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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Investigating the Effects of Future Climate on Arbuscular Mycorrhizal Fungi in a Belgian Pear Orchard Ecosystem

Chloë Vercauteren

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :

Prof. dr. Nadejda SOUDZILOVSKAIA

MENTOR :

Mevrouw Vera CLAESSENS

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Chloë Vercauteren[1], Vera Claessens[1], Francois Rineau[1], Rik Verdonk[1], Sabin Taranu[2], and Nadejda A. Soudzilovskaia[1]

[1]Centre for Environmental Sciences, Universiteit Hasselt, Campus Diepenbeek, Agoralaan Gebouw D, 3590, Diepenbeek, Belgium
[2]Water and Climate Department, Vrije Universiteit Brussel, Faculty of Engineering, Pleinlaan 2, 1050, Brussels, Belgium

*Running title: *Future Climate Effects on Mycorrhizal Fungi*

To whom correspondence should be addressed: Nadejda A. Soudzilovskaia, Tel: +32 (11) 26 82 62; Email: nadia.soudzilovskaia@uhasselt.be

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ABSTRACT (235 words)

Climate change significantly impacts soil microbial communities, including arbuscular mycorrhizal fungi (AMF), which are essential for plant nutrient uptake, stress tolerance, and overall ecosystem functioning. This study addresses key knowledge gaps by examining the distinct responses of two major AMF functional groups, rhizophilic and edaphophilic, to realistic ambient and future climate simulations in Belgian pear orchards. By combining spore counts and metabarcoding, both dormant and active stages of AMF were captured, providing a comprehensive view of community dynamics.

The findings reveal that future climate does not alter overall AMF abundance but drives phenological shifts. Both principal response curve (PRC) and relative abundance analyses derived from metabarcoding data identified rhizophilic AMF as the dominant group, consistent with the climate-induced phenological shifts in community composition. This suggests that shifts in their relative abundance exert a disproportionate influence on the broader AMF community structure. Furthermore, rhizophilic AMF exhibited increased phenological stability under future climate, as evidenced by both spore and relative abundance analyses. This persistence may result from weakened dormancy signals, alterations in host plant phenology, or the ruderal life-history traits of rhizophilic families.

Future studies should expand these findings through multi-year, multi-site studies to explore how warmer winters, shifting host phenology, and life-history traits shape AMF responses, expanding across diverse hosts and AMF types. This research advances the understanding of climate-driven AMF dynamics in agriculture, offering insights for sustainable crop production and soil fertility under future climate.

INTRODUCTION

Since the onset of the Industrial Revolution, atmospheric CO_2 concentrations have risen from 278 ppm in 1750 to 420 ppm in 2023, a 51% increase, significantly amplifying the greenhouse effect (WMO, 2024). As a result, global temperatures from 2014 to 2023 averaged 1.20°C (±0.12°C) above pre-industrial (1850–1900) levels, with the annual average in 2023 reaching 1.45°C (±0.12°C) above pre-industrial conditions (WMO, 2023). Global warming is predicted to lead to major shifts in weather patterns, intensifying heatwaves, prolonging droughts, and increasing both the frequency and severity of heavy rainfall (IPCC, 2022). It has also driven compound events, such as simultaneous drought and extreme heat that heighten wildfire risks. This trend is expected to persist and intensify as global temperatures continue to rise (IPCC, 2022).

Climate-driven shifts pose serious challenges to both natural and agricultural ecosystems. Terrestrial plants are sessile organisms, meaning they cannot migrate to evade environmental stressors. Therefore, it is crucial to understand their adaptive mechanisms to climate change, such as adaptations to water and nutrient limitations, as well as resistance to plant pathogens (Baldrian *et al.*, 2022; Cotton, 2018). A key adaptation strategy involves forming symbiotic relationships with arbuscular mycorrhizal fungi (AMF), which can mitigate various environmental stresses, including abiotic factors related to climate change (*e.g.*, heat and drought) (Baldrian *et al.*, 2022).

These fungi form symbiotic associations with up to 80% of terrestrial plant species, including economically valuable crops, such as fruit trees that hold significant value for the European agricultural industry (Cotton, 2018). AMF play a crucial role in various plant functions, such as enhancing phosphorus (P) uptake and improving plant resistance to soil-borne pathogens, heavy metals, and water stress (Cotton, 2018). Research indicates that AMF can contribute up to 90% of the plant's P supply (Begum *et al.*, 2019), making this AM fungal symbiosis a critical mechanism for improving crop adaptation to P-limited conditions. To facilitate symbiosis, AMF develop both extraradical and intraradical hyphal structures (Figure 1). Extraradical hyphae extend beyond the root system, expanding soil exploration and thereby increasing P availability and translocation, ultimately enhancing plant nutrition and growth (Brundrett & Tedersoo, 2018). Extraradical structures that extend beyond the plant roots include spores, which are reproductive units dispersed in soil. In contrast, intraradical structures are formed inside the plant roots and include hyphae that are involved in root colonization; arbuscules, which serve as the primary sites for nutrient and carbon exchange; vesicles, specialized for lipid storage; and spores (Somoza *et al.*, 2024; Antunes *et al.*, 2025).

To understand how AMF symbiotic structures form and function over time, it is essential to consider their life cycle. This cycle comprises two major phases (Antunes *et al.*, 2025). First, the asymbiotic phase, where dormant spores are activated, followed by spore germination in response to suitable biotic and abiotic conditions and the exploration of the soil for a suiting host (Antunes *et al.*, 2025) (Figure 2). Secondly, the symbiotic phase, includes four stages: (a) initiation of root colonization, (b) formation of intraradical structures, (c) development of extraradical mycelium, and (d) spore dispersal and dormancy (Figure 2)

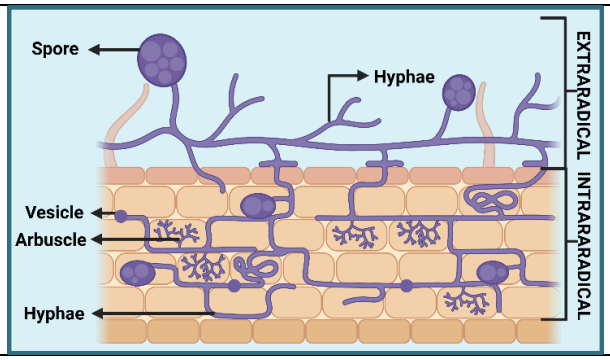


Figure 1— Plant Root Colonization by Arbuscular Mycorrhizal Fungi (AMF): Extraradical and Intraradical Structures. Figure made using BioRender.com.

(Antunes *et al.*, 2025). Upon host perception the AM fungus attaches to the root and forms a hyphopodium, through which a hypha penetrates the root epidermis and reaches the cortex (Stage a) (Antunes *et al.*, 2025). There, the intraradical mycelium continues to develop, colonizing the root cortex, and differentiating into arbuscules, spores, vesicles, and coils, depending on the taxon (Stage b) (Antunes *et al.*, 2025). Once colonization is established, the AM fungus extends into the surrounding soil, forming an extensive extraradical mycelial network (Stage c) (Antunes *et al.*, 2025). Finally, AMF completes its cycle with the release of spores into the soil, facilitating its dispersal and entry into dormancy, with the potential for future colonization once favorable conditions return (Stage d) (Antunes *et al.*, 2025). So, spores are essential for the long-term survival of AMF and the re-establishment of mycorrhizal networks following disturbances (Horsch, Antunes & Kallenbach, 2023).

As each phase of the AMF life cycle involves distinct processes of colonization, growth, and reproduction, different functional groups have evolved life-history strategies that emphasize particular stages to optimize their survival and ecological roles. Rhizophilic and edaphophilic AMF, the two predominant AM fungal functional groups, rely on distinct life-history strategies to fulfill their specific roles in relation to host plants (Horsch, Antunes & Kallenbach, 2023). Notably, *Gigasporaceae* adopt an edaphophilic (*i.e.,* soil-loving) strategy, prioritizing biomass allocation to extraradical hyphae for long-term persistence in stable environments, acquiring growth-limiting resources to recolonize when conditions become favorable (Figure 2, Stage c) (Antunes *et al.*, 2025). These AM fungi ensure survival in low-stress and low-disturbance environments by delaying sporulation to support dormancy (Stage d) and by maintaining the slow-growing competitor strategy that thrives in mature ecosystems (Hart & Reader, 2002; Hart & Reader, 2005; Staddon *et al.*, 2003; Maherali & Klironomos, 2007; Chagnon *et al.*, 2013). In contrast, *Glomeraceae* employ a rhizophilic (*i.e.,* root-loving) and ruderal strategy, prioritizing rapid intraradical colonization (Stage b) (Antunes *et al.*, 2025). These AM fungi are adapted for colonization in both highly disturbed and low-stress environments by fast re-establishment of mycorrhizal networks using high hyphal turnover and frequent reproduction (Stage d) at the expense of low biomass allocation to extraradical hyphae (Stage c). This strategy enables rapid plant colonization and nutrient acquisition, particularly in high-disturbance environments (Alguacil *et al.*, 2010; Verbruggen & Kiers, 2010; Ma *et al.*, 2018).

Prior research indicates that AM fungi promote plant growth under abiotic stress conditions. Plants inoculated with AMF exhibit enhanced resilience to various environmental stressors, including nutrient, alkali, and cold stress, salinity, drought, and extreme temperatures (Brundrett & Tedersoo, 2018; Baldrian *et al.*, 2022; Asato *et al.*, 2023). This enhanced stress tolerance facilitates higher crop yields per hectare across a wide range of agricultural species (Brundrett & Tedersoo, 2018). In addition to improving plant health, AMF contribute to soil carbon fixation through biogeochemical cycling, a process known as the 'sink effect'. Estimates suggest that mycorrhizal mycelium sequesters 13.12 Gt eCO_2 annually, accounting for approximately 36% of the CO_2 emissions from fossil fuels. This highlights the vital role of AMF in global carbon dynamics and ecosystem stability (Soudzilovskaia *et al.*, 2015; Soudzilovskaia *et al.*, 2019; Hawkins *et al.*, 2023).

Given their substantial impact on both plant health and ecosystem functioning, it is crucial to examine how AMF communities are affected by climate change. As climatic shifts are becoming a growing concern, research into how shifting climatic variables affect AMF communities is getting more critical. For example, water availability, a climatic factor influenced by climate change, can shape the carbon budgets of plants, thereby affecting the carbon allocated to AMF and altering their community composition (Heuck *et al.*, 2024). For instance, severe water stress can cause AMF re-establishment, as it is a disturbance factor that could favor rhizophilic AMF (Heuck *et al.*, 2024). Findings indicate that AMF exhibit significant sensitivity to changing climatic parameters, which can affect their diversity, distribution, and functions within ecosystems both directly and indirectly (Cotton, 2018; Weber *et al.*, 2019). Indirect effects arise from climate-driven changes in host plants, soil properties, and nutrient availability (Cotton, 2018; Weber *et al.*, 2019).

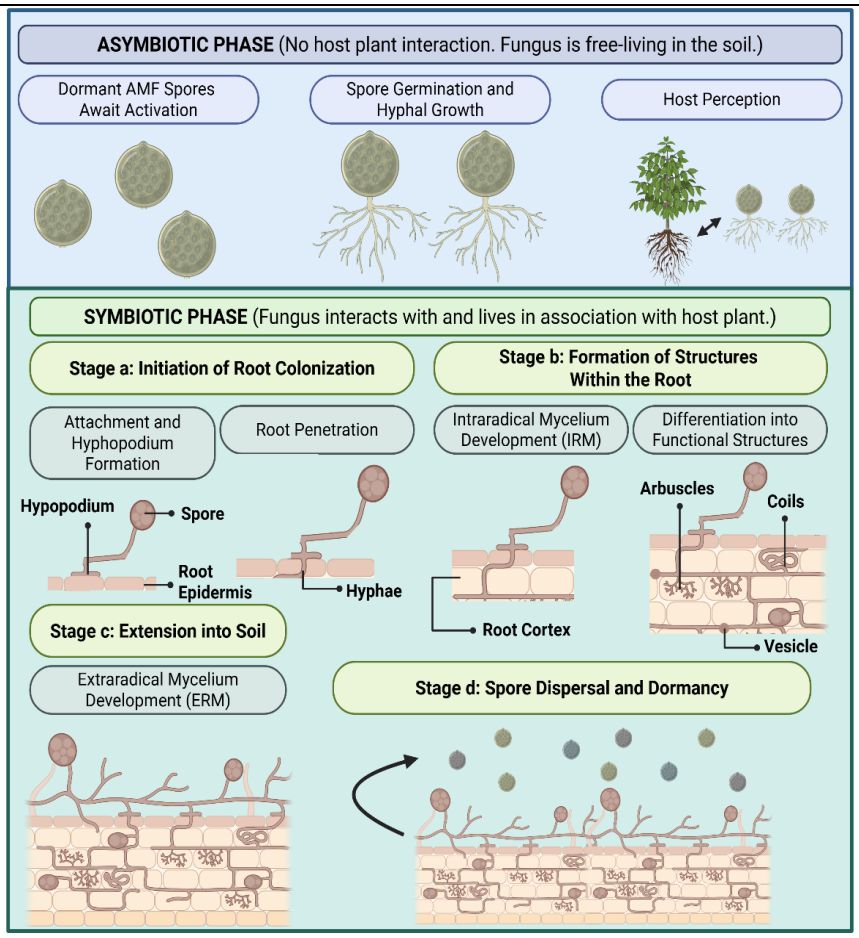


Figure 2— Diagram of the Arbuscular Mycorrhizal Fungal (AMF) Life Cycle. Figure made using BioRender.com.

