# INVESTIGATING THE ABILITY OF ARBUSCULAR MYCORRHIZAL FUNGI TO MITIGATE THE NEGATIVE EFFECTS OF WARMING AND DROUGHT ON NATIVE PERENNIAL FORBS

by

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# DEDICATION

My thesis is dedicated to my parents, Mari and Bill Eggers. Thank you for your constant love, support, and encouragement. I could not have done this without you.

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# ABSTRACT

The ability of arbuscular mycorrhizal fungi (AMF) to mitigate the negative effects of warming and drought on plant hosts is known for crop species but is poorly understood for native, perennial forbs. Examining the indirect influence of AMF on forbs' responses to these stressors will provide a more complete understanding of how native forbs will be affected by climate change. In an experimental greenhouse study, we inoculated two native forb species (*Achillea millefolium* and *Linum lewisii*) with three separate AMF species (*Rhizophagus clarus*, *Claroideoglomus etunicatum*, and *Gigaspora rosea*), then exposed plants, including an uninoculated control treatment, to varying degrees of drought and heat stress in a factorial design. We tested the effects of warming or drought treatments on plants' physical, floral, phenological, and physiological traits, including biomass, height, floral abundance, flower size, first date of flowering, floral scent, and photosynthetic performance. For both forbs, AMF ameliorated the negative effects of drought and warming on plant survival and vegetative growth, but the magnitude of effect was specific to the forb species, climate treatment, and AMF inoculant. AMF also produced changes in forb phenology, floral scent (volatile organic compounds), and flowering success and duration, which have broad implications for plant-pollinator interactions and the links between belowground and aboveground symbioses. Together, these results indicate that AMF can assist native forbs in surviving, growing, and reproducing in a warmer and drier climate.

# CHAPTER ONE

## INTRODUCTION

Terrestrial ecosystems will be challenged by both the direct and indirect consequences of anthropogenic climate change. As temperatures continue to rise and drought worsens in severity and frequency, plants respond directly to environmental change by modifying their physiology, phenology, and distribution (Kivlin et al., 2013; Parmesan and Hanley, 2015). Many plant populations have gradually shifted their ranges toward cooler northern latitudes and higher elevations (Jackson and Overpeck, 2000). Warmer spring temperatures and earlier snow melt have led to shifts in phenology for a diverse range of plant taxa, including herbaceous, graminoid, and woody species (Richardson et al., 2013; Rudgers et al., 2014). Elevated atmospheric $CO_2$ has increased plant photosynthetic rate and therefore total biomass for many woody species (Curtis and Wang, 1998). However, this body of research has not assessed the complex, indirect influence of the belowground ecosystem on plant response to climate change.

Soil biota play an important role in the relationship between climate and plants. Mycorrhizal fungi, for example, can enhance plant uptake of mineral nutrients and increase water supply, providing resistance to warming and drought stress (Al-Karaki and Al-Raddad, 1997; Augé, 2004). Fungal hyphae are finer in diameter than plant root hairs, allowing for greater access to nutrients, such as phosphorous and nitrogen, and moisture in the soil (Drew et al., 2003). Additionally, fungi are considered the most effective soil biota in stabilizing soil structure by creating a network of mycelium that holds soil particles together via physical entanglement (Augé, 2004). This creates an ideal environment for microaggregate formation and improved porosity, influencing soil water holding capacity (Augé, 2004). The combination of mycelial diameter and improved soil structure can increase fungal uptake of water and nutrients,

allowing plants to maintain a steadier water balance and fix more carbon during warming or drought stress (Augé, 2001, 2004). In return for this aid, plants allocate photosynthetically-produced carbohydrates to mycorrhizal symbionts (Öpik et al., 2006).

Arbuscular mycorrhizal fungi (AMF) form the most common mycorrhizal symbioses, associating with roughly 200,000 plant species (Barber and Soper Gorden, 2015). Research has shown that AMF can increase the biomass, phosphorous uptake, and photosynthetic activity of climate-stressed host plants (Al-Karaki and Al-Raddad, 1997; Augé, 2001; Wu et al., 2013). AMF have also been shown to modify plant floral traits, such as flower size, flower abundance, phenology, and floral volatile organic compounds, which in turn can impact plant-pollinator interactions (Bennett and Meek, 2020; Ceccarelli et al., 2010; Gange and Smith, 2005; Lazzara et al., 2017; Pendleton, 2000; Poulton et al., 2001, 2002; Varga and Kytöviita, 2010; Wolfe et al., 2005). However, research to date has not thoroughly assessed the combined effects of climate stress and AMF association on native forbs, especially on floral traits that inform fitness and reproduction. This research gap limits our understanding of the impacts of climate change on native plants, as well as indirect impacts on plant-pollinator interactions. My thesis provides evidence that AMF can help mitigate the negative effects of warming and drought on two native, perennial forbs, but the magnitude of effect is specific to the forb species, climate treatment, and AMF inoculant.

