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# Rooted in the Past: The Effect of Soil Legacy and Mycorrhizal Symbiosis on Red Clover and Soil Properties

GEO 511 Master's Thesis

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# I. Abstract

Global food security is increasingly threatened by climate change and the reliance on conventional, high-input synthetic fertilization, which degrades physical soil structures, depletes nutrients, and diminishes native soil microbiomes. While organic amendments and microbial bioinoculants offer a sustainable alternative, it remains unclear how the long-term legacy of different fertilization strategies impacts the establishment and success of newly introduced microbes. Therefore, this thesis investigates how distinct soil legacies (soils with mineral or organic fertilisation) influence the efficiency of arbuscular mycorrhizal fungi (AMF) inoculation in restoring soil health parameters and maintaining crop yields. This was evaluated by linking a long-term field trial with various organic fertilizers (e.g. composts, digestate, slurry, mineral-, control) to a controlled greenhouse experiment, where post-harvest soils were inoculated with a promising AMF strain (*Rhizoglomus irregularis*, SAF22), grown with clover *(Trifolium pratense)* and assessed using a multidimensional approach. For the field trial, the initial yield gap by the mineral fertilized control was (statistically) closed by the third season, though this may partly reflect crop-specific sensitivities. In the greenhouse, AMF inoculation universally increased plant growth and health across all treatments. Crucially, a symbiotic paradox emerged based on soil legacy: the mineral fertilized soil enabled the highest AMF root colonization but provided the lowest relative growth benefit, whereas nutrient-bound compost legacies restricted new fungal establishment but maximized the plant's actual growth response. This happened because mineral fertilizers suppress native microbes, creating an empty niche, whereas organic soils maintained competitive native communities that drove the plant to rely heavily on the tripartite relationship of AMF, the host plant and nitrogen fixing rhizobia bacteria. The findings ultimately show that transitioning to organic fertilization not only builds long-term soil quality, but it creates the necessary ecological foundation to maximise the benefits of microbial symbioses, offering a potentially effective strategy to sustainably replace synthetic inputs.

# II. Acknowledgments

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# V. List of Abbreviations

| | |
|---|---|
| AMF | Arbuscular Mycorrhizal Fungi |
| ANOVA | Analysis of Variance |
| BNF | Biological nitrogen fixation |
| C | Carbon |
| CoFEE | Compost Field Experiment Ehrendingen |
| DNA | Deoxyribonucleic acid |
| EA | Elemental Analyzer |
| GLM | Generalised Linear Model |
| MD | Mycorrhizal dependency |
| MGR | Mycorrhizal growth response |
| N | Nitrogen |
| NM | non-mycorrhizal |
| P | Phosphorus |
| PERMANOVA | Permutational Analysis of Variance |
| PGPR | Plant growth promoting rhizobacteria |
| Potential CEC | Potential Cation Exchange Capacity |
| $R^2$ | Coefficient of determination |
| SAF22 | *Rhizoglomus irregulare* strain SAF22 |
| SHI | Soil Health Index |
| SOC | Soil organic carbon |
| SOM | Soil organic matter |
| S-Value | Sum of exchangeable bases |
| SynCom | Synthetic Microbial Community |

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# 1 Introduction

## 1.1 Intensive agricultural management problems

A rapidly growing human population, projected to reach over 9 billion people by 2050, is putting unprecedented pressure on the global agriculture and food security (Brito et al., 2021; Tahat et al., 2020). To meet this rising demand, the industrialization of agriculture during the "Green Revolution" in the late 1960s had the goal of maximising crop production through higher yielding crop varieties, intensive mechanical tillage, and massive applications of synthetic chemical fertilizers (Bhunia et al., 2021; Samantaray et al., 2024). However, this pursuit of short-term productivity has come at a severe ecological and economic cost. Today, conventional high-input agriculture is trapped in an unsustainable dependency on synthetic agrochemicals (Kuila & Ghosh, 2022). It is driven by a depletion of finite resources (such as phosphate rocks) and global political instabilities, the cost of inorganic fertilizers has increased significantly by up to 300% in recent years, which places a high financial burden on farmers worldwide (Kuila & Ghosh, 2022; Sabater et al., 2025).

Fundamentally, conventional agriculture disrupts the natural nutrient cycles of the soil microbiome and leads to a shift from a self-sustaining, low-input ecosystem to a highly vulnerable, high-input synthetic system (Kuila & Ghosh, 2022). This poses several problems, such as a loss of the ability to naturally suppress diseases by a widespread reduction of beneficial soil microbiota (Kuila & Ghosh, 2022). In naturally healthy ecosystems, a diverse abundance of soil microbiota keeps harmful pathogens and organisms manageable through natural predation and competition (Sabater et al., 2025; Tahat et al., 2020). With the decrease in soil biodiversity by intensive farming, modern cultivated become highly susceptible to infections and diseases (Kuila & Ghosh, 2022). As a consequence, farmers are forced to rely even more heavily on the application of toxic agrochemicals (such as fungicides, pesticides and nematicides) to control leaf, seed and soil-borne pathogens (Kuila & Ghosh, 2022; Sabater et al., 2025). This creates a seemingly endless cycle, where chemicals actively degrade the beneficial microbes in the soil, such as arbuscular mycorrhizal fungi (AMF), and are eliminating the natural mechanical barriers and pathogen resistance that the microbiome would normally provide, thereby creating an ecosystem imbalance (Kuila & Ghosh, 2022). Beyond biological degradation, the dependency on synthetic fertilizers creates environmental pollution, salinization, acidification and nutrient-use inefficiency (Y. Liu et al., 2024; Verzeaux et al., 2017). While massive amounts of synthetic fertilizers are applied for securing high yields, it is estimated that approximately 50% of applied nitrogen (N) and 80-90% of applied phosphorus (P) can never be used by the crops and are ultimately lost to the environment (Bender et al., 2023; Solangi et al., 2023). N can leach into groundwater as highly mobile nitrate and volatilizing into the atmosphere as nitrous oxide, a strong greenhouse gas (GHG) (Haider et al., 2025; Verzeaux et al., 2017). Recent trials in Switzerland using a lysimeter setup in simulated arable soils (to assess nutrient uptake and N loss through leaching and gaseous emissions) show that simplified soil microbe communities exhibit a 65% increase in N leaching loss and a doubling in GHG emissions compared to soils with a robust and diverse microbiome (Bender et al., 2023) Synthetic P can rapidly bind with iron, aluminium, calcium and magnesium, forming insoluble complexes in the soil (Solangi et al., 2023). This leads to a long-term buildup of legacy P that plants cannot access (Solangi et al., 2023). For Switzerland, it was shown that the vast majority of conventionally managed soils is well above the national threshold for P deficiency due to long-term high-input fertilization (Lutz et al., 2023). In natural soil ecosystems, plants rely on AMF and bacteria to dissolve and transport the chemical nutrient structures to the roots (Dukare et al., 2022; Rubin & Görres, 2020). However, through a continued application of synthetic P and intensive mechanical tillage actively suppress and destroy the native fungal networks and destroy the macro-aggregate structure of the soil (Ghorui et al., 2024; Loaiza Puerta et al., 2018).

Moreover, the physical degradation of the soil structure leads to topsoil erosion, soil compaction and a decline of the soils biological foundation, soil organic matter (SOM) (Kacprzak et al., 2022; Loaiza Puerta et al., 2018). S By destroying the soil structure, it can no longer hold water, meaning that the highly fertilized topsoil is essentially washed away with surface runoff and leaching into lakes, rivers and groundwater, causing water eutrophication which results in strong growth of toxic algae, loss of aquatic life and the contamination of drinking water (Airee & Mukherjee, 2025; Rubin & Görres, 2020). Furthermore, global climate change accelerates this degradation, with an increased frequency of extreme weather events, such as heatwaves, heavy rainfall and droughts, which weaken plant physiological functions and decreases the water use efficiency (Ostadi et al., 2022).

## 1.2 Soil Health in Agriculture

Soil health is an essential and widely discussed term modern agronomy and soil science, which represents a historical paradigm shift from viewing soil only as a growing medium to recognizing it as a complex, dynamic biological ecosystem (Doran & Zeiss, 2000) Today, soil health is widely defined after Lehmann et al. (2020: 1): “Soil Health is the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans, and connects agricultural and soil science to policy, stakeholder needs and sustainable supply chain management”. It is important to distinguish this concept from seemingly similar terms, for example “soil fertility”, which focuses on maximizing crop production for human use, and “soil quality” evaluates a soil’s fitness for a specific anthropogenic purpose (Lehmann et al., 2020; Tahat et al., 2020). Soil health is a broader, intrinsic ecological concept, focussing on long-term ecological sustainability (Doran & Zeiss, 2000; Lehmann et al., 2020). Healthy soils are essential for both human and environmental well-being because they simultaneously balance multiple ecosystem services, including sustainable plant production, water quality regulation, providing nutrient dense crops and mitigating climate change via carbon sequestration (Doran & Zeiss, 2000; Lehmann et al., 2020). Because soil functions as a living ecosystem, its health cannot be measured by a single metric, but instead must evaluated through the integration of interconnected physical, chemical, and biological parameters (Bertola et al., 2021; Doran & Zeiss, 2000). Ultimately, so establishing a baseline for soil health is the necessity for sustainable agriculture, as healthy soils provide the resilience required to combat and withstand climate change.

### 1.2.1 Multidimensional Indicators of Soil Health

Quantifying physical parameters enables an understanding of soil structural stability, which determines both its resistance to degradation and its efficiency in water infiltration and retention (Doran & Zeiss, 2000). The key metrics include: soil aggregate stability, bulk density, topsoil depth, porosity, infiltration rates and water holding capacity (Diacono & Montemurro, 2010; Lehmann et al., 2020; Tahat et al., 2020). A crucial metric used in this study is the wet aggregate stability which measures how well soil particles bind together to resist from rainfall and mechanical tillage (Loaiza Puerta et al., 2018). Importantly, physical structure does not exist alone, it is fundamentally reliant on SOM, which is the central biological foundation of the soil (Diacono & Montemurro, 2010). High aggregate stability indicates a robust soil structure which is essential for root growth and an efficient movement of water and gases through the soil profile (Diacono & Montemurro, 2010). Furthermore, structural parameters like water infiltration and water holding capacity are vital for evaluating how soils can respond to extreme weather events, which are becoming more frequent due to climate change(Gao et al., 2023; Loo et al., 2022)

For chemical parameters, the focus shifts to evaluating the nutritional status, potential toxicity, and elemental balance

of the soil environment. Core metrics include soil pH, electrical conductivity (salinity), cation exchange capacity (CEC), total organic C, and the concentrations of plant-available macro- and micronutrients (Doran & Zeiss, 2000; Lehmann et al., 2020; Paradelo et al., 2023). A focus on nutrients is important for plant growth and the establishment of a successful symbiosis with microbial inoculants. Supplying these nutrients solely through synthetic fertilizers is costly and heavily reliant on finite global resources such as mined rock phosphate (Mpongwana et al., 2023; Paradelo et al., 2023; Powers & Thavarajah, 2019). However, chemical metrics are also vital indicators of anthropogenic soil degradation. The heavy reliance on synthetic agrochemicals and conventional irrigation actively influences soil acidification and salinization that represent drivers of chemical soil health loss (Bhunia et al., 2021; Y. Liu et al., 2024).

The evaluation of biological parameters aims to quantify the activities of "biological engineers" that drive nutrient cycling, organic matter decomposition and natural disease suppression (Lehmann et al., 2020; Tahat et al., 2020). These parameters include soil respiration microbial biomass, active enzymatic activities, pathogen abundance, and the functional diversity of bacteria, fungi, earthworms, and nematodes (Bertola et al., 2021; Doran & Zeiss, 2000; Lehmann et al., 2020; Tahat et al., 2020). Soil biodiversity is crucial as it shows the soil's ability to support symbiosis with beneficial microbial communities that supports crop success (Bender et al., 2023; Samantaray et al., 2024). Importantly, the biological activity of AMF directly connects back to physical soil health through the secretion of glomalin which acts as a biological 'glue' that binds and stabilizes physical soil macroaggregates (Loaiza Puerta et al., 2018; Onyeaka et al., 2024; Rillig et al., 2002).

A major achievement of the modern soil health framework is the acknowledgment that the chemical and physical parameters alone cannot sustain agriculture or explain every ecosystem function of the soil but the biological parameters are needed to represent the full dynamics of a soil (Lehmann et al., 2020). However, little researchers actually provide a full soil health assessment and usually focus on some key parameters, which means that mostly the biological ones are heavily underrepresented in current research (Lehmann et al., 2020). Some modern publications try to integrate different variables into a single, comprehensive Soil Health Index (SHI), for example by averaging normalized z-scores with parts of the three main parameters of soil health (Edlinger et al., 2025; Rog et al., 2025). However, as it was also experienced in this thesis, measuring and evaluating every single possible variable that would influence a complete soil health assessment is extremely time consuming and requires several perspectives with various areas of expertise, which is why most research focuses on some critical ones, rather than trying to assess everything (Lehmann et al., 2020). Due to the expertise of the research group this thesis was conducted in, it was mainly focused on selected biological variables, with the evaluation of AMF and nodules of rhizobia, plant performance data and some physicochemical parameters of the soil. This only captures a fraction of the essential variables, but it still can give an insight into how the soil behaves and as how healthy it can be seen (see **4.2**).

### 1.2.2 Restoration Strategies

Management strategies to restore degraded soil must include practices that combines all three parameters of soil health. The conventional agriculture's heavy reliance on synthetic agrochemicals has led to a large degree of soil degradation, environmental pollution, and a disruption of natural nutrient cycles, a primary strategy for restoration requires the transition to organic-based fertilization and soil ecological engineering (Bender et al., 2023; Kumar et al., 2023). The literature categorizes restoration strategies broadly into two main approaches: untargeted management (altering

agricultural practices) and targeted management (direct application of specific biological inoculants) (Bertola et al., 2021).

Untargeted management includes organic fertilizers and conservation practices, such as replacing synthetic inputs with an application of organic fertilizers, such as composts, digestates and biochar (Bertola et al., 2021; Enaime et al., 2023). Returning the residues of nutrients and C in these amendments can establish a circular nutrient economy that minimises greenhouse gas emissions and closes nutrient loops (Enaime et al., 2023; Paradelo et al., 2023). Unlike highly soluble synthetic fertilizers, the organic amendments slowly release nutrients and refill SOC, while physically stabilizing soil macro-aggregates (Diacono & Montemurro, 2010; Gao et al., 2023; Loaiza Puerta et al., 2018). To maximise the benefits, organic inputs should be paired with conservation agriculture, such as reduced tillage and incorporating cover crops to protect the hyphae of beneficial fungi and sustain microbial populations (Loaiza Puerta et al., 2018; Tahat et al., 2020; Vermeire et al., 2024). Together, they can form a tripartite symbiosis (see **1.4**).

## 1.3 Arbuscular Mycorrhizal Fungi (AMF)

AMF are obligate, mutualistic, symbiotic fungi belonging to the phylum Glomeromycota (Gough et al., 2022). They can form symbiotic relationships with over 80% of terrestrial plants. The relationship likely has co-evolved for over 400 million years and it is indicated that this was a crucial step that allowed early plants to transition from water to land (Oruru & Njeru, 2016; van der Heijden et al., 2015). Preparations for the AMF symbiosis start with the plant exuding *strigolactones* into the rhizosphere to trigger the germination of fungal spores and hyphal branching. Fungal spores then secrete *Myc* factors (lipochitooligosaccharides) that prepare the plants cells for colonization. This process is known as "SYM pathway" and is a highly conserved genetic pathway, meaning this ancient set of genes is still found almost unchanged today (Samantaray et al., 2024; van der Heijden et al., 2015).

### 1.3.1 Morphology and Resource Acquisition

After colonizing the host plants roots, they start to form specialized tree-like structures inside the root's cortex called arbuscules. Arbuscules act as a bidirectional hub for exchanging nutrients and carbon between the plant and the fungi. Additionally, AMF form vesicles which in contrast to arbuscules serves as storage organs, mainly for lipids and carbon (Kuila & Ghosh, 2022; van der Heijden et al., 2015). The symbiotic relationship can be understood as a massive extension of the plants root system in the soil, where the fungi's extraradical hyphal network has the ability to penetrate microscopic soil particles due to being up to 100 times finer than plant roots, and thereby mobilizing immobile nutrients in the soil and making them available for the plant. For this, AMF secrete organic acids, acid/alkaline phosphates and proteases to resolve bound inorganic and organic nutrients, such as P(Kuila & Ghosh, 2022; Samantaray et al., 2024; van der Heijden et al., 2015). In return, the plant can give up to 30% of its photosynthetic carbohydrates and fats to the fungi (Kuila & Ghosh, 2022; Samantaray et al., 2024). In addition to P, AMF use high-affinity transporters located in the mycelia, to forage for highly immobile inorganic N (ammonium & nitrate) (Verzeaux et al., 2017; Zhang et al., 2024). AMF can also collect up to 30% of the organic N found in decomposing organic matter, despite being

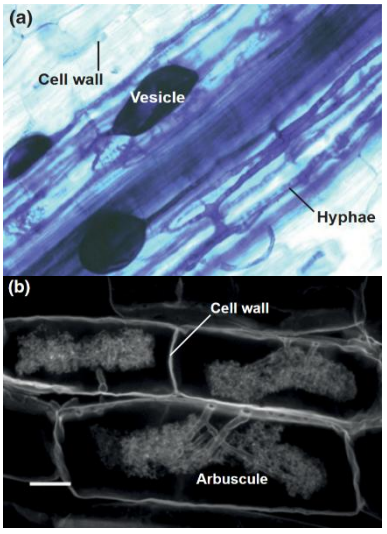



***Figure 1: AMF morphology.****(a) Clover root colonized by Glomus intraradices, picture by Marcel G. A van der Heijden. (b) Pisum sativum root cells with arbuscles. Picture by Peterson et al. (2004) and NRC press. Both taken from Marcel G.A. van der Heijden et al.*

obligate biotrophs. When taken up, the N is transported through the hyphal network (as amino acids) and taken up by the host plant's root cells as ammonium (Thirkell et al., 2016; Verzeaux et al., 2017).

### 1.3.2 Mycelial Connectivity and Stress Resilience

Most AMF are not host-specific and are able to simultaneously colonize multiple plants and by that they can form a large interconnected underground network, while plant roots can also be colonized by multiple different mycorrhizal fungi (van der Heijden et al., 2015). It is suggested that these networks, also called 'wood-wide-webs' function like a 'biological market' and are interchanging nutrients between the plants and fungi and allocate more nutrients to the plants providing the most C and vice versa (van der Heijden et al., 2015). The symbiosis goes beyond just nutrient exchange, as AMF can be a strong protection mechanism against several abiotic and biotic stress factors.

Abiotically, they can help to chemically and physically alter environmental conditions, such as reducing stress caused by temperature extremes, balance varying nutrient levels, increase tolerance in low-pH soils, improving the drought tolerance and protects against certain levels of salinity and can even capture toxic elements (i.e. heavy metals) within the field and make it immobile (Kuila & Ghosh, 2022; Samantaray et al., 2024).

Additionally, AMF also provide some protection against biotic factors, where they can generally increase the resistance against pests and soil-borne diseases, increase tolerance against nematodes and combat pathogenic fungi in the rhizosphere. AMF will directly compete with pathogens for nutrients and space, while also producing polysaccharides and phenolic compounds that thicken cell walls. Some AMF even produce antifungal and antibacterial antibiotics that inhibit pathogens and have the ability to indirectly trigger the plants defence system by plant-mediated mechanisms to reduce damage from soil borne-diseases (Kuila & Ghosh, 2022). With these mechanisms of reducing stress and optimizing nutrient uptake, AMF can significantly increase the accumulation of secondary metabolites, not just primary, thereby improving the nutritional value of crops (Bertola et al., 2021; Kuila & Ghosh, 2022).

### 1.3.3 Soil Architecture and Parasitism

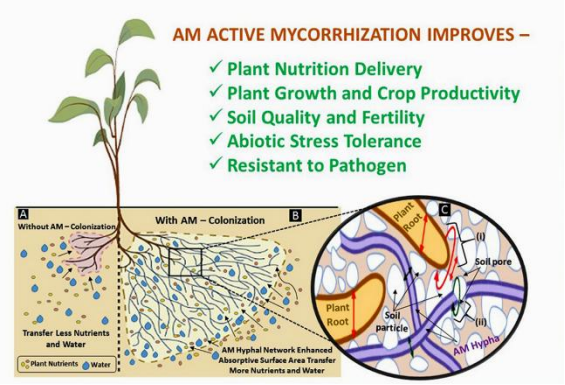



***Figure 2: Schematic representation of AMF functions****. (A) without AMF, (B) with AMF. Taken from Kuila and Gosh (2022), CC-BY-NC-ND license.*

AMF are described as "biological engineers" that directly influence soil health by providing stronger soil structure with the hyphal network, exuding a protein called glomalin, which can bind soil particles into more stable aggregates (Morton et al., 2014). This can increase the porosity of the soil, enhance water-holding capacity and prevent top-soil erosion and contribute to long-term carbon sequestration (Kuila & Ghosh, 2022; Oruru & Njeru, 2016; van der Heijden et al., 2015).

However, while AMF symbiosis is often viewed as only beneficial, it is not a guaranteed success and brings some complications depending on the environmental circumstances. The AMF-host relationship can only be really successful if the plant is limited by nutrients (like P), which can be described as mutualism-parasitism continuum (Thirkell et al., 2016; van der Heijden et al., 2015). For example, in highly productive and healthy soils, or in high P-input soils, the high carbon costs by the AMF can far succeed the nutritional benefits it can provide and ultimately result in a carbon drain and can lead to a negative growth response (Rog et al., 2025).

In extremely N-deficient soils, AMF may prioritize its own survival and start to immobilize N for their own growth

rather than sharing with the host, resulting in a 'parasitic shift' of the relationship (Thirkell et al., 2016; Verzeaux et al., 2017). The mechanisms explained in the 'biological market' is not flawless, as host plants often have problems excluding fungi from the carbon trade that do not provide nutritional value (van der Heijden et al., 2015). Because the successful relationship between AMF and host is strictly context dependent and heavily relies on existing soil health, a baseline crop productivity, native pathogen abundance, and the specific crop genotype, a commercial field application remains inconsistent and unpredictable (Lutz et al., 2023; Rog et al., 2025).

## 1.4 Rhizobia Bacteria

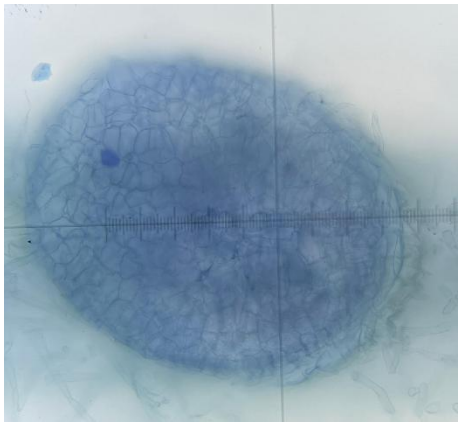


***Figure 3: Rhizobia bacteria nodule at 200x magnification.*** *Picture taken from roots of red clover with microscopy, by Cedric Gehrer.*

Rhizobia are a group of plant growth promoting rhizobacteria (PGPR) that nodulate roots and establish mutualistic symbiotic relationships mainly with leguminous plants, such as peas, soybeans and clover (Mpongwana et al., 2023; Samantaray et al., 2024). At first, the host plant releases flavonoid compounds into the rhizosphere (secondary metabolites), after which a nodulation expression is started in compatible rhizobial strains. This releases nodulation factors causing the plant's root hair to curl, leading to the formation of protective structures called nodules (Samantaray et al., 2024). This protected, micro-aerobic environment enables the bacteria to start fixating nitrogen by converting atmospheric dinitrogen gas ($N_2$) into Ammonium ($NH_3$) (Gough et al., 2022; Oleńska et al., 2022; Samantaray et al., 2024). The host plant provides photosynthetic carbohydrates to sustain the energy-demanding nitrogen fixating process, which is called biological nitrogen fixation (BNF). With BNF, rhizobia are essential in the global nitrogen cycle that can help in reducing the use of synthetic nitrogen fertilizer (Gough et al., 2022; Oleńska et al., 2022; Samantaray et al., 2024). Even though rhizobia exist freely in the soil without a host plant, only within a nodule of the host they differentiate into bacteroids which enables the BNF. Furthermore, rhizobium fertilizers can minimize nitrogen runoff and the associated GHG emissions of conventional fertilizers (Samantaray et al., 2024).

Beyond nitrogen fixation, rhizobia can enhance root architecture, assist in nutrient acquisition and provide stress resilience by neutralizing ethylene and sequestering heavy metals in root tissues (Oleńska et al., 2022; Samantaray et al., 2024).

A rather important part of this thesis is the so-called 'tripartite symbiosis' of rhizobia, host plant (clover) and AMF due to the rhizobia having a high demand of P for BNF and themselves are not able to acquire enough P on their own (Gough et al., 2022; Samantaray et al., 2024). Hence, AMF provide sufficient P to the Rhizobia and the plant, to sustain the BNF process and the rhizobia provide the plant with nitrogen to sustain the carbon costs for the AMF symbiosis. This means that the afore mentioned 'parasitic shift' of AMF in N deficient soils is no longer a point of concern due to rhizobia constantly fixing nitrogen and effectively eliminating the nitrogen limitation (Gough et al., 2022; Samantaray et al., 2024; Thirkell et al., 2016). Now, the plant has the nutrients to effectively enhance its photosynthesis and produce an abundant amount of carbon. It's now no longer N-limited, nor C-limited and the AMF can be fully symbiotic, creating a positive feedback loop (Gough et al., 2022; Verzeaux et al., 2017).

## 1.5 Mycorrhizal Growth Response (MGR)

Mycorrhizal growth response (MGR), which is also referred to as mycorrhizal dependency (MD) in older literature,

is an established metric used to quantify how much the host plant responds and relies on the AMF symbiosis for achieving maximum growth or yield (Cheeke et al., 2019; Tawaraya, 2003; van der Heijden, 2002). Because an effective plant-microbe symbiosis is essential for a soil ecosystem, MGR can be used as an indicator to quantify the biological soil health (Rog et al., 2025). The MGR can be calculated by comparing the yield or biomass of a group of plants inoculated with AMF and a control group of plants that is grown in the same, controlled conditions without the inoculation of AMF (Cheeke et al., 2019; Tawaraya, 2003; van der Heijden, 2002). The MGR is more meaningful in controlled greenhouse conditions, as in field-conditions the MGR is highly variable due to a variety of uncontrolled biotic and abiotic factors. In a study by Lutz *et al.* (2023) where maize was inoculated with AMF in a large-scale field experiment, results of MGR showed a range from negative 12% growth reduction to a 40% growth increase. This variation can be explained by plant pathogens (mainly fungal) in the soil, which show the problematic of applicability of MGR in field-conditions (Lutz et al., 2023; Rog et al., 2025). MGR is highly influenced by plant genetics, fungal root morphology, the baseline soil health conditions, community aspects, climate and biotic as well as abiotic stressors (Cheeke et al., 2019; Köhl et al., 2016; Lutz et al., 2023; Rog et al., 2025; Tawaraya, 2003). The consensus is that the MGR is highest in degraded, low-fertility soils. However, this is not strictly the case, as some studies have shown. For example, in a study by Köhl, Lukasiewicz, & van der Heijden, 2016 it is shown that AMF can benefit plants even in fertile soils through fungal adaptability. Certain AMF strains, such as *Rhizoglomus irregularis*, can adapt to intensive agricultural practices and a range of soil parameters and are therefore compatible with high-nutrient soils. They can compete and establish with native fungal communities, without shifting into a parasitic carbon-drain (Köhl et al., 2016). AMF strains that originated from high-richness P soils, they may be already adapted to these exact conditions (high P), and shift their role to supply whatever lack of other nutrients (such as N) are limiting the plant's growth (Gamper et al., 2005). In a recent study by Boussageon *et al.* (2025) it is also shown that many commercially available AMF inoculants are of low quality and fail to effectively colonize the roots and establish a symbiosis, showing the need for a promising AMF strain, such as SAF22, to be tested in more trials.

### 1.5.1 Responses on *Trifolium Pratense* (Red Clover)

***Figure 4: Flowering Red clover shoot at harvest.*** *Picture taken by Cedric Gehrer.*

*Trifolium pratense* serves as the plant species of the second part of this thesis, and it shows to be of particular interest regarding AMF symbiosis and rhizobia interactions. Red clover is consistently considered to be a remarkably responsive plant species to symbiotic relations (Köhl et al., 2016). Among grassland species, red clover shows one of the highest possible MP with its MGR approaching 100%, according to a study by van der Heijden (2002). This means that red clover is almost entirely dependent on AMF for optimal growth, but also if a compatible strain is inoculated the growth benefits are massive, even when inoculated in unsterilized field soils that contain native fungal DNA. In a study by (Köhl et al., 2016), where across multiple agricultural soils red clover reached an MGR ranging from 33% to 51%. Apart from AMF, red clover relies on its association with rhizobia, that colonize the root endosphere. In agricultural practice, the highly effective N-fixing relationship makes red clover an important fertility-building crop (Wahdan et al., 2021). It is frequently used in crop rotations as a sustainable method to introduce N in low-input systems, as its decomposing residues can release 40-70% of the total plant N back into the soil (Wahdan et al., 2021). However, using this N-fixing potential in field conditions requires a functioning soil microbiome. The extreme MGR that red clover reaches is not entirely

based on AMF, but again a tripartite relationship of AMF-clover-rhizobia, where a study by Shelby *et al.* (2016) showed that the entire *Trifolium* genus (true clovers) do only associate with one specific biovar: *Rhizobium leguminosarum bv. trifolii*, which relies heavily on the AMF relationship to function. The P-demand for this specific rhizobia is so high that the colonization of true clovers can fail in phosphorus-limited soils unless the plant is simultaneously colonized by AMF (Shelby et al., 2016). Therefore, to successfully use red clover as a biological fertilizer in crop rotations, field management practices must preserve AMF networks to ensure that clover receives enough P to sustain the N-fixation process.