Although research on the effects of altered water availability on AMF communities is more abundant than drought-focused studies, findings remain controversial, inconsistent, and context-dependent (Cotton, 2018). Further, the effects of increased rainfall on AMF diversity vary, with increases, decreases, and no change being reported (Cotton, 2018). Nevertheless, both drought and increased rainfall have been shown to influence AMF community composition (Cotton, 2018). In contrast to increased rainfall, drought has been reported to negatively affect AMF, leading to reduced root colonization, decreased extraradical hyphal density, and altered abundances of rhizophilic and edaphophilic AMF (Weber *et al.*, 2019). Moreover, while most studies show that increased atmospheric CO_2 does not significantly affect AMF richness or diversity, it does alter community composition, thereby changing community structure (Cotton, 2018). However, this shift is primarily driven by changes in carbon allocation to AMF, favoring rhizophilic over edaphophilic AMF, rather than broader climatic factors (Cotton, 2018).

A critical aspect of AMF dynamics under climate change that has gained increasing attention is the role of AMF spores (Wolf *et al.*, 2003; Zhang *et al.*, 2016; Kilpeläinen *et al.*, 2020). Spores, as reproductive units, enable AM fungi to establish and maintain contact with plant roots, which is essential for their survival, particularly in harsh environmental conditions, such as drought and extreme heat (Kilpeläinen *et al.*, 2020; Ahammed & Hajiboland, 2024). AMF spores can remain dormant in soil until favorable conditions trigger their germination, allowing them to recolonize plant roots when conditions become favorable (Giovannetti, 2000). Seasonal climatic variations that affect host plant phenology can, consequently, alter the timing of AMF root colonization and nutrient exchange, impacting plant health and ecosystem productivity (Asato *et al.*, 2023).

With projected temperature increases by 2040, enhanced CO_2 assimilation and transport to roots may delay AMF dormancy onset (Gray & Brady, 2016). However, these effects are taxon-dependent, with research indicating that rhizophilic fungal spores are often more resilient to environmental stressors, such as elevated CO_2, suggesting their potential to dominate in future climates (Wolf *et al.*, 2003). Moreover, C allocation from the plant to AMF varies across different phenological phases. As climate change affects plant phenology, it can alter C allocation to AMF, which may in turn impact spore phenology, since spore production requires a significant amount of carbon (Birgander & Olsson, 2017). Thus, climate change could alter the timing of key AMF developmental stages (Figure 2), making it crucial to understand these phenological shifts to predict their future ecological roles in ecosystems and inform agricultural practices in a changing climate.

Previous research on the impacts of climate change has mainly focused on individual climatic parameters, such as temperature increases and changes in precipitation, creating a gap in understanding the joint effects of a realistic climate change scenario that exposes ecosystems to a full suite of environmental stresses (Cotton, 2018; Zhang *et al.*, 2016; Hu *et al.*, 2022). This study aims to address this gap by exploring the response of the AMF community in a pear orchard agricultural system to the future climate scenario projected for Belgium (2042-2046). To simulate climate change under the predicted Belgian climate and assess its impact on AMF abundance, functional group composition and phenological dynamics, the state-of-the-art Ecotron facility at Hasselt University was used (Figure 3). The Ecotron enables precise control over key climatic parameters, such as soil temperature, air humidity, and atmospheric CO_2 levels, enabling the replication of both ambient (2013–2018) and future (2042-2046) climate conditions. By simulating full climate scenarios rather than individual climatic parameters, the Ecotron bridges the gap between controlled laboratory experiments and field studies. This innovative approach provides valuable insights into the ecological impacts of climate change, particularly in understanding the complex interactions among environmental drivers and AMF communities (Hasselt University, n.d.).

Addressing the gaps in current research on AMF responses to a full climate change scenario, this study examines how AMF associated with pear (*Pyrus communis* L.), a crop of major economic importance in Europe, respond to the projected 2042-2046 climate conditions in Belgium, based on the worst-case Representative Concentration Pathway (RCP 8.5 scenario) (Copernicus Climate Change Service, n.d.; IPCC, 2014). We hypothesized that the AMF community in a pear orchard will exhibit more rapid and pronounced phenological changes under future climate conditions (RCP 8.5, 2042-2046), as well as a significant shift in AMF functional group composition and an increased dominance of rhizophilic AMF, but no change in overall AMF abundance.

Figure 3— Ecotron Facility Maasmechelen, National Park Hoge Kempen (NPHK), Belgium

MATERIAL AND METHODS

The study aimed to assess climate change impacts on arbuscular mycorrhizal fungi (AMF) their abundance, functional group composition and phenological dynamics across key pear tree phenophases (*i.e.*, fruit growth, harvest, and dormancy). Therefore, we examined pear tree rhizosphere soil samples under both ambient (2013-2018) and future (2042-2046) climate conditions following the worst-case Representative Concentration Pathway (RCP 8.5) scenario.

Climate manipulations—This study is part of the broader QPear experiment, which aims to evaluate the impact of climate change expected in Belgian Limburg on pear tree growth, fruit quality, and orchard ecosystem functioning. Hereto, the state-of-the-art Ecotron facility was employed, operated by Hasselt University (Hasselt University, n.d.). This facility consists of 12 enclosed macro-scale sun-lit climate chambers (167 m^3) designed to precisely regulate and monitor key climatic parameters, including air and soil temperature, air humidity, atmospheric CO_2 levels, precipitation, groundwater content, and windspeed. Each climate chamber includes an atmospheric compartment and a lysimeter housing a soil-canopy column, enabling real-time monitoring of ecosystem processes (Figures 3 and 4) (Rineau *et al.*, 2019; Roy *et al.*, 2021). A detailed description of the macro-scale Ecotron facility is provided by Rineau *et al.*, (2019).

In late-autumn 2021, twelve adult pear trees, each approximately three meters tall, were excavated, along with their corresponding intact soil cylinders from an experimental orchard at "PCFruit" (ProefCentrum Fruitteelt) in Limburg province, Belgium. The trees were then placed into six macro-scale lysimeters (two meters in diameter and 1.5 meters in depth), with two trees per lysimeter. Subsequently, the trees were grown for one year in open air within the lysimeters, exposed to ambient climatic conditions. This pre-treatment facilitated the acclimatization of both the trees and the soil communities to the lysimeter environment. In January 2022, the lysimeters containing the trees were transported to the Ecotron, where they were exposed until 2024 to one of the two climatic treatments for Belgium under the RCP8.5 scenario: a typical climate from the 2013-2018 period or a typical climate from the period of 2042-2046. Each climate treatment was applied to three lysimeters, providing three biological replicates per condition.

The climate scenarios were generated by selecting a simulation from an ensemble of dynamically downscaled regional climate model (RCM) outputs, selected for its accurate representation of present-day climate variables of the region of interest and its alignment with future multi-model mean projections. This methodology incorporated the co-variance of climate variables, natural climate variability, and extreme events, providing a robust framework for generating realistic climate simulation in ecosystem manipulation experiments (Roy *et al.*, 2021; Vanderkelen *et al.*, 2020). Further details on the simulation of climate projections are provided in Supplementary Methods 1.

Sampling and Sample Processing—Rhizosphere soil sampling was conducted in 2024 at three key phenological time points of pear tree growth: fruit growth (16/07), harvest (17/09), and tree dormancy (26/11) (Figure 4). To account for technical replicates in each climate chamber, three rhizosphere soil samples were taken from random locations within each lysimeter at a depth of 0-30 cm. Sampling occurred within a radius of 60 cm around both tree trunks, with one sample taken near each trunk and a third sample positioned between the trunks, yielding a total of 54 soil samples. Rhizosphere soil was collected from both the soil directly surrounding the roots and the soil adhering to the roots. Sampling depths and tree phenological stages (i.e., fruit growth [leaves and fruits present], harvest [pears harvested, leaves still present], and dormancy [leaves fallen]) were documented, and sampled areas were labelled after each sampling event to prevent re-sampling at the same location. After collection, soil samples were thoroughly mixed, cleared of debris and roots, and stored at -20°C in labeled plastic bags for further analysis. Additionally, the soil samples where aliquoted into smaller fractions for the different analyses.

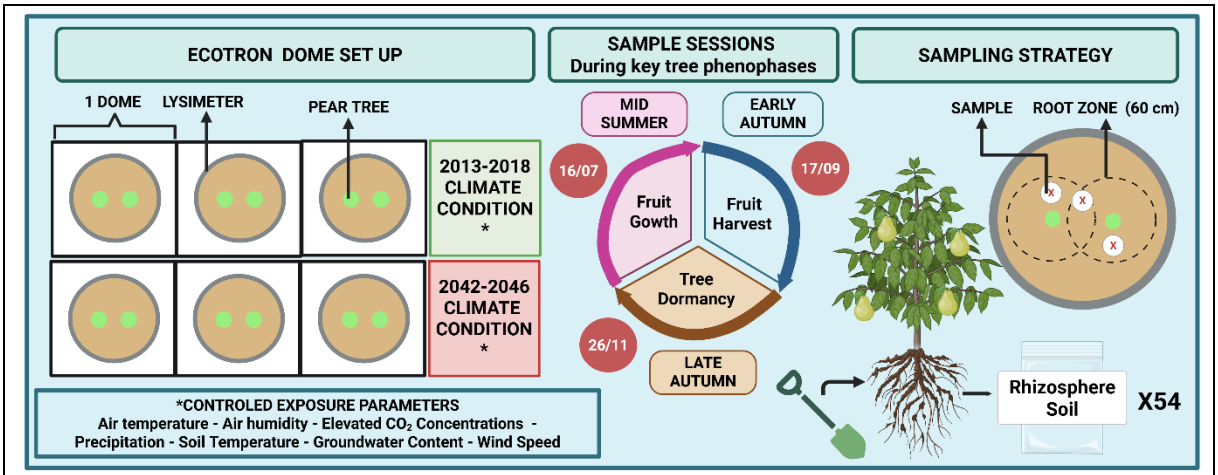


Figure 4—ECOTRON Dome Setup, Sample Sessions, and Sampling Strategy – White circles with a red cross indicate random sampling locations at each time point. Figure made using BioRender.com.

AMF Spore Extractions and Quantification—AM fungal spores were extracted from the 54 processed rhizosphere soil samples using the Ultrasound Wet-Sieving Technique (UWST) method, chosen based on the pilot experiments described in Supplementary Methods 2. Subsequently, spore visualization was performed by administering 200 µL of spore suspension from the primary AM fungal spore extractions on a microscope slide, with digital images captured using the Nikon SMZ800N stereomicroscope at 30× or 40× magnification and processed with NIS Elements software (Suppl. Figure 1). Each sample was visualized in triplicate to ensure accuracy.

Thereafter, the number of spores per 200 µL of spore solution was determined for each sample using ImageJ software, allowing for the quantification of the total AMF spore number (TSN) per gram of soil. TSN was determined using the following formula: $TSN = \frac{SN \times W}{S}$, where SN indicates the AMF spore numbers in one milliliter of spore suspension, W denotes the total volume of water used (20 mL), and S corresponds to the amount of soil processed (one gram). SN was initially determined for 200 µL of suspension and subsequently extrapolated to one milliliter.

