Investigating the Effects of Future Climate on Arbuscular Mycorrhizal Fungal Spore Dynamics in a Belgian Pear Orchard Ecosystem

Chloë Vercauteren[1], Vera Claessens[1], François Rineau[1], and Nadejda A. Soudzilovskaia[1]

[1]Centre for Environmental Sciences, Universiteit Hasselt, Campus Diepenbeek, Agoralaan Gebouw C - B-3590 Diepenbeek

*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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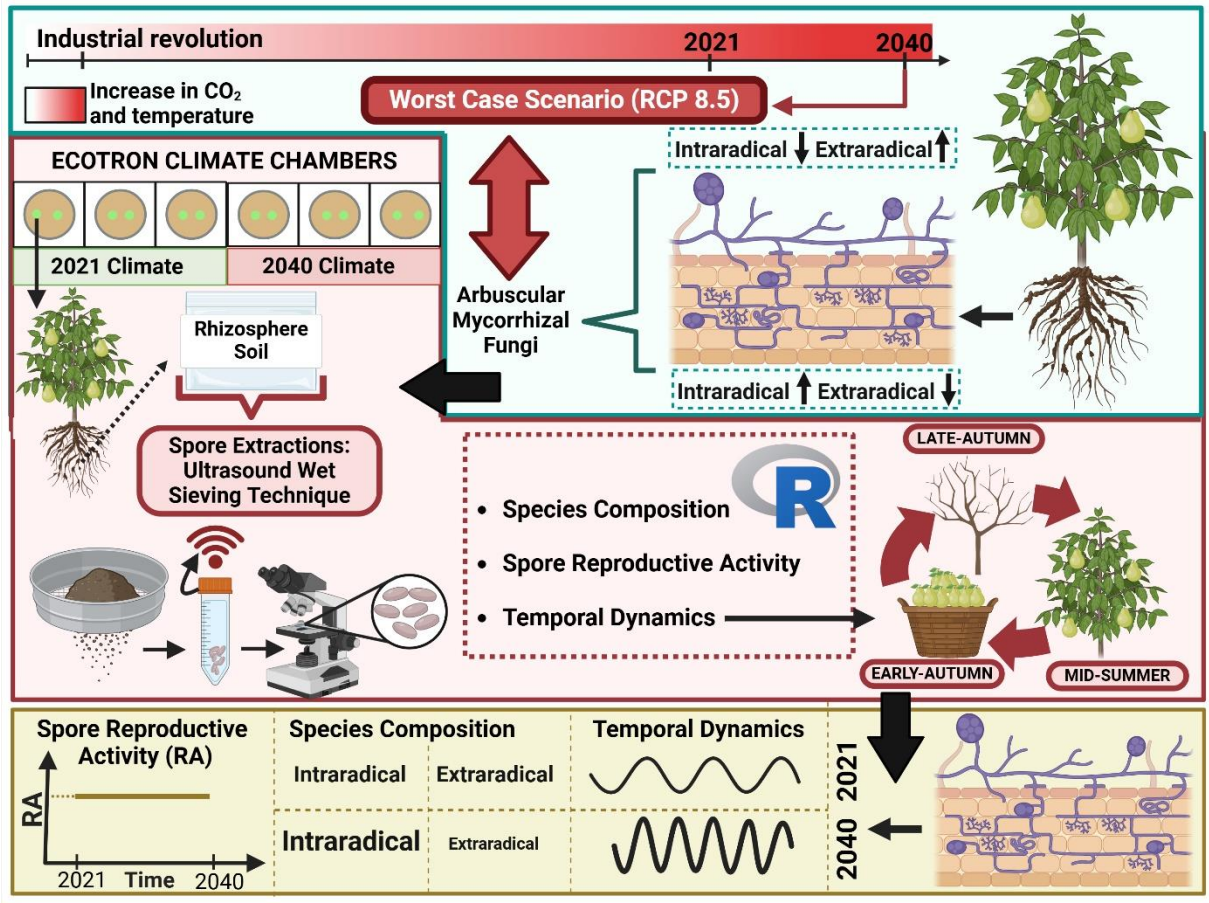
Graphical Abstract



ABSTRACT (218 words)

Climate change poses a critical threat to both global natural and agricultural ecosystems, significantly affecting essential soil microbial communities, including arbuscular mycorrhizal fungi (AMF). These fungi form essential symbiotic relations with most terrestrial plant species, including economically significant ones such as fruit trees. As AMF are sensitive to climatic fluctuations, climate change influences AMF community species composition, diversity, ecological functions, and potentially even the temporal dynamics. Jointly this affects nature and temporal dynamics of AMF interactions with host plants. Although research has explored the impact of individual climate variables on AMF, a major knowledge gap remains in regard to how multiple climate parameters, exhibiting realistic climate change, simultaneously affect AMF community dynamics. This study aims to address this gap by examining the response of AMF to the worst-case climate change scenario (RCP8.5) forecasted for 2040. Using the state-of-the-art Ecotron facility, both ambient (2018) and future (2040) climate conditions were simulated for a pear orchard ecosystem, with six pear trees cultivated under each set of climatic conditions. We assessed climate change impact on AMF spore reproductive activity species composition (i.e., ratio intraradical over extraradical), and temporal dynamics, revealing patterns of dormancy and activity, and providing insights into the shifts in AMF community phenology induced by climate change. Our research advances the understanding of climate-driven dynamics of AMF in agricultural systems, providing insights into sustainable crop production and soil fertility under future climate conditions.

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 [1]. 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 [2]. This global climate change is predicted to lead to major shifts in weather patterns, intensifying heatwaves, prolonging droughts, and increasing both the frequency and severity of heavy rainfall [3]. Additionally, it has driven compound events, such as simultaneous drought and extreme heat, which heighten wildfire risks. This trend is expected to persist and intensify as global temperatures continue to rise [3]. These climate-driven shifts pose serious challenges to natural and agricultural ecosystems. As terrestrial plants are sessile organisms, unable to migrate to evade environmental stressors, it is crucial to understand their adaptive mechanisms to climate change, such as adaptations to water- and nutrient-limitations and to plant pathogens. A critical plant strategy to adapt to nutrient limitation stress involves a symbiotic relation with arbuscular mycorrhizal fungi (AMF) [4].