## 1.6 Research Gap

Sustainable agriculture relies on long-term application of organic fertilizer treatments that are widely recognized for improving soil health and preserving the native microbial communities, including native AMF diversity, compared to conventional, high-input agriculture (Maeder et al., 2002; Oehl et al., 2004). However, the nutrient properties and mineralization levels left behind in the soil are highly different, meaning the fertilizer treatments should have distinct physical, chemical and biological legacies in the soil (W. Liu et al., 2019). These varying organic legacies, as well as the mineral N-fertilizer influences, form highly variable soils for subsequent crops and the microbiomes in multi-crop rotations (Gollner et al., 2011; W. Liu et al., 2019). As shown previously, legumes, such as red clover, are frequently integrated into the multi-crop rotations to naturally (re)fertilise degraded soils (Wahdan et al., 2021). Due to this ability to release large amounts of N back into the soil during decomposition, red clover acts as a fertility-building crop and reduces the reliance on synthetic N inputs (Wahdan et al., 2021). Red clover shows an extreme mycorrhizal dependency (MD) and relies heavily on the tripartite symbiosis of AMF-plant-rhizobia to maximise growth and restore minerals such as N in the soil (Köhl et al., 2016; van der Heijden, 2002; Wahdan et al., 2021). However, it's unclear which type of long-term organic fertilization legacy provides an optimal microbial and nutritional baseline for crops (Köhl et al., 2016; Rog et al., 2025). Maximizing growth with commercial AMF strains is viewed as a biofertilization strategy, yet field-inoculations yielded highly variable results and depending strongly on the baseline soil health, native pathogen abundance and environmental 'noise', such as varying weather, rainfall and temperature (Lutz et al., 2023; Rog et al., 2025). Furthermore, in controlled experiments, the benefits of AMF inoculation are often researched in artificial, hydroponic or sterilized soils, which remove the native microbiome and show zero competition for the inoculated AMF strain. Consequently, they fail to represent the biological reality of agricultural fields (Köhl et al., 2016; McLaughlin et al., 2023). It is also suggested that high mineral N-inputs can reduce AMF efficiency, while organic matter can enhance the AMF effectiveness and the N-transfer (Thirkell et al., 2016).

There is a lack of controlled studies that evaluate the legacies of organic and synthetic fertilizers in the establishment success of a newly inoculated AMF strain (Köhl et al., 2016). Therefore, there is an urgent need for integrated studies that evaluate how different, long-term fertilization strategies interact with newly introduced AMF strains to support dependent crops. This thesis addresses the gap by focussing on the soil legacy effects but also looking specifically at the soil-AMF interaction to provide a short-term base for more long-term inoculation trials.

## 1.7 Objectives, Research Questions and Hypotheses

This master thesis investigates long-term fertilization effects of different organic fertilizer treatments and mineral fertilization strategies on a multi-crop rotational agricultural field and link these *in-situ* to a complementary greenhouse experiment. The study uses the framework of the Compost Field Experiment Ehrendingen (CoFEE), started in 2019 by the Swiss Research Institute for Agriculture (Agroscope). The thesis expands the research scope with the integration

of AMF in a controlled greenhouse experiment, by establishing a link from the field to the greenhouse to examine the interaction effects between different soil legacies and the manual inoculation of a promising AMF strain (*Rhizoglomus irregularis*, SAF22).

The approach consists of two parts, the first is an evaluation of organic and mineral fertilization effects on the baseline soil health and plant performance within the ongoing CoFEE trial and the second is a Greenhouse experiment. In which a controlled pot experiment using unsterilized, post-harvest soils taken from the CoFEE site. To show the effects of fertilization history, six specific soil legacies were selected: four distinct organic fertilizer treatments, one unfertilized control and one fully mineral N-fertilized control (no organic fertilizer). These six soil legacies were crossed with two inoculation states: inoculated AMF - SAF22, and non-mycorrhizal (NM). *Trifolium pratense* was selected as a host plant due to its relationship with rhizobia bacteria and its high mycorrhizal responsiveness, which is often significantly lower in commercial crops like sunflowers and wheat. This strong responsiveness combined with its suitability for greenhouse cultivation allowed for an accurate assessment of the soil's inoculation potential.

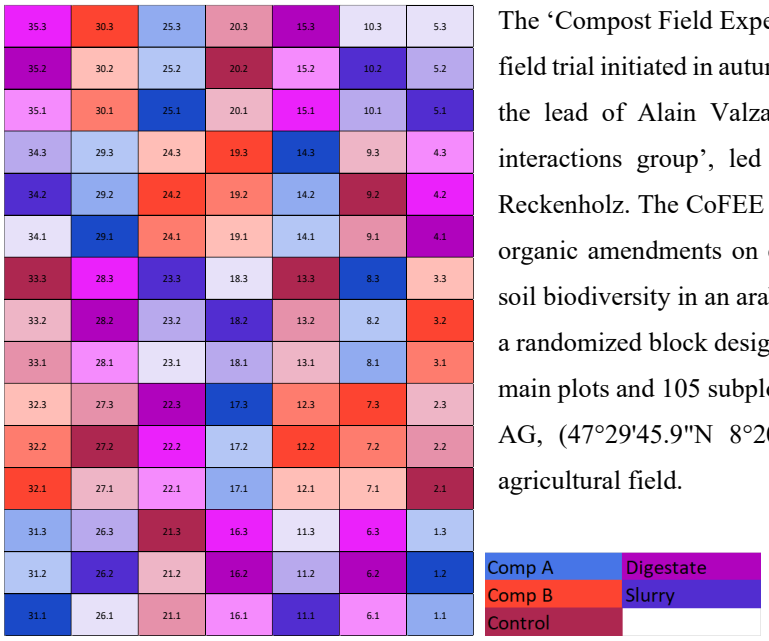
The general objective is to show and isolate how the distinct organic fertilizer treatment legacies influence the microbial competition and to determine whether a specific AMF inoculation can overcome native priority effects to enhance plant productivity, tripartite symbiosis and fertilization efficiency in organically managed soils compared to a soil relying on a mineral N-fertilization. With the goal of enhancing sustainable management strategies and providing farmers with a possible answer on which organic fertilization strategy to use and if AMF inoculation can be a viable addition to the fertilization practices.

To address these objectives, this thesis has the following research aims across two experiments in the field and greenhouse: (1) How do different organic fertilizers affect soil quality and plant performance?, (2) How do soils from different fertilizer treatments influence plant performance in a controlled environment?, (3) Does AMF inoculation significantly enhance nutrient uptake and plant growth, and in which soils is this most pronounced?, and (4) Do Rhizobia modify plant responses to AMF inoculation?. I hypothesize that (1) Crops in compost plots will exhibit greater biomass and nutrient uptake than other treatments, (2) Soils from compost treatments will show higher organic C and AMF effects than other treatments in greenhouse conditions, (3) Plants in compost treatments will show the highest growth response to MGR compared to other treatments, and (4) AMF inoculation will significantly enhance plant health and performance.

# 2 Material and Methods

This thesis used methods according to the plant-soil interactions groups protocols and standard protocols of Agroscope. The following section explains all the methods used in this thesis, for field data and greenhouse data creation and evaluation. Methods include soil sampling and analysis, harvest strategy, plant data analysis and fungal data analysis. All methods are done by the author with help of group members, except part of soil analysis (was done by Agroscope soil laboratory) and field data gathering for the tested years was done by Alain Valzano-Held.

## 2.1 Compost Field Experiment Ehrendingen (CoFEE) - Experimental Site and Design




The 'Compost Field Experiment Ehrendingen (CoFEE)' is a long-term field trial initiated in autumn of 2019 and managed by Agroscope, under the lead of Alain Valzano-Held with support from the 'Plant-Soil interactions group', led by Marcel van der Heijden at Agroscope Reckenholz. The CoFEE trial aims to investigate the effects of various organic amendments on carbon sequestration, nutrient efficiency and soil biodiversity in an arable farming system. The trial is established in a randomized block design with seven replicates (blocks), making it 35 main plots and 105 subplots in total. The site is located in Ehrendingen AG, (47°29'45.9"N 8°20'06.5"E) and uses an established organic agricultural field.

***Figure 5:Overview of experimental plot and subplots of CoFEE experiment.*** *Numbers show the different subplots in a randomized setup, with three colour hues per treatment, most saturated colour shows 100% N-fertilised plots, intermediate saturated hues show 50% N-fertilised treatments and least saturated hues show 0% N-fertilised treatments. This thesis focused on 0% N-fertilised for all treatments and additionally the control plots with 100% N-fertilised treatments. Source: Alain Valzano-Held (Agroscope).*

### 2.1.1 Treatments and Experimental Setup

The experiment follows a split-plot design using five main organic treatments and three mineral N fertilization levels: **Main organic treatments**; **Control**: No organic fertilizer application, **Compost A**: High Quality Compost enriched with 30% biochar (Bionika), **Compost B**: Standard agricultural recycling compost (Leureko), **Solid Digestate**: remaining material of anaerobic digestion of biogas production (Leureko), **Slurry**: Conventional cattle slurry sourced from a local farm. **Mineral Fertilization Levels**: Each main plot is subdivided into three levels of mineral N supplementation: 100% (full recommended dose), 50%, and 0% (no mineral N) (Richner & Sinaj, 2017).

All plots were subject to a standardized crop rotation. Following the initial fertilizer application in 2019, the rotation started with winter wheat (2019–2020), followed by a cover crop of *Phacelia* (2020–2021), silage maize (2021), a cover crop of black oat (2021–2022), and sunflower (2022). After the second compost and digestate application in 2022, the sequence continued with winter wheat (2022–2023), a cover crop mixture (UFA express; 2023–2024), silage maize (2024), a subsequent cover crop of UFA express (2024–2025), and finally sunflower in 2025 (Figure **7**).

***Figure 6: Experimental site of CoFEE plots after application of fertilisers****. Picture by Alain Valzano-Held (2020).*

### 2.1.2 Management and Timeline

The trial started in 2019 with the initial application of 100 $m^3$/ha for compost A, compost B and solid digestate. Slurry plots received 12 $m^3$/ha (targeted at 35kg N/ha). Mineral fertilizer and slurry were applied annually in 1-3 doses, while the compost treatments were applied at the start of the trial and again in 2022. All the applications followed the standard 'Ökologischer Leistungsausweis' (ÖLN) / 'Proof of ecological performance' practices with winter wheat as the first crop, with the exception of maize which was cultivated herbicide-free in both crop rotations.

### 2.1.3 Fertilization Strategy

The N requirements for each crop were calculated according to the 'Grundlagen für die Düngung landwirtschaftlicher Kulturen in der Schweiz / Swiss principles of fertilization in agricultural crops' (GRUD 2017) (Richner & Sinaj, 2017). For the control, compost and digestate treatments, mineral N was used to reach the target N levels (mainly ammonium nitrate 24%N, 5%Mg, 7%S and occasionally Urea 47%N for maize). In the slurry treatment, a combination of organic and mineral sources was used to reach the target N levels. Minor deviations of actual slurry total N concentrations occurred compared to the other treatments.

***Figure 7: CoFEE field pre-harvest of sunflowers in September 2025.*** *Picture by Cedric Gehrer.*

### 2.1.4 Soil Sampling and Harvest

First, the soil was sampled for the wet aggregate stability analysis, taking soil cores by hand with a soil auger to a depth of 6cm, transferring the samples into a plastic bag and storing them at the cold room at Agroscope. Three soil cores were taken per plot for representativeness and put in the same bag. This was done for all the subplots (105) in the field, meaning 315 soil core samples were taken. Additionally, at a different day, soil core samples were taken at 0-20cm, 20-50cm and 50-90cm (three soil horizons) with the help of a hydraulic pressure auger on a large tractor to even reach the depth. This system was able to automatically sort the horizons in containers which then had to be emptied and put into pre-labelled bags, having the plot number and horizon on it. To have a representative sample, 5 samples were taken per plot, meaning it was 525 times sampled to have the final 315 soil core samples of the deeper horizons. With the help of one Agroscope employee and a civilian service provider (ZIVI), the sampling was done in one day. After sampling, all cores were transported to the University of Zürich (Irchel and stored in the cold room at 4°C. The three horizon samples were collected for later analysis by the trials project leader and are not considered for this thesis. However, the 0-6cm samples were analysed on wet aggregate stability.

***Figure 8: Tractor on CoFEE field with sampling system attached.*** *Picture by Cedric Gehrer.*

Sunflower heads, where at least 4 sunflower heads (2x per plot) in the same row of the plot were sampled. This amounts to approximately 840 sunflower heads harvested. The sunflower heads are at the time of thesis writing not yet analysed and are therefore not considered. However, already analysed harvest data of 2020 (winter wheat), 2021 (maize) and 2022 (sunflower) was provided.

### 2.1.5 Wet Aggregate Stability

Wet aggregate stability analysis was conducted on the 0-6cm soil core samples for five treatment groups: Control 0N, Slurry 0N, Digestate 0N, Compost A 0N, Compost B 0N. The analysis was done at the Irchel campus of the University of Zürich, in the soil lab of the 'Agroecology and Plant-Microbiome Interactions' group using a nested sieve method originally reported from Yoder (1936) and modified from Kemper and Rosenau (1986) following the parameters of the 'OSU Soil Fertility Lab' (2021) protocol and adapted to the machine (Retsch AS 200) that was used (OSU Soil Fertility Lab, 2021). The analysis was done on 50g of air-dried soil that had been sieved to < 8mm. The sample was placed on a stack of nested sieves with mesh sizes of 2000, 500, 250 and 53µm in an analytical vibratory sieve shaker (Retsch AS 200). In the machine the sieve stack got sprayed on with constantly running water and oscillated for 10minutes at a rate of 30 oscillations per minute without a pre-wetting stage. Following the wetting cycle, the sieves were disassembled and the soil fractions, including any rocks retained on the 2000µm sieve, were transferred to labelled tin containers using a spray bottle with deionized water and a funnel. Samples were afterwards dried in a 40° C oven, until all water was evaporated. The dry weight of each fraction per sample was recorded using an analytical scale and put into an excel file, in order to calculate the mean weight diameter (MWD) using the calculation method of the protocol. Due to having almost no rocks in the samples, the adjustment for rock weight was not performed.

***Figure 9: Wet aggregate stability testing setup.*** *With nesting sieves inserted. Picture by Cedric Gehrer.*

### 2.1.6 Soil Analysis and Harvest Data

The following soil physiochemical properties were determined according to the standard reference methods of the Swiss Federal Agricultural Research Station (Agroscope, 2026). Soil texture was partitioned into clay (<2 µm) and silt (2–50 µm) fractions. Soil pH was measured in deionised water, and soil organic carbon (SOC, 'Corg') was determined by oxidation with dichromate. Total C (C-tot) and total N (N-total) were measured via elemental analysis. Humus content was derived from organic carbon measurements, and the fine earth fraction (KOF) was quantified to determine the proportion of the soil matrix below 2mm. These data were provided as final mass percentages (Mass.-%) for subsequent statistical evaluation.

### 2.1.7 Statistical Analysis

All data handling, visualisation and statistical analysis for the field evaluations were conducted using R (version 4.4.1; R Core Team., 2024) within the RStudio integrated development environment (v2024.09.0). The packages include 'dplyr', 'tidyr', 'stats', 'ggplot2', 'ggpubr', 'car', 'agricolae' (Fox & Weisberg, 2019; Kassambara, 2026; Mendiburu, 2023; R Core Team., 2024; Wickham, 2016; Wickham et al., 2024, 2026). Significance for all statistical tests was defined at an alpha level of 0.05.

The field data were evaluated for differences across the treatment blocks using parametric analysis. Prior to analysis, residuals were evaluated for normality using the Shapiro-Wil test and variance homogeneity were tested using the Levene's test ('car' package). Variables that met parametric assumptions were analysed using one-way or two-way analyses of variance' (ANOVA) to see significant main effects of soil legacy and interactions of soil legacy and treatments. Following significant ANOVA results, post-hoc pairwise comparisons were conducted using Tukey's Honestly Significant Difference (HSD) tests ('agricolae' package) to identify specific treatment differences and assign significance letters.

## 2.2 Greenhouse Experiment

The complete greenhouse experiment was set up and conducted at the Greenhouse of the 'Agroecology and Plant-Microbiome' group at the University of Zurich, Campus Irchel at Strickhofstrasse 48, Building 83.

### 2.2.1 Soil Source and Pot Preparation

The soil used for the greenhouse experiment was taken from the CoFEE trial field. For isolating the soil legacies for the greenhouse experiment, a subset of the CoFEE plots was selected. Due to the time constraints of this thesis, it was focused entirely on subplots receiving no mineral nitrogen fertilization (0% N), except for an additional fully fertilized control of the 'Control 100N' plots that received 100% of the recommended dose of mineral N fertilizer. Thus, six distinct soil legacy treatments were defined for the greenhouse trial:1. 'Control 0N' (0% mineral N fertilizer), 2. 'Slurry' (0% mineral N fertilizer), 3. 'Digestate' (0% mineral N fertilizer), 4. 'Compost A' (0% mineral N fertilizer), 5. 'Compost B' (0% mineral N fertilizer), 6. 'Control 100N' (100% mineral N fertilizer).

***Figure 10: Sieving setup for soil.*** *Done at Agroscope. Picture by Cedric Gehrer.*

Soil sourcing was done on September 30, 2025, shortly after the harvest of the sunflower cover crop. From each of the seven replicate blocks per treatment, topsoil samples (approximately 0–20 cm depth) were collected. For ensuring representativeness, three sub-samples were taken

per subplot using a shovel and subsequently combined. At Agroscope, the six combined field soils were sieved through a 5 mm mesh and a sieving machine. The sieve and brush were carefully cleaned and dried between the different soils, preventing cross-contamination. Following the sieving process, each of the six legacy soils was mixed at a 1:1 ratio with sand using a cleaned concrete mixer. The addition of 50% sand was done to improve soil aeration, prevent compaction in the greenhouse pots, and facilitate the root washing process during plant harvest easier. The prepared soil-sand mixtures were stored in a cold room at 4°C until one day prior to the start of the greenhouse experiment to prevent a cold-shock of the plants.

### 2.2.2 Experimental Plant - Seedling preparation

***Figure 11: Germinated seedlings in nursery pot.*** *Picture by Cedric Gehrer.*

Red clover (*Trifolium pratense*) was chosen as the plant for the trial due to its high dependency on and responsiveness to arbuscular mycorrhizal fungi (Köhl et al., 2016; van der Heijden, 2002). For avoiding disturbance in the soil microbiome and physical structure of the potting mixture through subsequent thinning of seedlings after germination, an initial pre-germination step was done in four pots with an abundance of seedlings, ensuring enough healthy seedling at the experiment start. The four pots were filled with the uninoculated 'Control 0N' soil-sand mixture. These nursery pots were irrigated, one week prior to the experiment start, with deionized water and left to germinate for one week with plastic wrap and small holes on top to avoid drought

### 2.2.3 Experimental Setup

The greenhouse experiment was set up on October 13, 2025, as a fully factorial 'Randomized Complete Block Design' (RCBD) with two main factors: Soil Legacy (see **2.2.1**) and AMF Inoculation (with and without AMF inoculum) (Hartung et al., 2019). Each of the 12 treatment groups was replicated eight times, resulting in a total of 96 pots. Approximately 1liter black plastic pots were used for the experiment and a cut out mesh was put at the bottom, so no substrate falls through the pots. For the NM control treatments, pots were filled with 800 g of the respective legacy soil-sand mixture. For the AMF treatments, pots were filled with 760g of the legacy soil-sand mixture and exactly 40 g (± 1 g) of the AMF inoculum substrate, which was thoroughly homogenized into the soil to ensure even distribution, was added. Four holes were poked into the soil of each prepared pot, and four healthy, equally developed *T. pratense* seedlings from the nursery pots were carefully transplanted into them. The pots were arranged into eight randomized blocks on sliding greenhouse tables, where each block contained exactly 12 pots (one from every treatment). The position of the pots was fully randomized and changed once in week 4. During the first three days of the experiment, any seedlings that failed to establish and died, were immediately replaced with healthy reserve seedlings from the nursery pots to maintain a constant plant density of four plants per pot.

***Figure 12: One block of seedlings after day 1.*** *Picture by Cedric Gehrer.*

The plants were grown for a total period of eight weeks. Pots were visually inspected every two-three days. Emerging weeds were manually removed to prevent competition. The plants were irrigated approximately every two days, or as needed based on visual assessment, strictly using deionized water to prevent any conflicting nutrients or pollution

from tab water. No supplementary fertilization was applied throughout the entire duration of the eight-week experiment. The watering aimed to maintain acceptable soil moisture, ensuring the plants did not experience severe drought stress. The greenhouse chamber conditions were as follows: Temperature around 20-25°C, 16 hours of light (6:00-22:00) with a light intensity of 500 mmol and the humidity was 50%.

### 2.2.4 Growth Period and Pest Management

***Figure 13: Clover in GH chamber.*** *Picture was taken shortly before harvest by Cedric Gehrer.*

During the eight-week growth period, height measurements were done at week 4 (November 3, 2025) and week 6 (November 19, 2025). Plant height was measured from the soils surface with a folding ruler to the highest shoot tip, and the total number of trifoliate leaves per plant. These intermediate measurements were taken to monitor early plant development prior to the anticipated establishment of the mycorrhizal symbiosis (week 4) and to assess morphological differences once AMF colonization was expected to be highly active (week 6).

During the measurements in week 4, an infestation of thrips was detected. Thrips are small insects that considered highly destructive pests that cause damage to plants by feeding on plant tissue, and also indirectly lead to higher potential of diseases (Omkar, 2021). To prevent severe plant damage and secure the viability of the bioassay, an immediate intervention became necessary. In consultation with the laboratory management, a commercially available, organic contact insecticide (Pyrethrum FS) was applied. The decision to apply an insecticide was explicitly made to reflect realistic agronomic field conditions, where pest management is a standard and necessary practice to prevent harvest loss. The insecticide was diluted to a concentration of 0.05% in deionized water and applied homogeneously across all 96 pots using a spray bottle until the foliage was thoroughly wetted, strictly following the manufacturer's guidelines.

***Figure 14: Visible Thrips damage after pesticide application.*** *Picture by Cedric Gehrer.*

After the treatment, the plants showed a temporary slowdown in growth and brown spots on the leaves, which are typical signs of thrips damage. However, the pesticide effectively controlled the pests, and the plants recovered afterwards. In week 6, a small number of thrips reappeared evenly across the different treatments. Therefore, a second and final Pyrethrum FS application was carried out using the same method to ensure all pests were eliminated before the final two growth weeks and prevent the premature death of the plants.

### 2.2.5 Harvest Preparation and Shoot Biomass Harvest

The experiment was harvested on December 9 and 10, representing exactly eight full weeks of plant growth. Prior to the harvest, a representative selection of pots covering all treatment combinations was photographed for documentation. For preventing any microbial cross-contamination between the pots, separated harvesting station and pre-labelled containers were set up for AMF and NM pots. Plant shoots were cut slightly above the soil surface using scissors. The entire above-ground biomass of all four plants per pot, including any detached leaves, was put in a 250

mL laboratory beaker to record the total shoot fresh weight using a precision laboratory scale. The shoot material was then placed into paper oven bags and dried in an oven at 40 °C until completely dry to determine the final shoot dry weight. The shoot-dry weight was recorded in an excel spreadsheet using the same analytical scale as before.

***Figure 15: Side-by-side view of two treatment blocks.*** *Native NM (left) and inoculated AMF (right). Picture by Cedric Gehrer.*

### 2.2.6 Root Harvest and Nodule Score

Following the shoots, the remaining soil and roots were emptied into a large plastic container to carefully separate the substrate from the root systems. The combined roots of the four plants were thoroughly washed with a 2mm mesh-sieve under running water until all adhering soil and sand particles were removed. The cleaned roots were gently blotted dry with paper towels and the total root fresh weight was recorded with the precision scale, again using the 250ml laboratory beaker. Because *T. pratense* forms symbiotic relationships with nitrogen-fixing rhizobia (see **1.5.1**), a root nodule scoring was done immediately after weighing. The nodules on the upper and, if necessary, lower parts of the root system were counted and assigned a score ranging from 1 to 5, based on the method described by Unkovich (2008). After nodule scoring, the entire root system of each pot was cut into roughly 1 cm fragments and thoroughly mixed to ensure homogeneity and representativeness. Then, a subsample of these root fragments was transferred into a 2 mL Eppendorf tube and shortly after the completion of the harvest frozen for subsequent quantitative PCR (qPCR) analysis. A second subsample was placed into a 15 mL Falcon tube, filled with ethanol, and stored at room temperature for AMF root colonization staining for microscopy. Any remaining root material was discarded. Because the root systems were prioritized for these analyses, root dry weight was not possible to measure due to being used for staining and DNA quantification. Additionally, a representative sample of the mixed potting substrate from each pot was collected in a 50 mL Falcon tube and stored at -20 °C for potential future soil analyses and the remaining soil was trashed. These post-harvest soil samples were not analysed at the time of this thesis due to time constraints.

***Figure 16: Harvest procedure in six pictures.*** *1. (top left) Emptying of pot with roots, 2. Washing process of roots, 3. Nodulation score assessment, 4. Cutting of roots into approx. 2cm length, 3. Mixing of roots, put in Eppendorf 2ml tube for qPCR, 6. Rest of root mass in 15ml Falcon tube for microscopy.*

### 2.2.7 Chlorophyll Analysis

To assess the photosynthetic capabilities of the plants, chlorophyll content was quantified using the leaves of the dried shoots only. First, the dried plant material was finely ground using a Qiagen TissueLyzer (Qiagen, Germany, manufactured by Retsch). For ensuring accurate final concentrations, about 50 mg (max. 54 mg) of the ground plant tissue was weighed with analytical lab scale and the exact weight was recorded in an excel spreadsheet. This weight was used to calculate the exact amount of buffer needed per sample.

The subsequent extraction followed a modified protocol adapted for small amounts of dried tissue (Vernon, 1960). An extraction buffer consisting of 90% acetone and 10% 0.2 M Tris-HCl (pH 8.0), pre-cooled to -20 °C, was utilized. The buffer was added to the tissue at a ratio of 50 µL per 10 mg of dry weight (e.g., 250 µL buffer for 50 mg tissue). The mixture was vortexed and then sonicated for 5 minutes in an ice-cooled water bath to maintain a temperature of approximately 4 °C. After sonication, the samples were centrifuged at 14,800 rpm for 5 minutes at 4°C.

The supernatant was pipetted into a 96-well plate for spectrophotometric quantification. The extracted samples were diluted 1:10 with the pre-cooled extraction buffer (e.g., 10 µL of sample added to 90 µL of buffer). Absorbance was measured at wavelengths of 649 nm and 665 nm using a spectrophotometer (Spectra max M2), with the pure extraction buffer serving as the blank. The resulting values were put in an Excel spreadsheet for calculating the chlorophyll a and b values. The concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll *a*+*b* in the extract (µg/mL) were calculated using the following equations:

$$Chlorophyll\ a = 11.63\ \times A_{665} - 2.39\ \times\ A_{649}$$
$$Chlorophyll\ b = 20.11\ \times A_{649} - 5.18\ \times\ A_{665}$$
$$Chlorophyll\ a + b = 6.45\ \times A_{665} +\ 17.72 \times A_{649}$$

After calculation of chlorophyll in the unit of mg/ml solution, the values were calculated into mg/g dry weight and were transformed into a CSV table for subsequent statistical analysis.

***Figure 17: Chlorophyll analysis in pictures****. Sonicating water bath with ice (left), and pipetting into 96-well plate setup (right). Picture by Cedric Gehrer.*

### 2.2.8 Root Staining

For manually visualizing fungal structure in the roots, the preserved root samples were cleared and stained using a modified ink-staining protocol by Alain Valzano-Held (2022). All washing and solution-exchange steps were done using a vacuum system fitted with custom lids (with hole and mesh) in the 15 ml falcon tubes, allowing for rapid suction of liquids directly from the falcon tubes without losing any root material. Initially, the 70% ethanol solution was removed, and the roots were rinsed with 8–10 mL of deionized water. For making the plant roots transparent, 8–10 mL of 10% potassium hydroxide (KOH) was added. The samples were incubated in a water bath at 80 °C for 20 minutes, fitting for the fine roots of *Trifolium pratense*. Following the incubation, the KOH was again removed using the vacuum system, and the roots were thoroughly washed at least two times with deionized water. The roots were subsequently stained by adding 8–10 mL of a natural ink-based staining solution (5% blue ink in standard kitchen vinegar) and subsequently incubated again in the 80 °C water bath for 20 minutes. The staining solution was discarded, and the roots were washed two times with water. Finally, the stained roots were submerged in 8–10 mL of 50% glycerol for storage. In this solution, the roots could stay almost indefinitely.