Spore Species Identification—Spore lengths were measured using ImageJ for identification and quantification of functional groups (*i.e.*, Rhizophilic and Edaphophilic). Edaphophilic spores were distinguished from Rhizophilic based on size, with Edaphophilic spores typically measuring > 150 µm and Rhizophilic spores < 150 µm (INVAM, n.d.; Muiruri *et al.*, 2022; Biosci *et al.*, 2021).

Metabarcoding_Library Preperation—DNA from the rhizosphere soil samples were extracted, quantified, amplified, cleaned, indexed, and prepared for sequencing using standard procedures, as detailed in Supplementary Methods 3. The final DNA library was sent to Novogene Co., Ltd. for sequencing.

Statistics—Differences in AM fungal spore abundance, community composition, and functional group responses across climate scenarios and phenological stages were assessed using multiple statistical approaches, detailed in Supplementary Methods 4. TSN, along with edaphophilic and rhizophilic spore counts, were analyzed using negative binomial generalized linear mixed models (GLMMs), with post-hoc comparisons to evaluate treatment effects. Principal Response Curves (PRC) and Redundancy Analysis (RDA) were used on metabarcoding data to investigate phenological shifts in AMF family composition under climate treatments, with family contributions and indicator taxa identified through species scores and IndVal analysis. To assess changes in overall mycorrhizal community structure, relative abundance data were analyzed using Bray–Curtis dissimilarity and permutational multivariate analysis of variance (PERMANOVA), testing for effects of climate, timepoint, and their interaction.

RESULTS

AMF Spore Abundance across Climate Scenarios and Phenological Stages—The estimated marginal means (EMMs) obtained from the arbuscular mycorrhizal fungal (AMF) spore counts did not differ significantly ($p > 0.05$) between the ambient (2013–2018) and future (2042-2046) climate conditions at each phenological time point (Figure 5). However, phenological variations were significant ($p < 0.05$) within both climates (Figure 5). Specifically, under ambient climate, mid-summer exhibited the highest EMM spore count (8.25, 95% CI: 7.25–9.25), which was significantly higher than late-autumn (7.62, 95% CI: 6.62–8.61; $p = 0.004$) and marginally higher than early-autumn (7.80, 95% CI: 6.80–8.80; $p = 0.064$). Similarly, under future climate, mid-summer EMM spore counts (8.25, 95% CI: 7.26–9.25) were significantly higher than late-autumn (7.51, 95% CI: 6.52–8.51; $p = 0.001$), but not significantly different from early-autumn (7.85, 95% CI: 6.85–8.85; $p = 0.100$). No significant differences were found between early- and late-autumn EMM spore counts in either climate condition (ambient: $p = 0.598$; future: $p = 0.200$). These model-derived estimates differ somewhat from the raw data distributions due to data transformations and covariate adjustments made during model fitting (See Supplementary Figure 4).

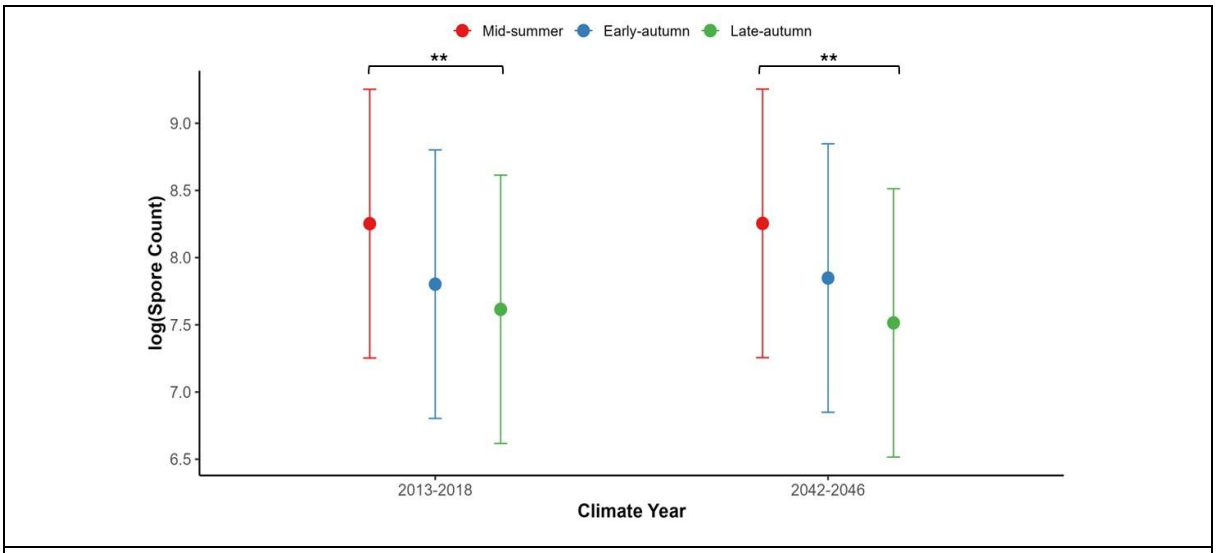


Figure 5—Model-Based Estimated Marginal Means (EMMs) of AMF log(Spore Count) across Climate Scenarios (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn). Points represent EMMs; lines indicate 95% CIs. ** denotes significance at $p < 0.005$ across timepoints within each climate.

AMF Functional Group-Specific Spore Dynamics—Next, the estimated marginal means (EMMs) of AMF spore counts were analyzed by functional group (i.e., rhizophilic and edaphophilic). Across both climates and all phenological time points, rhizophilic AMF consistently exhibited significantly higher EMM spore counts compared to edaphophilic AMF ($p < 0.0001$) (Figure 6A and 6B). Edaphophilic EMM spore counts remained stable across phenological time points in both climates, with no significant differences detected ($p > 0.302$) (Figure 6A). In contrast, Rhizophilic mid-summer EMM spore counts were significantly higher in mid-summer compared to early- ($p = 0.012$) and late-autumn ($p = 0.001$) under ambient climate (Figure 6B). However, this pattern weakened under future climate, with only significant differences found between mid-summer and late-autumn ($p = 0.028$). Model estimates differ slightly from raw data due to transformations and covariate adjustments (see Supplementary Figure 4).

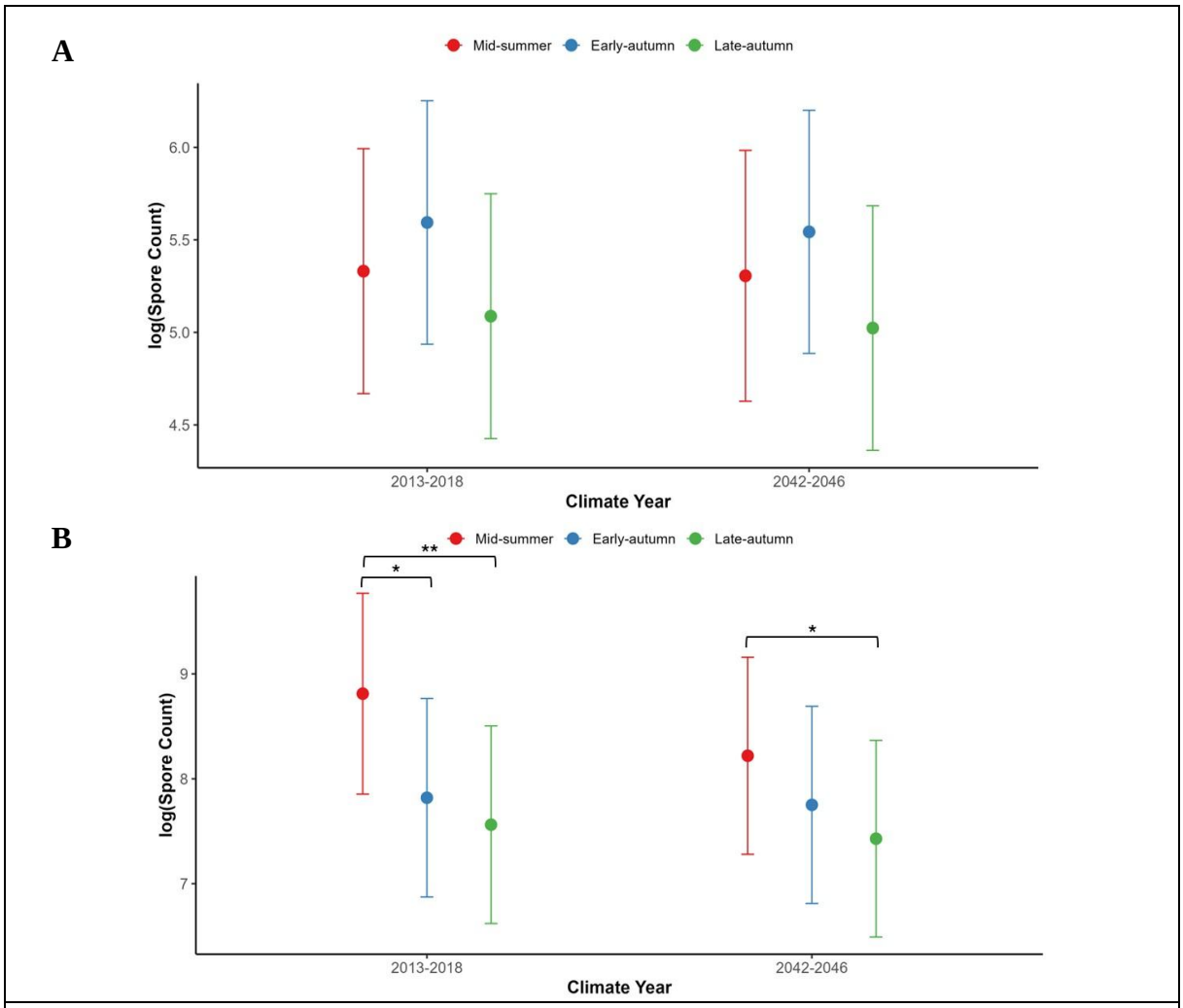


Figure 6—Model-Based Estimates Marginal Means (EMMs) of (A) Edaphophilic and (B) Rhizophilic AMF log(Spore Counts) across Climate Scenarios (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn).
* indicates significance at $p < 0.05$, and ** indicates significance at $p < 0.005$ across timepoints within each climate.

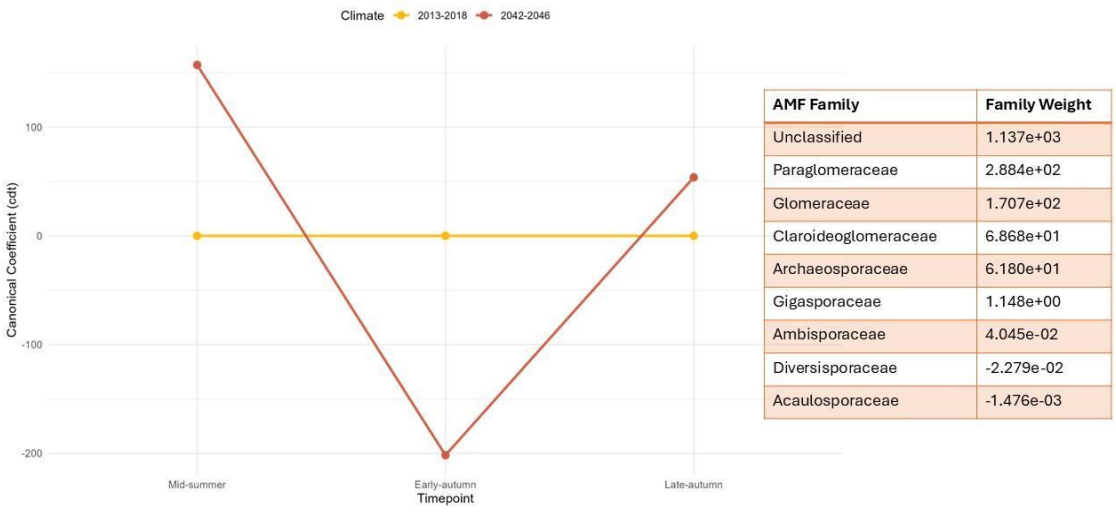
Climate Effects on AMF Community Composition Using Principal Response Curve (PRC) Analysis—PRC analysis showed a significant effect of climate treatment on mycorrhizal community composition at the family level. The primary constrained axis (RDA1) explained a substantial portion of treatment-related variation ($F = 11.39$, $p = 0.014$), with a variance of 8.4×10^9 compared to higher residual variance (3.02×10^{10}), indicating that much variation remains unexplained by climate alone. A significant climate × timepoint interaction ($F = 5.64$, $p = 0.003$) suggests that phenological shifts in mycorrhizal communities differ under future climate scenarios.