References Cited

Al-Karaki, G. and A. Al-Raddad. 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7: 83-88.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

Augé, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84: 373-381.

Barber, N. A. and N. L. Soper Gorden. 2015. How do belowground organisms influence plant–pollinator interactions? Journal of Plant Ecology 8: 1-11.

Bennett, A. E. and H. C. Meek. 2020. The influence of arbuscular mycorrhizal fungi on plant reproduction. Journal of Chemical Ecology 46: 707-721.

Ceccarelli, N., M. Curadi, L. Martelloni, C. Sbrana, P. Picciarelli and M. Giovannetti. 2010. Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. Plant and Soil 335: 311-323.

Curtis, P. S. and X. Wang. 1998. A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113: 299-313.

Drew, E., R. Murray, S. Smith and I. Jakobsen. 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant and Soil 251: 105-114.

Gange, A. C. and A. K. Smith. 2005. Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. Ecological Entomology 30: 600-606.

Jackson, S. T. and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26: 194-220.

Kivlin, S. N., S. M. Emery and J. A. Rudgers. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany 100: 1445-1457.

Lazzara, S., M. Militello, A. Carrubba, E. Napoli and S. Saia. 2017. Arbuscular mycorrhizal fungi altered the hypericin, pseudohypericin, and hyperforin content in flowers of Hypericum perforatum grown under contrasting P availability in a highly organic substrate. Mycorrhiza 27: 345-354.

Öpik, M., M. Moora, J. Liira and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. Journal of Ecology 94: 778-790.

Parmesan, C. and M. E. Hanley. 2015. Plants and climate change: complexities and surprises. Annals of Botany 116: 849-864.

Pendleton, R. L. 2000. Pre-inoculation by an arbuscular mycorrhizal fungus enhances male reproductive output of Cucurbita foetidissima. International Journal of Plant Sciences 161: 683-689.

Piao, S., Q. Liu, A. Chen, I. A. Janssens, Y. Fu, J. Dai, L. Liu, et al. 2019. Plant phenology and global climate change: Current progresses and challenges. Global Change Biology 25: 1922-1940.

Pischl, P. H. and N. A. Barber. 2017. Plant responses to arbuscular mycorrhizae under elevated temperature and drought. Journal of Plant Ecology 10: 692-701.

Poulton, J., R. Koide and A. Stephenson. 2001. Effects of mycorrhizal infection, soil phosphorus availability and fruit production on the male function in two cultivars of Lycopersicon esculentum. Plant, cell & environment 24: 841-849.

Poulton, J. L., D. Bryla, R. T. Koide and A. G. Stephenson. 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. New Phytologist 154: 255-264.

Pugnaire, F. I., J. A. Morillo, J. Peñuelas, P. B. Reich, R. D. Bardgett, A. Gaxiola, D. A. Wardle and W. H. Van Der Putten. 2019. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. Science advances 5: eaaz1834.

Richardson, A. D., T. F. Keenan, M. Migliavacca, Y. Ryu, O. Sonnentag and M. Toomey. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology 169: 156-173.

Rudgers, J. A., S. N. Kivlin, K. D. Whitney, M. V. Price, N. M. Waser and J. Harte. 2014. Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. Ecology 95: 1918-1928.

Varga, S. and M. M. Kytöviita. 2010. Gender dimorphism and mycorrhizal symbiosis affect floral visitors and reproductive output in Geranium sylvaticum. Functional ecology 24: 750-758.

Wolfe, B. E., B. C. Husband and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8: 218-223.

Wu, Q.-S., A. K. Srivastava and Y.-N. Zou. 2013. AMF-induced tolerance to drought stress in citrus: a review. Scientia Horticulturae 164: 77-87.

# CHAPTER TWO

## INVESTIGATING THE ABILITY OF ARBUSCULAR MYCORRHIZAL FUNGI TO MITIGATE THE NEGATIVE EFFECTS OF WARMING AND DROUGHT ON NATIVE PERENNIAL FORBS

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## Introduction

The global climate is projected to change rapidly over the next century, impacting aboveground and belowground ecosystems. Shifting precipitation patterns, increasing atmospheric carbon dioxide, and rising temperatures have and will continue to affect terrestrial ecosystems, including plant communities across North America (Hostetler et al., 2021; Whitlock et al., 2017). Plants can respond directly to environmental change by modifying their physiology, phenology, and distribution (Parmesan and Hanley, 2015; Piao et al., 2019). Considerable research has focused on the direct response of plants to climate change (Parmesan and Hanley, 2015). However, this body of research focuses mainly on aboveground changes, with less emphasis on the complex, indirect influence of the belowground ecosystem on plant response to climate change.