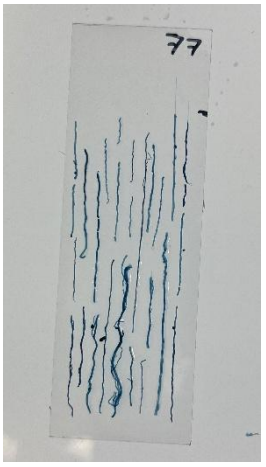



***Figure 18: Example of finished microscopy plate.*** *Sample is number 77, a replicate of 'Digestate'. Picture by Cedric Gehrer.*

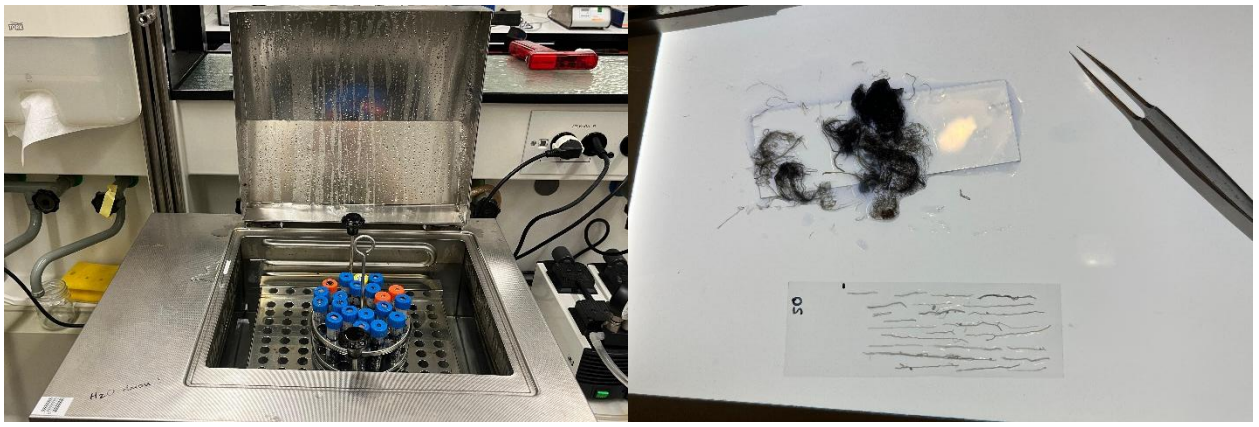


**Figure 19: Root staining in pictures.** *Incubation with ink-staining solution (left), and microscopy plating (right). Pictures by Cedric Gehrer.*

### 2.2.9 Mycorrhizal Colonization - Microscopy

Mycorrhizal colonization was quantified microscopically using glass slides and a microscope (Leica DM 3000 LED) The stained roots were aligned horizontally and parallel to the length of the microscope slides with a drop of glycerol and covered by a cover glass and sealed using histofluid. For each sample, exactly 100 crossings were evaluated with the microscope. One intersection with the microscope, looking approximately in the middle of the lens at 200x magnification, over a root fragment counts as a crossing. To evaluate the colonization, a scoring system was applied at each intersection (McGonigle et al., 1990). Intersections were categorized into mutually exclusive groups based on the dominant fungal structure observed: (1) empty (no AMF structures present), (2) exclusively hyphae, (3) exclusively arbuscules, (4) exclusively vesicles, (5) a vesicle-combination (if vesicles were present alongside other structures), and (6) a hyphae-combination (if hyphae and arbuscules were co-occurring, the intersection was conservatively scored as hyphae). The total colonization rates [%] were then calculated by adding the counted AMF

parts and dividing the number of intersections.

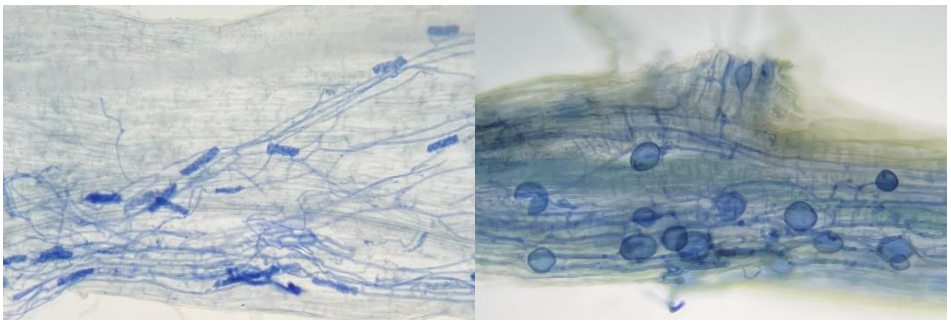


***Figure 20: Example of microscopy.*** *Visible through a 100x magnification (switched from 200x for thesis). Arbuscules and hyphae (left), vesicles, hyphae and soe arbuscules (right). Picture by Cedric Gehrer, trough the microscope lens.*

### 2.2.10 DNA Extraction and Quantification

The clover roots in the 2ml Eppendorf tubes were freeze-dried using a Lyophilizer Lyovac (SRK), then the roots were ground with two glass-beads per tube, using a FastPrep-24 5G (*MP Biomedicals*, USA, Irvine) and were ground at 6m/s for 1 minute. Approximately 20mg, (max. 30mg) of the tissue powder was put into new 2ml Eppendorf tubes for extraction. DNA was extracted using a modified protocol of the Nucleospin 96 Plant II kit (Macherey-Nagel). A master mix of Lysis Buffer PL1 was prepared and 510µL of this mix was added to each sample using a 10ml stepper. The samples were then again homogenized using the FastPrep at 6m/s for 30 seconds and subsequently incubated in a 65°C water bath for 30 minutes. Resulting Lysates were cleared in a centrifuge at 16,000 × g for 5 minutes. 500 µL of binding buffer PC was pre-dispensed into each well of an MN Square-well Block. The cleared lysate was subsequently transferred to the block and mixed using an electric multichannel pipette and afterwards transferred to a NucleoSpin Plant II binding plate and mounted on a vacuum manifold. The samples were drawn through the silica membrane via vacuum pressure, adjusted to a flow rate of 1-2 drops per second and sequentially washed with 400µL of Buffer PW1 and twice with 700µL of Buffer PW2 under vacuum. For drying out the silica membrane, the plate was sealed with gas-permeable foil and centrifuged at 6,000 × g for 10 minutes. DNA was eluted into a rack of tube strips using Buffer PE which was preheated to 65°C. For the first elution, 50µL of Buffer PE was dispensed directly onto the membrane, incubated to room temperature, and centrifuged at 6,000 × g for 2 minutes. This step was repeated with a second 50µL of Buffer PE. Concentrations and purity of nucleic acids were measured using a NanoDrop spectrophotometer at an absorbance of 260nm, using 2µL of sample per measurement. Afterwards. The extracted DNA was diluted to 1:10 with Milli-Q water (e.g. 10µL DNA into 90µL water) in a 96-well PCR plate, using a pipetting robot (Brand) at the Genetic Diversity Center (GDC) at ETH Zürich, for a working concentration of 1ng/µL for subsequent qPCR analysis.

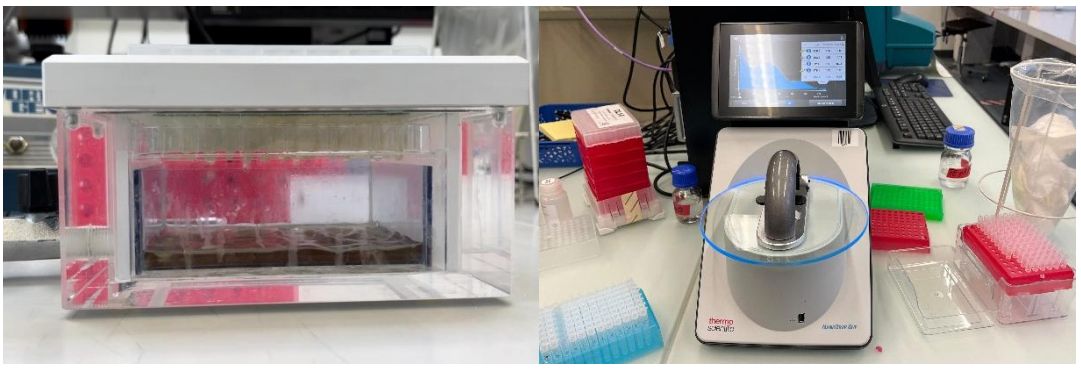


***Figure 21:Two DNA extraction steps.*** *Vacuum manifold (left), Nanodrop One (right). Pictures by Cedric Gehrer.*

### 2.2.11 Quantitative PCR (qPCR)

QPCR can quantify the absolute quantity of fungal DNA in the samples, resulting in absolute copy numbers of DNA. This was done following the methods described by Bodenhausen et al. (2021). Two distinct primer sets were utilized depending on the quantification target of the fungal DNA. For an assessment of general AMF in the samples, a broad-spectrum 18S rRNA gene primers AMG1F and AM1 were used, while for the strain-specific AMF; the SAF22 primer pair AlkF and 22R were used.

***Figure 22: QPCR system (CFX Opus 384).*** *Picture by Cedric Gehrer.*

For both approaches, the fungal signal was normalized relative to the host plant using a single copy reference gene, glyceradehyde-3-phosphate dehydrogenase (GAPDH). QPCR was performed in triplicate 20µL reactions, where each reaction contained 1x HOT FirePol EvaGreen qPCR Mix Plus, 250nM of each forward and reverse primer and 0.3% bovine serum albumin (BSA) and approximately 10ng of normalized template DNA. The reactions were run using a real-time qPCR system (CFX Opus 384, Bio-Rad Laboratories, Inc., USA, Hercules), with the following cycling conditions: preheated at 98°C, an initial denaturation step at 95°C for 15 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 62°C for 30 seconds, and elongation at 72°C for 20 seconds. A final melting curve analysis was performed from 65°C to 95°C in 0.5°C increments holding for 5 seconds. For SAF22, the steps were in the same order (preheating at 98°C, 40 cycles of denaturation at 95°C for 12s, annealing at 63°C for 40s and elongation at 72°C for 20s).

### 2.2.12 Soil Analysis

Prior to analysis, soil samples were completely dried in the oven at 40°C and sieved with a 2mm sieve (around 30g of soil per treatment) and a small subsample of the soils (around 2.5g) was ground to a fine powder using a ball mill for the total carbon (C) and total nitrogen (N) and humus analysis. The samples were then submitted to the soil laboratory at Agroscope and the physicochemical properties of the soil were determined following the Swiss standardized reference methods (Agroscope, 2026). Plant-available nutrients were extracted using an ammonium acetate-EDTA at a pH of 4.65 solution at a 1:10 ratio (AAE10 method). Following extraction, plant-available P was quantified using spectrophotometry, while the concentrations of extractable calcium (CA), magnesium (Mg) and potassium (K) were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The potential Cation Exchange Capacity (CEC) and base saturation were determined using a barium chloride extraction buffered at pH 8.1. Base cations (Ca, Mg, K, and Na) and exchangeable acidity (H) were quantified from this extract. Additionally, SOC (‘Corg’) and humus content were evaluated to characterize the organic profile of the soil. Due to standard total C and total N values were omitted from the primary analysis, due to a defect, these parameters were analysed independently via dry combustion using an elemental analyser (EA) according to standard protocols of Agroscope. Approximately 30-35 mg of the homogenized soil powder was precisely weighed into tin capsules and carefully sealed to prevent sample loss and subsequently loaded into the autosampler for analysis.

***Table 1: Results from soil analysis.*** *Analysis was done with substrate from 50:50 sand-soil mixture pre-growth period.*

| Parameter | Method | Unit | Control 0N | Slurry | Digestate | Compost A | Compost B | Control 100N |
|---|---|---|---|---|---|---|---|---|
| Carbon | Elemental Analyzer | C % | 0.89 | 1.07 | 0.8 | 1.04 | 1.04 | 0.81 |
| Calcium | Soil CEC Cations | cmol+/kg | 6.5 | 5.4 | 3.7 | 9.3 | 8 | 7 |
| Corg | Soil Corg | Mass.-% | 1.13 | 1.52 | 1.13 | 1.42 | 1.49 | 1.02 |
| Humus | Soil Organic Carbon | Mass.-% | 1.95 | 2.62 | 1.94 | 2.45 | 2.56 | 1.77 |
| Potassium | Soil CEC Cations | cmol+/kg | 0.13 | 0.16 | 0.2 | 0.2 | 0.19 | 0.11 |
| potentielle CEC | Soil CEC | cmol+/kg | 8.03 | 6.55 | 4.75 | 11.42 | 9.83 | 8.28 |
| Base saturation | Soil CEC | Mass.-% | 87.55 | 90.84 | 87.37 | 88.62 | 88.81 | 91.55 |
| Magnesium | Soil CEC Cations | cmol+/kg | 0.39 | 0.38 | 0.23 | 0.59 | 0.53 | 0.46 |
| Sodium | Soil CEC Cations | cmol+/kg | 0.01 | 0.01 | 0.02 | 0.03 | 0.01 | 0.01 |
| Phosphorus | AAE10 | mg/kg DS | 45.9 | 43.7 | 53.4 | 48 | 52.3 | 46.4 |
| pH-Value | Soil pH H2O | - | 6.19 | 6.52 | 6.39 | 6.48 | 6.18 | 5.53 |
| Nitrogen | Elemental Analyzer | N % | 0.07 | 0.11 | 0.06 | 0.1 | 0.1 | 0.06 |
| Hydrogen | Soil CEC H | cmol+/kg | 1 | 0.6 | 0.6 | 1.3 | 1.1 | 0.7 |
| Calcium (ICP) | AAE10 with ICP | mg/kg DS | 8004 | 7760 | 7720 | 7724 | 7310 | 7417 |
| Magnesium (ICP) | AAE10 with ICP | mg/kg DS | 116.8 | 130.2 | 130.4 | 124.5 | 136.5 | 125.8 |
| Potassium (ICP) | AAE10 with ICP | mg/kg DS | 37.8 | 66.6 | 68.7 | 52.2 | 71.4 | 42.4 |
| S-Value | Soil CEC | cmol+/kg | 7.03 | 5.95 | 4.15 | 10.12 | 8.73 | 7.58 |

### 2.2.13 Statistical Analysis

All data handling, visualisation and statistical analysis for the greenhouse data were conducted using R (version 4.4.1; R Core Team., 2024) within the RStudio integrated development environment (v2024.09.0). The packages include 'dplyr', 'tidyr', 'stats' 'ggplot2', 'ggpubr', 'car', 'emmeans', 'multicomp', 'multicompview', 'factoextra', 'vegan', 'agricolae' (Fox & Weisberg, 2019; Graves et al., 2024; Kassambara, 2026; Kassambara & Mundt, 2026; Lenth & Piaskowski, 2026; Mendiburu, 2023; Oksanen et al., 2026; R Core Team., 2024; Wickham, 2016; Wickham et al., 2024, 2026). Significance for all statistical tests was defined at an alpha level of 0.05. Data preparation prior to statistical analysis of qPCR data was done using a pre-made script of Elias Barmettler, PhD at the plant-soil interaction group.

The residuals of all continuous response variables were evaluated for normality using the Shapiro-Wilk test and the Levene's test for the homogeneity of variances. Variables meeting both parametric assumptions were analysed using two-way ANOVAs ('car' package). Variables showing right-skewness or moderate heteroscedasticity were $\log_{10}$ transformed prior to analysis. Significant main effects or interactions were evaluated using post-hoc pairwise comparisons with estimated marginal means ('emmeans' package) with Tukey adjustments. For response variables with non-normal distributions, Generalised Linear Models (GLMs) were used. Right-skewed data were modelled using a Gamma GLM with a logit-link function and conversely, discrete ordinal data (e.g. nodule score) were modelled using a Quasi-Poisson GLM, and proportional data with a Quasi-binomial GLM (Pádua et al., 2015; Thonar et al., 2014). Pearson's correlation coefficients (r) were calculated to evaluate the relationships between the soil chemistry on fungal establishment success, with model fits quantified by the coefficients of determination ($R^2$) to see the proportion of variance explained by soil parameters. A multivariate 'Principal Component Analysis' (PCA) was done using the 'prcomp' function and the 'factoextra' package. Validation of visual PCA clusters and the quantification of soil legacy and AMF inoculation was done using a Permutational Multivariate Analysis of Variance (PERMANOVA) based on Euclidean distances ('adonis2' function of 'vegan' package) with 999 permutations. Any significant PERMANOVA interactions were validated with a pairwise post-hoc comparison, adjusted via BH method to control

the false discovery rate. Finally, to test if abiotic soil conditions influenced the ecological shift, soil chemical parameters were post-hoc fitted to the PCA using the 'envit' algorithm.

# 3 Results

## 3.1 Part 1: Field Framework

The results for the field trial generally revealed that there were almost no significant differences between the organic treatment groups, but a significant difference of the control 100N group compared to the organic treatment groups. It must be noted that the yield data covers three years of harvests, including the 2020 harvest with winter wheat as the crop, and the soil data is from 2022 post-harvest of winter wheat. Unfortunately, the recent 2025 harvest data of sunflowers and the post-harvest soil data were not yet available at the time of this thesis.

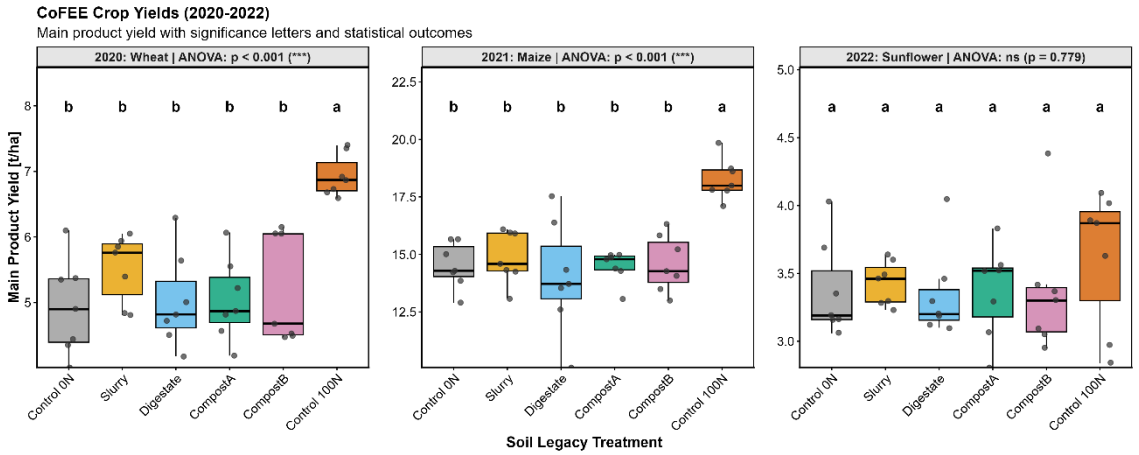



***Figure 23**:**Field crop yields across three seasons (2020-2022).** Boxplots show the main product yield (t/ha) for wheat (2020), maize (2021), and sunflower (2022). Median and interquartile ranges are shown, with individual replicates as points (n=7, with jitter to prevent overlapping). Statistical significance was tested via one-way ANOVA for each year. Tukey post-hoc tests show significant pairwise differences (p < 0.05) with different letters, coloured by legacies.*

### 3.1.1 Field Yield

To analyse the effect of the soil legacy on the field crop yields across three consecutive cropping seasons (2020–2022), one-way ANOVAs were performed for each year, as the data's residuals met the parametric assumptions ($p > 0.05$) for normality (Shapiro-Wilk) and homogenous variances (Levene) for all three years.

The results show that soil legacy had a highly significant impact on wheat yield in 2020 (ANOVA: $F(5,36) = 9.725$, $p < 0.001$) and maize yield in 2021 (ANOVA: $F(5,36) = 9.290$, $p < 0.001$). Post-hoc pairwise comparison shows that the 'Control 100N' legacy produced significantly higher yields (group 'a', Mean = 6.9 t/ha for wheat, and 18.3 t/ha for maize) compared to all other treatments. There were no significant differences in yields among the organic treatment groups (Slurry, Digestate, Compost A & B) and the 'Control 0N' group in 2020 and 2021 (all group 'b'). Mean yields in 2020 ranged from 4.9 t/ha ('Control 0N') to 5.5 t/ha ('Slurry'), and in 2021 from 14.0 t/ha ('Digestate') to 14.9 t/ha '(Slurry').

Interestingly, by the third season (2022, sunflower), this yield advantage for the mineral fertilization almost

disappeared. ANOVA indicates that soil legacy no longer had a significant impact on the sunflower yield for the year 2022 ($F(5,36) = 0.493$, $p = 0.779$). Visually, the multi-panel plot confirms this shift, with all treatments producing statistically similar yields (all grouped as 'a', Mean ranges from 3.3 t/ha to 3.6 t/ha). However, the absolute yield is still highest for 'Control 100N' with 3.62 t/ha, while the organic treatments ranged from 3.35 t/ha ('Digestate') to 3.43 t/ha ('Slurry'). Notably, across all three years, none of the organic treatments performed significantly better than the unfertilized 'Control 0N'.

### 3.1.2 Field Soil Quality Parameters

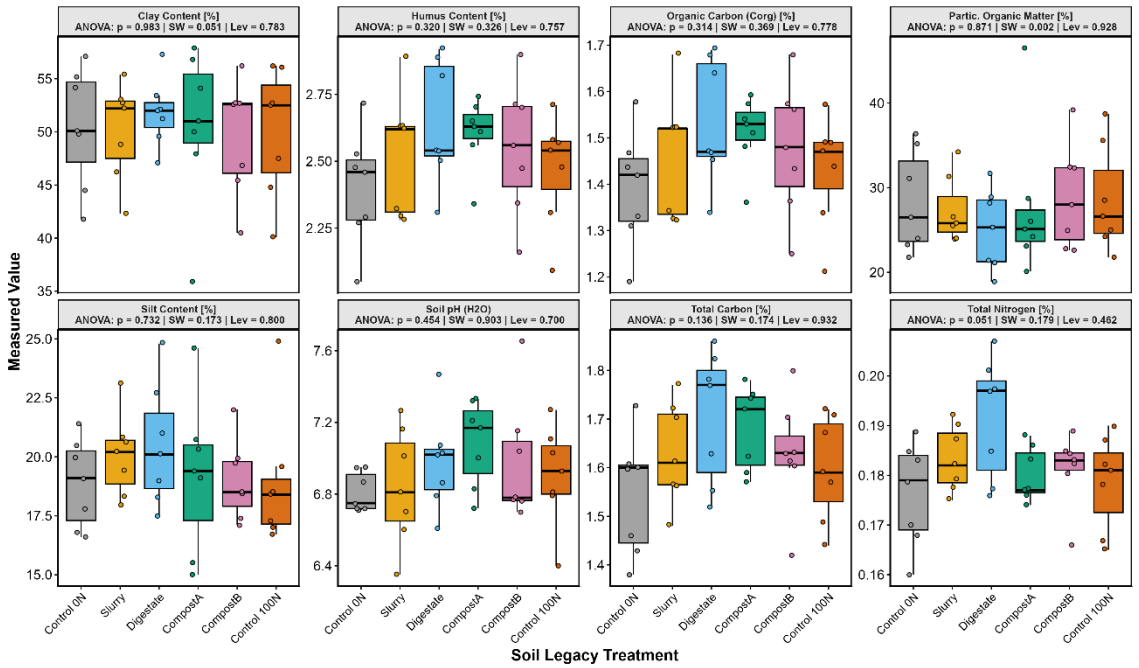



***Figure 24: Comprehensive field soil quality parameters (CoFEE 2022).*** *Panels show the measured values for eight physicochemical soil properties across the soil legacies (n=42). Boxplots show the median and interquartile ranges, with individual replicates as points (with jitter to prevent overlapping). Statistical significance was tested with one-way ANOVAs for each panel, coloured by treatment.*

For establishing a baseline of soil quality of the CoFEE field (for 2022), eight soil physicochemical properties were evaluated: Clay and silt content, humus content, organic C (Corg), particulate soil organic matter, soil pH, total C and total N. Prior to analysis, parametric assumptions were tested, which generally were met for all parameters (Shapiro-Wilk and Levene, $p > 0.05$), with the exception of particulate organic matter (Shapiro-Wilk, $p = 0.002$). Because the p-value (One-Way ANOVA, $F(5, 36) = 0.362$, $p=0.871$), was particularly non-significant no further non-parametric testing was done as it would not change the statistical outcome.

The analysis showed that the long-term fertilization (over 3 years for this data) had no significant differences across any of the tested physical or chemical parameters (all $p > 0.05$). A marginally, non-significant trend for 'Total N' is observed (One-Way ANOVA, $F(5, 36) = 2.467$, $p=0.051$), where 'Digestate' shows the highest median concentration, no pairwise post-hoc test were done due to a non-significant main effect. These results indicate that the field soils remained statistically largely uniform regarding in their basic physicochemical composition 3 years prior to the greenhouse experiment. Visually and statistically, the results show that any potential differences in plant performance in 2022 of CoFEE yields cannot be due to differences in baseline soil texture or carbon pools.

### 3.1.3 Field Aggregate Stability: Mean Weight Diameter (MWD)

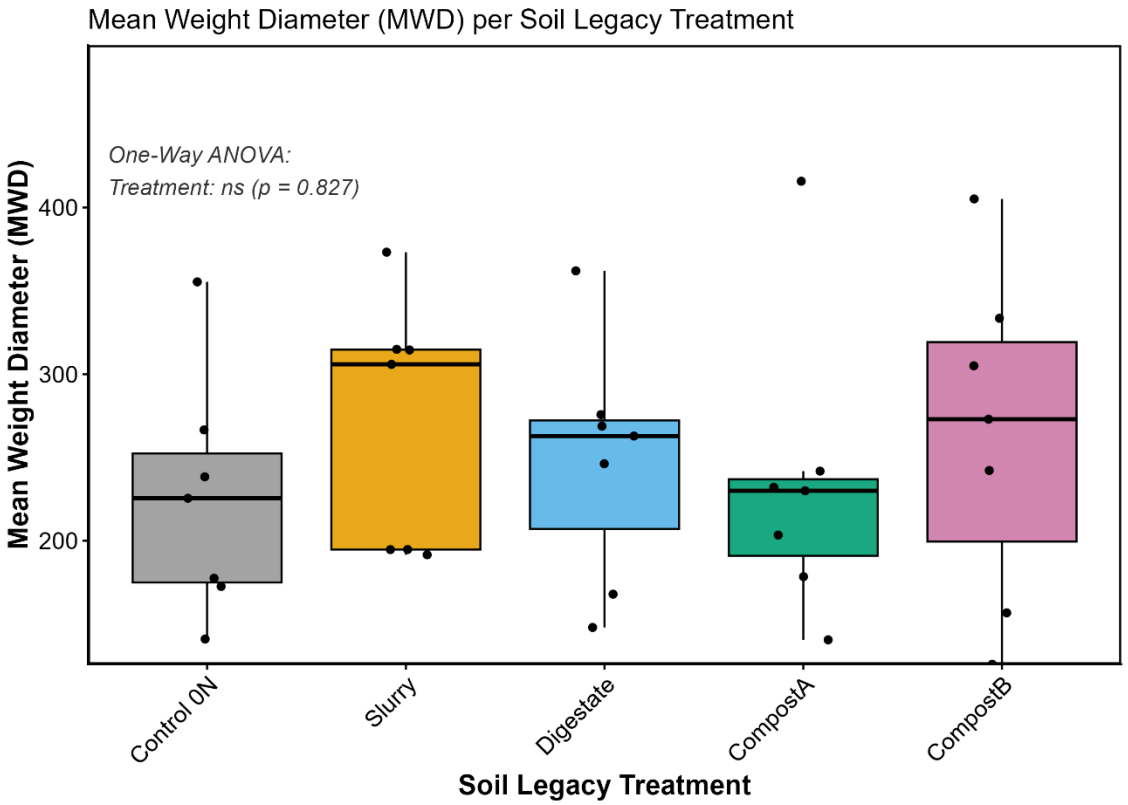



***Figure 25: Soil aggregate stability (field) per soil legacy****. Boxplots show the mean weight diameter (MWD) of topsoil aggregates (0-6cm) collected from the CoFEE field trial. Median and interquartile ranges are shown, with individual replicates as points (n=7 per treatment, with jitter to prevent overlapping). Statistical significance was tested using one-way ANOVA. 'Control 100N' data is not included in this analysis.*

Soil physical health was assessed via the soil aggregate stability, specifically the mean weight diameter (MWD) of the field soil aggregates for topsoil (0-6 cm depth). It was tested for parametric assumption (Shapiro-Wilk: W = 0.970, p = 0.429) and homoscedasticity (Levene's test: F(4,30) = 0.232, p = 0.918) for a one-way ANOVA to test for differences between the soil legacies.

The results showed no significant differences in MWD between any of the treatment groups (One-Way ANOVA, F(4,30) = 0.371, p = 0.827), indicating that all tested soils showed a statistically comparable physical quality.
Visually, 'Slurry' had the highest MWD (Mean= 269.94), SD = 74.64), while 'Control 0N' had the lowest MWD (Mean = 225.26, SD = 71.86). Notably, 'Compost B' had the second highest MWD (Mean = 263.07) but a very high intra-treatment variability (SD_MWD = 97.98).
It should be noted that the results for the 'Control 100N' group are omitted due to time constraints, even though the samples were collected. Overall, the results indicate that despite the three-year application of fertilizer treatments, macroscopical physical structure of the field soil remained largely unchanged across the treatments.

## 3.2 Part 2: Greenhouse Experiment Results

This experiment aimed to evaluate how long-term fertilization legacies (mineral vs. organic) influence the establishment and symbiotic efficiency of a newly introduced bioinoculant (*Rhizoglomus irregularis* SAF22) on red

clover (*Trifolium pratense*). Summarizing the experiment, the mineral fertilized legacy showed the highest AMF root abundance but had the lowest relative growth benefits. Conversely, organic compost legacies (high native competition) suppressed the establishment of the inoculant but maximised the plant's growth response. Native NM plant performance was highly influenced by the respective soil legacy, while AMF-inoculation significantly elevated all treatments in growth and chlorophyll content. AMF-inoculation universally increased rhizobial nodulation across all soil legacies, likely by providing P for the high-energy demanding BNF. Soil P availability was the primary driver of fungal establishment success, where higher P concentrations suppressed both native NM and SAF22 abundance.

