89  1     ns
11 Bonavau      Overall_Bioma… Contr… Morti…     8     8    -0.160   12.0 0.876 1     ns
12 Bonavau      Overall_Bioma… Contr… Dual …     8     8    -1.44    14.0 0.173 1     ns
```

Table S7: T-tests of all varieties to determine whether biomass varied significantly between AMF and control samples. No varieties and treatments showed significant changes with adjusted p-values with the Bonferroni correction.

```
> anova_model <- aov(MGR ~ Inoculum * Origin, data = data_mgr)
> anova_results <- Anova(anova_model, type = "III")  # Type III for unbalanced data
> print(anova_results)
Anova Table (Type III tests)

Response: MGR
                Sum Sq Df F value  Pr(>F)
(Intercept)       52.3  1  0.3702 0.54450
Inoculum         258.0  2  0.9134 0.40498
Origin          1301.9  2  4.6092 0.01251 *
Inoculum:Origin  554.5  4  0.9815 0.42197
Residuals      12287.1 87
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S8: Two-Way ANOVA to test origin and quality and on MGR. Only origin was a significant factor.

	Variety_Name	Inoculum	Average_MGR	SE
1	Every	AMF	-3.7617691	5.390743
2	Every	Mortierella	1.2560881	3.270748
3	Every	Dual Inoculation	-3.9530314	2.819794
4	Montalbano	AMF	-3.7829723	3.091324
5	Montalbano	Mortierella	-0.6037020	5.468403
6	Montalbano	Dual Inoculation	1.8964128	3.201803
7	Campesino	AMF	-17.0738085	6.346303
8	Campesino	Mortierella	-6.5568403	4.406524
9	Campesino	Dual Inoculation	-15.6024237	4.188994
10	Bonavau	AMF	0.1677844	3.876823
11	Bonavau	Mortierella	0.1726697	4.431133
12	Bonavau	Dual Inoculation	5.5220072	2.826695

Table S9: Overview of MGR values of different winter wheat varieties and treatments used.

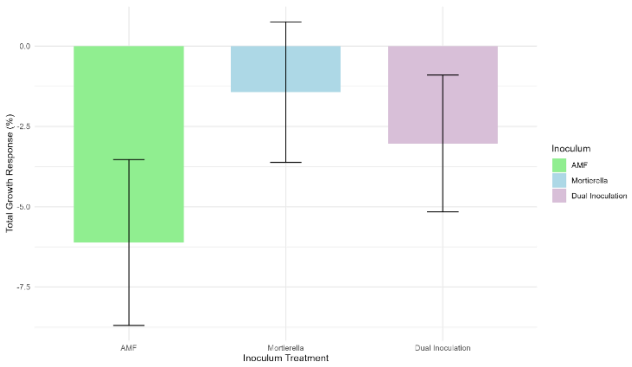


Figure S10: Total growth response of each treatment across all samples.

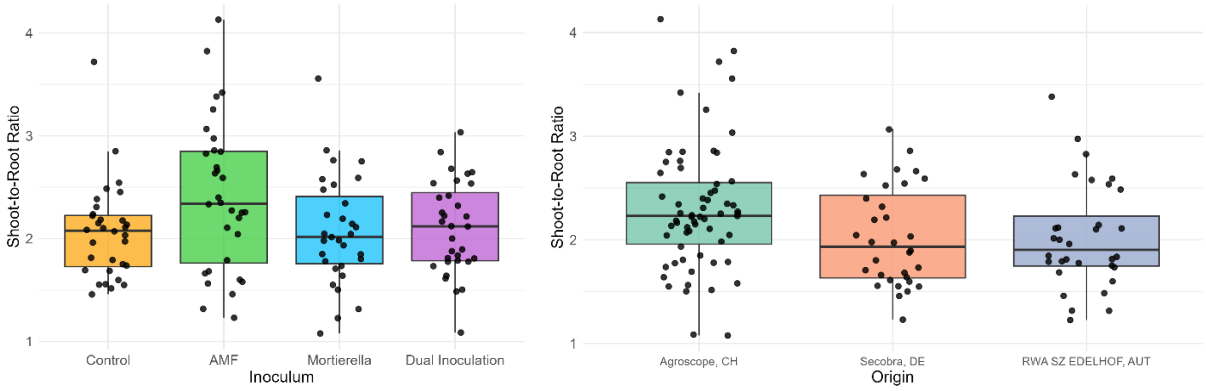


Figure S11: Whisker plots of shoot-to-root ratio for treatments (left) and origin (right).

```
> pearson_test <- cor.test(data_mgr$MGR, data_mgr$ratio_diff, method = "pearson")
> print(pearson_test)

        Pearson's product-moment correlation

data:  data_mgr$MGR and data_mgr$ratio_diff
t = -13.792, df = 94, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.8750591 -0.7387853
sample estimates:
       cor
-0.8180978

>
> lmm_model <- lmer(MGR ~ ratio_diff + (1 | Variety_Name), data = data_mgr)
> summary_lmm <- summary(lmm_model)
> print(summary_lmm)
Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTe
st']
Formula: MGR ~ ratio_diff + (1 | Variety_Name)
   Data: data_mgr

REML criterion at convergence: 643.1

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.48548 -0.67099 -0.05302  0.57205  2.49688

Random effects:
 Groups       Name        Variance Std.Dev.
 Variety_Name (Intercept) 18.68    4.322
 Residual                 43.05    6.561
Number of obs: 96, groups:  Variety_Name, 4

Fixed effects:
            Estimate Std. Error       df t value Pr(>|t|)
(Intercept) -1.83142    2.26533  3.00733  -0.808    0.478
ratio_diff  -0.44302    0.03008 92.15035 -14.728   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S10: Pearson's product-moment correlation and LMM showing a strong correlation between growth response and S:R ratio difference between control and inoculated samples. Inoculation induced S:R ratio changes significantly correlated with growth response.

```
> # Create a distance matrix using Euclidean distance
> distance_matrix <- dist(data_clean %>% select(Overall_Biomass, shoot_root_ratio),
method = "euclidean")
>
> # Run PERMANOVA to test if varieties cluster significantly
> permanova_result <- adonis2(distance_matrix ~ Variety_Name, data = data_clean, pe
rmutations = 999)
>
> # Print PERMANOVA results
> print(permanova_result)
Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = distance_matrix ~ Variety_Name, data = data_clean, permutations =
999)
          Df SumOfSqs      R2      F Pr(>F)
Model      3   14.583 0.24177 13.073  0.001 ***
Residual 123   45.734 0.75823
Total    126   60.317 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S11: PERMANOVA showing a strong negative correlation between overall biomass and S:R ratio, indicating varieties with higher S:R ratios grew worse.

Declaration of Originality

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

Seengen, 24.04.2025 Signature: 

Matriculation Number: 19-765-429 Name, Family Name: Dylan Baumann