These fungi form symbiotic associations with up to 80% of terrestrial plant species, including economically valuable crops such as fruit trees, which hold significant value for the European agricultural industry [4]. 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 [4]. To facilitate this symbiosis, AMF develop both extraradical and intraradical structures (Figure 1). The extraradical structures include the mycelium, which extends beyond the root system, expanding soil exploration and thereby increasing P availability and translocation, ultimately enhancing plant nutrition and growth [8]. Research indicates that AMF can contribute up to 90% of the plant's P supply [6]. Additionally, extraradical structures, which extend beyond the plant roots into the soil, consist of spores that are reproductive units dispersed in the soil, and hyphae that extend from the mycelium to aid in nutrient uptake. In contrast, intraradical structures are formed inside the plant roots and include hyphae, which are involved in root colonization, arbuscules, which serve as the primary sites for nutrient and carbon exchange, and vesicles, specialized for lipid storage [9]. Besides

nutrient uptake, fungal hyphae can accelerate the decomposition process of soil organic matter, improving soil quality by influencing its structure and texture, and thereby plant health [8].

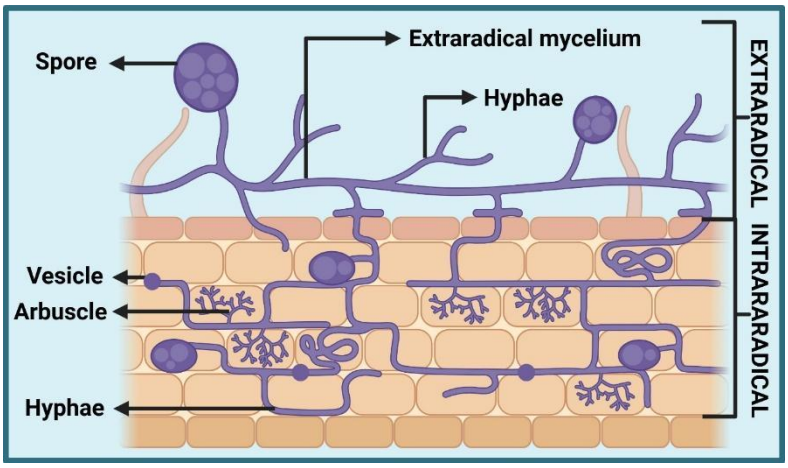


Figure 1: Plant Root Colonization by Arbuscular Mycorrhizal Fungi (AMF): Extraradical and Intraradical Structures. *Figure created with BioRender.com.*

Earlier research has already demonstrated that AMF promote plant growth under abiotic stress conditions by mediating complex signaling pathways between the plant and the fungus. Plants inoculated with AMF exhibit enhanced resilience to various environmental stressors, including salinity, drought, nutrient stress, alkali stress, cold stress, and extreme temperatures. This increased stress tolerance translates into higher crop yields per hectare across a wide range of agricultural species [8]. In addition to improving plant health, AMF may also indirectly influence atmospheric CO_2 fixation through biogeochemical cycling, contributing to soil carbon storage—a process known as the 'sink effect'. Estimates suggest that that mycorrhizal mycelium sequesters 13.12 Gt CO_2e each year, or about 36% of annual CO_2 emissions from fossil fuels, highlighting the vital role of these fungi in global carbon dynamics and ecosystem stability [9,10]. Given their substantial impact on both plant health and ecosystem functioning, it is crucial to examine how AMF communities, particularly AMF spores, are affected by climate change. With climate change becoming an increasing concern, research on how shifting climatic variables impact AMF communities has become increasingly critical.

Research has shown that AMF exhibit significant sensitivity to changing climatic parameters, which can both directly and indirectly affect their diversity, distribution, and functions within ecosystems. Indirect effects arise from climate-driven changes in host plants, soil properties (e.g., pH), and nutrient availability [4,11]. Although research attempts to understand the effects of altered water availability on AMF communities are more abundant compared to studies of the effects of drought, the findings remain controversial, often inconsistent, and context-dependent [4]. Furthermore, the effects of increased rainfall on AMF diversity vary, with increases, decreases, and no change being reported [4]. Nevertheless, both drought and increased rainfall have shown to influence AMF community composition in certain ecosystems [4]. In contrast to increased rainfall, drought has been reported to negatively affect AMF [11]. Further, most studies indicate that increased CO_2 does not significantly affect AMF richness or diversity; however, it alters community composition, thereby changing the community structure. Nevertheless, this shift is primarily driven by changes in carbon allocation to AMF, favoring Glomeraceae species while disadvantaging Gigasporaceae, rather than broader climatic factors [4].

A critical aspect of AMF dynamics under climate change that has gained increasing attention is the role of AMF spores [12-14]. Research suggests that in cold and temperate climates higher temperatures promote AMF colonization. As reproductive units, spores enable AMF to establish contact with plant

roots, which is essential for their survival, particularly in harsh environmental conditions, such as drought or extreme temperatures [15, 16]. AMF spores can remain dormant in the soil until favorable conditions trigger their germination, allowing them to colonize plant roots when conditions are optimal [17]. Consequently, seasonal climatic variations that influence host plant phenology can affect the timing of root colonization and thereby nutrient exchange, ultimately impacting plant health and ecosystem productivity [18]. With projected temperature increases by 2040, enhanced CO_2 assimilation and transport to roots may delay AMF dormancy onset [19]. Yet these effects are taxon-dependent. Research has shown that spores of *Glomeraceae* species are often more resilient to environmental stressors, such as elevated CO_2, which suggests their potential to become more dominant in future climate [12]. Thus, climate change could alter the temporal dynamics of AMF, making it crucial to understand these shifts in order to predict their future ecological roles in soil ecosystems and their potential implications for agricultural practices in a changing climate.

Previous research has predominantly focused on the effects of individual climatic parameters, creating a gap in understanding the joint impacts of full climate exposure. This study aims to address this gap by exploring a comprehensive climate scenario for 2040 in Belgium. The scenario is based on a Representative Concentration Pathway (RCP), as defined by the Intergovernmental Panel on Climate Change (IPCC) in their Fifth Assessment Report of 2014 [20]. These RCPs represent standardized greenhouse gas concentration pathways that project future climate outcomes, ranging from low-emission scenarios with active mitigation (RCP 2.6), through two intermediate pathways (RCP 4.5 and RCP 6.0), to a high-emission scenario (RCP 8.5). The RCP 8.5 scenario (i.e., worst-case emission pathway), characterized by a continuous rise of greenhouse gas emissions throughout the 21st century, projects a mean global temperature increase of +2.0°C by 2046-2056 and of +3.7°C by 2081-2100, leading to more rapid warming and more significant climate change[20].