### 3.2.1 Plant Performance: Shoot Biomass

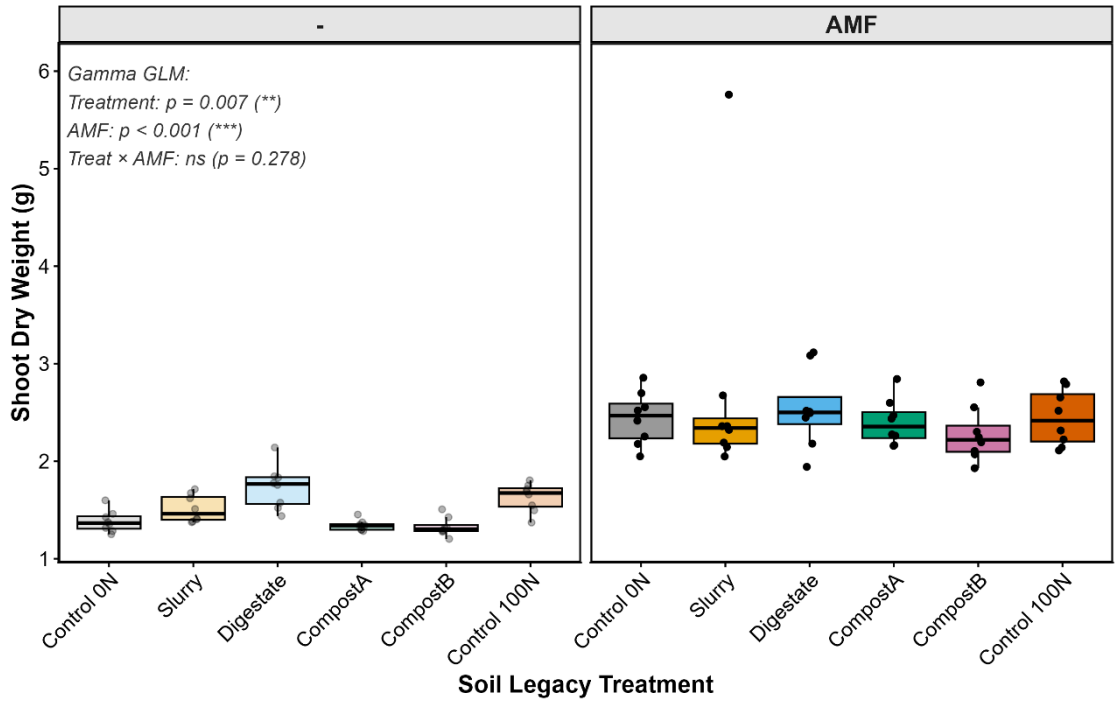



***Figure 26: Shoot dry weight across soil legacies.*** *Boxplots show the shoot dry weight (g), native NM (left) and SAF22-inoculalted (right). Median and interquartile ranges are shown, with replicates as points (with jitter to prevent overlapping) Statistical significance was tested via a Gamma GLM, coloured by legacy with NM in lighter hues.*

Total shoot dry weight was quantified after harvest to evaluate the influence of soil legacy and AMF on plant performance. Parametric as well as non-parametric assumptions failed across multiple transformations due to strong non-normality. Consequently, a Generalised Linear Model (GLM) specifying a Gamma distribution with a log-link function was used, to provide a strong fit for the right-skewed data.

The results show that the soil legacy had a significant independent main effect on plant growth ($F(5, 84) = 3.431$, $p=0.007$). SAF22-inoculation main effects were highly significant ($F(1, 84) = 226.814$, $p < 0.001$), showing that the AMF strain boosted the plant performance across all treatment groups significantly. Crucially, no significant interaction was seen between the soil legacy and the AMF inoculation ($F(5, 84) = 1.286$, $p = 0.278$). This lack of statistical interaction means that AMF inoculation promoted shoot biomass across all soil legacies, increasing the overall average from 1.49g in the native community group to 2.47g in the AMF inoculated group.

Independent of the overall AMF-driven increase, the soil legacy effects significantly influenced the plant biomass. Averaged across both main groups (NM and SAF22), plants grown in 'Digestate' (Tukey post-hoc letter 'a'), treatment

soil produced the highest overall dry biomass, while plants grown in 'Compost B' (Tukey post-hoc 'b', $p < 0.05$) treatment soil produced the lowest dry biomass. Interestingly, both compost legacies produced lower overall yields than the 'Control 0N' group (see Figure **S 5** for detailed post-hoc statistics).

### 3.2.2 Shoot Wet Weight

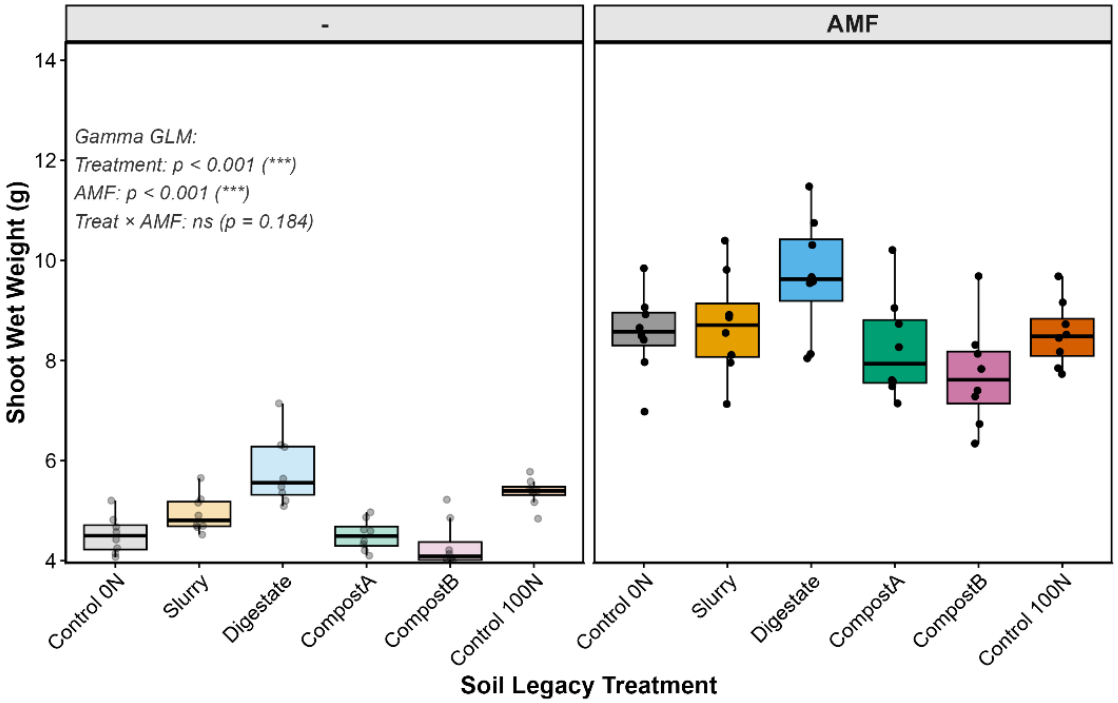



***Figure 27: Shoot fresh weight across soil legacies.*** *Boxplots show the shoot wet weight (g), native NM (left) and SAF22-inoculalted (right). Median and interquartile ranges are shown, with replicates as points (with jitter to prevent overlapping) Statistical significance was tested via a Gamma GLM, coloured by legacy with NM in lighter hues.*

Scientifically, the total dry weight is showing the absolute accumulation of carbon in the plant, however a plant can also be small but dense. Therefore, the plant's wet weight is equally important, as in commercial agriculture, harvest yields rely on fresh weight. A higher wet weight could reflect the hydraulic conductivity and water uptake, thereby indicating a greater capacity to hold water in the plant cells. Prior to analysis, the results were tested for parametric assumptions (Shapiro-Wilk and Levene's > 0.05) to decide for a fitting model. As both values (Shapiro: $p = 0.012$, Levene's: $p = 0.033$) violated the assumptions, Gamma GLM was used.

The results showed that AMF is the primary driver of plant growth, where the SAF22 inoculation increased the shoot wet weight across all groups (GLM: $F(1,84) = 713.95$), $p < 0.001$). Furthermore, there was no significant interaction effect between the AMF and soil legacy (GLM: $F(5, 84) = 1.55$, $p = 0.184$), meaning that the proportional growth benefit of the SAF22-AMF strain was consistent regardless of soil legacy.

Independent of the inoculation, soil legacy had a highly significant effect on the fresh biomass (GLM: $F(5, 84) = 13.28$, $p < 0.001$). Again, when averaged across both main groups (AMF and NM), plants grown in 'Digestate' treatment soil produced the highest fresh weight, significantly outperforming plants grown in 'Compost B' treatment soil, which yielded the lowest fresh weight (Tukey post-hoc, $p < 0.05$). It must be noted that, statistically the treatments were much more separated than for dry biomass, showing three significance groups ('a', 'b', 'c') (see Figure **S 1** for detailed post-hoc statistics).

### 3.2.3 Plant Performance: Mycorrhizal Growth Response (MGR)

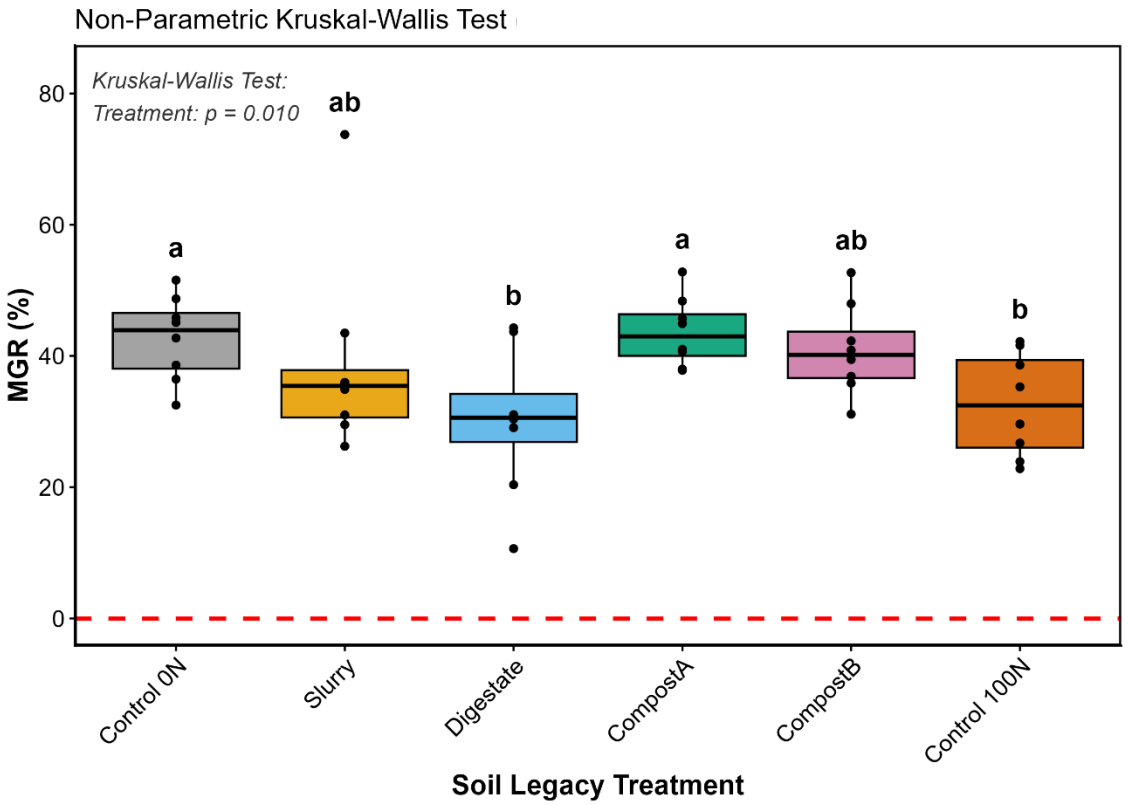



***Figure 28: Mycorrhizal growth response (MGR) across soil legacies**. Boxplots show relative growth benefit (%) of SAF22-inoculated plants compared to native NM group, calculated using bounded symmetry method (Köhl et al., 2016). The red dashed line at 0% shows no growth response. Median and interquartile ranges are shown, with individual replicates as points. Statistical significance was done via a non-parametric Kruskal-Wallis test (p = 0.010). Post-hoc pairwise differences are based on BH adjustment and show significant differences of legacies in different letters, coloured by legacies.*

MGR is arguably the main metric for evaluating plant performance regarding AMF inoculation. The MGR for the AMF is calculated according to (Köhl et al., 2016), where the responses can scale from -100% to +100%, to prevent the inflation of positive treatment effects. Due to a bounded metric violating the assumptions of normality for ANOVA where the variance would decrease for treatments approaching the boundaries, MGR was analysed using the non-parametric Kruskal-Wallis test and Benjamini-Hochberg post-hoc pairwise comparisons.

The result showed a significant effect of soil legacy on the MGR (Kruskal-Wallis: H(5) = 15.14, p=0.010). Post-hoc pairwise comparison shows that the 'Control 0N' (MGR_median = 43.9%) and 'Compost A' (MGR_median = 43.0%) treatment groups resulted in the highest symbiotic growth response (group 'a'). Although 'Control 0N' showed a slightly higher MGR_median, 'Compost A' had a lower standard deviation (SD_MGR = 5.26) than 'Control 0N' (SD_MGR = 6.43). 'Control 100N' (MGR_median = 32.5) and Digestate (MGR_median = 30.6) showed the lowest MGR of the compared groups (group 'b'). Interestingly, 'Slurry' (group 'ab') had the highest in-treatment variability (SD_MGR = 15.0), with a value range of 26.2% (min) to 73.8% (max).

### 3.2.4 Correlation of Soil Productivity and Symbiotic Benefit

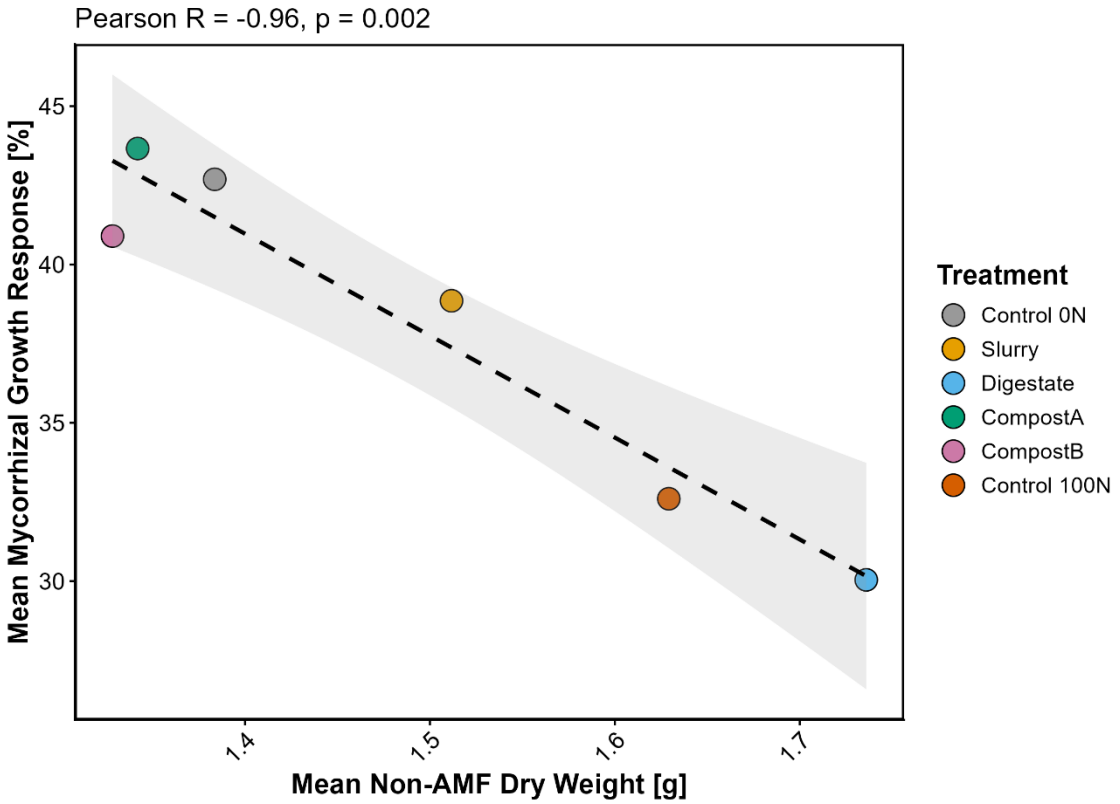



***Figure 29: Correlation between soil productivity and symbiotic benefit.*** *Pearson linear regression comparing the mean NM (native) shoot dry weight (g) against the mean MGR (%). Data points show the aggregated treatment means for each legacy. The dashed black line shows the linear regression fit, with the shaded grey region representing the 95% confidence interval.*

For evaluating the relationship between the baseline soil productivity and the symbiotic benefit of the AMF inoculant, a Pearson correlation was used following the methodology described by Rog et al. (2025). Native NM dry shoot mass was used as the independent variable representing soil productivity. This approach avoids the mathematical coupling and that would occur when using the dependent, inoculated biomass and shows an important connection of a plant trait to an ecosystem function.

When analysing the individual biological replicates (n=48), the analysis showed a highly significant, negative correlation between the native NM dry shoot biomass, and the MGR ($R = -0.484$, $p < 0.001$). Crucially, when this relationship is evaluated with aggregated values (averaging replicates within each legacy), the negative correlation becomes very strong (see Figure **29**), ($R=-0.96$, $p=0.002$). This confirms that soils with a lower plant productivity (‘Compost B’, ‘Compost A’, ‘Control 0N’) had a proportionally higher relative growth benefit from the SAF22 inoculation and conversely, in more productive legacies (‘Control 100N’, ‘Digestate’), the AMF response was significantly reduced. wha

### 3.2.5 Fungal Performance: AMF Root Colonization with qPCR and Microscopy

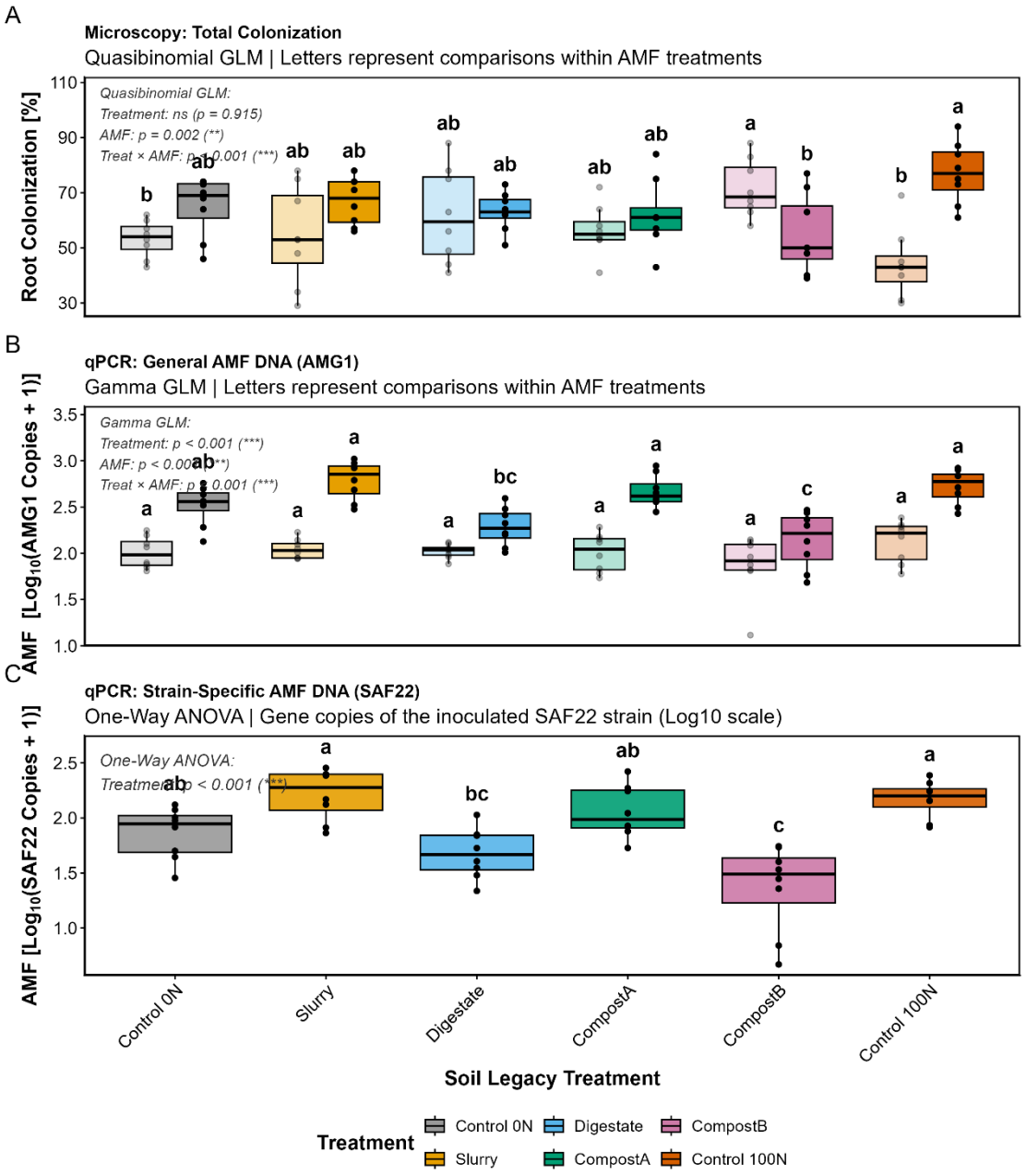



***Figure 30: Fungal root establishment across soil legacies.*** *(A) Macroscopic total root colonization (%) evaluated via visual microscopy. (B)Molecular quantification of General AMF DNA (Log$_{10}$ copies). (C) Molecular quantification of SAF22 abundance (Log$_{10}$ copies). Boxplots showing the median and interquartile ranges with individual replicates as points. Coloured by legacy, with NM group in lighter hues. Statistical significance was determined via quasi-binomial GLM (A), Gamma GLM (B), and one-way ANOVA (C). Post-hoc pairwise differences showed by different letters within the respective AMF treatments*

For evaluating if and how soil legacies influence the symbiotic establishment between AMF and plant roots, the total root colonization was first quantified via microscopy (plot A). Due to the values representing percentages (0-100%) a quasi-binomial GLM was used. The analysis showed a highly significant interaction effect of the soil legacies and AMF inoculation (GLM: $F(5, 84) = 7.26$, $p < 0.001$), showing that the colonization success of AMF is strictly dependent on the soil legacy. In NM organic legacies, specifically ‘Compost B’ (71.5%) and ‘Digestate’ (61.8%)

showed the highest colonization rates, whereas the mineral 'Control 100N' legacy had the lowest colonization rate (44.2%). However, this pattern shifted completely when looking at the SAF22 group. Now, 'Control 100N' had the highest overall colonization (77.2%) while 'Compost B' showed the lowest colonization (54.9%).

AMF establishment was further validated molecularly using qPCR for general AMF abundance (AMG1 primer, plot B) and strain-specific abundance (SAF22, plot C). For statistical analysis, a Gamma GLM was used for general abundance to account for right-skewed data, while the strain-specific abundance was evaluated with a one-way ANOVA on $Log_{10}$ transformed data. Similar to the microscopy results, the general AMF abundance ($F(5, 84) = 4.56$, $p < 0.001$), again showing a highly significant interaction effect. While the native copy numbers stayed relatively low for native fungal communities (1.87-2.13 $Log_{10}$ copies), the addition of the SAF22 strain caused a substantial increase in fungal DNA across all treatments (2.14-2.79 $Log_{10}$ copies). Interestingly, the SAF22 specific qPCR analysis confirms the successful establishment of SAF22 (ANOVA: $F(5, 42) = 12.54$, $p < 0.001$). Consistent with the inoculated microscopy result, the SAF22 abundance was highest in 'Slurry' and 'Control 100N' treatment soils and significantly lowest in 'Compost B'.

### 3.2.6 Fungal Performance: Correlation of AMF abundance of Microscopy vs. qPCR

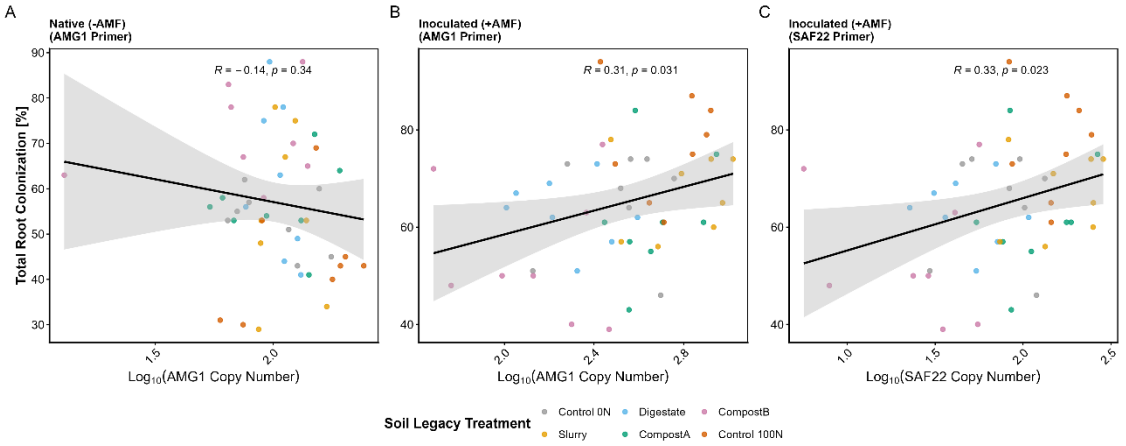



***Figure 31: Validation of methods comparing visual microscopy to molecular quantification (qPCR).*** *Scatter plots showing Pearson's linear correlations between total root colonization (%) and $Log_{10}$ transformed DNA copy numbers. (A) Relationship for native NM treatments (AMG1 primers), (B) inoculated treatments (AMG1 primers) and (C) inoculated treatments, strain-specific (SAF22 primers. Data points represent individual replicates coloured by legacy. Soild black lines indicate the linear regression fit, with grey shaded areas representing the 95% confidence intervals. Pearson correlation coefficients (R) and p-values are shown for each plot.*

For validating the accuracy of the microscopy results against the qPCR results, Pearson's linear correlations were performed. These regressions compared the visually assessed total root colonization percentages against the $Log_{10}$-transformed molecular copy numbers for both the general (AMG1) and strain-specific (SAF22) DNA. Overall, molecular DNA abundance significantly correlated with visual root colonization only in the AMF inoculated treatments, while native communities showed no statistical relationship.

In the native NM treatments, linear regression showed no significant relationship between the AMG1 $Log_{10}$ copy numbers and total root colonization (plot A: $R = -0.14$, $p = 0.34$). However, for the SAF22 groups, a significant positive linear correlation is shown between the visual total colonization rate and the general (AMG1) abundance (plot B: $R = 0.31$, $p = 0.031$). Also, this could be confirmed when looking at the correlation with the SAF22 qPCR data and total root colonization, as there is again a significant positive linear correlation (plot C: $R = 0.33$, $p = 0.023$) visible.

Coefficient of Determination ($R^2$) values were also calculated (general AMF, plot B: $R^2 = 0.098$, and SAF22, plot C: $R^2 = 0.105$), indicating that around 10% of the total variance in visual root colonization is statistically explained by the molecular AMF DNA abundance.