After correcting for multiple comparisons, none of the pairwise comparisons between treatment groups remained statistically significant (adjusted $p > 0.05$).This aligns with the finding that Indicator Species Analysis (IndVal.g) identified no families as exclusive indicators for individual climate × timepoint combinations, suggesting that no single fungal family strongly defines a particular treatment or season. Nevertheless, *Glomeraceae* (*i.e.*, rhizophilic AMF) showed a significant association with the majority of groups combined ($p = 0.033$) (Figure 7).

Further, the PRC plot visualizes the differences between future and ambient climate scenarios across the phenological time points (Figure 7). The strongest deviation was a sharp negative

shift in early-autumn (-201.7 cdt), along with positive deviations in mid-summer (157.1 cdt) and late-autumn (53.75 cdt). The greatest family weight is attributed to unclassified AMF, which may belong to any functional guild, but are currently unidentified. These unclassified AMF are the primary drivers of the shifts seen in the PRC plot. However, among the classified groups, rhizophilic AMF families (*Paraglomeraceae* 2.884e+02, *Glomeraceae* 1.707e+02, and *Claroideoglomeraceae* 6.868e+01) showed the highest family weights, contributing most to these shifts. Edaphophilic AMF families (*Gigasporaceae* 1.148e+00 and *Diversisporaceae* -2.279e-02) and ancestral AMF families (*Archaeosporaceae* 6.180e+01, *Ambisporaceae* 4.045e-02, and *Acaulosporaceae* -1.476e-03) exhibited lower weights.

Overall, the PRC suggests that families with high positive weights (*e.g.*, *Paraglomeraceae* and *Claroideoglomeraceae*) tend to increase in seasons with positive canonical coefficients (cdt) (mid- and late-autumn), while negatively weighted families (*e.g.*, *Diversisporaceae*) may increase in early-autumn where the community deviates negatively. Additionally, the PRC shows an increase in community turnover in early-autumn.



AMF Family	Family Weight
Unclassified	1.137e+03
Paraglomeraceae	2.884e+02
Glomeraceae	1.707e+02
Claroideoglomeraceae	6.868e+01
Archaeosporaceae	6.180e+01
Gigasporaceae	1.148e+00
Ambisporaceae	4.045e-02
Diversisporaceae	-2.279e-02
Acaulosporaceae	-1.476e-03

Figure 7— Principal Response Curve (PRC) on the Effects of Climate on Arbuscular Mycorrhizal Fungal (AMF) Community Composition at the Family Level Across Climate Conditions (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn). Canonical coefficients (cdt) are displayed for each timepoint, alongside family weights for each AMF family.

Climate Effects on AMF Community Composition Assessed by Relative Abundance and Permutational Multivariate Analysis of Variance (PERMANOVA)—PERMANOVA using Bray-Curtis dissimilarity confirmed a significant effect of Climate, Timepoint (*i.e.*, phenological timepoint), and their interaction on AM fungal community composition (R^2 = 0.12, p = 0.018). Individual effects of climate or timepoint were not significant within climate groups (p = 0.93 for timepoint within future climate), but their combined influence suggests a context-dependent response that varies over time.

Family-level linear models, followed by pairwise comparisons using EMMs, revealed several AMF families with significant changes in relative abundance across timepoints and/or climates. The AMF families were grouped by functional traits into rhizophilic (*Glomeraceae*, *Paraglomeraceae*, and *Claroideoglomeraceae*), edaphophilic (*Diversisporaceae* and *Gigasporaceae*), and ancestral (*Archaeosporaceae*, *Ambisporaceae*, and *Acaulosporaceae*) groups. Across all timepoints and climate scenarios, overall relative abundance followed the functional hierarchy of unclassified > rhizophilic > edaphophilic > ancestral AMF. However, distinct temporal and climate-driven patterns emerged within each group. The findings are visualized by the relative abundance plot (Figure 8).

Claroideoglomeraceae and *Glomeraceae* display clear seasonal sensitivity under ambient climate conditions, but this pattern is diminished or lost under future climate scenarios, with abundances remaining more constant throughout the year, reflecting a dampening of their phenology. *Claroideoglomeraceae* showed a significant increase in relative abundance from mid-summer to early-autumn under current climate (estimate = –0.050, p = 0.028), but this phenological increase was lost under future conditions. Under current climate *Glomeraceae* significantly increased from mid-summer to late-autumn (estimate = –0.048, p = 0.031).

Gigasporaceae decreased over the seasons under future climate. In future climate *Gigasporaceae* showed a significant higher abundance in mid-summer compared to markedly lower abundances in early (estimate = 0.001, p = 0.038) and late-autumn (estimate = 0.001, p = 0.050), indicating a pronounced seasonality with peak occurrences in mid-summer that declines over the growing season. Further, *Gigasporaceae* are significantly more abundant in future climate than under ambient climate (estimate = –0.001, p = 0.020; mid-summer). *Diversisporaceae* seem to be the most consistently represented edaphophilic family, appearing across all timepoints in both climates, except ambient early-autumn (Figure 8).

Paraglomeraceae and *Archaeosporaceae* showed a climate-driven response rather than a shift in their seasonal patterns. Both showed a significant increase under future climate conditions. Specifically, *Paraglomeraceae* showed a significant increase in early-autumn (estimate = –0.103, p = 0.017), while *Archaeosporaceae* showed a significant increase in late-autumn (estimate = –0.026, p = 0.040). Figure 8 shows that under future climate *Ambisporaceae* (late-autumn) and *Acaulosporaceae* (early-autumn) increased in relative abundance. Notably, several families identified as significant in relative abundance analyses, including *Paraglomeraceae*, *Glomeraceae*, and *Claroideoglomeraceae*, also contributed substantially to community composition shifts in the PRC analysis (Figure 7), suggesting their dual roles in both abundance dynamics and community-level responses to climate change.

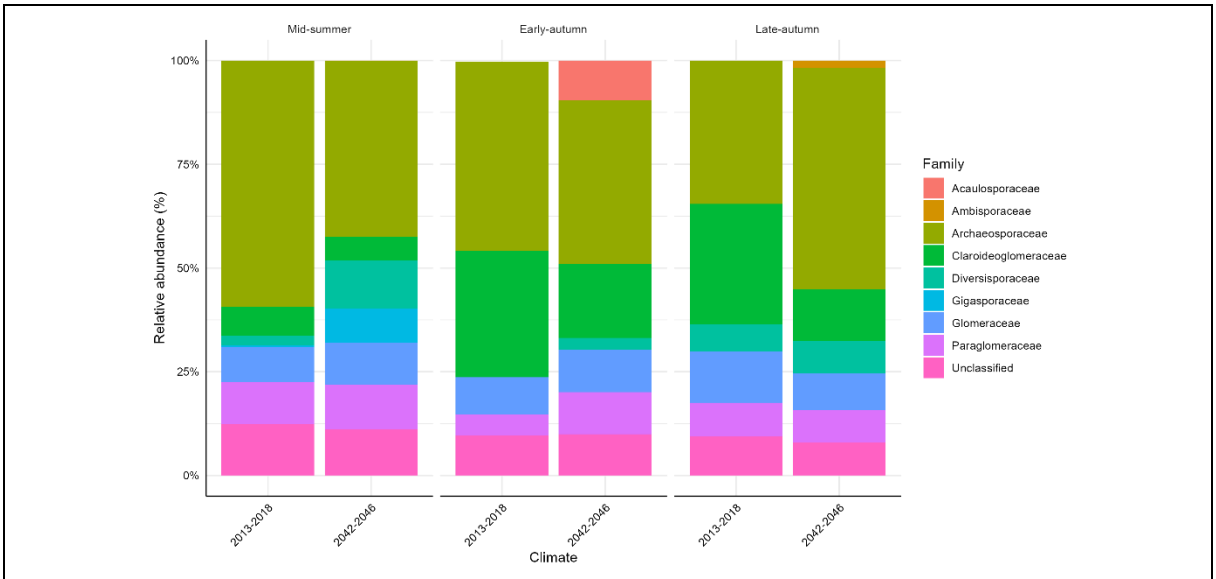


Figure 8—Relative Abundance of AM Fungal Families Across Climate Conditions (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn) Based on Mean Community Composition

DISCUSSION

Climate change increasingly disrupts natural and agricultural ecosystems, intensifying the need for effective plant adaptation strategies (WMO, 2024; WMO, 2023; IPCC, 2022). Arbuscular mycorrhizal fungi (AMF) play a crucial role in supporting plant resilience, making the understanding of how future climate conditions affect AMF dynamics essential for developing sustainable agricultural practices (Baldrian *et al.*, 2022; Cotton, 2018).

Despite growing interest in AMF, there remain significant gaps in understanding how distinct functional groups, such as edaphophilic and rhizophilic AMF, respond to future climate conditions. This study fills that gap by using the Ecotron facility to simulate a full future climate scenario, going beyond prior research that focused on individual climate factors (Cotton, 2018; Zhang et al., 2016; Hu et al., 2022). It compares ambient (2013–2018) and projected future (2042–2046) climates across key pear tree phenological stages (mid-summer, early-autumn, and late-autumn).

The study examined shifts in total AMF spore abundances, as well as changes in edaphophilic and rhizophilic AMF spore abundances, using AMF spore count data. Since spores represent dormant structures awaiting activation, it complements the data obtained by metabarcoding, which contains data on both active and inactive AMF. This dual approach provides insights into both current and potential future AMF communities (Antunes *et al.*, 2025; Giovannetti, 2000; Birgander & Olsson, 2017). This comprehensive analysis enhances the understanding of climate change induced shifts in AMF abundance, community dynamics, and phenology. These phenological changes could offer valuable insights into changes in carbon allocation, considering that spore production requires significant carbon input from host plants (Giovannetti, 2000; Birgander & Olsson, 2017; Antunes *et al.*, 2025).

Climate Change and AMF Functional Group Abundance—AMF consists of two main functional groups, rhizophilic and edaphophilic AMF. These groups differ in their strategies for nutrient acquisition, host interaction, as well as in their functions (Horsch, Antunes & Kallenbach, 2023). Environmental changes, like climate change, can alter their relative abundances, potentially impacting ecosystem functioning. For instance, a prior study emphasized the critical role of water availability in shaping AMF community structure, suggesting that climate-driven changes in temperature and moisture can differentially impact AMF functional groups, leading to shifts in community composition (Weber et al., 2019). Our results demonstrate that rhizophilic AMF consistently exhibit significantly higher spore abundance compared to edaphophilic AMF. However, this trend is shared across both climate conditions (Figures 6A and 6B), indicating no effect of future climate. The relative abundance patterns of AMF families (Figure 8) reinforce these findings, with rhizophilic families (*Glomeraceae*, *Paraglomeraceae*, and *Claroideoglomeraceae*) consistently exhibiting higher relative abundances than edaphophilic ones (*Gigasporaceae* and *Diversisporaceae*) across both climate conditions. These results indicate that rhizophilic AMF produces more numerous spores compared to edaphophilic AMF regardless of the climate period. It was initially hypothesized that climate change would further increase the dominance of rhizophilic AMF due to their greater resilience to environmental stress (Antunes *et al.*, 2025), thereby intensifying shifts in functional group composition. However, the findings did not support this hypothesis.

This result stands in contrast to a few scattered studies that examined individual climatic parameters, often showing inconsistent or contrasting results. For example, a study found that warming and elevated CO_2 significantly influenced AMF abundance, including spore production (Hu *et al.*, 2022). Another study found that warming initially increased AMF spore abundance, but this effect reversed when temperatures exceeded four degrees Celsius. AMF responses varied with the level of warming and CO_2, with warming effects diminishing at higher temperatures while CO_2 effects intensified (Hu et al., 2022). A global meta-analysis found that elevated temperatures reduced AMF spore density and diameter, but increased hyphal length. Although fewer and smaller spores were produced, fungal hyphae exhibited increased growth, which could improve nutrient and water absorption (Zhang *et al.*, 2016). Lastly, a review on the effects of altered precipitation and elevated CO_2 on AMF spore density showed varying results, with only elevated CO_2 having no significant effect on AMF abundance (Cotton, 2018). Our results, as hypothesized, suggest that under a multi-factorial future climate scenario, total AMF spore abundance remains stable, contradicting expectations based on isolated factor manipulations.