In this study, the worst-case RCP8.5 scenario for Belgium in 2040 is used, as it is expected to result in the most severe ecological effects. To simulate this comprehensive climate scenario and assess its impact on AMF communities, we used the state-of-the-art Ecotron facility at Hasselt University (Figure 2). The Ecotron offers precise control over key climatic parameters, such as soil temperature, air humidity, and CO_2 levels, enabling the replication of both ambient (2013–2018) and future (2040) climate conditions. By simulating the full climate scenario 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 their effects on AMF communities [21] .

Figure 2: Ecotron Facility Maasmechelen, National Park Hoge Kempen (NPHK), Belgium

Addressing the gaps in current research on AMF responses to climate change, this study assesses how AMF spores, specifically associated with pears (*Pyrus communis L.*), respond to the projected 2040 climate conditions in Belgium, based on the worst-case RCP 8.5 scenario. This research focuses on the species diversity, community composition, and temporal dynamics of AMF spores under these climate conditions. The study hypothesizes that the AMF spore community in a pear orchard will exhibit more rapid and pronounced temporal changes under future climate conditions (RCP 8.5, 2040), with a significant shift in spore community composition, including an increased dominance of *Glomeraceae* species. Moreover it is hypothesized that overall community diversity remains unchanged compared to ambient conditions.

MATERIAL AND METHODS

We examined pear tree rhizosphere soil samples under both ambient (2013-2018) and future (2040, RCP8.5) climate conditions, addressing climate change impacts on AMF community diversity, composition, and AMF community dynamics across different pear tree phenophases (i.e., temporal dynamics), such as fruit growth, harvest, and dormancy. We sampled rhizosphere soil at three different timepoints during summer and autumn to capture phenological shifts in AMF community composition.

Climate manipulations.
This study is part of the broader QPear experiment, which aims to evaluate the impact of the projected 2040 Belgian climate under the RCP 8.5 scenario on pear tree growth, fruit quality, and orchard ecosystem functioning in Europe, compared to ambient conditions from 2013-2018. To replicate these conditions, the innovative Ecotron infrastructure in Maasmechelen, operated by Hasselt University, was utilized. This facility entails advanced macro-scale (167 m^3) sun-lit climate chambers designed to precisely regulate key climatic parameters, including air and soil temperature, air humidity, CO_2 levels, precipitation, groundwater content, and windspeed (Figure 1, https://www.uhasselt.be/en/instituten-en/cmk-centre-for-environmental-sciences/infrastructure/ecotron). Detailed description of the macro-scale Ecotron facility is provided by Rineau et al (2019) [21]. In late autumn 2021, twelve adult pear trees, each measuring three meters in height, were excavated along with 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 1.6 meters in depth), with two trees per lysimeter. Subsequently the trees were grown for one year in lysimeters in open air, exposing them to ambient climatic conditions. This pre-treatment facilitated the acclimation of both the trees and the soil to the lysimeter environment.

In January 2022, the lysimeters containing the trees were transported to the Ecotron, where they were exposed to one of two climatic conditions until December 2024: a typical climate of 2013-2018 period, or a typical year of 2040 climate. The climate scenarios were generated by (MAKE NE SUMMARIZING SENTENCE BASED on REF and REF), ensuring three biological replicates per condition. The experiment included two exposure periods: from 2022 to 2023 and from 2023 to 2024.

Sampling and Sample Processing.
Rhizosphere soil sampling was conducted in 2024 at three key phenological stages (summer and autumn) of the pear tree: fruit growth (16/07), harvest (17/09), and dormancy (26/11) (Figure 1). 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 60 cm radius of both three trunks, with one sample taken near each trunk and a third positioned between trunks, yielding a total of 54 soil samples. Sampling depths and three phenological states (i.e., fruits growing, pears an or leaves present on tree, leaves fallen, or pears harvested) were documented, and sampled regions were labeled to prevent resampling at the same location. After collection, soil samples were thoroughly mixed, cleared of debris and roots, and stored in labeled plastic bags for further analysis.

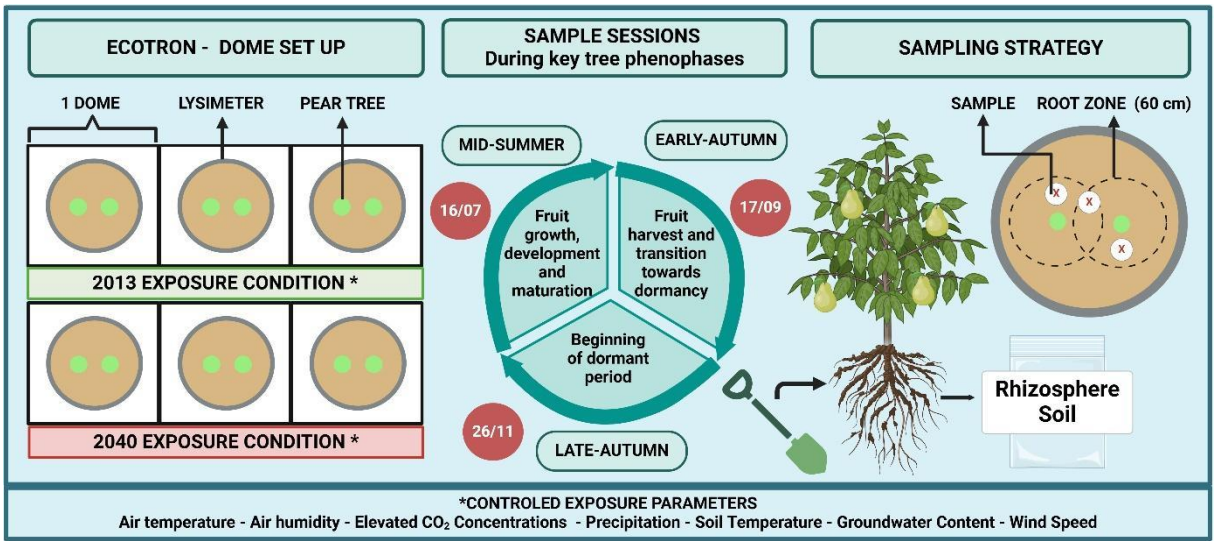


***Figure 3: ECOTRON Dome Setup, Sample Sessions, and Sampling** Strategy – White circles with a red cross indicate samples randomly collected at each sampling point to ensure unbiased data collection.*

Pilot Experiments – To optimize spore extraction techniques, two pilot experiments were conducted comparing the Ultrasound Wet-Sieving Technique (UWST) and Ultrasound Centrifuge Technique (UCT) [18]. Both methods were tested using a combination of Mycorrhiza Mix ("Mycorrhiza Mix" Snelkiemende Endomycorrhiza 50Gr—by Dutch Garden Seeds) and bulk soil samples, collected near a pear orchard with a low spore density, to assess their efficiency in spore recovery. The first pilot experiment aimed to identify the most effective technique, while the second focused on the impact of ultrasound exposure on spore integrity and recovery. These experiments guided the development of the final protocol, which employed UWST with ultrasound exposure to enhance spore extraction and recovery. The ultrasound effectively facilitated the separation of AMF spores from soil particles, optimizing the overall process and the subsequent visualization of spores. A full description of the pilot experiments can be found in Supplementary Methods Text 1.