### 3.2.7 Total Chlorophyll content and Rhizobia Nodule Score

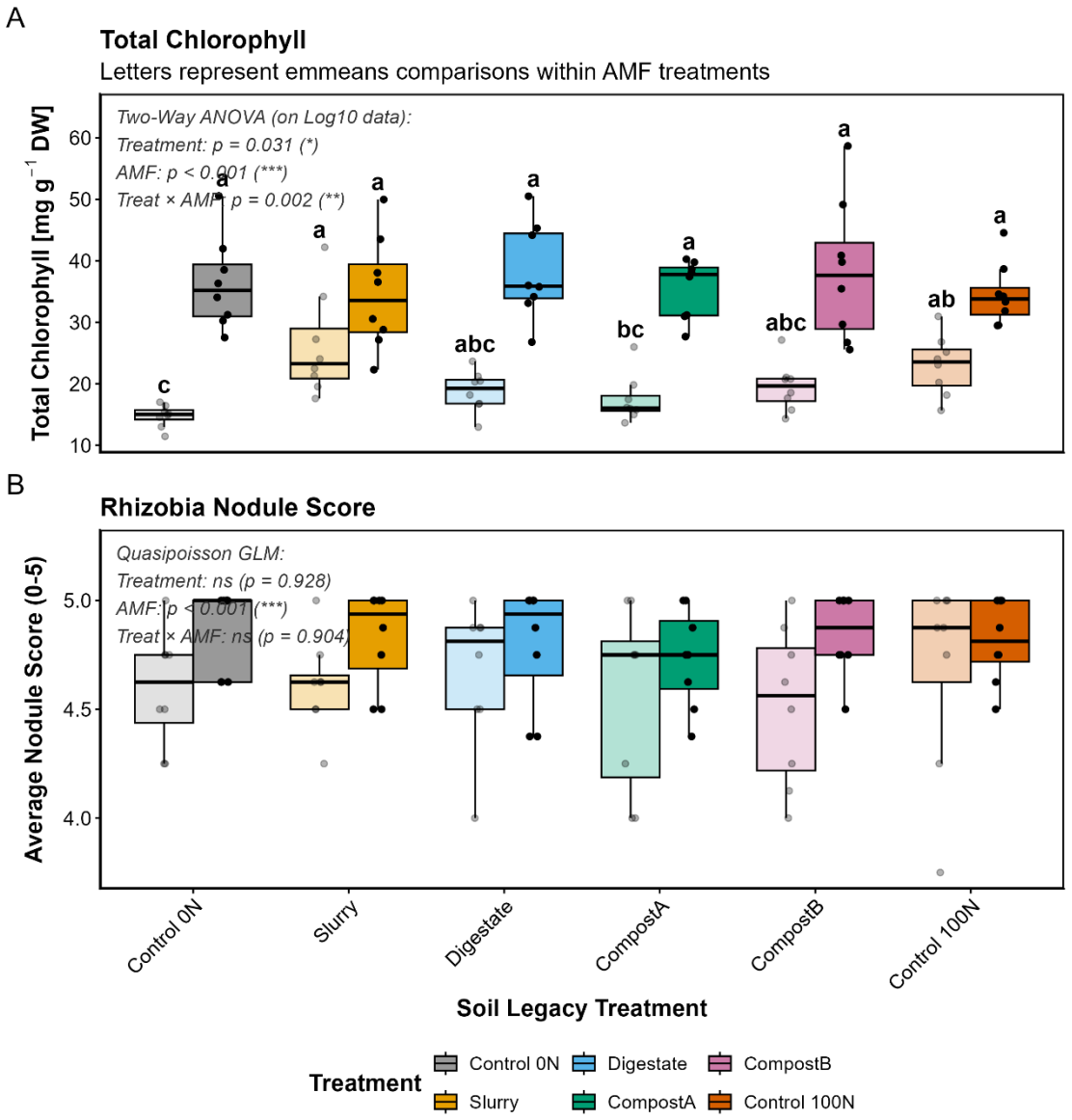



***Figure 32: Plant physiological performance across soil legacies.*** *(A) Total leaf chlorophyll content (mg $g^{-1}$ dry weight (DW). (B) Visual rhizobial root nodule score (0-5) evaluated at harvest. Boxplots show the median and interquartile ranges, with individual replicates as points (with jitter to avoid overlapping). Coloured by legacy, with NM group in lighter hues. Significance was determined via two-way ANOVA (chlorophyll data is $Log_{10}$ transformed) and Quasi-Poisson GLM (nodules). Post-hoc pairwise differences (emmeans) showed by different letters for native treatments.*

For evaluating plant health in the greenhouse trial, the physiological impact of soil legacy and AMF inoculation on the host plant's photosynthetic capacity was evaluated with total leaf chlorophyll content in mg/g dry weight (DW). Prior to analysis right-skewed data was $Log_{10}$ transformed to fulfil the assumptions of normality and homoscedasticity for a two-way ANOVA (Shapiro-Wilk: W = 0.987, p = 0.478, Levene's Test: F(11,84) =1.04, p = 0.416). The analysis showed a highly significant interaction of soil legacies and AMF inoculation (Two-Way ANOVA: F(5,84) = 4.26, p

= 0.002) which is driven by a large main effect of the SAF22 strain inoculation ($F(1, 84) = 200.354$, $p < 0.001$) with a significant effect of soil treatment ($p = 0.031$). In the native groups, the chlorophyll values are strictly dependent on the soil legacies, where 'Control 100N', and 'Slurry' showed the highest chlorophyll values (26.1 mg/g DW and 23.0 mg/g DW), while 'Control 0N' had the lowest values (14.7 mg/g DW). However, with the SAF22 inoculation the legacy influences were completely erased and with all the values almost doubling to the range of 34.5 mg/g DW to 38.2 mg/g DW). This shows the capability of the SAF22 strain to completely maximising the photosynthetic capacity regardless of the soil legacy, where for example the 'Control 0N' group experiencing a drastic increase of over 21 mg/g DW.

For evaluating if and how the soil legacies and the AMF symbiosis influenced the secondary symbiotic relationship with the nitrogen-fixing bacteria, the average nodule scores were visually quantified at the harvest (plot B).
The nodule scores represent bounded, right-skewed ordinal count data, they were analysed using a Quasi-Poisson GLM with a dispersion parameter to account for the biological variance. In contrast to the fungal colonization metrics, the rhizobial nodulation showed no significant interaction effects ($F(5, 84) = 0.31$, $p = 0.904$), nor any significant effect of soil legacy ($F(5, 84) = 0.271$, $p = 0.927$). However, nodulation scores were influenced by a highly significant main effect of the AMF inoculation ($F(1,84) = 11.85$, $p < 0.001$). Due to having no interaction, no soil legacy can be identified as a having a stronger effect than others. Average nodule scores in NM soils had a range between 4.52 and 4.69, whereas the SAF22 inoculated groups had a slightly higher range of 4.73 to 4.86.

### 3.2.8 Plant Performance: Plant Height over the Growth Period

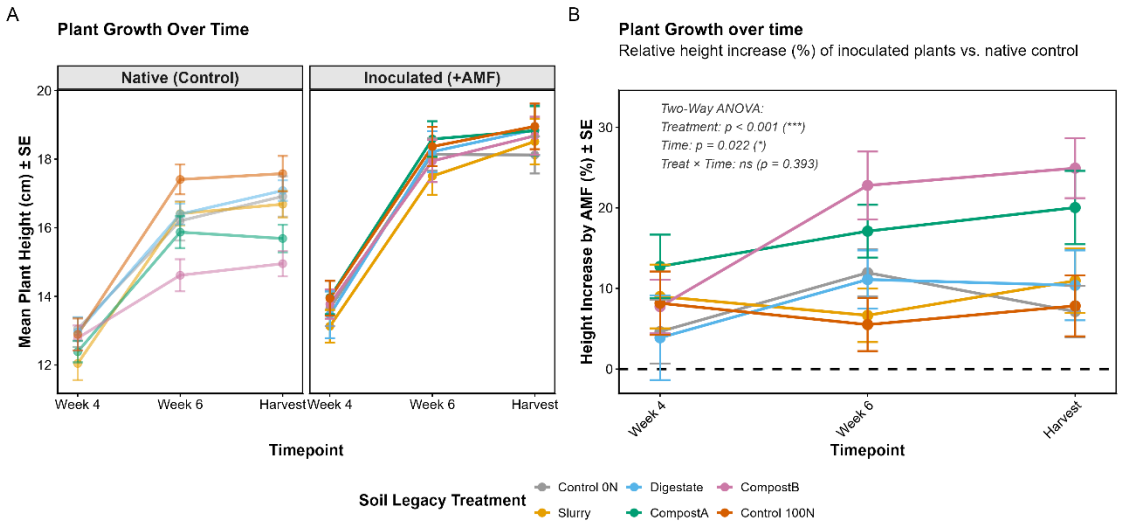



***Figure 33:Plant height and relative symbiotic benefit over time.*** *(A) Absolute mean plant height (cm) across growth period at week 4, week 6, and week 8 (harvest), separated by main group, native NM (left) and SAF22 inoculated (right). (B) Relative height increase (%) by the SAF22 inoculation compared to native NM for each legacy over time. Data points show the mean, with whiskers showing the standard error (± SE), coloured by legacy with native community in lighter hues (A). Statistical significance was tested (B) via two-way ANOVA.*

Plant height was recorded over the growth period at week 4, week 6 and at harvest. Overall, the amount of growth by the AMF symbiosis increases over time and was highly significantly influenced by the soil legacy. During the growth

period, height measurements have been recorded at week 4, week 6 and at harvest. The soil legacy strongly influenced the height of the plants in the native community, however, SAF22 inoculation erased the difference in all treatments, raising all to similar heights. Specifically, 'Compost B' having the shortest plants but showed the highest relative height boost over time (18.49%, Tukey group 'a'), followed by 'Compost A' (16.63%, group 'ab') and 'Control 100N' the highest plants but the lowest relative growth benefit (7.17%, group 'c'). All other treatments showed intermediate results ('Slurry', 'Digestate', and 'Control 0N') with statistically similar height benefits ranging from 7.91% to 8.87% (all group 'bc'). While the main effects of treatment ($p < 0.001$) and Time ($p = 0.002$) were significant, their interaction was not ($p = 0.393$).

### 3.2.9 Greenhouse Soil Analysis

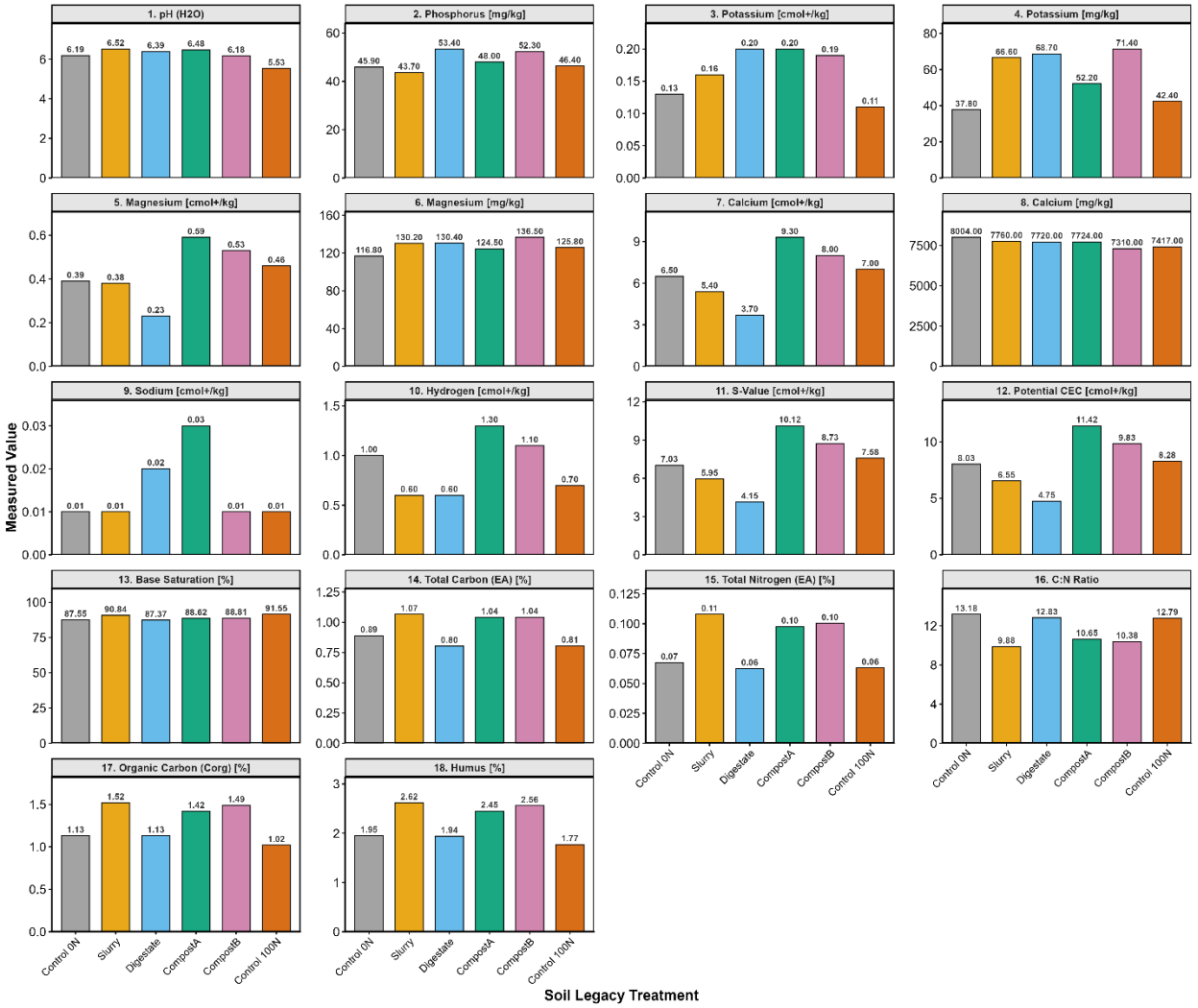



***Figure 34: Bar plots of 18 soil quality parameters measured prior to inoculation.*** *(n=1), coloured by treatment. Potassium, Mg, and Ca are shown in two metrics, with mg/kg representing the absolute concentration and cmol+/kg (centimoles of positive charge per kilogram) indicating the exchangeable cations bound to the CEC. EA stands for Elemental Analyzer.)*

The baseline physicochemical properties of the unsterilized greenhouse potting mixture were analysed prior to the greenhouse experiment. Overall, organic compost amendments established the highest exchange capacities and exchangeable Calcium (Ca) levels, were 'Digestate' and the 'Control 100N' showed lower exchange capacities. 'Compost A' and 'Compost B' recorded the highest potential cation exchange capacity (CEC: 11.42 and 9.83

cmol+/kg, respectively) and the highest S-Value (10.12 and 8.73 cmol+/kg). The compost treatments also maintained slightly below neutral pH values (6.48 and 6.18) and the most exchangeable Ca (9.30 and 8.00 cmol+/kg). P concentrations showed the highest levels in 'Digestate' (53.40 mg/kg) and 'Compost B' (52.30 mg/kg). Conversely, 'Digestate' had the lowest CEC (4.75 cmol+/kg), S-Value (4.15 cmol+/kg), and Ca (3.70 cmol+/kg), while the 'Control 100N' had the absolute lowest pH (5.53). Base saturation remained largely consistent across all treatments, ranging only slightly from 87.37% ('Digestate') to 91.55% ('Control 100N').

Regarding soil organic matter and nutrient cycling metrics, it is observable that the 'Slurry' and 'Compost' treatments consistently accumulated the highest C, N, and humus, while 'Control 100N' and 'Digestate' were comparatively depleted. 'Slurry' showed the absolute highest values for total C (1.07%), total N (0.11%), organic C (1.52%), and humus (2.62%). 'Compost A' and 'Compost B' showed similar values in total C (1.04%), total N (0.10%), organic C (1.42%-1.49%), and humus (4.45-2.56%). Consequently, these three treatments had the lowest C:N ratios, with 'Slurry' (9.88), 'Compost B' (10.36), and 'Compost A' (10.65). In contrast, 'Control 100N' and 'Digestate' had the lowest values across all organic metrics, for total N (0.06%), organic C (1.02% and 1.13% respectively). Due to the nitrogen depletion relative to C, this resulted in 'Control 0N', 'Digestate' and 'Control 100N' having the highest C:N ratios (13.18, 12.83, and 12.79, respectively).

### 3.2.10 Phosphorus as a Predictor of AMF Establishment

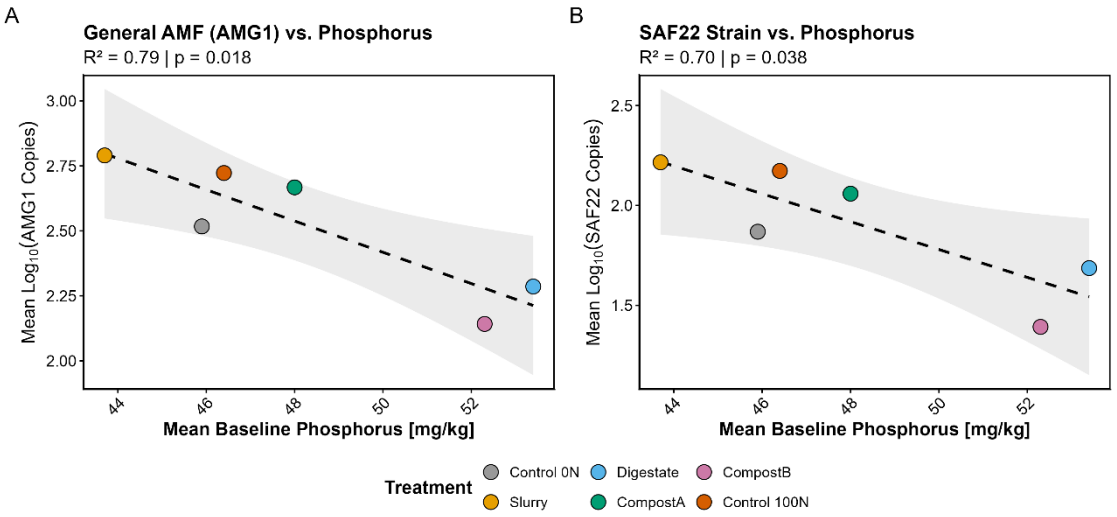



***Figure 35: Linear regression of AMF abundance against baseline soil P.*** *Plot (A) general AMF (AMG1 copies) and (B) strain-specific SAF22 copies evaluated against the mean baseline P concentrations (mg/kg). AMF copy numbers are Log₁₀ transformed. Data points represent the aggregated means for each soil legacy (n=6), coloured by legacy. Dashed line indicates the linear regression fit, with shaded grey regions representing 95% confidence intervals.*

For evaluating the effect of P on fungal establishment, linear relationships were calculated for the aggregated treatment means (n=6) for both general AMF and strain-specific AMF. AMF abundance was $Log_{10}$ transformed for the analysis and evaluated against mean baseline P concentrations (mg/g) of the soil legacies. The analysis showed a strong, significant negative correlation between the mean P values and the AMF abundance of general AMF copy numbers, with P explaining 79% of the variance ($R^2$ = 0.79, p = 0.018, plot A). A similarly strong and significant negative correlation was shown for the establishment of the SAF22 inoculant ($R^2$=0.70, p = 0.038, plot B). Specifically, the 'Slurry' and 'Control 100N' treatments showed the lowest P levels and allowed for the highest AMF abundances, whereas the P-rich 'Digestate' and 'Compost B' strongly restricted both native and inoculated AMF growth. This

confirms that across the soil legacies, higher concentrations of P consistently corresponded to a significant reduction in both native NM and SAF22 abundance.

### 3.2.11 PCA: Plant-AMF Performance by Soil Legacy

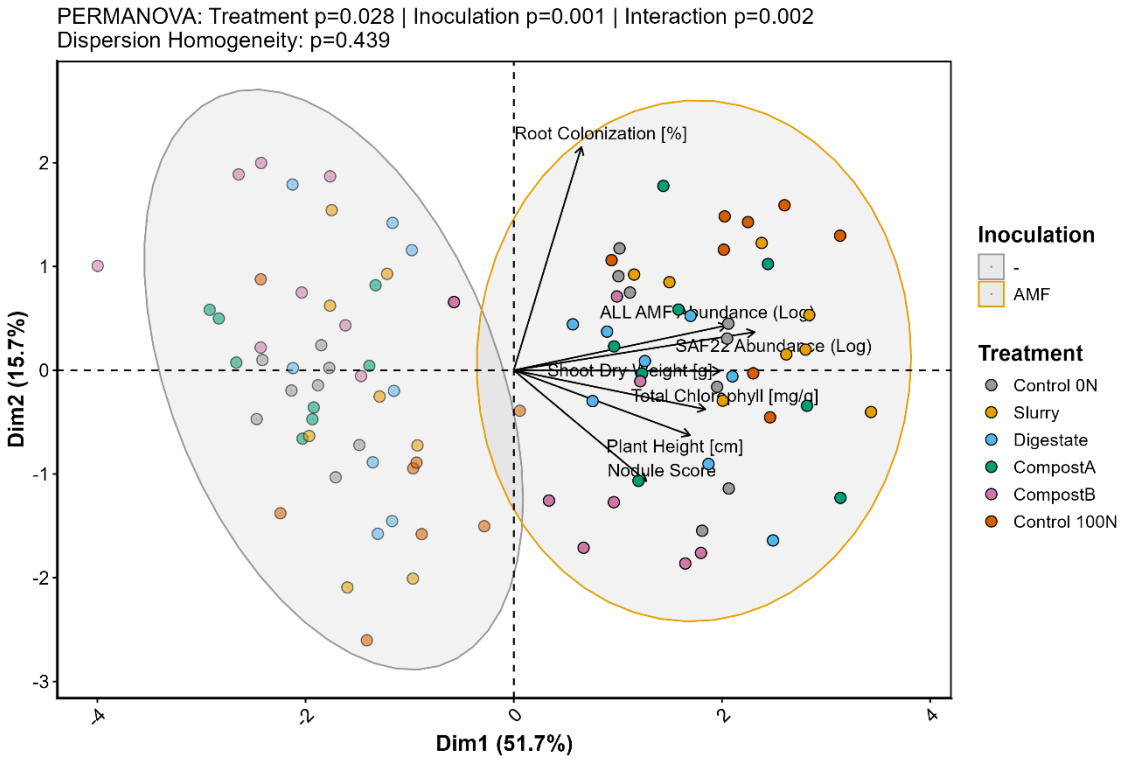



***Figure 36: Principal Component Analysis (PCA) of plant and fungal performances across soil legacies.*** *Data points represent independent biological replicates coloured by soil legacy. Shaded ellipses show the two main groups: native (grey) and SAF22-inoculated (yellow). Black vectors show the direction and influence of the standardized response variables. The two axes (Dim1, Dim2) capture the ecosystem variance. Spatial clustering was confirmed via PERMANOVA.*

For synthesizing the overall impact of soil legacies and AMF inoculation across all measured physical, physiological and molecular parameters, this multivariate PCA was calculated. Also, the initial soil abiotic conditions (soil chemical parameters, e.g. pH, total N, P) were fitted post-hoc ('envfit' w. 999 permutations) to test if the soil chemistry correlated with long-term trends. All response variables on the PCA were scaled for equal weighting across disparate units (e.g. mass percentages and $Log_{10}$ copy numbers). Furthermore, a permutational multivariate analysis of variance (PERMANOVA) using Euclidean distances, followed by pairwise comparison with Benjamini-Hochberg adjustments, was used to statistically validate the visual clustering observed within the PCA coordinate space. Visually, the PCA captured 67.5% of the ecosystem variance across the first two principal components (PC1: 51.7%, PC2: 15.8%).

The PERMANOVA showed that the SAF22-inoculation fundamentally changed the ecosystem, being the absolute driver of change in the system, accounting for 43.8% ($R^2$ = 0.438, F(1,84) = 83.32, p = 0.001) of the multivariate variance. In contrast, the soil legacy had a significant but minor main effect ($R^2$ = 0.050, F(5, 84) = 1.902, p = 0.028). Furthermore, a highly significant interaction between Treatment and AMF ($R^2$ = 0.070, F(5, 84) = 2.660, p = 0.002) shows that the ecosystem shift caused by the AMF inoculation was dependent on the soil legacy.

Visually, the PCA shows the two main clusters for the greenhouse experiment, the native NM community and the SAF22 groups. However, post-hoc pairwise PERMANOVA confirms that while the AMF-inoculation shifted everything to the right, the plants can be divided into sub-groups based on the soil legacy. 'Control 100N' and 'Slurry' showed a strong shift to the right, and statistically they cluster together to a single, almost identical group (p-adj. = 0.706). Also, 'Control 100N' showed a vertical shift from the NM cluster to the SAF22 one, whereas 'Compost B' showed an opposite vertical shift. In the AMF cluster, 'Compost B' formed its own isolated group with no clustering with other groups (p-adj. = 0.002). Detailed pairwise statistics for all combinations are provided in the appendix (see Figure **S 4**). The vector fitting ('envfit') showed that the soil chemistry baseline did not cause the groupings ($p > 0.05$ for all variables).

# 4 Discussion

This master thesis investigated the impact of long-term fertilisation of various fertiliser legacies on soil physicochemical properties and the influence of AMF on plant performance. The research was divided into a field assessment of the CoFEE trial and a controlled greenhouse experiment using red clover as the study plant. Firstly, it was tested whether the fertilisation changed the yield and properties of the soil profile and secondly, the effect of AMF on plant performance was tested and conversely if and how the soil legacies influenced this relationship. The following discussion interprets the results with a focus on the greenhouse findings and subsequently combines it with the field findings. A last part discusses what the findings mean for the larger agronomic picture and shows practical implications of this study.

## 4.1 Overview of Key Findings, answers to research questions and hypotheses

Evaluation of the CoFEE yield data across three seasons (2020–2022) showed that 'Control 100N' initially produced the highest harvest yields for both the 2020 wheat and 2021 maize seasons. This showed that a short-term mineral N-fertilization had the largest immediate effect on harvest yield, while the organic and control treatments did not significantly differ from each other (ANOVA, Tukey post-hoc, all group 'b') (Figure 3). Although being not statistically different from the other treatments in group 'b', 'Slurry' showed a substantially higher yield tendency during these initial years. Interestingly, by the third cropping season (2022, sunflower), this mineral yield advantage disappeared completely, with all treatments, including the organic legacies, equalizing and showing no significant differences in harvest yield (ANOVA, all group 'a'). Soil was not completely analysed in terms of the three main parts of soil health, focussing the analysis on physicochemical properties. The results (of 2022) show that there was no significant difference across all treatments for '0-20cm' (Figure 4) as well as for deeper layers (see Appendix Figure 18). Furthermore, no significant differences are seen in wet aggregate stability for MWD for all treatments (except 'Control 100' was not included in the analysis due to time constraints). Therefore **hypothesis 1** for the field must be rejected, as composts did not exhibit greater biomass nor nutrient uptake than other treatments across the evaluated timeframe.

However, looking at the greenhouse trial, plant performance was for one significantly increased by AMF and secondly significantly influenced by the soil legacy, where it was highest in biomass (dry & fresh weight, Figure **26**, Figure **27**) for 'Digestate' across both main groups (SAF22 and NM, Tukey post-hoc, group 'a'). Conversely, 'Compost B' produced the lowest plant biomass (dry & fresh weight) across both main groups (Tukey post-hoc, group 'b' respectively 'c'). Soil analysis for the pot mixture soils (50:50 soil/sand) showed that not the composts ('rank' 2, respectively 3), but 'Slurry' had a marginal lead in soil organic C (SOC), which means that **hypothesis 2** must be

rejected with the note that no statistical significance was be tested as only one sample per pot soil could be analysed (n=1), due to an overload of pending samples in the soil laboratory. Organic management using cattle slurry can lead to high total C and soil structure improvements (Loaiza Puerta et al., 2018). Interestingly, 'Control 100N' showed the lowest values across the board for SOC and pH which would align with findings from literature (Abd-Alla et al., 2023).

Nutrient uptake and plant growth were significantly increased by AMF, showed with the MGR (Figure **28**), where 'Control 0' and 'Compost a' (non-parametric post-hoc, group 'a') showed median MGRs of 43.9% and 43%, respectively. In contrast, 'Digestate' and 'Control 100N' showed the lowest (but still relatively high) MGR (non-parametric, post-hoc, group 'b', with 30.6 % and 32.5% respectively). 'Slurry' and 'Compost B' treatments showed intermediate responses (post-hoc group 'ab', 35.4% and 40.2%, respectively). Therefore, **hypothesis 3** is partially supported, while compost treatments exhibited relative high AMF effects, 'Control 0N' had the highest MGR and shared the post-hoc group with 'Compost A'. Interestingly, the high relative growth benefit almost inversely correlated with the fungal establishment. Microscopy and qPCR results showed that 'Control 100N' had the highest establishment of AMF with SAF22 inoculation, while 'Compost B' had the lowest. This hints at a disconnection of colonization and mutualistic benefits. Regarding plant health, AMF overall did significantly enhance the total chlorophyll across all treatments and the rhizobia nodulation score (Figure **32**). The chlorophyll content was dependent on the soil legacy, with NM plants showing clear differences in the amount of chlorophyll, where 'Slurry' showed the highest content (Tukey post-hoc, group 'a'), but when inoculated, the plants were all elevated and physiologically equalized to the same amount of chlorophyll (Tukey post-hoc, all group 'a'). However, in nodulation score no dependence of soil legacy was noted, but AMF overall provided a highly significant increase for all treatments, where no 'best' group can be identified. Due to these results and looking again at total biomass (dry & wet weight, Figure **26**, Figure **27**) and MGR (Figure **28**), **hypothesis 4** is fully supported.

### 4.1.1 Soil Legacy on Soil Quality

It is necessary to establish the reason for a 50:50 sand-soil mixture for the pot experiment in the greenhouse, as this must naturally have a distinct influence on the soil environment. This was done as it is standard practice in many greenhouse studies, specifically for mycorrhizal studies (Alayafi et al., 2025; Bodenhausen et al., 2021; Boussageon et al., 2025; Cheeke et al., 2019; Kasper et al., 2019). The sand provides an essential physical support for root development and an uniform AMF inoculum distribution without introducing conflicting components, as sand is chemically inert (Alayafi et al., 2025). Moreover, it serves as a measure against compaction or waterlogging, providing macropores for drainage and aeration, making the environment easier for root expansion and hyphal networks. Additionally, this step of diluting the baseline nutrient pool of the soil is necessary, as it makes the plants more dependent on AMF colonization for nutrient sourcing (Kasper et al., 2019). If 100 % natural field soil was used, the nutrients could have been to concentrated and the plant would reject a strong AMF colonization to save on C (Rog et al., 2025). The sand forces the plant into a slight nutrient deficiency which makes it possible to accurately determine the MGR in the greenhouse. Notably, the soil analysis was conducted using the sand-soil mixture, not the natural soil.