The persistent dominance of rhizophilic AMF spore abundance can be explained through their distinct sporulation strategies. Rhizophilic

AMF (*e.g.*, *Glomeraceae*) typically produce numerous small spores and rapidly colonize host roots, with little investment in external hyphal networks (Gío-Trujillo & Alvarado-López, 2024). This strategy facilitates fast exploitation of host-derived resources and efficient sporulation without the high carbon costs of maintaining extensive extraradical mycelium (Gío-Trujillo & Alvarado-López, 2024; Weber *et al.*, 2019). In contrast, edaphophilic AMF (e.g., *Gigasporaceae*) produce generally fewer, larger spores and invest more energy in forming extensive hyphal networks, resulting in slower growth and delayed establishment (Gío-Trujillo & Alvarado-López, 2024; Weber *et al.*, 2019).

These contrasting sporulation strategies fit within rhizophilic and edaphophilic life-history traits, linked to the competitor-stress tolerator-ruderal (C–S–R) framework. This framework is further explained in the section "*Climate Change and AMF Phenological Dynamics: AMF Functional Group Abundances*" linked to AMF resource allocation strategies. This framework helps explain the consistently higher relative abundance of rhizophilic AMF compared to edaphophilic AMF (Figure 8). Rhizophilic AMF adopt a ruderal strategy, which enables rapid colonization and allows them to thrive in frequently disturbed or rapidly changing environments (Antunes *et al.*, 2025; Alguacil *et al.*, 2010; Verbruggen & Kiers, 2010; Ma *et al.*, 2018; Wolf *et al.*, 2003). Conversely, edaphophilic AMF adopt a slower, more competitive strategy better suited to stable ecosystems, which may limit their capacity to dominate in short-term or rapidly changing environments (Bruce *et al.*, 1994; Xu *et al.*, 2017). Our findings suggest that climate change does not simply increase the dominance of rhizophilic AMF, indicating that the response of AMF functional groups to environmental shifts is more complex and context-dependent than previously expected.

Climate Change and AMF Phenological Dynamics—Although climate change did not significantly affect AMF functional group abundances, it did alter their phenological dynamics. The timing of carbon allocation within the plant plays a central role in perennial systems, where phenological activity typically peaks in late summer and early autumn (Hallmark *et al.*, 2024: Chapin *et al.*, 1990). During this period, plants shift their carbon allocation from aboveground growth to belowground structures (Kaiser *et al.*, 2010). After completing vegetative growth and fruiting, fruit-bearing plants likely increase carbon allocation to their roots and symbiotic partners like AMF (Yang et al., 2020). Although mainly documented in forest systems, this pattern can be explained by the reduced carbon demand following fruit production, allowing enhanced allocation to belowground AMF (Medeiros et al., 2023; Hupperts *et al.*, 2017). Carbon allocation to AMF also varies with plant developmental stage (Salmeron-Santiago *et al.*, 2021), supporting root maintenance and nutrient uptake in preparation for dormancy (Hupperts *et al.*, 2017; Bago et al., 2000). AMF sporulation may align with this increase in host-derived carbon, enabling AMF to reproduce when carbon availability is highest (Yang et al., 2020). This is vital because AMF spore production represents a major carbon investment, spores are rich in lipids that fuel energy-intensive metabolic processes during germination, including gluconeogenesis, lipid turnover, and amino acid synthesis (Bago et al., 2000). This high metabolic cost underscores the importance of host-derived carbon allocation.

Climate induced shifts in plant phenology can modify the timing and extent of this carbon allocation from plants towards AMF (Antunes *et al.*, 2025; Birgander & Olsson, 2017; Giovannetti, 2000). Such changes can influence spore production, given that sporulation is a carbon-intensive process (Antunes *et al.*, 2025; Birgander & Olsson, 2017; Giovannetti, 2000; Wolf *et al.*, 2003; Baldrian *et al.*, 2022). Alterations in AMF activity can, in turn, affect soil health, plant health, crop productivity, and overall ecosystem functioning (Cera *et al.*, 2021; Baldrian *et al.*, 2022; Cotton, 2018). Previous research has already evidenced that climate change influences soil organism phenology (Asato *et al.*, 2023). Therefore, understanding how climate-driven factors interact with AMF phenology is essential for predicting future shifts in the crucial AMF-plant symbioses and their broader ecological impacts.

Climate Change and AMF Phenological Dynamics: Overall AMF Abundance— Overall AMF abundance refers to the total amount of all AMF present, encompassing all species and functional groups without distinction. Our

results on overall AMF spore production reveal a significantly higher spore abundance in mid-summer compared to late-autumn (Figure 5). Nevertheless, these patterns remain consistent across climate conditions, indicating no effect of future climate on overall AMF spore phenology (Figure 5). The peak in mid-summer spore counts aligns with previous findings suggesting that higher temperatures enhance AMF sporulation and colonization in temperate regions (Smith & Read, 2008; Kilpeläinen *et al.*, 2020). Higher temperatures (20–26°C) are found to favor AMF colonization and sporulation, likely due to enhanced root growth and increased nutrient availability (Kilpeläinen *et al.*, 2020).

Climate Change and AMF Phenological Dynamics: AMF Functional Group Abundances—Although there is no evidence of climate-induced shifts in overall AMF spore phenology, our results reveal shifts in the functional group phenology. Rhizophilic AMF spores, under ambient climate, exhibited significantly higher spore counts in mid-summer compared to early and late-autumn, indicating a mid-summer peak in spore production. However, in future climate, this pattern was only present between mid-summer and late-autumn. These changes suggests a climate-induced shift in rhizophilic spore production dynamics, reflecting a response to climate change, resulting in a trend towards a more even distribution of spore production (*i.e.*, stabilization trend) in future climate.

On the other hand, edaphophilic AMF already demonstrated a stable spore distribution, which persisted under future climate conditions, indicating that the spore production of this group is less influenced by phenological changes and potentially more resistant to phenological shifts under climate change. The observed phenological shift in rhizophilic AMF spore production is further supported by the community composition analyses, including Principal Response Curve (PRC) and Permutational Multivariate Analysis of Variance (PERMANOVA) results on metabarcoding data.

The PERMANOVA results revealed that the phenological dynamics between ambient and future climates differ, as it revealed a significant effect of climate treatment on AMF community composition at the family level with a significant climate × timepoint interaction (Figure 7). However the majority of the variance remained unexplained by climate alone, this suggests that climate-driven phenological shifts arise from interactions rather than from climate or time point alone. In addition, none of the pairwise comparisons remained significant after correction for multiple testing, meaning that differences between specific climate × timepoint combinations are subtle and potentially complex. The AMF functional groups in the PRC analysis, identified through metabarcoding, included rhizophilic (*Glomeraceae*, *Claroideoglomeraceae*, and *Paraglomeraceae*), edaphophilic (*Gigasporaceae*, *Diversisporaceae*), and ancestral (*Archaeosporaceae*, *Ambisporaceae*, *Acaulosporaceae*) AMF families.

Three key phenological shifts were detected from ambient towards future climate: a pronounced negative deviation in early-autumn, and positive deviations in mid-summer and late-autumn (Figure 7). These shifts were primarily driven by unclassified AMF, whose functional guilds remain unidentified. Among the classified taxa, rhizophilic AMF were the main drivers of these shifts, as they contained the highest weights in the PRC, such as *Paraglomeraceae* (2.884e+02) and *Glomeraceae* (1.707e+02) (Figure 7).

In contrast, edaphophilic AMF families, including *Gigasporaceae* (1.148e+00), and ancestral AMF families, for example *Acaulosporaceae* (-1.476e-03), contributed less to community change. The indicator species analysis showed that although no AMF taxa were exclusively associated with particular climate and timepoint combinations, the family *Glomeraceae* emerged as a significant indicator, strongly associated with most sampling groups. Overall, these findings highlight that the abundance patterns of rhizophilic families align closely with the overall climate-induced phenological shifts observed in the community composition. This means that phenological shifts in their relative abundance can disproportionately shape overall AMF community structure, so even small changes may strongly influence its reorganization under future climate scenarios.

The relative abundance analysis (Figure 8) corroborates the PRC results, reinforcing the dominant role of rhizophilic AMF in driving climate-related phenological shifts in community composition. Since rhizophilic AMF families carried the strongest weights in the PRC analysis, it is possible that observed shifts in other functional groups may partly reflect cascading effects driven by relatively small changes within the rhizophilic AMF group. For example, *Gigasporaceae* exhibit enhanced mid-summer relative abundance followed by a rapid seasonal decline, indicating an increase in early-season presence under future climate change (Figure 8).

On the contrary, *Paraglomeraceae* and *Archaeosporaceae* showed a climate-driven response rather than a shift in their seasonal patterns, showing increased abundances towards future climate (Figure 8). The *Archaeosporaceae* family is classified within the ancestral functional group, characterized by low biomass production and a lack of strong preference for either intraradical or extraradical growth (Weber *et al.*, 2019). They likely represent the original form of AMF (Powell *et al.*, 2009). Unlike the other functional groups, their ecological role is not well understood, but they may indirectly benefit plants by enhancing AMF diversity and community function (*i.e.*, functional complementarity) (Maherali & Klironomos, 2007).

The relative abundance analysis supports the spore analysis findings by indicating a phenological stabilization of rhizophilic AMF under future climate conditions. The *Claroideoglomeraceae* and *Glomeraceae* families exhibited clear seasonal fluctuations under ambient climate, but this phenological pattern was dampened under future climate conditions, where their abundances remained more stable year-round. Specifically, *Claroideoglomeraceae* increased significantly from mid-summer to early-autumn under current climate, while *Glomeraceae* showed a significant rise from mid-summer to late-autumn.

We initially hypothesized that future climate would cause more rapid and pronounced phenological shifts in the AMF community by enhancing CO_2 assimilation and root transport, potentially delaying dormancy (Gray & Brady, 2016; Keeler *et al.*, 2021). However, rather than a rapid, pronounced shift, our findings suggest that future climate conditions appear to redistribute AMF activity, primarily through the dampening of seasonal peaks in rhizophilic AMF and the emergence of a more stabilized phenological pattern. This pattern of rhizophilic AMF stabilization may be explained by the following possibilities:

(1)Warmer winters may weaken environmental cues, such as temperature drops or resource scarcity, which normally trigger dormancy. This may alter AMF dormancy patterns, leading to more consistent fungal activity and evenly distributed spore production across seasons (Kilpeläinen *et al.*, 2020; Ahammed & Hajiboland, 2024; Giovannetti, 2000; Keeler *et al.*, 2021; Dumbrell *et al.*, 2011).

(2) Warmer winters can cause shifts in host plant phenology that in turn influences AMF dynamics (Cotton, 2018; Weber *et al.*, 2019; Dumbrell *et al.*, 2011). As plants adapt to longer growing seasons and altered growth patterns due to climate change (Baldrian *et al.*, 2022; Cotton, 2018; Calanca *et al.*, 2023), the timing of AMF root colonization and nutrient exchange could become more consistent, contributing to more consistent spore production.

(3) PRC and abundance analyses both indicate that rhizophilic AMF are key drivers of AMF community dynamics, likely due to their ruderal life strategy (Grime, 1977; Heuck *et al.*, 2024). Their rapid growth and root-colonizing ability make them well-suited to climate-induced disturbance (Antunes *et al.*, 2025; Alguacil *et al.*, 2010; Wolf *et al.*, 2003). In contrast, slower-growing, competitive edaphophilic AMF are less responsive (Grime, 1977; Heuck *et al.*, 2024; Antunes *et al.*, 2025; Bruce *et al.*, 1994; Xu *et al.*, 2017). This concept is further explained in "*Connection To Life History Traits*". As a result, even small shifts in rhizophilic AMF under warming may cascade through the community, suppressing competitors and driving broader phenological changes, while rhizophilic AMF may stabilize.