Mycorrhizae, fungi that form symbiotic relationships with plants, play an important role by indirectly influencing plant response to environmental change (Kivlin et al., 2013). Mycorrhizal fungi can improve plant nutrition and growth by enhancing the uptake of mineral nutrients, such as phosphorus, zinc, and copper, as well as providing drought resistance through increased water uptake via an extensive network of fungal hyphae (Al-Karaki and Al-Raddad, 1997; Augé, 2004). In exchange, the host plant allocates photosynthetically produced carbohydrates to the fungal partner (Öpik et al., 2006). In terrestrial systems, arbuscular mycorrhizal fungi (AMF) form the most common mycorrhizal symbioses, associating with roughly 80% of vascular plants (Basu et al., 2015). AMF association can increase biomass, phosphorous uptake, and photosynthetic activity in climate-stressed host plants (Al-Karaki and Al-Raddad, 1997; Augé, 2001). AMF also can alter plant floral traits, such as flower size (Bennett and Meek, 2020; Gange and Smith, 2005; Wolfe et al., 2005; Varga and Kytöviita,

2010), flower abundance (Gange and Smith, 2005; Lazzara et al., 2017; Pendleton, 2000; Poulton et al., 2001, 2002), phenology (Poulton et al., 2002), and floral volatile organic compounds (VOCs) (Ceccarelli et al., 2010), which in turn can impact plant-pollinator interactions (Gange and Smith, 2005; Wolfe et al., 2005; Varga and Kytöviita, 2010).

However, as research to date has focused primarily on testing how AMF mediates the effects of climate stress on crop species or grasses, native forbs have received less attention (e.g. Mathur et al., 2018; Wu et al., 2013). This research gap limits our understanding of the impacts of warming and drought on natural plant communities, as well as indirect impacts on plant-pollinator interactions. Examining this complexity will give us a better understanding of how belowground symbiosis will directly affect native plants and indirectly affect pollinators in warmer and drier North American ecosystems.

Impact of AMF on temperature-stressed plants

The intensity and frequency of high temperatures are predicted to increase drastically over the next century, affecting plant morphological traits, biochemical processes, and physiological growth with major repercussions on natural and agricultural systems (Solomon et al., 2007). Fungal symbionts, including AMF, are expected to benefit plant hosts due to the heightened demand for nutrients imposed by increased plant metabolism in warmer conditions (Kivlin et al., 2013). Given the nature of mycorrhizal symbiosis, it is posited that AMF will enhance plant tolerance to abiotic stressors, such as increased temperatures (Mathur et al., 2018).

Research investigating the impacts of AMF on temperature-stressed plants has focused primarily on crop species. AMF inoculation is known to increase biomass, height, growth rate, and photosynthate production for maize, trifoliate orange, strawberry under temperature stress

(Wu et al., 2013). However, there has been minimal research on the potential for AMF to modify native plant traits and responses to warming in natural systems. Rudgers and colleagues began to address this knowledge gap through their investigation of the effects of warming on the composition of graminoids and their fungal partners in a field-based, high-elevation ecosystem (Rudgers et al., 2014). They found that experimental warming increased AMF colonization of two grass species (*Festuca thurberi* and *Achnatherum lettermanii*) and a shrub (*Artemesia tridentata*) but subsequent effects on plant traits were not measured. Rudgers et al. (2014) is one of few studies attempting to quantify the effects of AMF on temperature-stressed native forb communities.

Impacts of AMF on drought-stressed plants

A meta-analysis by Kivlin et al. (2013) found that AMF consistently reduced the negative effects of drought on plant biomass in both experimental greenhouse studies and natural field systems. The abundance of AMF was often inversely correlated with soil moisture, suggesting the importance of this symbiosis in water-stressed systems (Kivlin et al., 2013). Similarly, a meta-analysis by Worchel et al. (2013) found that AMF promoted growth in drought stressed C3 grasses, which have lower water use efficiencies than C4 grasses and therefore benefited more from AMF under drought; droughted and well-watered C4 grasses were unaffected by AMF.

There are several ways AMF can promote plant growth under drought. First, the mycelial network increasing root surface area and improving soil structure can increase fungal water and mineral uptake, allowing plants to maintain steadier water balance and fix more carbon during drought stress (Drew et al., 2003). Second, AMF improve phosphorous uptake during periods of drought (Al-Karaki and Al-Raddad, 1997; Augé, 2001). Additionally, AMF species vary in their

ability to aid plants under abiotic stress based on the extent of their hyphal networks and contributions of mycorrhizal-root water uptake, making some better than others at mediating drought conditions (Kivlin et al., 2013). Lastly, host plant genotypes can vary in the degree to which they are dependent upon AMF symbioses for nutrient uptake in dry soil conditions (Al-Karaki and Al-Raddad, 1997).

Although previous literature has shown that AMF can increase vegetative growth of host plants under drought stress, the response of physiological, floral, and phenological plant traits to drought and AMF is not well understood.