Spore Extractions and Spore Quantification—AMF spores were extracted from the 54 processed rhizosphere soil samples using the UWST method, selected based on the prior pilot experiment results. Spore visualization was performed by placing 200 µL of spore suspension from primary extractions on a microscope slide, with digital spore images being captured using the Nikon SMZ800N stereomicroscope at 30× or 40× magnification and processed with NIS Elements software. The procedure was performed in triplicate to ensure accuracy. Due to potential damage, degradation, or overlap with plant material, some spores could not be confidently identified. These uncertain spores were categorized separately as a distinct group for further analysis. Subsequently, the numbers of spores per 200 µL of spore solution were determined 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 1 mL of spore suspension, W denotes the total volume of water used (mL), and S corresponds to the amount of soil processed (g). *SN* was initially determined for 200 µL of suspension and subsequently scaled to 1 mL (i.e., multiplication by 5), ensuring *W* is also standardized to 1 mL. In addition to these measurements, the lengths of all visualized spores were measured using ImageJ and systematically documented to help in spore species identification.

Spore Species Identification— The images were analyzed to identify the AMF species present. Gigasporaceae spores were distinguished from other AMF species, such as Glomeraceae, based on size, with Gigasporaceae spores measuring >150 µm and Glomeraceae spores <150 µm.

Statistics –To assess differences in AMF spore counts between ambient (2013-2018) and future (2040, RCP 8.5) climate conditions and between seasons, data analysis was conducted using R version 4.4.2. The statistical approach employed a Negative Binomial Generalized Linear Mixed Model (GLMM), implemented using the package glmmTMB (version 1.1.5), with additional diagnostics performed using performance (version 0.10.3) and DHARMa (version 0.4.6). The previously calculated Total AMF Spore Numbers (TSN) were used as the response variable to assess spore counts.

Before model selection, data were checked for overdispersion by comparing the mean and variance of TSN, along with evaluating residual patterns using the DHARMa package. Given that variance exceeded the mean, a Negative Binomial GLMM (nbinom2) was chosen to account for overdispersion. The model included climate condition (ambient vs. future) and season (early autumn, late autumn and middle summer) as fixed effects, while Unit was included as a random effect to account for within-experiment variability. To assess statistical differences in spore counts between climate conditions within each season, post-hoc pairwise comparisons were conducted using emmeans(), applying Tukey's adjustment for multiple comparisons. Additionally, differences among seasons within each climate condition were analyzed using the same approach. For data visualization, predicted spore counts were extracted using emmeans() and plotted using ggplot2 (version 3.3.5). Bar plots and scatter plots were generated to illustrate observed and predicted spore counts across climate conditions and seasons. To compare raw spore counts, boxplots were created for each climate condition and season.

PRELIMINARY RESULTS

The pilot experiments aimed to optimize spore extraction techniques and assess the impact of ultrasound on spore recovery and integrity. The Ultrasound Wet-Sieving Technique (UWST) proved to be the most effective method for spore extraction, ensuring maximum recovery efficiency, while the Ultrasound Centrifuge Technique (UCT) showed satisfactory results but was not as effective as UWST. The second pilot experiment demonstrated that ultrasound exposure enhanced spore recovery without compromising spore integrity.

While table 1 indicates the p-values derived from the negative binomial generalized linear mixed model (GLMM), which assessed the relationship between spore counts (i.e., Total Spore Number) and the interaction between climate and season while accounting for the random effects of Units, the figures (4 and 5) indicate observed spore counts between both climates (2013 and 2040) during each of the key phenological time points (Early-autumn, Late-autumn, and Mid-summer) and between key phenological time points during each of the climate conditions. The results on spore count analysis indicate that spore counts did not show significant differences between the ambient (2013-2018) and future (2040) climate conditions within each season ($p > 0.8$) (Table 1A, figure 3 and 4). However, seasonal variations differed significantly within both climate conditions, as mid-summer displayed significant higher median spore counts compared to late-autumn and early-autumn ($p = 0.0241$ for 2013-2018 and $p = 0.0051$ for 2040) (Table 1B). Moreover, early-autumn showed significant higher spore counts compared to mid-summer ($p = 0.0241$ for 2013-2018 and $p = 0.0345$ for 2040) (Table 1B). This was not the case between climates, as seasonal patterns were evidenced to not differ between 2013-2018 and 2040 (Table 1B, Figure 3 and 4).

Table 1: Overview on significancy– *(A) Comparisons for Climate (2013-2018 compared to 2040) within Each Season (B) Comparisons for Seasons within Each Climate (2013-2018 and 2040)*

A.

SEASON	P-Value
Early-autumn	0.8471
Late-autumn	0.8465
Mid-summer	0.9142

B.