Connection To Life History Traits—Rhizophilic AMF were found to be more abundant than edaphophilic AMF, as detailed in the section "*Climate Change and AMF Functional Group Abundance*". Additionally, under future climate

conditions, rhizophilic AMF exhibit phenological stabilization, maintaining more consistent abundances across seasons. This pattern is supported by both spore analysis and relative abundance data (see section "*Climate Change and AMF Phenological Dynamics: AMF Functional Group Abundances*"). Further, PRC analysis highlights their strong influence on climate-induced phenological shifts, reflected in their dominant family weights (see the same section). Because of their dominance and functional importance, even modest shifts in rhizophilic AMF abundance can trigger cascading effects across other functional groups, influencing overall AMF community structure. This community-level reorganization, shaped by the responsiveness of rhizophilic families, underscores the adaptive strategies of AMF under climate stress and their critical role in sustaining plant-fungal symbioses and soil health under future climate.

It is crucial to evaluate this dominant role of rhizophilic AMF in light of their life-history traits and resource allocation strategies, as partly explained in the section "*Climate Change and AMF Functional Group Abundance*". Their ruderal strategy enables rapid colonization and allows them to thrive in frequently disturbed or rapidly changing environments (Antunes *et al.*, 2025; Alguacil *et al.*, 2010; Verbruggen & Kiers, 2010; Ma *et al.*, 2018; Wolf *et al.*, 2003). This strategy is closely linked to increased resource allocation towards absorptive hyphae, resulting in rapid hyphal growth and efficient phosphorus and carbon uptake, which enhances their ability to increase plant biomass (Bruce *et al.*, 1994; Bago *et al.*, 1998; Yang *et al.*, 2017, Gosling *et al.*, 2016). They also form anastomoses, connections between different fungal hyphae, facilitating integration into larger mycorrhizal networks that enhance nutrient exchange among plants (De La Providencia *et al.*, 2004). In contrast, edaphophilic AMF adopt a slower, more competitive strategy better suited to stable ecosystems, which may limit their capacity to dominate in short-term or rapidly changing environments (Bruce *et al.*, 1994; Xu *et al.*, 2017). They allocate more resources to transport hyphae (Hart & Reader, 2005; Souza *et al.*, 2005), while their investment in extraradical hyphae suggests potential advantages in stable environments (Hart & Reader, 2002; Hart & Reader, 2005; Staddon *et al.*, 2003; Maherali & Klironomos, 2007; Chagnon *et al.*, 2013). Unlike rhizophilic AMF, edaphophilic AMF tend to form anastomoses internally within their own hyphae, potentially limiting their functional connectivity and broader ecological role (De La Providencia *et al.*, 2004).

Furthermore, rhizophilic AMF are less sensitive to carbon limitation compared to edaphophilic AMF due to their lower investment in extensive external hyphal networks and their faster root colonization (Treseder et al., 2018; Gío-Trujillo & Alvarado-López, 2024). Compared to edaphophilic AMF, their smaller extraradical networks allow them to persist under reduced carbon availability (Treseder *et al.*, 2018). Studies show that when the positive effects of elevated CO_2 on plant growth diminish over time, carbon allocation to AMF declines, favoring stress resilient species, such as rhizophilic AMF (Heuck *et al.*, 2024). Consequently, they may gain an advantageous role within the community, leading to more consistent spore production across phenological time points (Cotton, 2018; Horsch *et al.*, 2023).

These life-history traits can be understood under the competitor, stress-tolerator, ruderal (C-S-R) framework, a resource-centric, focused framework with the capacity to form intermediate classifications (Heuck *et al.*, 2024). This framework classifies organisms into competitors, stress-tolerators, and ruderals based on how they allocate resources in response to environmental pressures (Grime, 1977). In AM fungi, competitors are characterized by extensive extraradical hyphal growth to acquire nutrients (*e.g.*, edaphophilic AMF) and ruderals by their short life cycles and rapid network establishment (*i.e.*, rhizophilic AMF) (Grime, 1977; Heuck *et al.*, 2024). Some ancestral AMF families may exhibit stress-tolerant traits such as low turnover and biomass production, but due to limited trait-specific data, they cannot yet be definitively classified within this framework (Grime, 1977; Heuck *et al.*, 2024). While promising, the C-S-R framework its applications remain limited by a lack of trait-specific data (Antunes *et al.*, 2025).

Research suggests that the C-S-R framework should not be viewed as a rigid classification but rather as a hypothesis-generating tool to guide trait-based research (Antunes *et al.*, 2025).

Recent work shows partial support for C-S-R-based predictions (Heuck *et al.,* 2024). For instance, ruderal taxa like *Glomeraceae* often increase under elevated CO_2, consistent with their fast-growing, carbon-responsive strategies (Heuck *et al.,* 2024).

Lastly, ecological differentiation also plays a role. Rhizophilic AMF tend to colonize younger roots, while edaphophilic taxa are more likely to establish in older roots over time, especially in perennial systems (Kil *et al.,* 2014; Vukicevich *et al.,* 2019). This niche partitioning may facilitate long-term coexistence, but in dynamic or disturbed systems, such as agricultural environments, rhizophilic AMF often emerge as the dominant group (Horsch, Antunes & Kallenbach, 2023).

Future Perspectives—The observed phenological shifts in AMF communities under future climate scenarios underscore the critical role of life-history strategies in integrating ecosystem responses to environmental change, particularly climate change. These findings deepen our understanding of AMF ecological adaptation and highlight the importance of incorporating mycorrhizal phenology and functional traits into predictions of climate change impacts on both natural and agricultural ecosystem resilience. While this study, conducted within Belgian pear orchards, offers valuable insights, its regional focus may limit the broader applicability of the results. Expanding research across diverse geographic regions and climatic conditions is essential to develop a more comprehensive understanding of AMF responses to global climate dynamics. Moreover, the study's relatively short temporal scope, based on a single year of sampling, may not fully capture the long-term, gradual effects of climate change on AMF abundance, community composition, and phenological patterns. Extended longitudinal studies are needed to assess the persistence and resilience of AMF communities throughout different stages of climate adaptation.

Furthermore, the integration of the continuously collected abiotic data from the Ecotron facility would enable a more detailed, mechanistic understanding of how climate factors influence AMF phenology, community dynamics, and functional group interactions. For example, changes in water availability driven by climate change can impact a plant's carbon allocation, influencing the carbon supplied to AMF and subsequently shifting their community composition (Heuck *et al.*, 2024).

Additionally, to further clarify the dominant role and stabilization trend of rhizophilic AMF, future research should investigate how warmer winters may weaken environmental cues that typically trigger fungal dormancy, potentially prolonging active periods. Studies should also examine how altered host plant phenology, such as extended growing seasons, shapes the timing and consistency of AMF colonization. Furthermore, trait-based approaches should explore how ruderal life-history strategies enable rhizophilic AMF to maintain activity and outcompete slower-growing taxa under climate stress. Together, these lines of research, integrating spore phenology, root colonization, and functional traits with plant phenology, will help uncover the drivers of phenological stabilization and clarify how small shifts in rhizophilic AMF affect broader community structure and functional group interactions.

Moreover, expanding research across a broader range of AMF host plants, including crops, herbs, and shrubs, will help assess the consistency of these patterns across ecosystems. Ultimately, extending this trait-based, phenological approach to other mycorrhizal types, including ectomycorrhizal (EcMF), ericoid (ErMF), and orchid mycorrhizal fungi (OrMF), will be key to understanding the full scope of mycorrhizal responses to climate change in both agricultural and natural systems.

This study did not explicitly investigate how phenological shifts in AMF activity might influence broader ecological processes such as soil carbon cycling, soil health, plant health, crop productivity, and overall ecosystem functioning. AMF produce glomalin, a glycoprotein that promotes the formation of soil aggregates and helps retain organic matter, improving soil structure, porosity, and water-holding capacity, essential factors for stabilizing soil carbon and maintaining soil fertility and health (Nautiyal *et al.,* 2019). Additionally, AMF improve plant health through enhanced nutrient uptake and increased resistance to soil-borne pathogens, which supports plant growth and stress resilience (Baldrian *et al.*, 2022; Cotton, 2018). Shifts in

AMF activity can thus influence crop productivity and, on a larger scale, alter ecosystem functioning by modifying nutrient cycling and plant–soil feedbacks critical for ecosystem stability. For example, AMF contribute significantly to soil carbon storage through biogeochemical cycling (Soudzilovskaia *et al.*, 2015, 2019; Hawkins *et al.*, 2023). Understanding these connections is essential for predicting how climate-driven changes in AMF phenology may ultimately affect ecosystem stability and resilience.

By revealing climate change–induced shifts in AMF community phenology this study offers crucial insight into how mycorrhizal dynamics respond to future climate. This is crucial for informing predictive models of ecosystem functioning under future climate scenarios, guiding sustainable land management, and maintaining soil health and fertility. Moreover, these findings enhance our understanding of how AMF support plant productivity and ecosystem resilience in agricultural systems.

CONCLUSION

This study provides new insights into how projected future climate conditions (2042–2046) affect arbuscular mycorrhizal fungal (AMF) abundance, phenology, and functional group composition in Belgian pear orchards. By integrating metabarcoding and spore analysis, it provides a comprehensive view of community-level and functional group-specific responses under realistic, integrated climate scenarios.

Key findings demonstrate that while the overall AMF spore abundance remained stable, functional group-specific phenological shifts occurred, with rhizophilic AMF emerging as key drivers of community-level reorganization under future climate conditions. Principal response curve (PRC) analyses and relative abundance data showed that rhizophilic families drove most climate-induced phenological shifts, while edaphophilic AMF, typically adapted to stable environments, showed limited responsiveness. These patterns align with the predictions of the Competitor-Stress Tolerator-Ruderal (CSR) framework. The rhizophilic AMF's ruderal traits (rapid colonization, lower carbon demands, and resilience in disturbed conditions) enable them to gain an increasingly dominant role under future climate stress.

Moreover, rhizophilic AMF displayed a phenological stabilization trend under future climate conditions, with both the spore production and the relative abundances of rhizophilic community members becoming more evenly distributed across the tested seasons. This trend may result from warmer winters weakening dormancy cues, altered host plant phenology extending their active periods, and the disturbance-tolerant growth strategies of this functional guild. This stabilization may reshape nutrient cycling dynamics, carbon fluxes, and plant–fungal interactions, with cascading effects on ecosystem functioning.

Future research should include long-term, multi-site studies that integrate abiotic climate variables to assess the resilience of climate-driven shifts in AMF communities. Investigating how warmer winters, altered host phenology, and ruderal strategies shape rhizophilic AMF responses, through integrated spore, colonization, and trait data, will clarify mechanisms of phenological stabilization. Expanding to diverse host plants and AMF types is key to predicting ecosystem responses under both agricultural and natural ecosystems. Additionally, the impact on soil carbon cycling, nutrient dynamics, and plant–soil interactions should be examined to better predict the effects on ecosystem resilience. Together, this will offer key insights for predicting ecosystem responses to future climate, guiding sustainable land management, and supporting soil health and ecosystem resilience.

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SUPPLEMENTARY METHODS 1: CLIMATE PROJECTIONS

The study's climate projections were generated by integrating both large-scale and regional models to estimate local conditions at a 15 km spatial resolution. The climate scenarios were based on a Representative Concentration Pathway (RCP), as defined by the Intergovernmental Panel on Climate Change (IPCC) in their Fifth Assessment Report of 2014 (Copernicus Climate Change Service, n.d.; IPCC, 2014), representing standardized greenhouse gas concentration pathways that project future climate outcomes. The RCP 8.5 scenario (*i.e.*, worst-case emission pathway) is characterized by a continuous rise of greenhouse gas emissions throughout the 21st century, projecting a mean global temperature increase of +2.0°C by 2046-2056 and of +3.7°C by 2081-2100 that leads to more significant climate change (Copernicus Climate Change Service, n.d.; IPCC, 2014). In this study, the RCP8.5 scenario for Belgium in 2042-2046 and 2013-2018 is used, as it is expected to result in the most severe ecological effects. The experiment included two exposure periods: 2022 to 2023 and from 2023 to 2024.

The model output consists of three-hourly data for air temperature, relative humidity, precipitation, and wind speed, which was downscaled to half-hour intervals through linear interpolation, in accordance with the Ecotron's operational scale. To account for atmospheric CO_2 levels, real-time measurements from the nearby Integrated Carbon Observation System (ICOS) were incorporated, with projections for 2040-2045 suggesting an increase of +133 µmol.mol−1. Soil water potential and air temperature were based on field data from the ICOS station.