## Addressing the Research Gap

Few studies have evaluated plant trait response to AMF and climate stress outside of biomass, height, and colonization. Measuring plant floral traits that inform fitness, reproduction, and interactions with pollinators in combination with climate treatments will provide a more comprehensive understanding of plant response. Currently, research on AMF inoculation of forb species has shown a generally positive influence on floral display, nectar quantity, and nectar quality (Gange and Smith, 2005; Keeler et al., 2021; Laird and Addicott, 2007; Pendleton, 2000; Poulton et al., 2001; Wolfe et al., 2005). There is less certainty about the effects of AMF on floral volatile organic compounds, but the potential for AMF modification of relative compound abundance is strong due to mycorrhizal changes in plant water and nutrient availability (Besmer and Koide, 1999; Ceccarelli et al., 2010). Additionally, a handful of studies have reported mixed, species-specific effects of AMF on plant-pollinator dynamics through floral trait changes. Association with AMF can increase pollinator visitation, shift the community of pollinator visitors, and change plant community competition among species (Cahill et al., 2008; Gange and

Smith, 2005; Wolfe et al., 2005). However, this body of research does not address how climate change impacts the relationships among AMF, floral traits, and pollinators. Research on how floral traits, such as flower size, floral volatiles, and phenology, are altered by AMF and climate manipulations is needed. Basic measurements of biomass or height will not provide a complete assessment of how plant populations and communities will change in the coming decades. Moving forward, research should include robust plant trait analysis, including floral traits that inform fitness and reproduction, to better understand the links between above and belowground species interactions.

# Methods

## Source and Materials for Plants

We used an experimental greenhouse study to test the relationship between forbs, AMF, and climate treatments (drought or temperature). Seeds of two native forbs (*Achillea millefolium*, yarrow; and *Linus lewisii*, wild blue flax) were bought from Montana plant nurseries and tested for germination success in growth chambers (at temperatures 25 °C, 28.1 °C, and 30.4 °C). These species were selected for their wide distribution in native plant communities across North America, difference in vegetative form, floral structure, and inflorescence type, and varied trait response to AMF inoculation (Burkle and Zabinski, in review).

In fall 2019, *Achillea millefolium* and *Linus lewisii* were seeded for the temperature treatment experiment with 5 seeds per cone-tainer (approximately 7cm diameter x 24cm deep). A total of 120 cone-tainers (hereafter, pot) were seeded for each forb species in the temperature treatment (240 total). In fall 2020, *Achillea millefolium* and *Linus lewisii* were seeded for the drought treatment experiment with 5 seeds per pot, for a total of 160 pots per species (320 total). Germinants were thinned to one individual plant (largest and most centered) per pot, roughly one to two weeks after seeding.

## AMF and Pot Preparation

Three AMF species, *Gigaspora rosea, Claroideoglomus etunicatum*, and *Rhizophagus clarus*, were selected to test the effect of mycorrhizal symbiosis on plant traits. AMF inoculants were purchased from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM), West Virginia, USA. Selection of AMF species was based on previous results

of AMF and the selected species' success in altering floral traits (Burkle and Zabinski, in review).

To prevent biological contamination in potting, all pots were washed in a 10% bleach solution and topsoil was mixed with a 3:1 ratio of soil to sand and pasteurized at 70 °C for one hour. Polyester mesh squares were placed into the bottom of pots to cover drainage holes and prevent mass soil leakage. From bottom to top, each pot was filled with 250 ml soil-sand mix, 250 ml soil-sand and respective inoculum mix (AMF inoculum concentration of 20ml/pot) and capped with 50 ml soil-sand mix. For each experiment, uninoculated controls (no AMF) consisted of pots filled entirely with 550 ml sterilized soil-sand mix.

Treatment Combinations

For the temperature experiment, treatment combinations included three separate species of AMF inoculum (*G. rosea, C. etunicatum, R. clarus*) and a sterile soil control, two species of native, perennial forbs (*A. millefolium* and *L. lewisii*), and three temperature growth chambers (25 °C, 28.1 °C, and 30.4 °C) in a factorial design. Each treatment combination (3 temperatures x 4 AMF treatments x 2 host plant species) had 10 replicates, for a total of 240 pots across all treatments. For the drought experiment, treatment combinations included three separate species of AMF inoculum (*G. rosea, C. etunicatum, R. clarus*) and a sterile soil control, two species of native, perennial forbs (*A. millefolium* and *L. lewisii*), and a well-watered or drought treatment, in a factorial design. Each treatment combination (2 watering regimes x 4 AMF treatments x 2 host plant species) had 20 replicates, for a total of 320 pots across all treatments.

Temperature Treatments

To investigate how warming temperatures interact with forbs and AMF, three temperatures were selected from the 2017 Montana Climate Assessment (Whitlock et al., 2017) to simulate average summer temperatures for Montana at present (25 °C), under business-as-usual projections for 2050 (28.1 °C), and under business-as-usual projections for 2100 (30.4 °C). Three separate plant growth chambers were set to 25 °C, 28.1 °C, and 30.4 °C, while photoperiod (16-hour), light intensity (200 $\mu$mol), and other environmental variables were held constant. Pots were placed in growth chambers immediately after seeding in October 2019. Because more water loss can occur due to evaporation under warmer temperatures, pots were watered to maintain uniform moisture levels across growth chambers; 15ml in the 25 °C, 40ml in the 28.1 °C, and 80ml in the 30.4 °C (temperatures hereafter called 25 °C, 28 °C, and 30 °C for simplicity). Racks of plants were rotated in growth chambers weekly to account for variability within each growth chamber. Starting December 2019, 20ml of ½ strength Hoagland's solution was applied to each pot once every two weeks.