COMPARISON	P-Value
Early-autum vs. Late-autumn (2013-2018)	0.8208
Early-autum vs. Mid-summer (2013-2018)	0.0241
Late-autum vs. Mid-summer(2013-2018)	0.0024
Early-autum vs. Late-autumn (2040)	0.8039
Early-autum vs. Mid-summer (2040)	0.0345
Late-autum vs. Mid-summer(2040)	0.0051

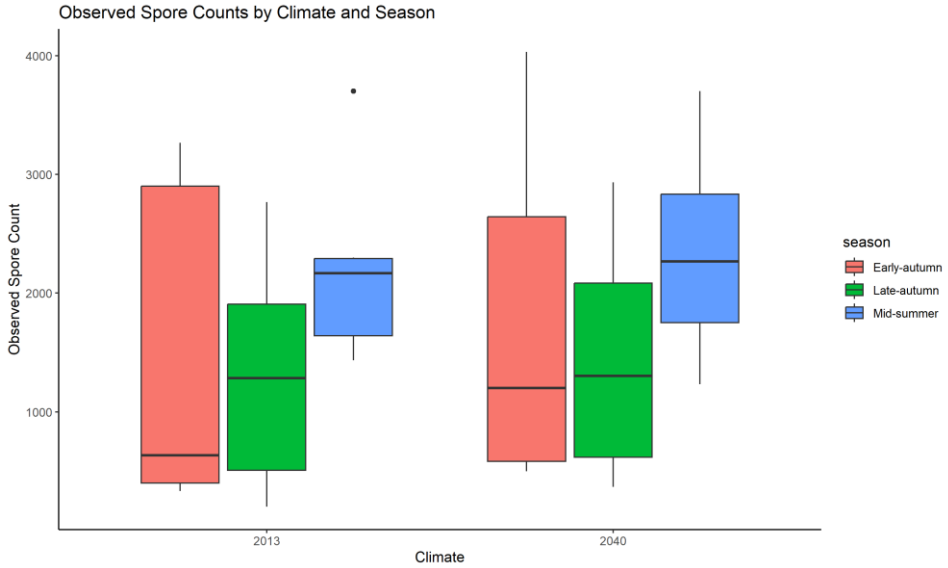


Figure 4: Comparison of observed spore counts between both climates (2013 and 2040) during each of the key phenological time points (Early-autumn, Late-autumn, and Mid-summer).

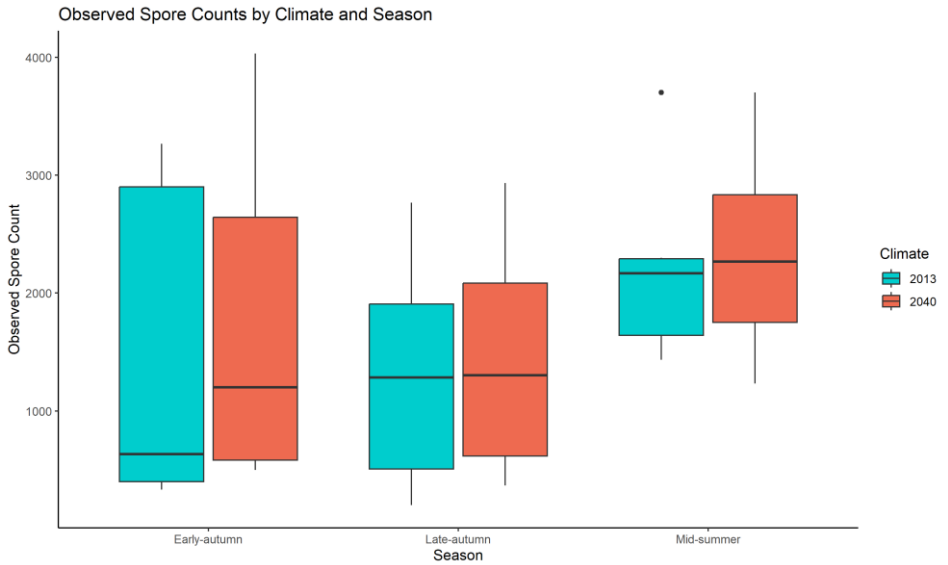


Figure 5: Comparison of observed spore counts between key phenological time points (Early-autumn, Late-autumn, and Mid-summer) for each of the climate conditions (2013 and 2040).

Additionally, the results from the Generalized Linear Mixed Model (GLMM) indicate that predicted spore counts did not differ significantly between both climate conditions nor in seasonal patterns between the climate conditions (Suppl. Figure 1 A and B). This was further evidences by the observed versus predicted spore counts (Suppl. Figure 2 A and B).

DISCUSSION

Climate change is a global challenge with far-reaching effects on both the environment and agriculture, impacting ecosystems worldwide and thereby societies. As the consequences of climate change become increasingly evident, we are observing shifts in weather patterns, ecosystem dynamics, and agricultural practices. Given that climate change is inevitable and plants, including crops, are sessile and unable to migrate, understanding potential adaptation strategies is essential. One such strategy involves Arbuscular Mycorrhizal Fungi (AMF), which play a crucial role in plant growth, health, and overall functioning. These soil fungi, which form symbiotic relationships with their host plants, enhance phosphate uptake and protect plants from soil pathogens, making them potential key players in helping plants adapt to the challenges posed by climate change [REF]. Therefore understanding the effects of climate change on key microbial communities that play essential roles in plant health and soil fertility is crucial. This study investigated the impact of projected climate change on arbuscular mycorrhizal fungi (AMF) in Belgian pear orchards, crucial for European agriculture, using a cutting-edge Ecotron facility to simulate both current (2013-2018) and future (2040) climate conditions under the RCP 8.5 scenario.

Research has shown that AMF are sensitive to climate conditions, with their diversity, distribution, and functions affected by direct climate variables and indirect changes in host plant physiology, soil properties, and nutrient availability. While individual parameters like drought, temperature, and CO2 levels influence AMF, the joint effects of these factors on AMF dynamics remain underexplored. This study aims to fill that gap by examining the full 2040 climate, rather than focusing on individual factors, and their impact on AMF, particularly the role of spores in adapting to new environmental conditions. Understanding these shifts is crucial for predicting how AMF may contribute to plant resilience under future climate scenarios.

Our results indicate that future climatic conditions do not significantly ($p > 0.8$) affect AMF reproductive capacity (i.e., spore abundance), indicated by spore count measurements during each of these climatic conditions. However, seasonal patterns show significant changes, particularly in mid-summer to early autumn ($p = 0.0241$ for 2013-2018 and $p = 0.0051$ for 2040) and mid-summer to late autumn ($p = 0.0241$ for 2013-2018 and $p = 0.0345$ for 2040) transitions (Table 1B). While these findings indicate critical insights into the phenological shifts of AMF communities within each climate condition, they are not different between the climatic conditions (i.e., 2013-2018 and 2040), indicating that climate change does not affect seasonal patterns. Nevertheless, community composition shows significant differences ($p = \ldots$), evidences by a shift in species composition; while Glomeraceae shows an increase in abundance Gigasporaceae shows a decrease.