SUPPLEMENTARY METHODS 2: SPORE EXTRACTION PILOT EXPERIMENTS

Pilot Experiments– Prior to performing spore extractions on the rhizosphere soil samples, two pilot experiments were conducted to optimize the spore extraction technique: Ultrasound Wet-Sieving Technique (UWST) and Ultrasound Centrifuge Technique (UCT). While conducting these techniques two substrate sources were utilized: a bulk soil sample collected near a pear orchard with a low spore density and a commercial Mycorrhiza Mix ("Mycorrhiza Mix" Snelkiemende Endomycorrhiza 50Gr—by Dutch Garden Seeds) containing AMF spores (Dutch garden seeds, n.d.). The protocols for UWST and UCT were based on previous research (Boyno *et al.*, 2023), with modifications to the sieve sizes used (1 mm, 100 µm, and 40 µm). The first pilot experiment aimed to identify the most effective technique, while the second focused on the impact of ultrasound exposure on arbuscular mycorrhizal fungal (AMF) spore integrity and recovery. These experiments guided the development of the final protocol, which employed UWST with ultrasound exposure, allowing for the optimization of spore extractions, while ensuring maximum recovery efficiency for subsequent analyses.

When using the UWST technique, a spore suspension was prepared by suspending one gram of Mycorrhiza Mix or a 1:1 mixture of Mycorrhiza Mix and bulk soil in dH_2O using a magnetic stirrer for 1 min. The suspension was then subjected to an ultrasound bath (30 sec at 28 kHz) and subsequentially filtered through 1 mm, 100 µm, and 40 µm sieves. The 100 µm and 40 µm sieve contents were collected, while the 1 mm sieve content was discarded.

Subsequently, the sieve contents were washed with dH_2O, exposed to ultrasound (30 sec at 28 kHz), and passed through the 40 µm sieve. These last steps were repeated three times. The final sieve content was washed with a 55% sucrose solution and centrifuged (seven minutes at 1500 rpm) to create gradient separation. The supernatant was then passed through a 40 µm sieve, and the retained sieve content was thoroughly washed to remove sucrose before being resuspended in 20 mL of MiliQ H_2O.

In contrast, while using the UCT method, a spore suspension was created by mixing one gram of Mycorrhiza Mix in 40 mL of dH_2O, using a magnetic stirrer (five minutes at four to five rpm), before being subjected to an ultrasound bath (30 sec at 28 kHz). The suspension was then centrifuged (three minutes at 3000 rpm), and the supernatant was passed through a 40 µm sieve. The final sieve content was collected using 25 mL of MiliQ H_2O.

Spore counts were determined, for both UWST and UCT techniques, by placing one milliliter of spore suspension at the center of a Petri dish, which was prepared with a measuring grid (Figure 4.A). Visualization was conducted using a stereomicroscope (Nikon bino SMZ 800) and images were captured for subsequent analysis in ImageJ (win64). The number of spores per milliliter of spore solution was determined using ImageJ analysis and quantified as outlined in the **Materials and Methods** section under *AMF Spore Extractions and Spore Quantification*.

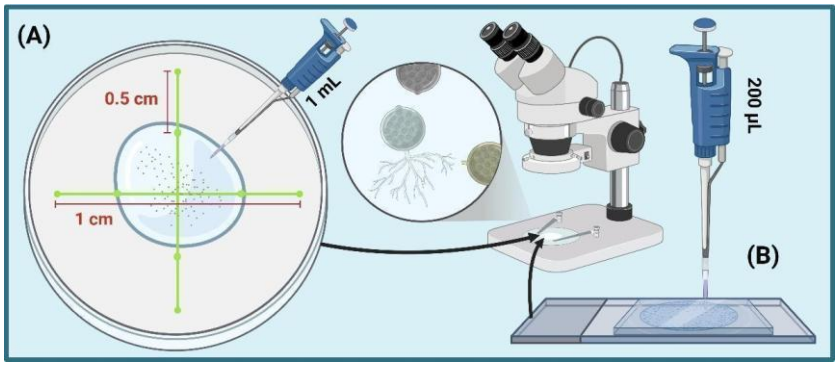
Pilot experiment 1— The first pilot experiment aimed to compare the UWST and UCT techniques to determine the most effective spore extraction technique. One gram of Mycorrhiza Mix in powdered form was obtained by sieving the Mycorrhiza Mix to isolate fine particles. This is important as subsequent analyses will utilize homogenized soil rather than the aggregate clusters present in the Mycorrhiza Mix. The UWST proved to be the most effective method for spore extraction, ensuring maximum recovery efficiency, while the UCT showed satisfactory results but not as effective as UWST (Suppl. Table1).

Pilot experiment 2—The second pilot experiment aimed at assessing the impact of ultrasound on spore integrity and recovery. One gram of powdered Mycorrhiza Mix underwent UWST without ultrasound exposure, serving as a control. Additionally, a 1:1 mixture of bulk soil (0.5 g) and powdered Mycorrhiza Mix (0.5 g) was subjected to UWST with ultrasound exposure. Ultrasound exposure showed to enhance spore recovery without compromising spore integrity.

Enhancement of Spore visualization— To optimize spore analysis, the visualization technique was refined using primary spore extractions from the rhizosphere soil samples. 200 µL of spore suspension was placed on a microscope slide and covered with a cover slip (Figure 4.B). Digital images of observed spores were captured using a stereomicroscope (Nikon SMZ800N) at 30× or 40× magnification and processed with NIS Elements software. The procedure was conducted in triplicate, ensuring three technical replicates for accuracy.

Supplementary Table 1—Overview of Total Spore Number (TSN), Spore Number Per One mL (SN), and Extract Volume (W; amount of water used) for the Ultrasound Wet-Sieving Technique (UWST) and Ultrasound Centrifuge Technique (UCT).

Technique	*SN*	*W*	*TSN*
UWST	*11*	*20*	*220*
UCT	*3*	*25*	*75*



Supplementary Figure 1—Spore visualization methods (A) Measuring grid on Petri dish with 1 mL of spore solution. (B) Microscopic slide with 200 µL of spore solution covered with a slide cover. (A-B) Both are visualized for arbuscular mycorrhizal fungal (AMF) spores using a stereomicroscope. Figure made using BioRender.com.

SUPPLEMENTARY METHODS 3: LIBRARY PREPARATION

In between all the following steps DNA quality was checked using Gel Electrophoreses 1.5%.

Library Preparation: DNA Extraction—The rhizosphere soil samples were grinded using liquid nitrogen for soil homogenization. DNA was subsequently extracted using the RNeasy PowerSoil kit, automated for MagMAX (Sofie, 2021). DNA quality and concentration were assessed using a NanoDrop spectrophotometer, and the extracts were diluted 20-fold prior to amplification by Polymerase Chain Reaction (PCR).

Library Preparation: Amplification PCR—The PCR Master Mix was prepared using the Q5® Hot Start High-Fidelity DNA Polymerase protocol (New England Biolabs., 2012) for 25 µL reaction volumes per well, with a 10% surplus to account for pipetting loss. Each 25 µl reaction contained: 17µL of nuclease-free water, 5µL of 5X Q5 Reaction Buffer (1X final concentration), 0.5 µl of 10 mM dNTPs (200 µM each), 0.625 µl each of 10 µM forward and reverse primers (0.25 µM final concentration), and 0.25 µl of Q5 Hot Start High-Fidelity DNA Polymerase (0.02 U/µl final). Subsequently, 24 µl of PCR Master Mix and 1 µl of DNA template were added to each well of a 96-well PCR plate placed on a cold block, repeated according to the number of samples. A blank was included containing 24µL PCR Mastermix and 1µL of nuclease-free water. Subsequently each well was mixed thoroughly and the 96 well plate was briefly centrifuged using a plate-spinner. PCR amplification was then performed under the following cycling conditions using the C1000 Touch Thermal Cycler (96-well): initial denaturation at 98 °C for 3 min; 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 7 min; hold at 4–12 °C. The amplification PCR targeted the V4 region of the small subunit (SSU) 18S rRNA gene (~300 bp amplicon) using the primers AMV4.5NF_il (forward) and AMDGR_il (reverse) (Van Geel et al., 2016). The V4 region is selected for its high variation across AMF species.

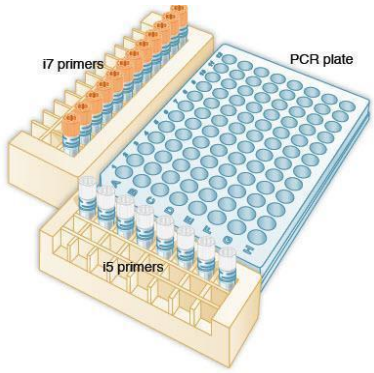
Library Preparation: PCR Cleanup—Post-PCR cleanup steps using AMPure XP beads were performed after the initial amplification PCR and after the index-PCR, to remove residual primers and primer dimers, as well as remaining PCR products. This was performed as described in the 16S Metagenomic Sequencing Library Preparation protocol for the Illumina MiSeq System (Novogene., n.d.), automated for MagMAX.

The amplification and index PCR were cleaned by initial centrifugation of the PCR plate for one minute to collect condensation. Subsequently, 60µl of AMPure XP beads and 50µl of 5x concentrated PCR buffer (50 mM Tris-HCl pH 8.5, 250 mM KCl, 7.5 mM $MgCl_2$) were added to 25µl PCR product and mixed thoroughly. Next, the solution was transferred to a new MagMAX deep-well 96-well plate and incubated at room temperature for five minutes

to enhance DNA-binding and recovery. Meanwhile, two deep-well wash blocks were prepared, each well containing 200 µl of 80% ethanol, along with an elution plate filled with 75 µl of 10 mM Tris-HCl (pH 8.5) per well. Subsequently, all plates were loaded into the MagMAX system in the specified order and orientation, as outlined in the MagMAX protocol AM1839spin DW. According to this protocol, the MagMAX instrument performs a binding step for five minutes, followed by two 15-second wash steps, 30 seconds of bead drying, and a four minute heated elution step.

Library Preparation: Indexing PCR—Prior to indexing, the PCR Master Mix was prepared using the Q5® Hot Start High-Fidelity DNA Polymerase protocol (New England Biolabs., 2012) for 25 µL reaction volumes per well, with a 10% surplus to account for pipetting loss. Each 25 µl reaction contained: 9.25 µL of nuclease-free water, 5 µL of 5X Q5 Reaction Buffer (1X final concentration), 0.5 µl of 10 mM dNTPs (200 µM each), 0.25 µl of Q5 Hot Start High-Fidelity DNA Polymerase (0.02 U/µl final). The dual indices (Nextera XT index kit v2), i-5 (S502, S503, S505, S506, S507, S508, S510, and S511) and i-7 (N701, N702, N703, N704, N705, N706, N707, N710, and N711), were centrifuged (Illumina, 2016). This indexing was performed as described in the 16S Metagenomic Sequencing Library Preparation protocol for the Illumina MiSeq System (Novogene., n.d.). 15 µl of PCR Master Mix was added, along with 5 µl of PCR product to each well, according to the number of samples. A blank was included containing 15 µL PCR Mastermix and 5 µL of nuclease-free water. Next, 2.5 µL of i-5 index, and 2.5 µL of i-7 index were added to all the PCR plate wells with regular replacement of hand gloves to protect index quality. Subsequently, each well was mixed thoroughly, and the 96-well plate was briefly centrifuged using a plate-spinner. PCR was then performed under the following cycling conditions using the C1000 Touch Thermal Cycler (96-well): initial denaturation at 98 °C for 3 min; 18 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 7 min; hold at 4–12 °C.

Lastly, Qubit 4 (Invitrogen™ Fluorometer) was used to obtain the final DNA concentration. The final DNA library was sent to Novogene Co., Ltd. for sequencing.



Supplementary Figure 2— Index plate set-up. Adapted from New England Biolabs.

SUPPLEMENTARY METHODS 4: STATISTICS

All statistical analyses were performed using R version 4.4.2.