Plants received temperature treatments for three months in the growth chambers from October 2019 to December 2019, before being vernalized in a climate-controlled chamber at approximately 4 °C with a 12-h photoperiod until March 2020. All individuals were then removed from the cold room and returned to their respective growth chambers set to the same temperature treatments (25 °C, 28 °C, and 30 °C). In June 2020, due to plants' slow growth rate, all pots were moved to a greenhouse with a day/night temperature regime of 26 °C/15 °C to promote growth and flowering. Plants remained in the greenhouse from June 2020 to December 2020 and racks were rotated weekly. Plants were watered to field capacity every other day and Hoagland's solution application continued. Beginning December 2020, plants were moved back

to the cold room (4 °C with a 12-h photoperiod) for vernalization until April 2021. In April 2021, the plants were returned to the greenhouse to promote growth and flowering before being harvested in October 2021.

Drought Treatments

To test how reduced soil moisture interacts with forbs and AMF, inoculated plants and a sterile soil control were treated with a post-establishment period of pulsed drought or watering to field capacity. Beginning October 2020, all plants were allowed to establish and grow for four months in the greenhouse (day/night temperature regime of 26 °C/15 °C), watered to field capacity on alternate days and fertilized with 20ml of ½ strength Hoagland's solution biweekly. In December 2020, individuals were then moved to the cold room (4 °C) and vernalized, before being returned to the greenhouse in March 2021. Plants were allowed to acclimate to greenhouse conditions for a month before the drought treatment began in April 2021.

To impose drought treatments, water was withheld from plants until the first signs of wilting were visible in ≥75% of individuals per group (on average, four days without watering). Droughted plants were then watered to field capacity. The following day, water was again withheld until the first signs of wilting were visible in ≥75% of individuals, repeating the cycle. First signs of wilting were defined as any visible loss of rigidity in vegetative tissue, which was assessed daily for all plants. Starting April 2021, plants received drought treatment throughout the duration of flowering until harvest in October 2021. This pulsed drought cycle aims to mimic natural drought conditions (Huberty & Denno, 2004) and while also allowing flowering to occur (plants subjected to continuous drought did not produce flowers, Burkle and Runyon 2016).

Due to the slow growth and poor establishment of *L. lewisii* in the uninoculated control treatment and in the *G. rosea,* and *R. clarus* inoculated soils, these plants did not receive drought treatment. However, *L. lewisii* inoculated with *C. etunicatum* did receive drought treatment due to their vigorous growth.

Measuring Plant Traits

Physical (plant height), floral (flower size, floral abundance, volatile organic compounds), phenological (first day of flowering, flowering duration), and physiological (Fv/Fm, photosynthetic performance) plant traits were measured to evaluate the effects of mycorrhizal and climate treatments. Plant height, flower size, and floral abundance were measured daily from the opening of the first flower to harvest in November 2021. Plant height (cm) was estimated as the distance from the soil surface to the tallest living part of the plant. Flower size was estimated by measuring length (a) and width (b) of each flower using digital calipers. These values were used to calculate floral area ($cm^2$) using the formula for the area of an ellipse, ($A = \pi ab$). *Achillea millefolium* plants produced a single umbel that grew in size over the course of the season, so flower size was estimated as the maximum length and width of the umbel produced by a plant by the end of the season. *Linum lewisii* plants produced new flowers every day, so flower size was measured as the length and width of each flower produced; floral area was averaged across all flowers produced by an individual plant. Floral abundance was calculated for *L. lewisii* as the number of new, open flowers produced by a plant during its lifetime. First day of flowering was measured as the number of days that elapsed between seeding and the production of the first flower. Flowering duration was calculated as the total number of days an individual had open flowers (not senesced).

Fv/Fm (ratio of variable to maximum fluorescence after dark adaptation), which measures the efficiency of Photosystem II (photosynthetic performance) and is a proxy for plant stress (i.e., high efficiency indicates low stress), was measured once at ambient greenhouse temperature for all living individuals from both the temperature and drought treatments shortly after peak flowering in the summer of 2021 (Murchie and Lawson, 2013). These measurements were taken predawn, to ensure leaves were dark-adapted, using a LICOR (LI-6800, Licor, Lincoln, NE, USA) clamped onto a single, healthy leaf.