These findings contrast with prior research examining AMF responses to individual or combined climatic parameters. Han et al. (2021) conducted a meta-analysis on AM fungal abundance and found that elevated CO_2 and warming significantly stimulated AM fungal abundance , with a critical threshold at a 4°C increase causing a decline. Furthermore, Zhang et al. (2016) demonstrated that elevated temperature and nitrogen addition suppress AMF spore density and diversity while increasing hyphal length density (HLD), potentially leading to a negative effect on AMF spore abundance. Interestingly, their findings suggest that AMF maintain their ecological role in supporting plant diversity and productivity despite these changes.

The observed stability in AMF reproductive capacity under future climate conditions could be explained by species-specific responses within the AMF community. As Zhang et al. (2016) noted, AMF can buffer plant communities against climate-induced degradation, potentially maintaining ecological functions even as individual species fluctuate in abundance. This resilience could account for our findings of seasonal shifts in spore abundance without significant overall differences between climate scenarios.

Our results indicate that shifts in AMF community composition could reflect similar ecosystem adaptations, where certain taxa like Glomeraceae become more dominant under changing conditions while others, like Gigasporaceae, decline.

The dynamics of arbuscular mycorrhizal (AM) fungi are critical for understanding ecosystem functioning, particularly in the face of environmental changes.

In the study by Cotton et al. (2015), the authors found that elevated atmospheric carbon dioxide (CO2) significantly altered the community composition of AM fungi, increasing the ratio of Glomeraceae to Gigasporaceae. This suggests that Glomeraceae may have a competitive advantage under higher CO2 conditions, likely due to their enhanced ability to adapt to changing environments. In contrast, Gigasporaceae did not exhibit a similar increase and showed vulnerability to changes, as noted in Alguacil et al. (2021), where a significant decrease in the abundance of Gigasporaceae was recorded under simulated climate warming and drought conditions. Their findings indicated that Gigasporaceae family members suffered dramatic reductions, with abundances decreasing by 67% and 77% in response to warming and rainfall reduction, respectively. This contrast in responses highlights the potential for Glomeraceae to dominate in future ecosystems, especially as climate change intensifies.

Moreover, the capacity for hyphal network formation further distinguishes these two families. Research by de la Providencia et al. (2005) suggests that Glomeraceae exhibit greater plasticity in forming anastomoses, allowing them to connect different mycorrhizal networks effectively. This ability to form extensive hyphal networks enhances resource allocation and nutrient exchange among plants (Smith & Read, 1997). In contrast, Gigasporaceae primarily form anastomoses within the same hypha, limiting their networking capabilities and potentially their ecological effectiveness. Given these differences, it is plausible that the superior networking abilities of Glomeraceae contribute to their increased abundance, as indicated in Table 2 of our findings, which provides evidence of their dominance over Gigasporaceae.

Furthermore, Cotton et al. (2015) emphasize the importance of interannual temporal dynamics in shaping AM fungal communities. Their findings indicate that large-scale interannual variations may have a more significant influence on community composition than atmospheric changes alone. This insight aligns with our observations, where fluctuations in the abundance of Glomeraceae and Gigasporaceae were noted over time, reinforcing the idea that natural environmental variations play a crucial role in determining AM fungal dynamics.

A key observation from our study is the seasonal stability of AMF spore counts during autumn, contrasted by an increase toward the summer period. The rise in mid-summer spore counts aligns with previous research indicating that higher temperatures enhance AMF sporulation and colonization in temperate regions (Smith & Read, 2008; Treseder et al., 2018). However, the absence of significant differences between current and future climate conditions suggests that AMF communities may exhibit a degree of resilience to climate change, at least within the temporal scale of our study.

Research has shown that spores of *Glomeraceae* species are often more resilient to environmental stressors, such as elevated CO_2, which suggests their potential to become more dominant in future climate [12].

CONCLUSION

This research is pivotal for uncovering climate-driven AMF dynamics in agricultural systems, offering valuable insights that will inform global change models and contribute to strategies for sustaining crop yields in an increasingly unpredictable climate.

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Supplementary Methods Text 1.

Pilot Experiments – Prior to performing spore extractions on the rhizosphere soil samples, two pilot experiments were conducted to optimize spore extraction technique: Ultrasound Wet-Sieving Technique (UWST) or 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. The protocols for UWST and UCT were based on previous research [22], with modifications to the sieve sizes used (1 mm, 100 µm, and 65 µm). These pilot experiments allowed for the optimization of spore extractions, 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 65 µm sieves. The 100 µm and 65 µm sieve contents were collected, washed with dH_2O, and exposed to ultrasound (30 sec at 28 kHz) three times. The final sieve content was washed with a 55% sucrose solution and centrifuged (7 min at 1500 rpm) to create gradient separation. The supernatant was then passed through a 65 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 bulk soil and one gram of Mycorrhiza Mix (1:1) in 40 mL of dH_2O, using a magnetic stirrer (5 min at 4-5 rpm), and subjected to an ultrasound bath (30 sec at 28 kHz). The suspension was then centrifuged (3 min at 3000 rpm), and the supernatant was passed through a 65 µm sieve. The final sieve content was collected using 20 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 **(NAME)**, and images were captured for subsequent analysis in ImageJ (win64). The number of spores per milliliter of spore solution was quantified through ImageJ analaysis.

Pilot experiment 1— The first pilot experiment aimed to compare UWST and UCT to determine the most effective spore extraction technique. One gram of Mycorrhiza Mix in powdered form, obtained by sieving to isolate fine particles, was used. This pilot experiment was crucial, as subsequent analyses will utilize homogenized soil rather than the aggregate clusters present in the Mycorrhiza Mix, ensuring a more standardized basis for evaluating both methods.

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. This comparison evaluated whether ultrasound exposure enhanced spore recovery while maintaining their 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 all observed spores on the slide were captured using a stereomicroscope (Nikon SMZ800N) at 30× or 40× magnification and processed with NIS Elements software **(VERSION)**. The procedure was conducted in triplicate, ensuring three technical replicates for accuracy.