The greenhouse trial evaluated how the soil legacies from a six-year fertilization trial affected the growth from the natural soils and how AMF inoculation interacted with these legacies and the host plant (*T. pratense*). Unfortunately, it was not possible to test several samples of the pot soil mixture, hence the soil analysis of the pot soil consisted of only one sample per treatment, hence no statistically significant statements can be made. However, trends are visible

as the soil was taken after the growth period of the 2025 sunflower crops in the CoFEE trial (Figure **34**). The mineral N fertilized 'Control 100N' treatment soil was consistently lowest in pH, total N [%], soil organic C [%], and second to last in potassium (K) [mg/kg]. The relative high acidity (5.53) is likely due to a known consequence of mineral N-fertiliser that is directly driven by the ammonium (Haider et al., 2025; Y. Liu et al., 2024). It is consistently shown that mineral-fertilized soils are more depleted of nutrients and show lower biological activity post-harvest. Because chemical fertilizers can be taken up by plants rapidly, due to the nutrients being already in a highly soluble inorganic form, which can be quickly dissolved in water and therefore are highly available (Airee & Mukherjee, 2025; Bhunia et al., 2021; Panday et al., 2024). However, plants likely cannot absorb them fast enough but they are also prone to being lost to the environment through leaching into groundwater, surface runoff or sublimation to the atmosphere, which is another reason for the high depletion found after a growth period (Bhunia et al., 2021; Panday et al., 2024). In contrast, the nutrients in organic fertilizers are physical and chemically bound in more complex organic molecules, which plants cannot uptake as fast and by that have to rely on microorganisms, such as bacteria and fungi to make the nutrients accessible in a process called mineralization (Diacono & Montemurro, 2010; Panday et al., 2024; Solangi et al., 2023). In the soil analysis of the greenhouse pot soils, this pattern is reflected in the fact that 'Slurry' shows the highest values in total and soil organic C and total N. The outcome is consistent with the management of the CoFEE trial, where cattle slurry was applied several times a year. The application frequency provided a continuous influx of C and N, surpassing the microbial decomposition rate, creating a steady supply of fresh N and C at the time of sampling (Bhunia et al., 2021; Diacono & Montemurro, 2010; Shivangi et al., 2024). It should be noted, that during the collection of soil from the CoFEE field, some random sunflower leftover parts were present on the ground, which were thoroughly removed, but still could have contributed to a higher SOC across all treatments. Though, some plots had substantially less sunflowers in the plot (i.e. 'Control 0N'). However, this is solely based on observation rather than data and is included for contextual purposes. Furthermore, compost treatments rank similarly high in these soil parameters but also showing the highest values in magnesium (Mg) [cmol+/kg], calcium (Ca) [cmol+/kg], potential CEC [cmol+/kg] and the s-value [cmol+/kg], confirming literatures findings that link long-term organic amendments application to a significant increase in the soil's potential CEC and the subsequent retention of base cations (Diacono & Montemurro, 2010). This is particularly relevant, as organic materials increase the SOC and thereby introduce weakly acidic functional groups that carry a high negative charge. This negative charge is the primary mechanism that allows the soil organic matter (SOM) to act as a chemical buffer and actively retaining positively charged nutrient cations (like Ca, MG and K), preventing them from leaching out (Diacono & Montemurro, 2010). Interestingly, 'Slurry' and 'Digestate' and 'Control 0N' ranked lower in these specific parameters than the mineral 'Control 100N' treatment. 'Digestate' showed the lowest values overall, ranking as the absolute worst in Ca, Mg, total C, potential CEC and the S-value, while also tying the 'Control 100N' for lowest remaining total N. This could be due to the anaerobic digestion, since the process uses large amounts of degradable C to produce biogas and the resulting digestate is depleted of the stable, humified carbon structures that are necessary to provide the functional groups enhancing CEC (Edlinger et al., 2025). Additionally, anaerobic digestion actively degrades the complex, fibrous organic matter needed to build soil aggregates or feed the microbes (Edlinger et al., 2025). It converts the organic nitrogen into highly soluble ammonium, causing a displacement of Ca and Mg cations and causing them to leach away and decreasing the overall S-value (Diacono & Montemurro, 2010). This soluble N behaves similar to a synthetic fertilizer with fast available N, and is prone to post-growth depletion via leaching and volatilization, failing to build long term legacy, which can explain the equally low levels of total N of 'Digestate' to 'Control 100N' (Diacono & Montemurro, 2010; Velthof et al., 2024). The C:N ratio measured confirm this depletion of total N relative to the C for 'Control 0N',

'Digestate' and 'Control 100N' and explains the post-harvest N values. This makes sense as 'Control 0N' did not receive N and confirms that for 'Digestate' and 'Control 100N' the N was highly available and rapidly consumed or leached. Both composts soils fall in the optimum C:N ratio balance (10-12) due to SOM mineralization affecting the nutrient immobilization (Diacono & Montemurro, 2010). Interestingly, the 'Slurry' had the lowest value (C:N = 9.88), which can be directly attributed to the specific field management of this treatment, where to achieve the recommended N dose, the organic 'Slurry' was enriched with mineral N. This combined application provided more highly available N than the microbial communities needed for decomposition and more than the plants were able to take up, resulting in a slight surplus of N (Diacono & Montemurro, 2010).

### 4.1.2 Plant Performance and Fungal Reliance

Going from the soil analysis to the plant growth results of the greenhouse trial shows a major finding; while the soil legacy was the secondary driver of plant growth, the AMF inoculation is the absolute main driver of plant size (dry & wet biomass and height). Regardless of soil legacy, the AMF-strain provided a universal boost to the growth of the host plant compared to the NM soil, significantly increasing the final plant shoot biomass (wet & dry) and relative plant height growth (see Figure **26**, Figure **27**, Figure **33**). Due to the statistical results for biomass (wet & dry), no interaction effect of AMF and treatment soil was recorded, meaning it must be looked at the average of both main groups to establish which treatment soils produced the highest biomass. 'Digestate' produced the heaviest plants across both main groups for dry and fresh weight (post-hoc, group 'a') across all groups with 2.1g and 7.51g, respectively. This aligns with the soil analysis that while digestates are not effective in building long-term soil quality, its nutrients are available rapidly and contribute to fast nutrient absorption and therefore high-volume growth. As established in section **4.1.1,** the anaerobic digestion produces highly soluble, plant-available N (ammonium), that acts similarly to a synthetic fertilizer (Bhunia et al., 2021). 'Digestate' outperformed even the 'Control 100N' treatment soil, which is not statistically better or worse than the other treatments (group 'ab', except 'Compost B') in dry biomass and is second in wet biomass (sole group 'ab'). Plants in 'Compost B' soil produced the lowest biomass for dry and fresh weight (group 'b' (1.74g), resp. group 'c' (5.76g)). As previously discussed, nutrients in composts remain bound in stable organic structures, where microbial mineralization is required in order to become plant-available, preventing a rapid absorption as it was seen in the 'Digestate' treatment soil (Diacono & Montemurro, 2010). It is also mentioned that depending on the specific compost's characteristics, microbes can actually compete with the plant for nutrients short-term, also explaining the lowest biomass for compost (Diacono & Montemurro, 2010).

Looking at the growth benefit of the AMF inoculation, we see an almost perfect inversed correlation of MGR and biomass (Figure **29**), where the observed MGR was highest in low-yielding soils (e.g. 'Control 0N', 'Compost A'. 'Compost B') and vice versa lowest in high-yielding soils (e.g. 'Control 100N' and 'Digestate'), which confirms the finding that the success of mycorrhizal inoculation depends on soil legacy. This is confirmed by the finding of Rog *et al.* (2025), that the potential of AMF inoculation for maximising yield potential is highest in soils with low productivity, while relative low returns for highly productive soil can be expected. Again, this can be explained by the amount of highly soluble and rapidly available nutrients (e.g. N, P, K) in 'Control 100N' and 'Digestate' that allow the plant to absorb these nutrients without having to share carbohydrates with the AMF, effectively inhibiting the symbiosis (Ghorui et al., 2024). Interestingly, even though 'Control 100N' and 'Digestate' had the lowest MGR (32.5% and 30.6% respectively), they still provide a massive agricultural benefit paired with AMF. Legumes (such as *T.pratense*) have high dependencies on AMF for optimal growth, due to the additional relationship with rhizobia,

which require a high amount of P to function (Gough et al., 2022). Meaning, even though red clover received highly available amounts of N through 'Control 100N' and 'Digestate', the plant still maintains the strong relationship with AMF to support its nodulation (Gough et al., 2022; A. Liu et al., 2020).

It is also observed for the relative height increase over time (Figure **33**), that again both composts showed the largest relative benefit by the AMF inoculation (Tukey post-hoc, group 'a' (Compost B) and 'ab'), showing that over the whole growth period, the composts received the largest height boost(18.49%, Tukey post-hoc, group 'a'), followed by compost B (16.63%. group 'ab'), while 'Control 100N' had the lowest (7.71%, group 'c'). Relative height benefit over time represents the same ecological principle as MGR, with the MGR using biomass and relative height uses the actual height of the plants over the growth period. Both metrics evaluate how much the plant depended on the fungi in the different soils. Again, the plant was highly dependent on the soil legacy, but the absolute harvest height was influenced mainly by the AMF. Interestingly, 'Control 0N' had the highest MGR (43.9%) but a relatively low growth benefit over time (7.91%). As a possible explanation, the distinction between nutrient content and availability as well as the carbon costs of tripartite symbiosis become important. The soil analysis showed that 'Control 0N' had only slightly higher values than in C and N than 'Digestate' and 'Control 100N', the N was likely bound to more complex SOM (like in composts) (Thirkell et al., 2016). Because of this low availability of nutrients, the NM plants experienced a starvation of nutrients, which then had a rather high C cost on the host plant for maintaining the tripartite symbiosis Takács *et al.*, 2018). This could have resulted in a higher competition of AMF and rhizobia for the C and reducing the fitness of rhizobia which fixed less atmospheric N, while the AMF still provided enough P to result in a higher overall biomass (high MGR) (Chien et al., 2024). Consequently, the plant may have had less N for height growth and therefore a relatively low height benefit over time. Moreover, the height of the plant visibly reached a plateau after week 6, which is likely because the clover reached its natural growth limit, indicated by some pots, where the plants reached the flowering stage (see Figure **4**). As noted by (Shelby et al., 2016), reaching the flowering stage usually means the plants shifted its energy from growth to reproduction.

### 4.1.3 Mechanisms of Plant Health

Plant health itself was inferred via the total amount of chlorophyll in the leaves and serves as a direct proxy for the plant's nutritional status and its stress resilience, as chlorophyll is the primary pigment which drives the harvesting of sunlight, which is also responsible for energy transfer and the electron transfer in photosynthetic organisms (Ostadi et al., 2022). Therefore, measuring the chlorophyll content serves as an estimate of the capability of the plant's photosynthetic potential and an increased amount of chlorophyll means that the plant has more energy available for plant growth and yield. (Alayafi et al., 2025; Hu et al., 2022). In the results (see Figure **32**), soil legacy significantly influenced the chlorophyll content, and the AMF showed a highly significant effect. Moreover, the interaction effect between soil legacy and AMF was significant meaning that the AMF did function entirely different based on the soil legacy between the main groups (AMF and NM). In the NM group, 'Slurry' showed the highest values (Tukey post-hoc, group 'a') and 'Control 0N' the lowest (Tukey post-hoc, group 'c'), hinting at a N limitation for chlorophyll in the plant (Chien et al., 2024). Therefore, soil legacy heavily influenced the chlorophyll concentrations in the leaves. This can be explained by the soil analysis (see Figure **34**) where 'Slurry' had the highest amount of total N and produced the highest chlorophyll levels. While for 'Control 100N' (group 'ab') and 'Digestate' (group 'abc') the low but highly available amounts of N mean that the clover was less limited and still could produce an intermediate amount of chlorophyll. The 'Control 0N' technically showed a slightly higher amount of N, than 'Control 100N' and 'Digestate' it was likely bound to SOM and therefore not easily available for the plant, resulting in the lowest baseline

chlorophyll content (Bhunia et al., 2021; Thirkell et al., 2016). Furthermore, nodulation serves as a secondary measure of plant health in clover as it represents the ability to secure the N supply, by sustaining its C trade with the nitrogen fixing bacteria (Sharma et al., 2020). No treatment effect for nodulation was observed. While baseline P levels across all soil legacies were likely sufficient for vegetative growth, BNF by rhizobia requires substantial higher amounts of P to sustain the energy-demanding process (Gough et al., 2022; Kuila & Ghosh, 2022). The roots are physically limited in how they can acquire P, as unlike mobile nitrate, phosphate is highly immobile and binds to soil particles with only a small amount accessible by roots (Powers & Thavarajah, 2019). The available P is consumed quickly by roots when touching creating a "depletion-zone" (Gough et al., 2022: 440). Meaning that regardless of which soil legacy, NM roots may have quickly experienced the same limitation.

However, in the SAF22 group, the nodulation was significantly increased by AMF as well as the chlorophyll contents. Interestingly, all legacies were 'equalized' to the same chlorophyll levels (Tukey post-hoc, all group 'a'). This is for one a massive boost in plant health by AMF but also with the findings of the nodulation score (see Figure **32**), it shows the clear influence of the tripartite symbiosis. While the baseline P allowed for a relatively high nodulation in NM treatment soils, the BNF is highly energy demanding (see **1.4**). Nitrogenase, which converts the atmospheric nitrogen into ammonia, is ATP dependent and is limited by P (Gough et al., 2022). Due to the high demand of energy, the concentration of P inside active root nodules can be three times higher than in other plant tissue (Gough et al., 2022). In the NM roots, it is likely that not enough P could be acquired by the roots to meet the high demand. However, with the introduction of the SAF22 strain, the hyphal network could extend beyond the depletion zone to access otherwise unavailable P (Alayafi et al., 2025; Gough et al., 2022; Rubin & Görres, 2020). By transporting this newly accessible P back to the plant, the AMF powered the energy-demanding nitrogenase process, which allowed the plant to significantly increase its nodulation (Gough et al., 2022).

### 4.1.4 Mechanisms of Fungal Symbiosis

Results of colonization and AMF abundance were done with microscopy and qPCR respectively (see **3.2.5**). A native AMF community could be observed in the NM main group. In this native main group, the microscopy results show that 'Compost B' had the highest total root colonization, while 'Control 100N' and 'Control 0N' had the lowest, which was entirely influenced by the soil legacy. These results indicate that the mineral fertilization actively supressed the native AMF colonization, which aligns with field observations that show that an intensive agriculture decrease in AMF activity and colonization (Boussageon et al., 2025; Verzeaux et al., 2017). In contrast, an application of organic amendments, especially ones that provide a slow and balanced release of nutrients (i.e. composts), create a more favourable environment, promoting root colonization by AMF (Verzeaux et al., 2017). Interestingly, while microscopy showed significant structural differences in native colonization across treatments, the qPCR results for the general AMF abundance showed no significant differences in native fungal DNA abundance. This contrast comes from the fundamental difference of physical presence and symbiotic activity of AMF, where molecular qPCR quantifies the absolute fungal DNA that is present in the samples but it cannot differentiate between active, physical symbiotic structures inside the roots, (such as arbuscules, vesicles or hyphae), which would provide insights into the physiology of the fungi (Bodenhausen et al., 2021). Additionally, differences between DNA quantification and microscopy of stained fungal roots are documented in the literature, where for example intraradical produced spores contribute to the DNA to a larger extent than intraradical hyphae (Köhl et al., 2016). Meaning that the DNA quantified by the general AMF qPCR were present in the soil network across all treatments, however they may have only actively established a functional symbiosis in more favourable environments such as in the 'Compost B' soil treatment. To evaluate this

relationship, linear regressions were calculated to compare the two methods. While the significant p-values prove that the visual microscopy accurately captured the AMF strains establishing inside the roots, the relatively low coefficient of determination ($R^2 \approx 10\%$) leaves a considerable amount of unexplained variance. This shows that there is some methodological gap between estimating a 3D volume on a 2D microscopic slide versus a precise, absolute molecular DNA extraction.

With the introduction of the SAF22 strain to the soil environments, the establishment differed substantially between soil legacies, where its success was mainly dependent on the existing colonization by the native AMF community, indicating a soil preference of the SAF22 strain. This shows a well-documented ecological phenomenon known as priority effect, which is described as "the influence of the order and timing of species arrivals in a specific habitat on subsequent community development" (Ghorui et al., 2024: 4). Meaning that the timing of root colonization plays a critical role, due to the primary colonizer gaining a major competitive advantage over the later arriving colonizer, by using the C resources of the roots early and thereby occupying the empty structural niche and inducing heavy competition (Thonar et al., 2014). Due to the favourable environment in 'Compost B', the native community could establish itself early and formed a robust symbiotic network. SAF22 faced strong competition for both physical root space and C resources inside the roots at the start of the inoculation. Literature further confirms that native fungal communities can outcompete, reduce or even eliminate introduced inoculants in the long term (Basiru & Hijri, 2022). It can be a reason for the difficulty in studying inoculants, where a strong native community inherently influences the outcomes inoculation success. This can be eliminated by sterilizing the soil, however this doesn't always mimic the real-world field conditions and can therefore provide results that may not be applicable in field conditions (Basiru & Hijri, 2022). Consequently, the native community competed heavily against the inoculant, resulting in 'Compost B' having the significantly lowest SAF22 DNA abundance (qPCR) and the lowest total root colonization (microscopy) in the SAF22 group. Conversely, in the 'Control 100N' and 'Slurry' treatments, the native AMF establishment was actively suppressed by the soil legacy and created the empty niche in the root system (Basiru & Hijri, 2022; Köhl et al., 2016). A reduced diversity of native fungal diversity and a reduced niche overlap have been shown to directly promote the establishment and long-term success of introduced inoculants (Basiru & Hijri, 2022). Due to the SAF22 strain being a highly aggressive generalist that is known to establish well in a wide range of soil types, it faced almost no competition from native fungi and was allowed to essentially exploit the root space (see **3.2.5**) (Lutz et al., 2023). Uniquely, 'Compost A' ranked as all other treatments equally high in group 'a' for the native group (general qPCR) but also ranked as high as 'Slurry' and 'Control 100N' for the SAF22 group (general qPCR) (see Figure **30**). This could be due to the biochar, which is highly porous and has a massive surface area for extraradical hyphae to infiltrate (Al-Wabel et al., 2018; Jabborova et al., 2025). The pores can protect the mycelium from environmental degradation and can form an organic-mineral complex that improves water retention, aeration and nutrient adsorption (Al-Wabel et al., 2018; Jabborova et al., 2025). This may have helped the extraradical fungal network in the rhizosphere to establish and resulted in the high total fungal DNA.

Looking back to the plant performance results (see **3.2.3**), an interesting growth paradox is observed. A treatment soil with the highest colonization by the SAF22 strain yielded the lowest relative growth benefits and vice versa. 'Control 100N' showed the highest SAF22 establishment but the lowest MGR and relative height benefit over time. In contrast, 'Compost B' had the absolute lowest SAF22 abundance but the highest MGR and relative height benefits over time. This finding is directly supported by recent large-scale field inoculation studies using the same *R.irregularis* SAF22 strain, which confirms that there is no correlation between the physical extent of root colonization by the inoculant

and the MGR (Lutz et al., 2023). It is shown that the symbiotic efficiency of AMF depends primarily on the nutritional demands of the host plant and the functional quality of the fungal community, rather than merely the physical abundance of fungi in the roots (Lutz et al., 2023). In the 'Control 100N' treatment, SAF22 colonized the roots extensively due to the empty niche, however because the plant had highly available mineral N, the fungal network showed less effective for the nutrient acquisition and therefore provided less relative growth advantage. 'Control 100' was likely less dependent on the fungal symbiosis. Therefore, the plant gave C to the fungi but received less benefit in return, resulting in a relatively lower MGR (Rog et al., 2025). In 'Compost B' and 'Control 0N', the AMF were very efficient in trading the slowly released, more complex nutrients meaning that the plant got a lot of benefits for its investment of C to the fungi (Rog et al., 2025).
The shift in colonization efficiency (see Figure **30**) shows that soil legacies heavily influenced the competition in the soil environment and while organic legacies promoted strong native abundance of AMF, they hinder the establishment of commercially inoculated strains.

Furthermore, linear regression revealed a significant, negative correlation between soil P availability and total AMF abundance for both the general community and the SAF22 strain (see Figure **35**). Where 'Digestate' and 'Compost B' have shown the highest P values in the soil analysis, they have the lowest mean copy numbers for both general and SAF22 specific abundance, while 'Slurry' has the lowest P and highest AMF abundance. The high $R^2$ value (0.79, 0.70) means that this single variable (P) can explain 70-79% of the biological variance. The symbiosis with AMF can cost the host plant up to 20% of their photosynthetic C and due to this high cost, the fungal symbiosis is maintained based on a high P demand (Kuila & Ghosh, 2022; Rubin & Görres, 2020). The high demand for P comes from its own demand and the sustaining of the relationship with rhizobia that also needs a high amount of P to sustain the ATP production for nitrogen fixation (Gough et al., 2022; Morton et al., 2014). Hence, if there is more P available to the plant without having to rely on AMF for P, the plants are less dependent on AMF which is reflected in the relatively low AMF abundance.

## 4.2 Synthesis of Field and Greenhouse Data

In previous sections, it was thoroughly established that the chemical and symbiotic mechanisms in detail which drive the plant performance in the greenhouse. However, as the soil is taken directly from the CoFEE trial, it is necessary to evaluate the biological findings in a broader context of the long-term trial. The available data showed that after three years of the distinct fertilization, no significant differences in bulk soil chemistry (clay, humus, texture, SOC, silt, pH, total C and total N, see Figure **24** and Figure **S1**) across all depth horizons (0-20, 20-50 and 50-90cm). Furthermore, after six years of trial duration, the physical structure of the topsoil (0-6cm) was measured with aggregate stability via the MWD and again no significant differences between the soil legacies were observed (no Control 100N evaluation, see Figure **25**). This seems counterintuitive that soils with statistical physicochemical profiles produced different plant and fungal behaviour in the greenhouse. The literature indicates that bulk soil properties, such as aggregate stability, total C and overall SOM reserves, are buffered and usually not very sensitive to show large changes over shorter time periods (Airee & Mukherjee, 2025; Diacono & Montemurro, 2010; Lehmann et al., 2020). Although, due to many results of the CoFEE trial are still being not fully analysed and the topsoil soil aggregate stability only refers to a depth of 6cm, the synthesis has to be taken with caution. In contrast to the physical and chemical properties (slowly evolving), the biological and symbiotic responses in the greenhouse were clearly observable. A PCA was performed on most of the analysed biological variables (root colonization, AMF abundance, plant height, dry biomass,

chlorophyll and nodulation), which showed a clear shift of the biological environment that is strongly influenced by the AMF interaction with soil legacies (Figure **36**). This difference might be explained by microbial communities (that were not assessed except AMF) which are sensitive to environmental changes and can act as an early indicator of soil quality (Bertola et al., 2021; Tahat et al., 2020). As established previously the ecosystem shift could have mainly been driven by the native mycorrhizal communities and the competition with the SAF22 strain, where 'Control 100N' made it possible for SAF22 to aggressively colonize the roots due to a suppressed native community due to synthetic inputs, while robust native communities showed high competition against the inoculant. Due to their sensitivity of changes in root exudates and micronutrients, microbial communities can show impacts of soil management long before the physicochemical changes become observable (Tahat et al., 2020). The PCA results show the biological shift with the field legacy changing the baseline functioning of the plant-soil system (significant treatment effect). More importantly, the soil legacy significantly influenced the system with the introduction of the SAF22 inoculant (significant interaction effect). As shown in most results and the summarizing PCA, the success of the AMF inoculant was not random as well as the growth benefits of the plants were not random but determined by the fertilization history. This proves that distinct agricultural management leaves a lasting impact that determines the future symbiotic potential. Thus, the findings show that biological indicators, especially the tripartite symbiosis (for legumes) or the microbe-plant relationship could offer a sensitive proxy for biological soil health indicators (Tahat et al., 2020).

As established previously in the introduction, it would be important to evaluate soil health trough a framework that integrates several physical, chemical, and biological properties into one coherent assessment, which some publications such as Edlinger et al. (2025) and Rog et al. (2025). A comprehensive soil health evaluation would highly be in the interest of this work however, within the timeframe and scope of this study, evaluating such a comprehensive framework, even though important, could simply not be made, as the parameters that were tested already used the majority of time available for the whole thesis. Therefore, it was not focused on establishing a calculation of an SHI but rather produce meaningful data for less parameters that all contribute to soil health. It can be inferred, however, from the plant health and mycorrhizal interactions, an important part of the soil microbiome, that the long-term compost applications maintained a more robust soil health despite high MGR, where a strong native abundance of AMF induced competition to the SAF22 strain. In this context, the high dependency does not indicate degradation, but rather a functional ecosystem where nutrients are organically bound and need the natural microbial nutrient cycling. Conversely, the lower MGRs observed do not just indicate healthy soils, but rather a more chemically exhausted and biologically depleted unbalanced legacy.

## 4.3 Agronomic Big Picture

As established, the biological and physicochemical metrics provide an understanding of soil health in agroecosystems, decision-making of farmers is ultimately driven by crop-productivity and economic viability. Regarding this, the CoFEE yield data showed that the only mineral N fertilized 'Control 100N' soil produced the highest crop yield short-term during the initial seasons. This explains why conventional and chemically intensive agriculture remains the global farming model due to a rapid, short-term productivity that maximizes crop biomass (Bhunia et al., 2021; Tahat et al., 2020). However, as the global demand for food is expected to double to feed a projected population of 9 billion people by 2050, attempting to meet this demand through an even larger intensification of agriculture is a massive threat (Bhunia et al., 2021). The human-induced degradation of soils by extensive cultivation, synthetic agrochemical and a depletion of SOM has already degraded approximately 40% of the world's agricultural land (Doran & Zeiss, 2000).

While the CoFEE yield was highest in the solely mineral fertilized treatment, relying on this conventional model of farming will make the soil increasingly more unproductive over time. The short-term yield maximization of the 'Control 100N' legacy also comes at a high economic and ecological cost, where the production of synthetic N fertilizers (Haber-Bosch) requiring a massive amount of energy and especially fossil fuels (Loo et al., 2022). Additionally, the agricultural sector is a major driver climate change, contributing to approximately 20-30% of worldwide anthropogenic GHG emissions (Panday et al., 2024; Shivangi et al., 2024). The climate change further threatens global crop productivity and soil degradation in a positive feedback loop (Shivangi et al., 2024).

The discussed short-term yield benefits of mineral fertilization come at large economic and geopolitical cost, due to conventional farming being largely dependent on fossil fuels (Loo et al., 2022). The availability of raw fertilizer materials is also largely influenced by geopolitical reasons (Paradelo et al., 2023). Furthermore, the synthetic P fertilizers almost entirely rely on a non-renewable finite resource of phosphate rock (Gough et al., 2022; Powers & Thavarajah, 2019). It is estimated that the global phosphate reserves could run out somewhen in the next 50 to 100 years (Gough et al., 2022; Powers & Thavarajah, 2019). The reserves are distributed unequally across the world, with for example Europe being entirely dependent on P imports to maintain its agricultural production (Velthof et al., 2024). Due to the common synthetic farming model and the essential properties of e.g. P for crop production, relying on the over application of fertilizers is entirely unsustainable and also traps farmers in a model where international conflicts and fluctuating energy markets threaten the food security and profitability of agriculture (Paradelo et al., 2023; Solangi et al., 2023). This is seen in large-scale conflicts such as the Russia-Ukraine war and the most recent war between USA and Iran, where for both, at least in Europe, prices for oil (petrol) and gas have increased drastically (Guo et al., 2022; Zaugg, 2026). Furthermore, the synthetic inputs are highly inefficient where a majority of the inputs leach into groundwater or volatilize into the atmosphere and are essentially wasted (Kuila & Ghosh, 2022; Loo et al., 2022). Besides pollution and wasteful management, the high-input application of synthetic fertilization also has high biological costs, because plants are less likely to spend much C for the symbiotic relationship with microbes. This, as discussed thoroughly, leads to a loss in beneficial soil microbiome and leaves plants more vulnerable to pathogens which in return leaves the farmers with no choice but to use more agrochemicals, such as pesticides to protect the crops due to the plants loosing part of the defence system (George & Ray, 2023; Tahat et al., 2020). The pesticides however also further degrade the soil microbiome, where for example certain fungicides strongly reduce AMF spore germination, hyphal development and root colonization (Bertola et al., 2021; Kuila & Ghosh, 2022).

Due to this abundance of problems regarding the conventional agricultural model, a global transition toward a circular nutrient economy becomes necessary to recycle nutrients such as P and N from waste, food processing, and animal manure back into the agricultural soil as for example organic amendments (Paradelo et al., 2023; Velthof et al., 2024). Furthermore, literature shows that organic amendments have the potential increase SOC, stabilize soil aggregates, reverse erosion and actively mitigate climate change by sequestering $CO_2$ from the atmosphere into the lithosphere (Lazcano et al., 2021; Panday et al., 2024; Shivangi et al., 2024). This could provide a slow-release nutrient supply that can decouple farmers from the volatile market of synthetic fertilizers.

The main critique for organic fertilizers is the "yield gap", which is well documented and means that organic crop yields initially range 5-34% lower than the conventional counterparts, which can also be clearly seen in the initial 2020 and 2021 CoFEE yield data (Panday et al., 2024). However, as previously established, the 2022 CoFEE yield data shows that the gap can naturally close, with organic legacies successfully almost matching the productivity of the

mineral control by the third season, where the evaluation of the yield data shows that even though the 'Control 100N' still produces the highest absolute yield, all organic treatments are in the same post-hoc group 'a' and are statistically speaking the same.

This shift of the CoFEE yield results shows the mechanisms behind the nutrient cycling, with the note that this cannot be taken for granted, as the different crops used in the three years have different responses to nutrient availability and this must be considered. Wheat and maize are highly demanding crops that respond strongly to an immediately available amount of N, whereas sunflowers have deep root systems and act as highly efficient nutrient scavengers (Alayafi et al., 2025; Bender et al., 2023; Haider et al., 2025; Rubin & Görres, 2020). This may have allowed the 2022 sunflower crop to efficiently use the deeper, slower releasing organic nutrient pools established over the previous three years. As discussed earlier, synthetic fertilizers supply an immediately available amount of N, which directly caused the yield maximization observed in 2020 and 2021, they fail to build long-term soil quality. In contrast, the complete equalization of the harvest yields by the 2022 season likely shows the delayed mineralization of the initial 2019 compost and digestate applications. Ultimately, this statistical equalization could provide evidence that regardless of the application frequency, organic amendments have the potential to eventually stabilize and match conventional yields, but it requires a tolerance of farmers for an initial yield reduction, and the possibility that this is strongly dependent of the chosen crop. Accelerating the closing of the yield gap without many synthetic fertilizers is part of active research and it could require the ecological engineering of the soil microbiome (Bender et al., 2023; Lutz et al., 2023). AMF and other beneficial microbes are more and more recognized as essential biological methods to help substitute chemical fertilizers (Haider et al., 2025; Kuila & Ghosh, 2022; Onyeaka et al., 2024). In organic systems, AMF extend the root structure past the root depletion zone to scavenge nutrients (Basiru & Hijri, 2022; Rubin & Görres, 2020). Additionally, as described before, the establishment of microbes (especially AMF) can strengthen the defence mechanisms against pathogen and enhance the resilience against drought and extreme temperatures, making it especially relevant with climate change in mind (Dukare et al., 2022). While the yield gap does exist with bioinoculants or biofertilizers, a combination of both could close this it together.