To collect floral volatiles, flowers were encased in 950ml polyethylene cups with domed lids (Dart Container Corporation, Mason, MI, USA). Portable volatile collection systems (Volatile Assay Systems, Rensselaer, NY, USA) pulled air (0.5L $min^{-1}$) for one hour through volatile traps containing 30 mg of the adsorbent HayeSep-Q (Restek, Bellefonte, PA, USA). Volatiles were collected from every flowering individual once during the day. Due to the difference in floral inflorescence structure, the number of flowers being sampled varied between *L. lewisii* and *A. millefolium*. For *L. lewisii*, an individual flower was encased, as compared to *A. millefolium* where the entire umbel was encased and sampled from. To account for this difference, volatile emissions were standardized by mean floral area encased and reported as VOC emissions per floral area per hour (ng $mm^{-2}$ $h^{-1}$). Volatiles were eluted from traps using 200 $\mu L$ of dichloromethane and 500 ng of *n*-nonyl-acetate was added as an internal standard. Samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer and separated on a HP-1ms column (30 m X 0.25 mm inside diameter, 0.25 $\mu m$ film thickness); helium was used as the carrier gas. The GC oven was maintained at 35 °C for 3 min and then increased by 5 °C $min^{-1}$ to 125 °C, then 25 °C $min^{-1}$ to 250 °C. Quantifications were made relative to the internal standard using ChemStation software (Agilent

Technologies, Wilmington, DE, USA). Compounds were identified using NIST 08 Mass Spectral Search Program (National Institute of Standards and Technology, Gaithersburg, MD, USA) and confirmed by comparing retention times and mass spectra with commercial standards, when available.

In November 2021, plants were destructively harvested. Aboveground shoots were cut, dried at 48 °C for three days, and weighed (grams).

AMF Colonization Analyses

To assess AMF colonization, washed root segments were cleared, stained, and mycorrhizal structures were identified using a dissecting scope. During destructive plant harvest, soil and root matter were separated from aboveground vegetation. Roots were thoroughly washed to eliminate soil and gravel bound in the mass. Five individuals per treatment group of *A. millefolium* and *L. lewisii* from the drought experiment and three replicates of *A. millefolium* and *L. lewisii* from the temperature experiment were sampled for identification of presence or absence of AMF. If a treatment group had <5 surviving replicates, all individuals were sampled. To collect representative root samples, twenty 1-inch sections were randomly selected from varied locations in the root mass of an individual and soaked in deionized water. Root segments were then cleared for 48 h in 10% KOH at room temperature, acidified for 12 h in 3% HCl, and stained with a Trypan blue/lactoglycerol stain. Roots were inspected with a dissecting scope to confirm presence or absence of AMF via identification of arbuscular vesicles, arbuscules, hyphae, and spores. AMF colonization of roots in both forb species was confirmed in *R. clarus* and *C. etunicatum*. While there was some evidence of colonization of *L. lewisii* by *G. rosea*, there was no evidence of functional mycorrhizal structures in *A. millefolium*. While some root

cells contained fungal structures, there was no evidence of hyphae extending outside of single cells. Lack of AMF colonization was confirmed for uninoculated control plants for both forb species in all but one replicate. One *L. lewisii* in the uninoculated control treatment was colonized by AMF, so its trait data were removed from any analysis or figures. Results are reported for all treatments. Root biomass was not measured due to the root-bound nature of nearly all plant individuals.

Statistical Analyses

We tested the effects of increased temperature or drought, in combination with AMF treatments, on *A. millefolium* and *L. lewisii* survival and flowering using separate generalized linear models (GLM) with a binomial family distribution. To test the main and interactive effects of AMF treatments and increased temperature or drought on *A. millefolium* and *L. lewisii* plant traits, we first performed separate multivariate analyses of covariance (MANCOVA). Biomass was square-root transformed for normality for drought-treated *A. millefolium.* Fv/Fm was $(\sin^{-1})^6$ transformed for drought-treated and temperature-treated *A. millefolium,* and $\sin^{-1}$ transformed for drought-treated *L. lewisii* for normality. For *A. millefolium* in the temperature experiment, biomass, first day of flowering, flowering duration, Fv/Fm, and max floral area were not significantly correlated ($P<0.05$) and were included in the MANCOVA. For *L. lewisii* in the temperature experiment, only max height and first day of flowering were included in the MANCOVA, due to significant correlation between all other variables ($P>0.05$). For *A. millefolium* in the drought experiment, only biomass and first day of flowering were included in the MANCOVA, due to significant correlation between all other variables ($P>0.05$). For *L. lewisii* in the drought experiment, biomass, first day of flowering, floral abundance, average

floral area, and Fv/Fm were not significantly correlated with one another ($P<0.05$) and were included in the MANCOVA.

Following a significant MANCOVA ($P<0.05$), the main and interactive effects of AMF with drought or temperature on each forb species were tested with a two-way ANOVA. We performed Tukey's HSD test following a significant ANOVA to identify pairwise differences in AMF and climate treatments (i.e., drought or temperature) for plant traits. For *A. millefolium* in the temperature experiment and *L. lewisii* in the drought experiment, several treatment groups had extremely low or zero survival; therefore, further analyses were not possible and we reported general patterns instead.