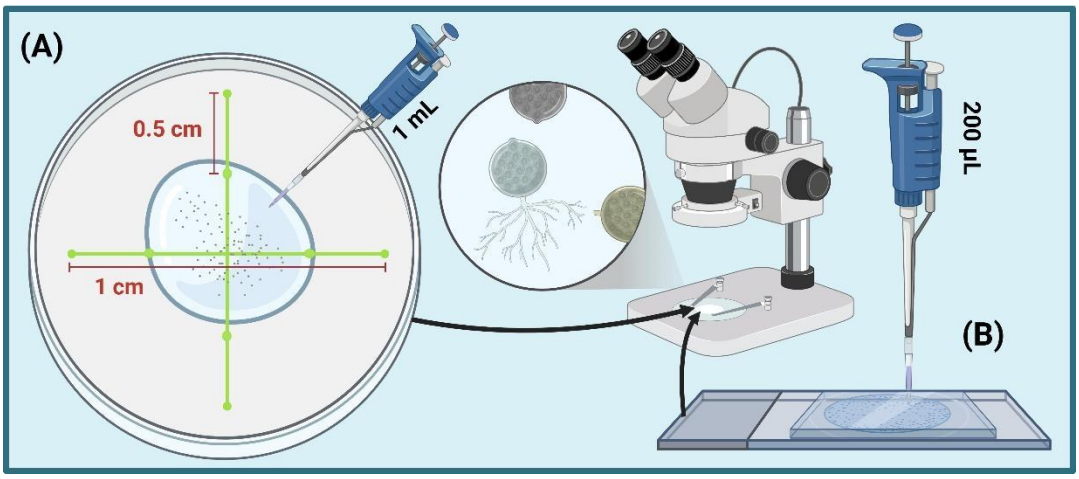
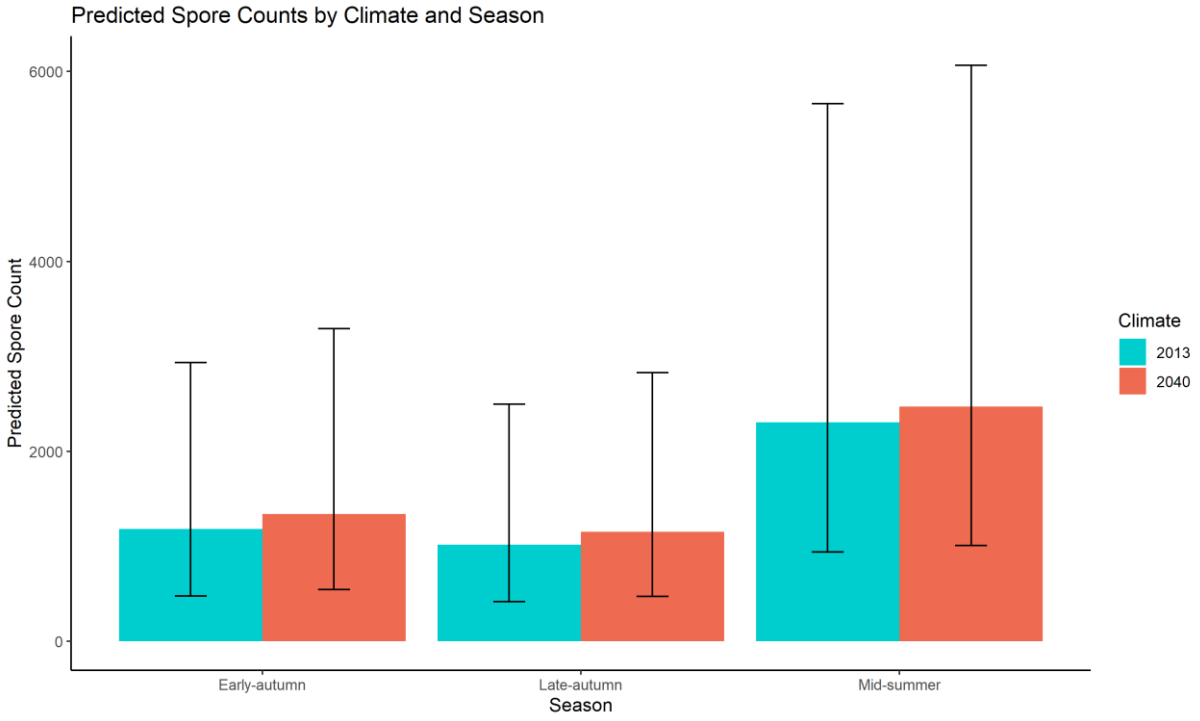


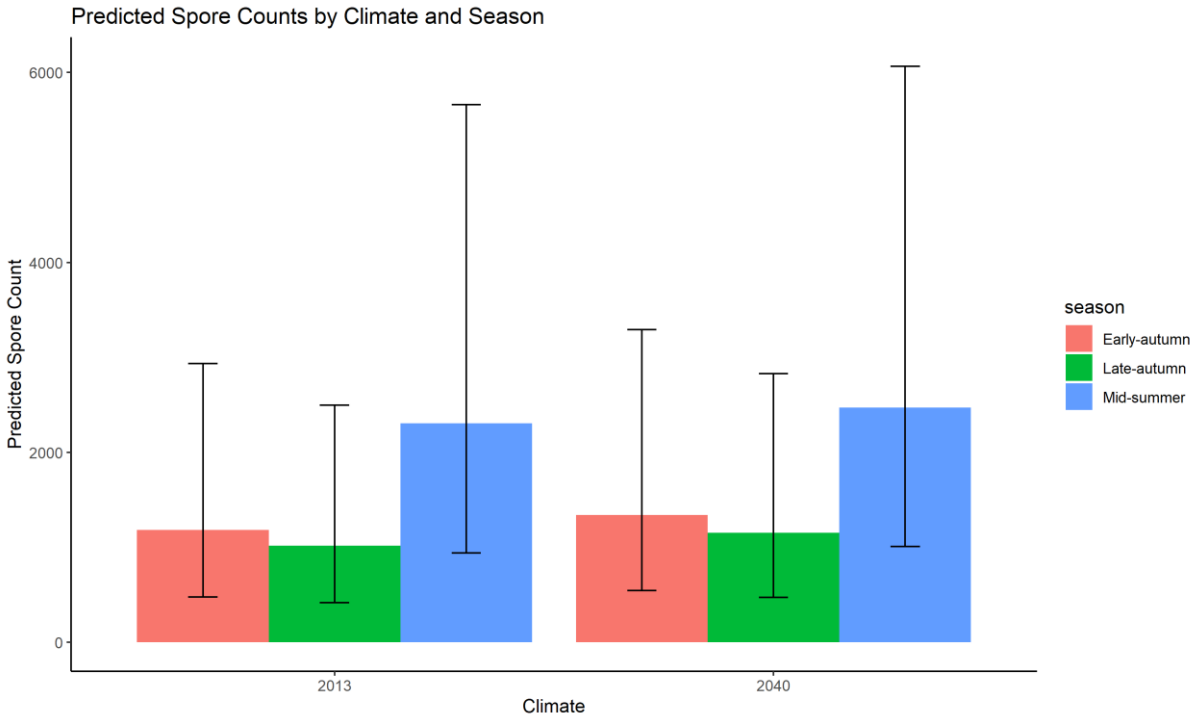
Figure S1: Spore visualization methods (A) Measuring grid on Petri dish with 1 mL of spore solution. (B) Microscopic slide with 200 µL of spore solution covered with a slide cover. (A-B) Both are visualized for arbuscular mycorrhizal fungal (AMF) spores using a stereomicroscope.