Statistics: Spore Abundances across Climate Scenarios and Phenological Stages Using Spore Counts—Total AMF spore numbers (TSN) were used as response variable in the statistical analysis. Before analysis, data were checked for overdispersion by comparing the mean and variance of TSN, along with evaluating residual patterns. As the variance exceeded the mean, a Negative Binomial Generalized Linear Mixed Model (GLMM) was implemented using the package glmmTMB (version 1.1.5, run through nbinom2 function of glmmTMB package), with additional diagnostics performed using performance (version 0.10.3) and DHARMa (version 0.4.6) packages. The model included climate condition (ambient vs. future), phenological time points (early-autumn, late-autumn and

mid-summer), and spore type (rhizophilic and edaphophilic) as fixed effects, with Unit (Lysimeter number) as a random effect to account for within-experiment variability. pH was tested as a fixed effect but did not significantly improve model fit (ANOVA comparison: $\chi^2 = 0.25$, $p = 0.61$) and was therefore excluded from the final models. pH values were obtained as described in supplementary methods 5.

To assess statistical differences in spore counts between climate conditions and phenological time points, post-hoc pairwise comparisons were conducted using estimated marginal means (emmeans()), applying Tukey's adjustment for multiple comparisons. Spore counts were visualized as Model-Based Estimates Marginal Means (EMMs) using log-transformed estimated mean and 95% CI, with separate plots showing total spore abundance and abundances specific to edaphophilic and rhizophilic AMF. P-values were derived using emmeans() based on the interaction between climate condition, phenological timepoint, and TSN in the full model, while accounting for Unit as a random effect. Separate models were run for rhizophilic and edaphophilic AMF.

Statistics: Principal Response Curves (PRC) Analysis—PRC analysis was conducted to investigate the effect of climate treatments on AM fungal family composition over phenological timepoints. The following packages were used: vegan package (version 2.6-10), phyloseq (version 1.52.0), dplyr (version 1.1.4), tidyr (version 1.3.1), and indicspecies (version 1.8.0). Metadata and amplicon sequence variant (ASV) count data were preprocessed and aggregated to the taxonomic family level using the tax_glom() function from phyloseq. Samples corresponding to the controls were excluded prior to analysis.

The PRC model used climate as treatment factor and phenological timepoints as temporal factor, accounting for repeated measures. Statistical significance of the treatment effect was assessed using permutation-based ANOVA with 999 permutations, a non-parametric approach suitable for ecological community data. Permutations involved randomly reshuffling the data to evaluate whether the observed treatment effect exceeded expectations under the null hypothesis. Redundancy Analysis (RDA) was applied to examine the interaction effects between climate treatment and phenological timepoint on community composition. Climate and Timepoint factors were coded as categorical variables. The significance of Climate and Timepoint effects and their interaction was evaluated via permutation ANOVA on the RDA model terms. To complement this, pairwise PERMANOVA tests were performed using the pairwise Adonis package (version 0.4.) to assess differences between climate × timepoint combinations, with p-values adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method via p.adjust().

To identify key mycorrhizal families driving community shifts, family contributions were derived from family scores on the RDA axes using the scores() function. These weights indicate the strength and direction of each family in association with the environmental gradients modeled in the RDA. For PRC, family weights reflect how closely each family's temporal response aligns with the overall community trajectory described by the PRC. Additionally, indicator species analysis (IndVal method) was performed to detect AMF taxa significantly associated with specific climate × timepoint combinations. A visualization of the PRC coefficients, RDA ordinations, and species contributions was generated using the ggplot2 package (version 3.5.2).

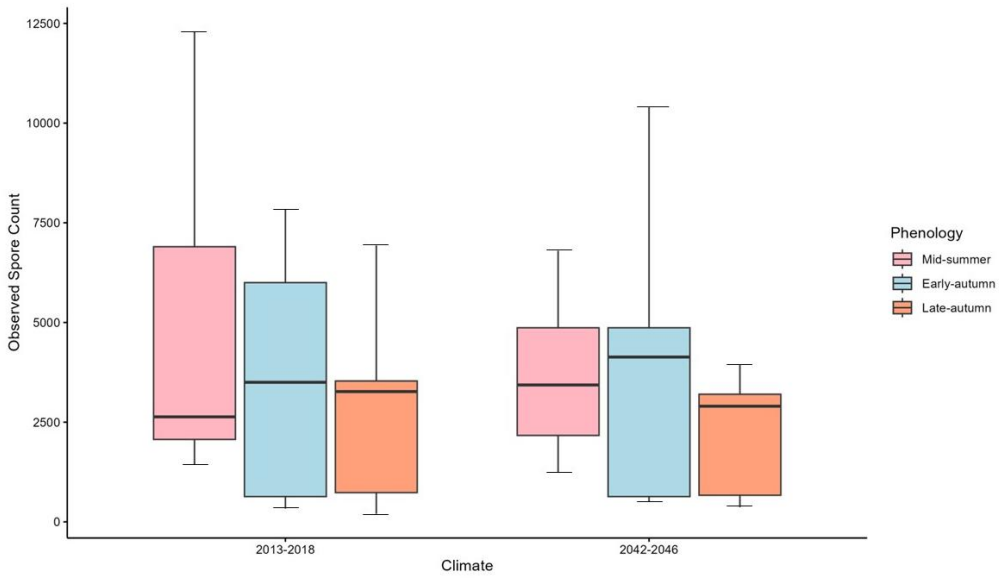
Statistics: Community Composition Analyses Using Relative Abundances and Permutational multivariate analysis of variance (PERMANOVA)— Community composition was analyzed using the phyloseq, vegan, and ggplot2 packages. Prior to analysis, ASVs detected in negative controls were identified and their counts were subtracted from the corresponding ASVs in the samples to correct for contamination. ASV count data was transformed to relative abundance using transform_sample_counts() to account for differences in sequencing depth. Relative abundance bar plots were generated using ggplot2, visualizing mycorrhizal family composition across climate treatments and phenological timepoints. Mean relative abundances were calculated by averaging the relative abundances of each AMF family across samples within each climate × timepoint group and subsequently expressed as percentages.

To assess treatment effects on overall community composition, Bray–Curtis dissimilarity matrices were calculated using distance(). PERMANOVA was conducted with adonis2(), using 999 permutations. The model included Climate, Timepoint, and their interaction (Climate * Timepoint). To examine individual contributions, an additive model (Climate + Timepoint + Climate:Timepoint) was tested. Additionally, within-treatment PERMANOVA was performed by subsetting the data (*i.e.,* to a specific climate scenario) to test for differences across timepoints. All p-values were derived from permutation-based testing (adonis2()), which is appropriate for non-parametric ecological data.

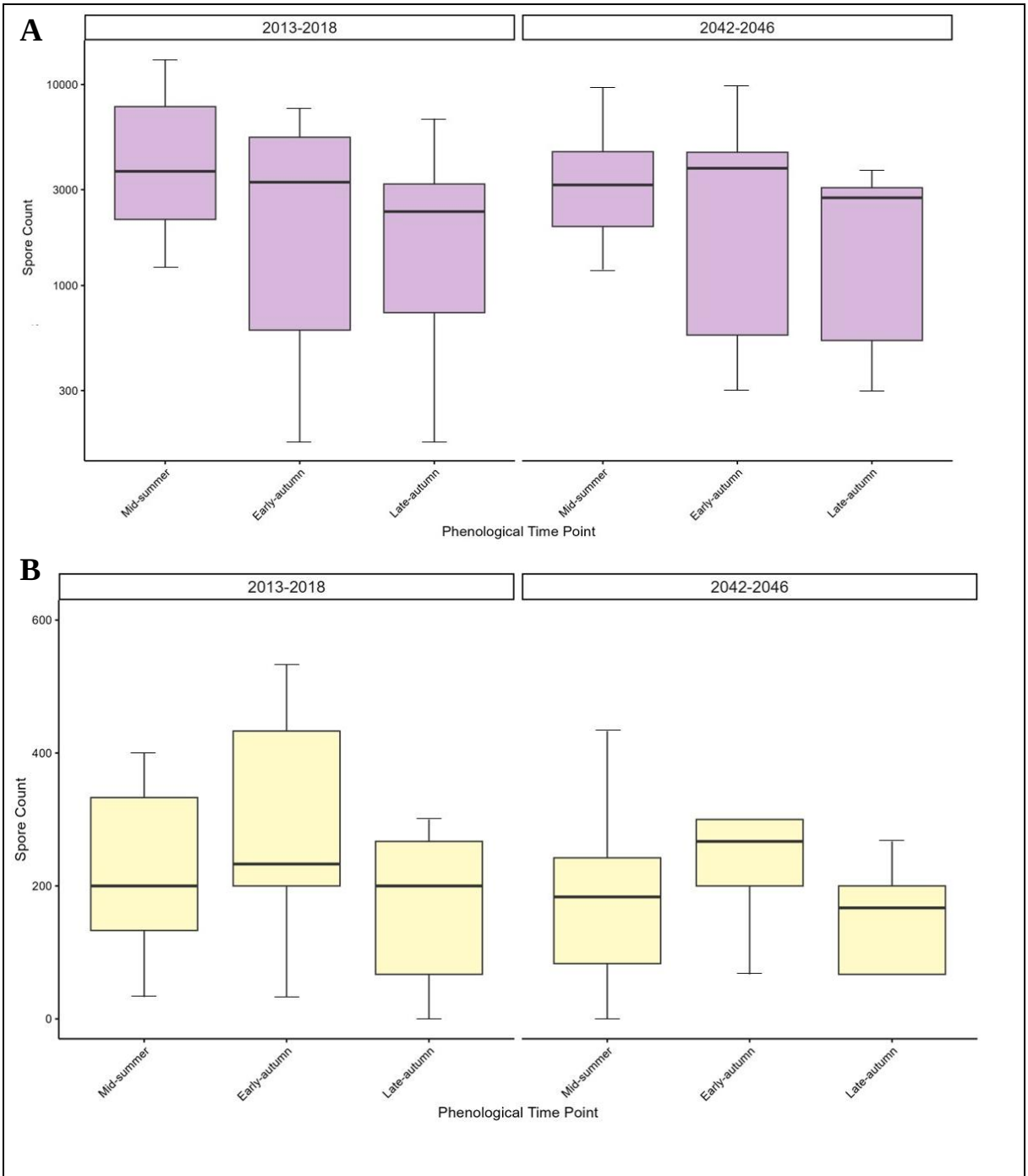
To assess climate and phenological effects on individual AMF families, relative abundance data were aggregated by Sample, Climate, Timepoint, and Family. Linear models (LMs) were fitted per family to test for main effects and interactions between Climate and Timepoint. Appropriate, linear mixed models (LMMs) were used to account for repeated measures (i.e., multiple timepoints per sample). Post-hoc pairwise comparisons were performed using emmeans() to identify differences within and between climate scenarios across timepoints, allowing the detection of family-specific responses.

SUPPLEMENTARY METHODS 5: SOIL PH ANALYSIS

Soil pH was determined using a colorimetric assay adapted from Weligama et al. (2022). Briefly, one gram of rhizosphere soil was mixed with five mL of 0.01M $CaCl_2$ (1:5 w/v) and shaken for one hour on a rotary mixer, followed by centrifugation at 2093 g for 10 minutes. To convert absorbance values to pH, a calibration curve was created using standard buffer solutions with known pH values. Three buffer systems were prepared covering the range pH 4.0 to 8.5: citrate buffer (0.1M, pH 4.0–5.5), phosphate buffer (0.1M, pH 6.0–7.5), and Tris buffer (0.1M, pH 8.0–8.5). For pH measurement, 200 µL of all the supernatants and buffer solutions were individually transferred in triplicate to a 96-well plate and mixed with 10 µL of a 25% diluted pH indicator dye (GHE pH Test Kit, 30 mL). The absorbance was then measured at 615 nm using a fluorescence plate reader (FLUOstar Omega; 96-well). The calibration curve was generated by plotting the absorbance values against the known pH values of the buffers. Positive (phosphate buffer, pH 7.0) and negative ($CaCl_2$ + dye only) controls were included.



Supplementary Figure 3—Raw Data on Overall Arbuscular Mycorrhizal Fungal (AMF) Spore Counts Across Climates (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn).



Supplementary Figure 4— Raw Data on (A) Rhizophilic and (B) Edaphophilic Arbuscular Mycorrhizal Fungal (AMF) Spore Counts Across Climates (2013–2018 [Ambient] and 2042–2046 [Future]) and Phenological Timepoints (Mid-Summer, Early-Autumn, and Late-Autumn).