## 4.4 Practical Implications

As research for an effective, practical inoculation is ongoing, an immediate measure is adjusting land management practices to preserve the soil's existing microbiome. AMF rely on an extensive, intact extraradical mycelial network to scavenge for nutrients and water (Basiru & Hijri, 2022; van der Heijden et al., 2015). Intensive conventional agricultural practices, especially mechanical tillage, are physically destroying these delicate hyphal networks which reduced the AMF diversity and the benefits to the crop (Kuila & Ghosh, 2022; Tahat et al., 2020). Therefore, transitioning to conservation agriculture, such as reduced tillage, is essential to preserve the structural integrity of the mycorrhizal network and enhance soil aggregation (Tahat et al., 2020; Vermeire et al., 2024). Furthermore, integrating leguminous cover crops and intercropping systems into crop rotations can provide the AMF with continuous living roots that sustain AMF population and maximise the rhizobial relationships for N acquisition (Alayafi et al., 2025; Oruru & Njeru, 2016).

Furthermore, to fully benefit from AMF, farmers could strategically reduce the application rates of highly soluble N and P fertilizers to induce a moderate nutrient demand which in return activates the plant's reliance on native and inoculated AMF (Kuila & Ghosh, 2022). As previously shown, (see **4.1.2**) high levels of soluble P reduce mycorrhizal symbiosis as the plant can absorb more P themselves (Ghorui et al., 2024). This aligns directly with the greenhouse findings, where the high availability of P suppressed the mutualism of host plant and AMF (see Figure **35**). This

practice could not only reduce the financial cost of synthetic fertilizers, but also minimize the environmental nutrient leaching while still maintaining crop yields (Ghorui et al., 2024).

For substituting the reduced chemical fertilizers, a circular economy could be implemented directly on the farm, by converting agricultural and animal waste into high-quality organic amendments (Enaime et al., 2023). The feasibility of using high-quality organic fertilizers was shown with the CoFEE findings, where the application of compost successfully equalized synthetic inputs and matched the yields by the third season. Furthermore, an application of biochar, produced by composting manure with biochar, could reduce GHG emissions and nutrient leaching during the composting (Gao et al., 2023). On the field, biochar increases the water holding capacity and can protect fungal hyphae and soil microbes from environmental degradation (Jabborova et al., 2025; Ramesh et al., 2025).

Finally, with the increasing adoption of commercial AMF bio-inoculants, it has been shown that many available products (up to 85%) fail to successfully colonize the plants roots or improve plant growth in field conditions (Boussageon et al., 2025). Practically, this means that it cannot be blindly relied on commercially available products until universal regulations and quality control are globally implemented (Ghorui et al., 2024). Native strains should be prioritized for now, which can be multiplied on-farm and are ecologically adapted to local soil conditions, offering a more reliable and cost-effective method for successful field inoculations (Arias Mota et al., 2024; Oruru & Njeru, 2016).

### 4.4.1 Research Limitations

This thesis successfully established fungal-plant interactions for SAF22 and red clover with a native microbiome baseline. However, certain limitations regarding field sampling and soil health assessment must be acknowledged. First, the CoFEE experimental site shows a noticeable slope, which introduces the potential for nutrient leaching between the different soil legacy plots as well as the management of the field could have led to an overlapping of the plots (i.e. tractor). To account for this, the initial plan was to take samples only from the plots (one for each evaluated legacy) at the top of slope. However, after discussions with the research group, it was decided based on the group's expertise to use a composite of every plot per legacy to ensure a higher representativeness of the entire field. This decision was consciously made with the trade-off that could have influenced results by the potential leaching of plots and may have led to a dilution of the physicochemical differences between the organic and mineral legacies. Moreover, as discussed, time constraints and data availability (i.e. CoFEE) shifted the focus away from soil health. This resulted in an analysis of parameters that highly influence soil health, but do not provide the big picture and it would be highly recommended to explore these extensively in further research.

### 4.4.2 Methodological Outlook and Experimental Design

If this thesis was conducted in a larger timeframe of over a year, future research could address these limitations through a targeted experimental design and build directly on the results of this work. For a reduction in environmental 'noise', potential future research should focus on fewer treatments in the greenhouse design, for example one unfertilized control, one fully synthetic fertilized control, one compost and due to the high usage in Swiss agriculture, a slurry treatment. Importantly, the future trials should focus again on unsterilized, native soil to more accurately replicate real-world priority effects and microbial competition (Ghorui et al., 2024; Thonar et al., 2014). While such an approach would build on the results of this work and look again into a specific application of SAF22 and could bring further valuable insights for such an application, other studies have shown that the efficiency of single-strain inoculants under real-world conditions can be largely unpredictable (Lutz et al., 2023). A more modern approach, although arguably

much more complex, could investigate the potential of Synthetic Microbial Communities (SynComs) for overcoming problems regarding single-strain applications, such as a strong competition with the native microbiome and the priority effect. SynComs are biologically engineered to mimic a highly resilient, natural microbiome by combining multiple microorganisms, such as AMF with *Trichoderma* and rhizobacteria, where the AMF can scavenge for nutrients (mainly P), *Trichoderma* could help protecting the host plant against pathogens by strengthening its resilience and also provide enhanced nutrient acquisition, and rhizobacteria would provide the N by BNF (Bertola et al., 2021; Boussageon et al., 2025; Gough et al., 2022). For maximising the agronomic relevance, the inoculation strategy could be tested on intercropping system, either with single-strain application or SynComs by using a more representative crop like wheat that would directly mirror the CoFEE field, combined with red clover to build on the results of this thesis and test for benefits via the shared networks of fungi (AMF and/or *Trichoderma*) and bacteria.

For soil health, a future trial could then test the soil microbiome and chemical parameters both pre-growth and post-growth periods to evaluate the initial nutrient availability and the subsequent scavenging potential for nutrients of the microbes. During the growth period, an assessment of leachate (run-off) from the greenhouse pots would provide quantitative data on how much N and P is essentially wasted, especially from conventional fertilization compared to the retention potential of organic soils (Bender et al., 2023). Furthermore, for the tracking of fungal colonization, the methodology should decide for one of the quantification methods (microscopy or qPCR), as both have their benefits and drawbacks. Due to the experience in this thesis, qPCR would be recommended, as it provides more accurate quantitative data, with the trade-off that no qualitative data can be collected on the developmental stage for the fungi in the root system.

If these approaches would prove to be highly successful in the controlled environment, a large research project that would probably be beyond any scope of a master thesis, could be launched with the goal to use these engineered communities and put them directly in either the CoFEE trial, or another suitable agricultural field and test if real-world environmental stressors, such as winter conditions, droughts and pathogens, could eventually provide a reliable and effective approach to sustain and maybe increase yields compared to high-input, conventional agricultural practices that could ultimately replace synthetic fertilizers.

# 5 Conclusion

This master's thesis examined the biofertilization potential of the SAF22 AMF strain on *T. pratense*, specifically investigating how long-term fertilization legacies of the CoFEE framework influence the symbiotic efficiency. Considering the ecological degradation caused by conventional agriculture, the aim of this work was to show how distinct fertilizer treatments influence fungal competition and to determine whether AMF inoculation can overcome native priority effects to enhance plant productivity, ultimately providing potential management strategies and identify when AMF inoculation serves as a viable agricultural addition. To address this, a greenhouse experiment was conducted using natural soil substrates from the CoFEE field trial.

The greenhouse experiment showed that both plant performance and fungal reliance are highly dependent on the soil's fertilization history. In the field, conventional mineral fertilization ('Control 100N') was highly successful in maximizing short-time harvest yield and absolute plant biomass during the initial seasons but failed to build long-term parameters of soil health. However, the initial yield gap in the field closed by the third season, where the delayed mineralization of the organic legacies almost caught up to the productivity of the mineral control, although being possibly related to the crop. When used in a controlled greenhouse environment, the rapidly available, nutrients decreased the dependence of the plant on AMF for nutrient acquisition. Conversely, complex organic amendments ('Compost A, B' and 'Slurry') drove the host plant to rely heavily on its symbiosis with AMF, but also rhizobia bacteria for N with the tripartite symbiosis. Consequently, AMF inoculation provided the highest relative growth (MGR) to plants grown in compost treatment soils, shown with an inverse correlation between soil productivity and symbiotic benefit. Even though the AMF strain struggled to physically establish in the roots of 'Compost B' due to a higher competition with more robust native fungal community and the subsequent priority effects, the environment showed a massive MGR. In contrast, the suppression of native fungi in mineral-fertilized soils created an empty niche that allowed SAF22 to colonize roots more extensively, yet this resulted in a minimal mutualistic benefit and a lower MGR. Regardless of the soil legacy or colonization extent, AMF acted as an equalizer for plant health, where it universally raised chlorophyll contents across all treatments and supplied the P necessary to significantly increase the rhizobia nodulation.

While this thesis showed the mechanisms of fungal reliance, the limits of the research must be acknowledged. The efficiency of the AMF inoculant was shown to be highly context dependent, having a highly aggressive generalist AMF strain (SAF22) and a highly mycorrhizal-dependent host plant. Therefore, the specific symbiotic dynamics evaluated cannot be applied universally to non-leguminous crops or different AMF inoculants. Furthermore, while the controlled greenhouse setting successfully isolated specific fungal-plant interactions, it is limited in translating the findings of the mutualistic benefits to highly diverse and varying, real-world field conditions where a broad range of biotic and abiotic stressors is found simultaneously.

Summarizing, organic amendments are essential for building long-term soil health and establishing a self-sustaining nutrient cycle, however microbial symbiosis need to be incorporated to accelerate the closing of the initial yield gap to conventional agriculture. Future research could directly build upon this work by replicating the tested SAF22 inoculation withing an actual field environment. Furthermore, the sequencing of the native microbes and a comprehensive soil analysis pre- and post-harvest could provide evidence of how tripartite symbiosis drives nutrient depletion and cycling across the different fertilizer legacies.

# 6 Literature


Abd-Alla, M. H., Al-Amri, S. M., & El-Enany, A.-W. E. (2023). Enhancing rhizobium–legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change. *Agriculture*, *13*(11), 2092. https://doi.org/10.3390/agriculture13112092

Agroscope. (2026). *Verzeichnis der Referenzmethoden* (Schweizerische Referenzmethoden Der Forschungsanstalten Agroscope, p. 11). Agroscope. https://www.agroscope.admin.ch/agroscope/de/home/themen/umwelt-ressourcen/monitoring-analytik/referenzmethoden.html

Airee, S., & Mukherjee, A. (2025). Impacts of farming management on soil health and water quality in Kentucky. *Agrosystems, Geosciences & Environment*, *8*(2), e70134. https://doi.org/10.1002/agg2.70134

Alayafi, A. A. M., Alharbi, B. M., Abdulmajeed, A. M., Alnusaire, T. S., ALrashidi, A. A., AL-Balawi, S. M., Khalaf Anazi, H., Alghanem, S. M. S., Al Zoubi, O. M., & Soliman, M. H. (2025). Arbuscular mycorrhizal fungi symbiosis enhances growth, nutrient uptake, and oil quality in sunflower–pumpkin under intercropping systems. *Frontiers in Plant Science*, *16*, 1598272. https://doi.org/10.3389/fpls.2025.1598272

Al-Wabel, M. I., Hussain, Q., Usman, A. R. A., Ahmad, M., Abduljabbar, A., Sallam, A. S., & Ok, Y. S. (2018). Impact of biochar properties on soil conditions and agricultural sustainability: A review. *Land Degradation & Development*, *29*(7), 2124–2161. https://doi.org/10.1002/ldr.2829

Arias Mota, R. M., De La Cruz Elizondo, Y., Ruelas Monjardín, L. C., & Perea-Rojas, Y. D. C. (2024). Dominant morphotypes of native arbuscular mycorrhizal fungi from coffee plantations and their propagation with trap plants. *International Journal of Plant Biology*, *15*(3), 744–756. https://doi.org/10.3390/ijpb15030054

Basiru, S., & Hijri, M. (2022). The potential applications of commercial arbuscular mycorrhizal fungal inoculants and their ecological consequences. *Microorganisms*, *10*(10), 1897. https://doi.org/10.3390/microorganisms10101897

Bender, S. F., Schulz, S., Martínez-Cuesta, R., Laughlin, R. J., Kublik, S., Pfeiffer-Zakharova, K., Vestergaard, G., Hartman, K., Parladé, E., Römbke, J., Watson, C. J., Schloter, M., & Van Der Heijden, M. G. A. (2023). Simplification of soil biota communities impairs nutrient recycling and enhances above- and belowground nitrogen losses. *New Phytologist*, *240*(5), 2020–2034. https://doi.org/10.1111/nph.19252

Bertola, M., Ferrarini, A., & Visioli, G. (2021). Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by -omics approaches: A perspective for the environment, food quality and human safety. *Microorganisms*, *9*(7), 1400. https://doi.org/10.3390/microorganisms9071400

Bhunia, S., Bhowmik, A., Mallick, R., & Mukherjee, J. (2021). Agronomic efficiency of animal-derived organic fertilizers and their effects on biology and fertility of soil: A review. *Agronomy*, *11*(5), 823.

https://doi.org/10.3390/agronomy11050823

Bodenhausen, N., Deslandes-Hérold, G., Waelchli, J., Held, A., Van Der Heijden, M. G. A., & Schlaeppi, K. (2021). Relative qPCR to quantify colonization of plant roots by arbuscular mycorrhizal fungi. *Mycorrhiza*, *31*(2), 137–148. https://doi.org/10.1007/s00572-020-01014-1

Boussageon, R., Eigenmann, M., Frommenwiler, J., & Van Der Heijden, M. G. A. (2025). Poor quality of commercial arbuscular mycorrhizal inoculants used for agriculture and home gardening. *Journal of Sustainable Agriculture and Environment*, *4*(4), e70107. https://doi.org/10.1002/sae2.70107

Brito, I., Carvalho, M., & Goss, M. J. (2021). Managing the functional diversity of arbuscular mycorrhizal fungi for the sustainable intensification of crop production. *Plants, People, Planet*, *3*(5), 491–505. https://doi.org/10.1002/ppp3.10212

Cheeke, T. E., Zheng, C., Koziol, L., Gurholt, C. R., & Bever, J. D. (2019). Sensitivity to AMF species is greater in late-successional than early-successional native or nonnative grassland plants. *Ecology*, *100*(12), e02855. https://doi.org/10.1002/ecy.2855

Chien, C.-C., Tien, S.-Y., Yang, S.-Y., & Lee, C.-R. (2024). The costs and benefits of symbiotic interactions: Variable effects of rhizobia and arbuscular mycorrhizae on Vigna radiata accessions. *BMC Plant Biology*, *24*(1), 780. https://doi.org/10.1186/s12870-024-05488-5

Diacono, M., & Montemurro, F. (2010). Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development*, *30*(2), 401–422. https://doi.org/10.1051/agro/2009040

Doran, J. W., & Zeiss, M. R. (2000). Soil health and sustainability: Managing the biotic component of soil quality. *Applied Soil Ecology*, *15*(1), 3–11. https://doi.org/10.1016/S0929-1393(00)00067-6

Dukare, A., Mhatre, P., Maheshwari, H. S., Bagul, S., Manjunatha, B. S., Khade, Y., & Kamble, U. (2022). Delineation of mechanistic approaches of rhizosphere microorganisms facilitated plant health and resilience under challenging conditions. *3 Biotech*, *12*(3), 57. https://doi.org/10.1007/s13205-022-03115-4

Edlinger, A., Herzog, C., Garland, G., Walder, F., Banerjee, S., Keel, S. G., Mayer, J., Philippot, L., Romdhane, S., Schiedung, M., Schmidt, M. W. I., Seitz, B., Wüst-Galley, C., & Van Der Heijden, M. G. A. (2025). Compost application enhances soil health and maintains crop yield: Insights from 56 farmer-managed arable fields. *Journal of Sustainable Agriculture and Environment*, *4*(1), e70041. https://doi.org/10.1002/sae2.70041

Enaime, G., Wichern, M., & Lübken, M. (2023). Contribution of biochar application to the promotion of circular economy in agriculture. *Frontiers in Agronomy*, *5*, 1214012. https://doi.org/10.3389/fagro.2023.1214012

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third). Sage. https://www.john-fox.ca/Companion/

Gamper, H., Hartwig, U. A., & Leuchtmann, A. (2005). Mycorrhizas improve nitrogen nutrition of *Trifolium repens* after 8 yr of selection under elevated atmospheric $CO_2$ partial pressure. *New Phytologist*, *167*(2), 531–542. https://doi.org/10.1111/j.1469-8137.2005.01440.x

Gao, S., Harrison, B. P., Thao, T., Gonzales, M. L., An, D., Ghezzehei, T. A., Diaz, G., & Ryals, R. A. (2023). Biochar co-compost improves nitrogen retention and reduces carbon emissions in a winter wheat cropping system. *GCB Bioenergy*, *15*(4), 462–477. https://doi.org/10.1111/gcbb.13028

George, N. P., & Ray, J. G. (2023). The inevitability of arbuscular mycorrhiza for sustainability in organic agriculture—A critical review. *Frontiers in Sustainable Food Systems*, *7*, 1124688. https://doi.org/10.3389/fsufs.2023.1124688

Ghorui, M., Chowdhury, S., Balu, P., & Burla, S. (2024). Arbuscular mycorrhizal inoculants and its regulatory landscape. *Heliyon*, *10*(9), e30359. https://doi.org/10.1016/j.heliyon.2024.e30359

Gollner, M. J., Wagentristl, H., Liebhard, P., & Friedel, J. K. (2011). Yield and arbuscular mycorrhiza of winter rye in a 40-year fertilisation trial. *Agronomy for Sustainable Development*, *31*(2), 373–378. https://doi.org/10.1051/agro/2010032

Gough, E. C., Owen, K. J., Zwart, R. S., & Thompson, J. P. (2022). The role of nutrients underlying interactions among root-nodule bacteria (Bradyrhizobium sp.), arbuscular mycorrhizal fungi (Funneliformis mosseae) and root-lesion nematodes (Pratylenchus thornei) in nitrogen fixation and growth of mung bean (Vigna radiata). *Plant and Soil*, *472*(1–2), 421–449. https://doi.org/10.1007/s11104-021-05254-8

Graves, S., Piepho, H.-P., Selzer, L., & Dorai-Raj, S. (2024). *multcompView: Visualizations of Paired Comparisons*. https://CRAN.R-project.org/package=multcompView

Guo, M., Zhang, Z., & Zhu, X. (2022). The impact of the Russian-Ukrainian war on oil markets in european countries and possible solutions. In F. Balli, A. Y. H. Nee, & S. A. Qalati (Eds), *Proceedings of the 2022 international conference on economics, smart finance and contemporary trade (ESFCT 2022)* (Vol. 226, pp. 296–304). Atlantis Press International BV. https://doi.org/10.2991/978-94-6463-052-7_34

Haider, S., Song, J., Bai, J., Wang, X., Ren, G., Bai, Y., Huang, Y., Shah, T., & Feng, Y. (2025). Toward low-emission agriculture: Synergistic contribution of inorganic nitrogen and organic fertilizers to GHG emissions and strategies for mitigation. *Plants*, *14*(10), 1551. https://doi.org/10.3390/plants14101551

Hartung, J., Wagener, J., Ruser, R., & Piepho, H.-P. (2019). Blocking and re-arrangement of pots in greenhouse experiments: Which approach is more effective? *Plant Methods*, *15*(1), 143. https://doi.org/10.1186/s13007-019-0527-4

Hu, Y., Pandey, A. K., Wu, X., Fang, P., & Xu, P. (2022). The role of arbuscular mycorrhiza fungi in drought tolerance

in legume crops: A review. *Legume Research - an International Journal*, (Of). https://doi.org/10.18805/LRF-660

Jabborova, D., Madan, A., Perveen, K., Khan, F., Flores-Castañón, N., Banerjee, A., Barasarathi, J., Mastinu, A., & Sayyed, R. (2025). Combined application of biochar and arbuscular mycorrhizae enhances growth and soil functionality in common bean under drought stress. *Journal of Sustainable Agriculture and Environment*, *4*(4), e70117. https://doi.org/10.1002/sae2.70117

Kacprzak, M., Kupich, I., Jasinska, A., & Fijalkowski, K. (2022). Bio-based waste' substrates for degraded soil improvement—Advantages and challenges in european context. *Energies*, *15*(1), 385. https://doi.org/10.3390/en15010385

Kasper, S., Christoffersen, B., Soti, P., & Racelis, A. (2019). Abiotic and biotic limitations to nodulation by leguminous cover crops in south texas. *Agriculture*, *9*(10), 209. https://doi.org/10.3390/agriculture9100209

Kassambara, A. (2026). *ggpubr: 'ggplot2' based publication ready plots*. https://CRAN.R-project.org/package=ggpubr

Kassambara, A., & Mundt, F. (2026). *factoextra: Extract and visualize the results of multivariate data analyses*. https://CRAN.R-project.org/package=factoextra

Köhl, L., Lukasiewicz, C. E., & van der Heijden, M. G. A. (2016). Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. *Plant, Cell & Environment*, *39*(1), 136–146. https://doi.org/10.1111/pce.12600

Kuila, D., & Ghosh, S. (2022). Aspects, problems and utilization of Arbuscular Mycorrhizal (AM) application as bio-fertilizer in sustainable agriculture. *Current Research in Microbial Sciences*, *3*, 100107. https://doi.org/10.1016/j.crmicr.2022.100107

Kumar, U., Raj, S., Sreenikethanam, A., Maddheshiya, R., Kumari, S., Han, S., Kapoor, K. K., Bhaskar, R., Bajhaiya, A. K., & Gahlot, D. K. (2023). Multi-omics approaches in plant–microbe interactions hold enormous promise for sustainable agriculture. *Agronomy*, *13*(7), 1804. https://doi.org/10.3390/agronomy13071804

Lazcano, C., Zhu-Barker, X., & Decock, C. (2021). Effects of organic fertilizers on the soil microorganisms responsible for N2O emissions: A review. *Microorganisms*, *9*(5), 983. https://doi.org/10.3390/microorganisms9050983

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth & Environment*, *1*(10), 544–553. https://doi.org/10.1038/s43017-020-0080-8

Lenth, R. V., & Piaskowski, J. (2026). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. https://CRAN.R-project.org/package=emmeans

Liu, A., Ku, Y.-S., Contador, C. A., & Lam, H.-M. (2020). The impacts of domestication and agricultural practices on legume nutrient acquisition through symbiosis with rhizobia and arbuscular mycorrhizal fungi. *Frontiers in Genetics*, *11*, 583954. https://doi.org/10.3389/fgene.2020.583954

Liu, W., Zhang, Y., Jiang, S., Murray, P. J., Liao, L., Li, X., & Zhang, J. (2019). Spatiotemporal differences in the arbuscular mycorrhizal fungi communities in soil and roots in response to long-term organic compost inputs in an intensive agricultural cropping system on the North China Plain. *Journal of Soils and Sediments*, *19*(5), 2520–2533. https://doi.org/10.1007/s11368-019-02244-3

Liu, Y., Lan, X., Hou, H., Ji, J., Liu, X., & Lv, Z. (2024). Multifaceted ability of organic fertilizers to improve crop productivity and abiotic stress tolerance: Review and perspectives. *Agronomy*, *14*(6), 1141. https://doi.org/10.3390/agronomy14061141

Loaiza Puerta, V., Pujol Pereira, E. I., Wittwer, R., Van Der Heijden, M., & Six, J. (2018). Improvement of soil structure through organic crop management, conservation tillage and grass-clover ley. *Soil and Tillage Research*, *180*, 1–9. https://doi.org/10.1016/j.still.2018.02.007

Loo, W. T., Chua, K.-O., Mazumdar, P., Cheng, A., Osman, N., & Harikrishna, J. A. (2022). Arbuscular mycorrhizal symbiosis: A strategy for mitigating the impacts of climate change on tropical legume crops. *Plants*, *11*(21), 2875. https://doi.org/10.3390/plants11212875

Lutz, S., Bodenhausen, N., Hess, J., Valzano-Held, A., Waelchli, J., Deslandes-Hérold, G., Schlaeppi, K., & Van Der Heijden, M. G. A. (2023). Soil microbiome indicators can predict crop growth response to large-scale inoculation with arbuscular mycorrhizal fungi. *Nature Microbiology*, *8*(12), 2277–2289. https://doi.org/10.1038/s41564-023-01520-w

Maeder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., & Niggli, U. (2002). Soil fertility and biodiversity in organic farming. *Science*, *296*(5573), 1694–1697. https://doi.org/10.1126/science.1071148

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular—Arbuscular mycorrhizal fungi. *New Phytologist*, *115*(3), 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x

McLaughlin, S., Joller, C., Siffert, A., Stirnemann, E. M., & Sasse, J. (2023). A versatile glass jar system for semihydroponic root exudate profiling. *Journal of Visualized Experiments*, (201), 66070. https://doi.org/10.3791/66070

Mendiburu, F. de. (2023). *agricolae: Statistical Procedures for Agricultural Research*. https://CRAN.R-project.org/package=agricolae

Morton, J. B., Benedito, V. A., Panaccione, D. G., & Jenks, M. A. (2014). Potential for industrial application of

microbes in symbioses that influence plant productivity and sustainability in agricultural, natural, or restored ecosystems. *Industrial Biotechnology*, *10*(5), 347–353. https://doi.org/10.1089/ind.2014.0014

Mpongwana, S., Manyevere, A., Mupangwa, J., Mpendulo, C. T., & Mashamaite, C. V. (2023). Foliar nutrient content responses to bio-inoculation of arbuscular mycorrhizal fungi and Rhizobium on three herbaceous forage legumes. *Frontiers in Sustainable Food Systems*, *7*, 1256717. https://doi.org/10.3389/fsufs.2023.1256717

Oehl, F., Sieverding, E., Maeder, P., Dubois, D., Ineichen, K., Boller, T., & Wiemken, A. (2004). Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia*, *138*(4), 574–583. https://doi.org/10.1007/s00442-003-1458-2

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Borman, T., Carvalho, G., Chirico, M., Caceres, M. D., … Weedon, J. (2026). *vegan: Community Ecology Package*. https://CRAN.R-project.org/package=vegan

Oleńska, E., Małek, W., Sujkowska-Rybkowska, M., Szopa, S., Włostowski, T., Aleksandrowicz, O., Swiecicka, I., Wójcik, M., Thijs, S., & Vangronsveld, J. (2022). An alliance of trifolium repens—Rhizobium leguminosarum bv. Trifolii—Mycorrhizal fungi from an old zn-pb-cd rich waste heap as a promising tripartite system for phytostabilization of metal polluted soils. *Frontiers in Microbiology*, *13*, 853407. https://doi.org/10.3389/fmicb.2022.853407

Omkar (Ed.). (2021). *Polyphagous pests of crops*. Springer Singapore. https://doi.org/10.1007/978-981-15-8075-8

Onyeaka, H. N., Akinsemolu, A. A., Siyanbola, K. F., & Adetunji, V. A. (2024). Green microbe profile: Rhizophagus intraradices—a review of benevolent fungi promoting plant health and sustainability. *Microbiology Research*, *15*(2), 1028–1049. https://doi.org/10.3390/microbiolres15020068

Oruru, M. B., & Njeru, E. M. (2016). Upscaling arbuscular mycorrhizal symbiosis and related agroecosystems services in smallholder farming systems. *BioMed Research International*, *2016*, 1–12. https://doi.org/10.1155/2016/4376240

Ostadi, A., Javanmard, A., Amani Machiani, M., Sadeghpour, A., Maggi, F., Nouraein, M., Morshedloo, M. R., Hano, C., & Lorenzo, J. M. (2022). Co-application of TiO2 nanoparticles and arbuscular mycorrhizal fungi improves essential oil quantity and quality of sage (Salvia Officinalis L.) in drought stress conditions. *Plants*, *11*(13), 1659. https://doi.org/10.3390/plants11131659

OSU Soil Fertility Lab. (2021). *Procedure for Wet Aggregate Stability (WAGS Protocol).* (Last revised 04/2021.). Ohio State University.

Pádua, C. S. de, Fatoretto, M. B., Menarin, V., Moral, R. de A., Demétrio, C. G. B., D'Alessandro, C. P., & Rojas, V.

A. (2015). Generalized linear mixed models: An application to fungi data. *Proceedings of the 60th World Statistics Congress of the International Statistical Institute*.

Panday, D., Bhusal, N., Das, S., & Ghalehgolabbehbahani, A. (2024). Rooted in nature: The rise, challenges, and potential of organic farming and fertilizers in agroecosystems. *Sustainability*, *16*(4), 1530. https://doi.org/10.3390/su16041530

Paradelo, R., Navarro-Pedreño, J., Glaser, B., Grobelak, A., Kowalska, A., & Singh, B. R. (2023). Potential and constraints of use of organic amendments from agricultural residues for improvement of soil properties. *Sustainability*, *16*(1), 158. https://doi.org/10.3390/su16010158

Powers, S. E., & Thavarajah, D. (2019). Checking agriculture's pulse: Field pea (Pisum Sativum L.), sustainability, and phosphorus use efficiency. *Frontiers in Plant Science*, *10*, 1489. https://doi.org/10.3389/fpls.2019.01489

R Core Team. (2024). *R: A Language and Environment for Statistical Computing.* (Version 4.4.1.) [Computer software]. https://www.R-project.org/.

Ramesh, K., Dinakarkumar, Y., Gujjula, K. R., Kotaru, L. S., Brindha Devi, P., Pazhamalai, V., & Ivo Romauld, S. (2025). Impacts of biomass-derived biochar on plant nutrition, soil function, and agricultural sustainability: A review. *Environmental Quality Management*, *35*(2), e70198. https://doi.org/10.1002/tqem.70198

Richner, W., & Sinaj, S. (Eds). (2017). Grundlagen für die Düngung landwirtschaftlicher Kulturen in der Schweiz (GRUD 2017). *Agrarforschung Schweiz, Spezialpublikation*, *8*(6), 276.