Supplementary Figure 1.

A.

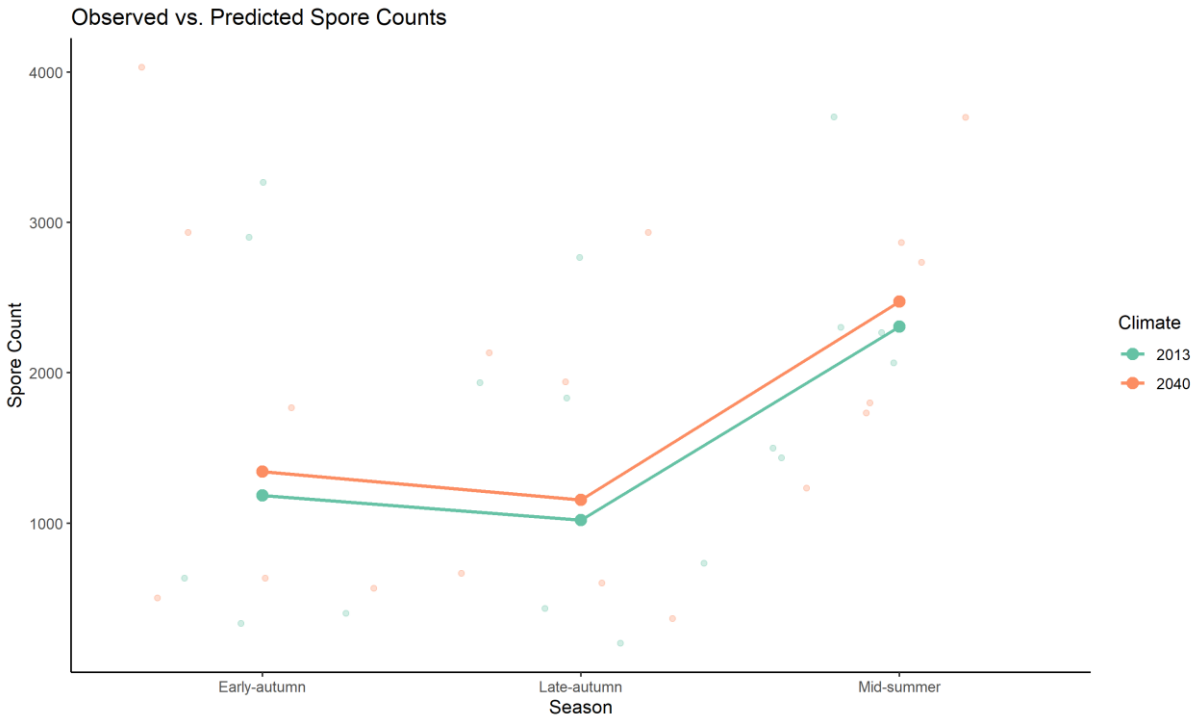


B.

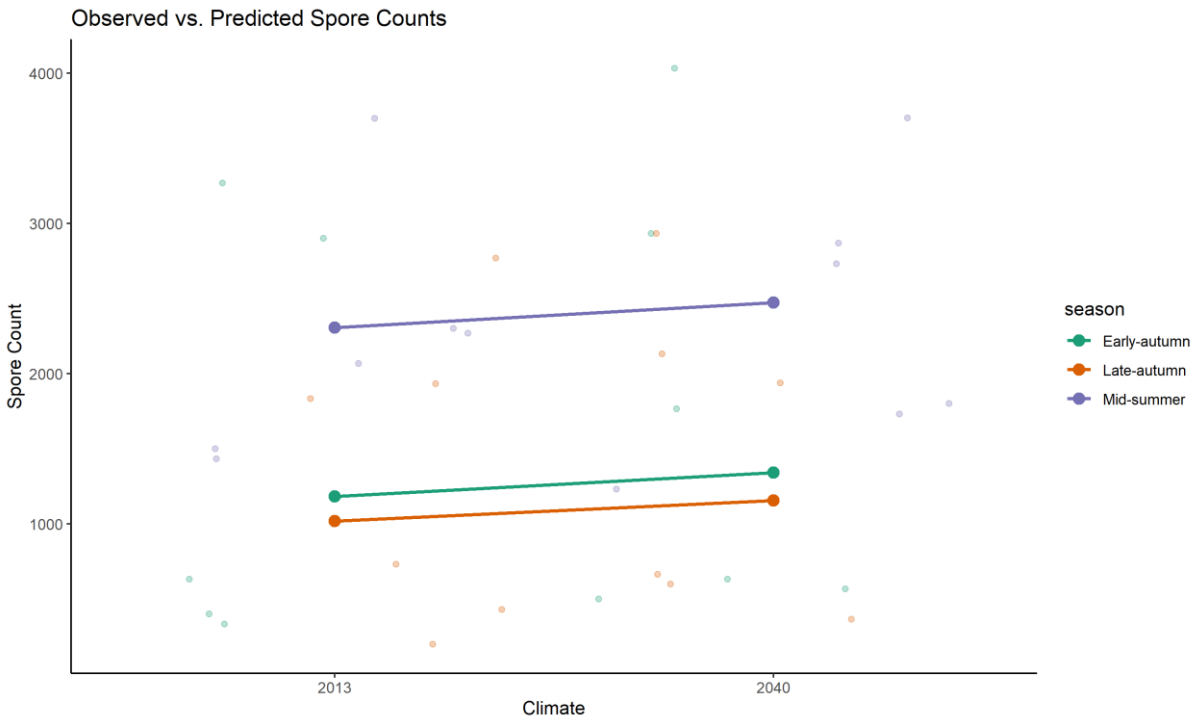


Supplementary Figure 2.

A.



B.


Observed vs. Predicted Spore Counts
Spore Count
4000
3000
2000
1000
2013
2040
Climate
season
Early-autumn
Late-autumn
Mid-summer