We tested the main and interactive effects of AMF and climate treatments on total volatile emissions for each forb species with a two-way ANOVA. The effects of AMF and climate treatments on the composition and dispersion (among-individual variation in composition) of volatile compounds for *A. millefolium* and *L. lewisii,* were tested using separate PERMANOVAs and distance-to-centroid ANOVAs ('adonis' and 'betadisper' in vegan package in R). We used nonmetric multidimensional scaling to visualize treatment effects.

Statistical Software Used. MANCOVAs and GLMs were performed in the statistical software, 'JMP' (Version 16.2.0). All other analyses were performed in the statistical software, 'R' (Version 1.2.1335).

# Results

## Temperature-treated *Achillea millefolium*

Survival, flowering, and plant traits. Of the 120 *A. millefolium* individuals in the temperature experiment, 40% survived (48 individuals). Survival was marginally affected by the interaction between AMF inoculation and temperature treatment, with strong main effects of AMF and temperature treatments (Table 1). Overall, AMF inoculation often increased survival while warmer temperatures decreased survival relative to the uninoculated, 25 °C control (Figure 1A). The *R. clarus* treatment mitigated the negative effects of increased temperature best, with relatively constant survival across temperature treatments (Figure 1A). *Claroideoglomus etunicatum* inoculated plants had high survival at 25 °C but were severely negatively affected by increasing temperatures (Figure 1A).

Of the 48 surviving *A. millefolium*, 10 individuals flowered; 6 from the *R. clarus* treatment, 3 from the *C. etunicatum* treatment, and 1 from the control treatment (Figure 1E). Whether an individual flowered was marginally affected by the interaction between AMF and temperature, with strong main effects of AMF (Table 1, Figure 1E). *Rhizophagus clarus* was the only AMF treatment that had flowering plants in the 28 °C and 30 °C temperatures.

*Achillea millefolium* plant traits were significantly affected by AMF treatments (Wilks' lambda = 0.0001, DF = 12, F = 12.648, P = 0.0036). Specifically, biomass, flowering duration, and Fv/Fm were significantly altered by AMF treatments but not by temperature treatments (Table 2). Mean biomass was 27% lower in the *G. rosea* treatment than the *R. clarus* treatment across all temperatures, but there were no differences between any AMF inoculants and the uninoculated control (Figure 2A). Flowering duration was reduced by 93% on average across

AMF treatments, compared to the single control individual that flowered (Figure 2I). Fv/Fm was 5% higher in *A. millefolium* inoculated with *C. etunicatum* and *G. rosea* than in uninoculated control plants (Figure 2E). First day of flowering and max floral area were not affected by AMF or temperature treatments.

Floral VOC total emissions and composition. Due to limited replicates, differences in total floral volatiles and floral VOC composition emitted by *A. millefolium* could not be analyzed.

Temperature-treated *Linum lewisii*

Survival, flowering, and plant traits. Of the 120 *L. lewisii* individuals in the temperature experiment, 64% survived (77 individuals). Survival was marginally affected by the interaction between AMF treatment and temperature treatment, with strong main effects of AMF and increased temperature (Table 1). Generally, *R. clarus* and *C. etunicatum* treatments increased survival, relative to *G. rosea* and the uninoculated control treatments (Figure 1B). *Rhizophagus clarus* had consistently high survival across all temperature treatments, with an average of 93% survival (Figure 1B). In contrast, as temperature increased, *C. etunicatum* and *G. rosea* treatments had increasingly reduced survival compared to *R. clarus* and uninoculated control plants (Figure 1B). On average, survival was reduced by 32-38% with increased temperatures (28 °C and 30 °C) compared to the 25 °C treatment (Figure 1B).

Of the surviving 77 *L. lewisii* individuals, 64 flowered. AMF and temperature treatments significantly affected the proportion of plants that flowered (Table 1). Across the three temperature treatments, proportion flowering was greatest in the *C. etunicatum* treatment (97%),

followed by the *R. clarus* (85%), control (71%), and *G. rosea* (46%) treatments (Figure 1F). AMF treatments of *C. etunicatum* and *R. clarus* had consistently high proportion flowering with increased temperature, while *L. lewisii* inoculated with *G. rosea* did not flower at the highest temperature (Figure 1F).

*Linum lewisii* plant traits were significantly affected by treatments (Wilks' lambda = 0.549, DF = 14, F = 2.59, P = 0.003). Max height was affected by AMF treatments, but not by temperature or their interaction (Table 3). On average, across the three temperature treatments, the *R. clarus* inoculated plants were 24% taller than control plants and 46% taller than *G. rosea* inoculated plants (Figure 2B). *Gigaspora rosea* inoculated plants were 24% shorter than *C. etunicatum* inoculated plants on average, but neither were significantly different than the uninoculated control (Figure 2B). AMF influenced the effect of temperature treatments on flowering phenology (Table 3). Specifically, the first day of flowering was delayed by a week on average (7.2 days) across the three temperature treatments in *C. etunicatum* inoculated plants grown in 28 °C, compared to uninoculated control and *R. clarus* inoculated plants grown in 25 °C, 28 °C, or 30 °C (Figure 2J).