Rillig, M. C., Wright, S. F., & Eviner, V. T. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant and Soil*, *238*(2), 325–333.

Rog, I., van der Heijden, M. G. A., Bender, F., Boussageon, R., Lambach, A., Schlaeppi, K., Bodenhausen, N., & Lutz, S. (2025). Mycorrhizal inoculation success depends on soil health and crop productivity. *FEMS Microbiology Letters*, *372*, fnaf031. https://doi.org/10.1093/femsle/fnaf031

Rubin, J. A., & Görres, J. H. (2020). Potential for mycorrhizae-assisted phytoremediation of phosphorus for improved water quality. *International Journal of Environmental Research and Public Health*, *18*(1), 7. https://doi.org/10.3390/ijerph18010007

Sabater, C., Neacsu, M., & Duncan, S. H. (2025). Harnessing beneficial soil bacteria to promote sustainable agriculture and food security: A one health perspective. *Frontiers in Microbiology*, *16*, 1638553. https://doi.org/10.3389/fmicb.2025.1638553

Samantaray, A., Chattaraj, S., Mitra, D., Ganguly, A., Kumar, R., Gaur, A., Mohapatra, P. K. D., Santos-Villalobos, S. D. L., Rani, A., & Thatoi, H. (2024). Advances in microbial based bio-inoculum for amelioration of soil health and sustainable crop production. *Current Research in Microbial Sciences*, *7*, 100251.

https://doi.org/10.1016/j.crmicr.2024.100251

Sharma, M. P., Grover, M., Chourasiya, D., Bharti, A., Agnihotri, R., Maheshwari, H. S., Pareek, A., Buyer, J. S., Sharma, S. K., Schütz, L., Mathimaran, N., Singla-Pareek, S. L., Grossman, J. M., & Bagyaraj, D. J. (2020). Deciphering the role of trehalose in tripartite symbiosis among rhizobia, arbuscular mycorrhizal fungi, and legumes for enhancing abiotic stress tolerance in crop plants. *Frontiers in Microbiology*, *11*, 509919. https://doi.org/10.3389/fmicb.2020.509919

Shelby, N., Duncan, R. P., Van Der Putten, W. H., McGinn, K. J., Weser, C., & Hulme, P. E. (2016). Plant mutualisms with rhizosphere microbiota in introduced versus native ranges. *Journal of Ecology*, *104*(5), 1259–1270. https://doi.org/10.1111/1365-2745.12609

Shivangi, S., Singh, O., Shahi, U. P., Singh, P. K., Singh, A., Rajput, V., Minkina, T., El-Ramady, H., & Ghazaryan, K. (2024). Carbon sequestration through organic amendments, clay mineralogy and agronomic practices: A review. *Egyptian Journal of Soil Science*, *64*(2), 581–598. https://doi.org/10.21608/ejss.2024.260719.1707

Solangi, F., Zhu, X., Khan, S., Rais, N., Majeed, A., Sabir, M. A., Iqbal, R., Ali, S., Hafeez, A., Ali, B., Ercisli, S., & Kayabasi, E. T. (2023). The global dilemma of soil legacy phosphorus and its improvement strategies under recent changes in agro-ecosystem sustainability. *ACS Omega*, *8*(26), 23271–23282. https://doi.org/10.1021/acsomega.3c00823

Tahat, M. M., Alananbeh, K. M., Othman, Y. A., & Leskovar, D. I. (2020). Soil health and sustainable agriculture. *Sustainability*, *12*(12), 4859. https://doi.org/10.3390/su12124859

Takács, T., Cseresnyés, I., Kovács, R., Parádi, I., Kelemen, B., Szili-Kovács, T., & Füzy, A. (2018). Symbiotic effectivity of dual and tripartite associations on soybean (glycine max L. Merr.) cultivars inoculated with Bradyrhizobium Japonicum and AM fungi. *Frontiers in Plant Science*, *9*, 1631. https://doi.org/10.3389/fpls.2018.01631

Tawaraya, K. (2003). Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition*, *49*(5), 655–668. https://doi.org/10.1080/00380768.2003.10410323

Thirkell, T. J., Cameron, D. D., & Hodge, A. (2016). Resolving the 'nitrogen paradox' of *arbuscular mycorrhizas*: Fertilization with organic matter brings considerable benefits for plant nutrition and growth. *Plant, Cell & Environment*, *39*(8), 1683–1690. https://doi.org/10.1111/pce.12667

Thonar, C., Frossard, E., Šmilauer, P., & Jansa, J. (2014). Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. *Molecular Ecology*, *23*(3), 733–746. https://doi.org/10.1111/mec.12625

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, R., Giller, K., Alves, B., & Chalk, P. (2008). *Measuring plant-associated nitrogen fixation in agricultural systems* (Australian Centre for International

Agricultural Research, Ed.). Australian Centre for International Agricultural Research (ACIAR).

Valzano-Held, A. (2022). *Wurzen Färben DE (Alains Methode)—V2* [Unveröffentlichtes Laborprotokoll]. Agroecology and Plant-Microbiome Interactions, University of Zurich.

van der Heijden, M. G. A. (2002). Arbuscular mycorrhizal fungi as a determinant of plant diversity: In search of underlying mechanisms and general principles. In M. G. A. can der Heijden & I. Sanders (Eds), *Mycorrhizal Ecology* (Vol. 157, pp. 243–265). Springer-Verlag.

van der Heijden, M. G. A., Martin, F. M., Selosse, M., & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, *205*(4), 1406–1423. https://doi.org/10.1111/nph.13288

Velthof, G. L., Cals, T. C. A., Van 'T Hull, J. P., Lesschen, J. P., Lessmann, M., Porre, R. J., Ros, M. B. H., Rietra, R. P. J. J., Schoumans, O. F., Veenemans, L., & Westerik, D. (2024). Managing organic resources in agriculture: Future challenges from a scientific perspective. *Frontiers in Sustainable Food Systems*, *8*, 1393190. https://doi.org/10.3389/fsufs.2024.1393190

Vermeire, M.-L., Thiour-Mauprivez, C., & De Clerck, C. (2024). Agroecological transition: Towards a better understanding of the impact of ecology-based farming practices on soil microbial ecotoxicology. *FEMS Microbiology Ecology*, *100*(4), fiae031. https://doi.org/10.1093/femsec/fiae031

Vernon, L. P. (1960). Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. *Analytical Chemistry*, *32*(9), 1144–1150. https://doi.org/10.1021/ac60165a029

Verzeaux, J., Hirel, B., Dubois, F., Lea, P. J., & Tétu, T. (2017). Agricultural practices to improve nitrogen use efficiency through the use of arbuscular mycorrhizae: Basic and agronomic aspects. *Plant Science*, *264*, 48–56. https://doi.org/10.1016/j.plantsci.2017.08.004

Wahdan, S. F. M., Tanunchai, B., Wu, Y., Sansupa, C., Schädler, M., Dawoud, T. M., Buscot, F., & Purahong, W. (2021). Deciphering *Trifolium pratense* L. holobiont reveals a microbiome resilient to future climate changes. *MicrobiologyOpen*, *10*(4), e1217. https://doi.org/10.1002/mbo3.1217

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2026). *dplyr: A Grammar of Data Manipulation*. https://CRAN.R-project.org/package=dplyr

Wickham, H., Vaughan, D., & Girlich, M. (2024). *tidyr: Tidy Messy Data*. https://CRAN.R-project.org/package=tidyr

Zaugg, S. (2026, April 1). *Folgen des Iran-Kriegs Weltwirtschaft im Schock: Haushalte immer stärker betroffen*. SRF. SRF News. https://www.srf.ch/news/international/folgen-des-iran-kriegs-weltwirtschaft-im-schock-haushalte-immer-staerker-betroffen

Zhang, C., Van Der Heijden, M. G. A., Dodds, B. K., Nguyen, T. B., Spooren, J., Valzano-Held, A., Cosme, M., & Berendsen, R. L. (2024). A tripartite bacterial-fungal-plant symbiosis in the mycorrhiza-shaped microbiome drives plant growth and mycorrhization. *Microbiome*, *12*(1), 13. https://doi.org/10.1186/s40168-023-01726-4

# 7 Appendix

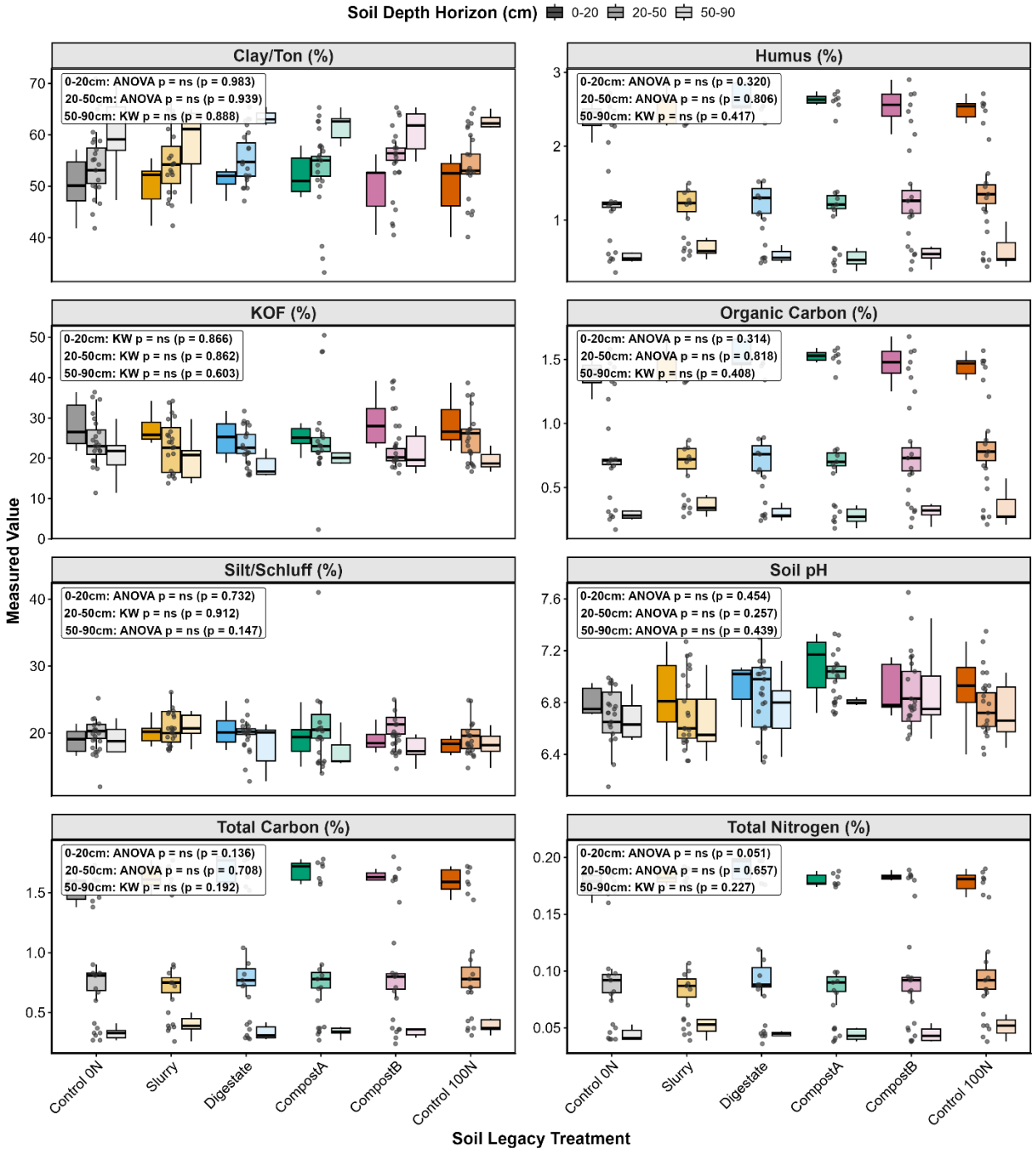



***Figure S 1:Panel of 8 physicochemical parameters in Boxplots for CoFEE (2022).*** *For all dephts (0-20, 20-50, 50-90), colored by treatment and different hues for the different depths. Statistical testing was done with two-way ANOVA or Kruskal-Wallis, highlighted in the top left corner of each panel. Coloured by treatment, and lighter hues for each depht.*

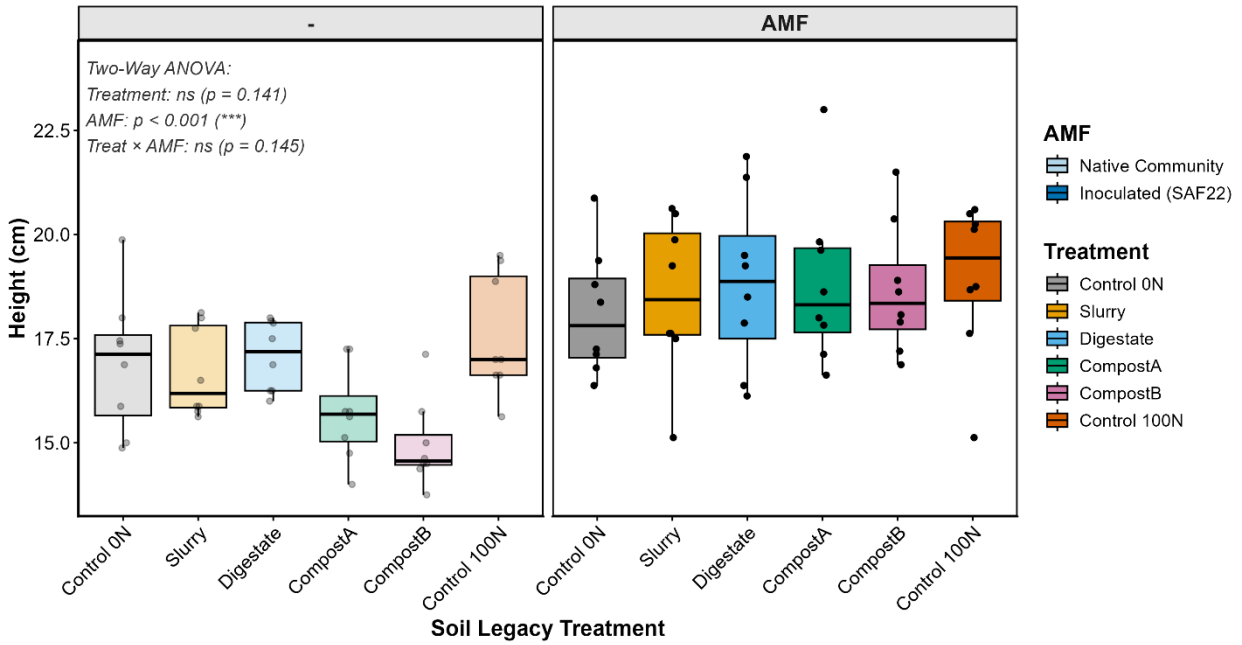



***Figure S 2:Separated boxplots of harvest height of clover.*** *Black line shows the median with whiskers showing the variance of the data, colored by treatment with lighter hues for native community group.*

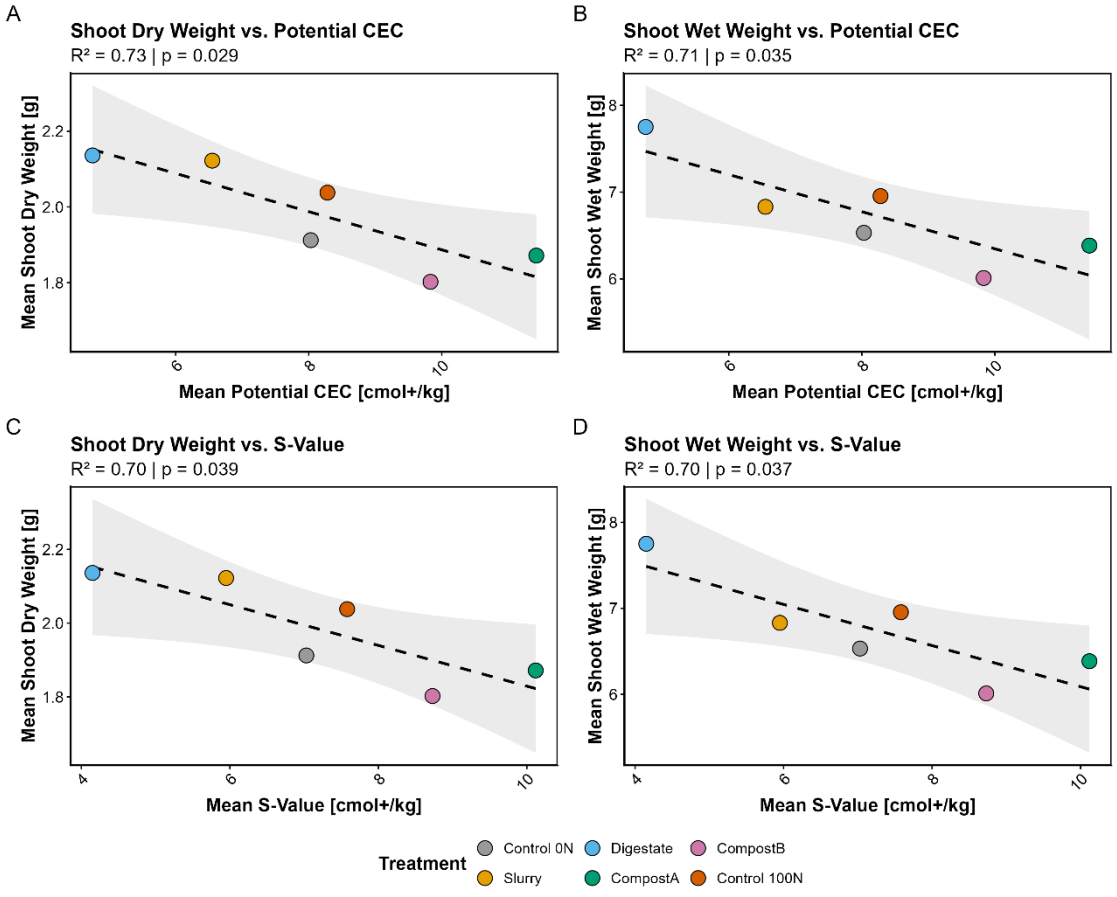



***Figure S 3: Shoot Dry & Wet Weight vs S-Value & Potential CEC.*** *Four regressions, showing shoot dry and shoot wet weight vs potential CEC and s-value, coloured by treatment.*

| Comparison Pairs | Df | Sum of Sq | F-Statistic | R-Squared | p-value (Raw) | p-value (BH Adj.) | Significance |
|---|---|---|---|---|---|---|---|
| Control 0N_- vs CompostA_- | 1 | 2.103394 | 0.81 | 0.054 | 0.467 | 0.505 | |
| Control 0N_- vs CompostB_- | 1 | 12.390274 | 4.64 | 0.249 | 0.001 | 0.002 | * |
| Control 0N_- vs Digestate_- | 1 | 3.540730 | 1.37 | 0.089 | 0.261 | 0.308 | |
| Control 0N_- vs Slurry_- | 1 | 4.836096 | 1.86 | 0.117 | 0.104 | 0.140 | |
| Control 0N_- vs Control 100N_- | 1 | 6.040349 | 1.88 | 0.119 | 0.117 | 0.151 | |
| Control 0N_- vs Control 0N_AMF | 1 | 55.843307 | 23.71 | 0.629 | 0.001 | 0.002 | * |
| Control 0N_- vs CompostA_AMF | 1 | 61.196886 | 24.08 | 0.632 | 0.002 | 0.003 | * |
| Control 0N_- vs CompostB_AMF | 1 | 40.704853 | 12.95 | 0.481 | 0.001 | 0.002 | * |
| Control 0N_- vs Digestate_AMF | 1 | 53.000784 | 19.05 | 0.576 | 0.002 | 0.003 | * |
| Control 0N_- vs Slurry_AMF | 1 | 78.138955 | 18.06 | 0.563 | 0.001 | 0.002 | * |
| Control 0N_- vs Control 100N_AMF | 1 | 76.770067 | 32.75 | 0.701 | 0.001 | 0.002 | * |
| CompostA_- vs CompostB_- | 1 | 5.969888 | 1.84 | 0.116 | 0.138 | 0.172 | |
| CompostA_- vs Digestate_- | 1 | 4.725253 | 1.50 | 0.097 | 0.200 | 0.240 | |
| CompostA_- vs Slurry_- | 1 | 4.140131 | 1.30 | 0.085 | 0.278 | 0.322 | |
| CompostA_- vs Control 100N_- | 1 | 9.870641 | 2.61 | 0.157 | 0.038 | 0.056 | |
| CompostA_- vs Control 0N_AMF | 1 | 57.351823 | 19.60 | 0.583 | 0.001 | 0.002 | * |
| CompostA_- vs CompostA_AMF | 1 | 64.692124 | 20.78 | 0.598 | 0.001 | 0.002 | * |
| CompostA_- vs CompostB_AMF | 1 | 44.295680 | 11.93 | 0.460 | 0.001 | 0.002 | * |
| CompostA_- vs Digestate_AMF | 1 | 56.150673 | 16.74 | 0.545 | 0.002 | 0.003 | * |
| CompostA_- vs Slurry_AMF | 1 | 80.953220 | 16.53 | 0.541 | 0.001 | 0.002 | * |
| CompostA_- vs Control 100N_AMF | 1 | 79.168705 | 27.15 | 0.660 | 0.001 | 0.002 | * |
| CompostB_- vs Digestate_- | 1 | 10.067108 | 3.13 | 0.183 | 0.028 | 0.042 | . |
| CompostB_- vs Slurry_- | 1 | 12.040451 | 3.72 | 0.210 | 0.011 | 0.017 | . |
| CompostB_- vs Control 100N_- | 1 | 27.004377 | 7.04 | 0.334 | 0.001 | 0.002 | * |
| CompostB_- vs Control 0N_AMF | 1 | 66.121581 | 22.12 | 0.612 | 0.001 | 0.002 | * |
| CompostB_- vs CompostA_AMF | 1 | 77.232529 | 24.33 | 0.635 | 0.001 | 0.002 | * |
| CompostB_- vs CompostB_AMF | 1 | 56.231359 | 14.89 | 0.515 | 0.001 | 0.002 | * |
| CompostB_- vs Digestate_AMF | 1 | 64.647286 | 18.93 | 0.575 | 0.001 | 0.002 | * |
| CompostB_- vs Slurry_AMF | 1 | 91.871996 | 18.53 | 0.570 | 0.001 | 0.002 | * |
| CompostB_- vs Control 100N_AMF | 1 | 83.797500 | 28.14 | 0.668 | 0.002 | 0.003 | * |
| Digestate_- vs Slurry_- | 1 | 3.746823 | 1.19 | 0.078 | 0.302 | 0.344 | |
| Digestate_- vs Control 100N_- | 1 | 7.750664 | 2.06 | 0.129 | 0.108 | 0.143 | |
| Digestate_- vs Control 0N_AMF | 1 | 40.670117 | 14.01 | 0.500 | 0.001 | 0.002 | * |
| Digestate_- vs CompostA_AMF | 1 | 48.130458 | 15.58 | 0.527 | 0.001 | 0.002 | * |
| Digestate_- vs CompostB_AMF | 1 | 29.414683 | 7.97 | 0.363 | 0.001 | 0.002 | * |
| Digestate_- vs Digestate_AMF | 1 | 37.534443 | 11.27 | 0.446 | 0.001 | 0.002 | * |
| Digestate_- vs Slurry_AMF | 1 | 60.949725 | 12.50 | 0.472 | 0.001 | 0.002 | * |
| Digestate_- vs Control 100N_AMF | 1 | 57.993218 | 20.05 | 0.589 | 0.001 | 0.002 | * |
| Slurry_- vs Control 100N_- | 1 | 4.082973 | 1.08 | 0.072 | 0.382 | 0.427 | |
| Slurry_- vs Control 0N_AMF | 1 | 39.422148 | 13.47 | 0.490 | 0.001 | 0.002 | * |
| Slurry_- vs CompostA_AMF | 1 | 45.861876 | 14.73 | 0.513 | 0.002 | 0.003 | * |
| Slurry_- vs CompostB_AMF | 1 | 24.800234 | 6.68 | 0.323 | 0.001 | 0.002 | * |
| Slurry_- vs Digestate_AMF | 1 | 35.869114 | 10.70 | 0.433 | 0.001 | 0.002 | * |
| Slurry_- vs Slurry_AMF | 1 | 61.900172 | 12.64 | 0.474 | 0.001 | 0.002 | * |
| Slurry_- vs Control 100N_AMF | 1 | 61.113278 | 20.96 | 0.600 | 0.001 | 0.002 | * |
| Control 100N_- vs Control 0N_AMF | 1 | 38.151333 | 10.82 | 0.436 | 0.001 | 0.002 | * |
| Control 100N_- vs CompostA_AMF | 1 | 41.803452 | 11.27 | 0.446 | 0.001 | 0.002 | * |
| Control 100N_- vs CompostB_AMF | 1 | 21.865664 | 5.07 | 0.266 | 0.001 | 0.002 | * |
| Control 100N_- vs Digestate_AMF | 1 | 34.533177 | 8.74 | 0.384 | 0.001 | 0.002 | * |
| Control 100N_- vs Slurry_AMF | 1 | 57.923207 | 10.54 | 0.430 | 0.001 | 0.002 | * |
| Control 100N_- vs Control 100N_AMF | 1 | 61.739698 | 17.57 | 0.557 | 0.001 | 0.002 | * |
| Control 0N_AMF vs CompostA_AMF | 1 | 2.281953 | 0.80 | 0.054 | 0.577 | 0.604 | |
| Control 0N_AMF vs CompostB_AMF | 1 | 8.029535 | 2.32 | 0.142 | 0.056 | 0.079 | |
| Control 0N_AMF vs Digestate_AMF | 1 | 2.769927 | 0.89 | 0.060 | 0.465 | 0.505 | |
| Control 0N_AMF vs Slurry_AMF | 1 | 3.975680 | 0.86 | 0.058 | 0.598 | 0.617 | |
| Control 0N_AMF vs Control 100N_AMF | 1 | 5.631985 | 2.11 | 0.131 | 0.073 | 0.100 | |
| CompostA_AMF vs CompostB_AMF | 1 | 12.338228 | 3.38 | 0.195 | 0.003 | 0.005 | * |
| CompostA_AMF vs Digestate_AMF | 1 | 5.597941 | 1.70 | 0.108 | 0.143 | 0.175 | |
| CompostA_AMF vs Slurry_AMF | 1 | 2.589405 | 0.54 | 0.037 | 0.857 | 0.857 | |
| CompostA_AMF vs Control 100N_AMF | 1 | 5.059702 | 1.78 | 0.113 | 0.127 | 0.161 | |
| CompostB_AMF vs Digestate_AMF | 1 | 3.168773 | 0.81 | 0.055 | 0.542 | 0.577 | |
| CompostB_AMF vs Slurry_AMF | 1 | 21.055641 | 3.88 | 0.217 | 0.002 | 0.003 | * |
| CompostB_AMF vs Control 100N_AMF | 1 | 23.782747 | 6.89 | 0.330 | 0.004 | 0.006 | * |
| Digestate_AMF vs Slurry_AMF | 1 | 10.135082 | 2.00 | 0.125 | 0.054 | 0.077 | |
| Digestate_AMF vs Control 100N_AMF | 1 | 11.331945 | 3.67 | 0.208 | 0.002 | 0.003 | * |
| Slurry_AMF vs Control 100N_AMF | 1 | 3.284367 | 0.71 | 0.048 | 0.712 | 0.723 | |

***Figure S 4: Pairwise statistics output for Permanova of PCA plot.***

| Plot 1: Tukey Post-Hoc for Shoot Dry Weight (Treatment Main Effect) | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Estimated Mean (g)** | **Standard Error** | **Lower CI (95%)** | **Upper CI (95%)** | **Sig. Letter** |
| Digestate | 2.098 | 0.087 | 1.877 | 2.345 | a |
| Slurry | 2.033 | 0.084 | 1.819 | 2.272 | ab |
| Control 100N | 1.996 | 0.082 | 1.786 | 2.231 | ab |
| Control 0N | 1.838 | 0.076 | 1.644 | 2.054 | ab |
| CompostA | 1.795 | 0.074 | 1.606 | 2.006 | ab |
| CompostB | 1.739 | 0.072 | 1.556 | 1.943 | b |

***Figure S 5: Detailed post-hoc statistics for shoot dry weight.***

| Plot 1b: Tukey Post-Hoc for Shoot Wet Weight (Treatment Main Effect) | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Estimated Mean (g)** | **Standard Error** | **Lower CI (95%)** | **Upper CI (95%)** | **Sig. Letter** |
| Digestate | 7.505 | 0.192 | 7.006 | 8.040 | a |
| Control 100N | 6.772 | 0.173 | 6.321 | 7.254 | ab |
| Slurry | 6.565 | 0.168 | 6.128 | 7.033 | b |
| Control 0N | 6.215 | 0.159 | 5.801 | 6.658 | bc |
| CompostA | 6.103 | 0.156 | 5.697 | 6.538 | bc |
| CompostB | 5.763 | 0.147 | 5.380 | 6.174 | c |

***Figure S 6:Detailed post-hoc statistics for shoot fresh weight.***

# Personal 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.

The use of AI-tools in this work was as follows:

- Google Gemini: brainstorming, summarizing, help in data analysis and interpretation with R
- Google Notebook LLM: mind-mapping, summarizing research papers, organizing research library
- Scite AI: literature search, citation checks

All underlying research and scientific conclusions are the sole work of the author.

Zürich, 24.04.2026 Signature: 

Matriculation Number: 18-916-411 Name, Family Name: Cedric Gehrer