Floral VOC total emissions and composition. Flowers of *L. lewisii* emitted the same 11 compounds, including eucalyptol, benzyl alcohol, and $\alpha$-pinene, in all treatments. Increased temperature and AMF treatment had no effect on total floral volatile emissions (Figure 2N, Table 3), composition (Figure 3A, Table 7), or dispersion (Figure 3A, Table 7).

Drought-treated *Achillea millefolium*

Survival, flowering, and plant traits. Of the 160 *A. millefolium* individuals in the drought experiment, 94% survived, but only 17% flowered. There were no effects of AMF treatment, drought treatment, or their interaction on either survival (Figure 1C, Table 1) or proportion of flowering individuals (Figure 1G, Table 1).

*Achillea millefolium* plant traits were significantly affected by AMF treatment (Wilk's lambda = 0.37, F = 2.898, P = 0.014). Mean biomass was 36% greater in the *C. etunicatum* treatment than in the *R. clarus*, *G. rosea,* or control treatments across the drought treatments (Figure 2C). First day of flowering was 68 days earlier in the *C. etunicatum* treatment compared to the control (Figure 2K). There were no significant differences in first day of flowering between the control treatment and *R. clarus* or *G. rosea* treatments (Figure 2K).

Floral VOC total emissions and composition. Flowers of *A. millefolium* emitted the same 26 volatile compounds in all treatments, including benzyl alcohol, p-cymene, and benzaldehyde, but the total emission of volatile compounds and composition (i.e. relative abundances) varied. Total VOC emission was affected by the interaction between AMF treatment and drought (Figure 2O, Table 4). Uninoculated control plants in the drought treatment had over three times greater total emissions, on average, compared to all other treatment groups (Figure 2O). Additionally, volatile composition was affected by AMF treatment, but not by drought or the interaction between AMF and drought (Table 7, Figure 3B). The compounds that contributed most strongly to this difference in total emissions were benzyl alcohol and p-cymene. The levels of benzyl alcohol and p-cymene were on average 19 times and 7 times greater, respectively, for uninoculated control plants in the drought treatment than all other treatments. Neither AMF nor

drought treatment significantly influenced the dispersion of floral compounds (Figure 3B, Table 7).

Drought-treated *Linum lewisii*

Survival, flowering, and plant traits. Of the 160 *L. lewisii* individuals in the drought experiment, 51% survived (82 individuals). Survival was affected by AMF treatment, drought treatment, and their interaction (Table 1). Plants in the *C. etunicatum* treatment had high survival in drought (95%) and well-watered treatments (90%) (Figure 1D). Plants in the *R. clarus*, *G. rosea*, and control treatments had 0% survival when droughted (Figure 1D). For well-watered plants, *R. clarus* treatment had the second greatest survival (88%), followed by *G. rosea* (60%) and uninoculated control (43%) treatments (Figure 1D).

The proportion of well-watered *L. lewisii* that flowered was affected by AMF treatment; the effect of the drought treatment and the interaction between AMF and drought could not be tested due to lack of surviving replicates in the drought treatment (Table 1). All plant individuals inoculated with *C. etunicatum* flowered, regardless of the drought treatment, while less than 3% of the surviving plants in the *R. clarus* treatment flowered (Figure 1H). No plants that survived in the well-watered control or *G. rosea* treatments flowered (Figure 1H). Drought treatment did not affect the proportion of *C. etunicatum* plants that flowered in well-watered versus drought treatments (Figure 1H).

*Linum lewisii* plant traits were affected by treatments (Wilk's lambda = 0.008, F = 23.2, DF = 15, P <0.0001). For well-watered plants, AMF inoculation affected biomass and Fv/Fm; the effect of drought treatment could not be tested in *R. clarus, G. rosea,* or control treatments due to limited replicates (Table 5). *Claroideoglomus etunicatum* treatment increased biomass in

well-watered plants by 4- to 9-fold, compared to the other AMF treatments including the uninoculated control (Figure 2D). Fv/Fm of well-watered *C. etunicatum* inoculated plants was 3-6% higher than any other AMF treatments including the uninoculated control (Figure 2H). Comparing droughted and well-watered *C. etunicatum* plants, drought treatment had a marginal effect on biomass and no effect on first day of flowering, floral abundance, average floral area, Fv/Fm, or total VOCs (Table 6). In general, droughted *C. etunicatum* plants had a greater biomass (14%) than well-watered *C. etunicatum* plants (Figure 2D).

Floral VOC total emissions and composition. Flowers of *L. lewisii* emitted the same 11 volatile compounds in all treatments, including eucalyptol, $\alpha$-pinene, and benzaldehyde. The lack of flowering individuals in control, *R. clarus*, and *G. rosea* groups prevented analysis the effects of AMF on total floral volatile emissions and composition. Across *C. etunicatum* inoculated plants, there were no significant differences in total emissions (Figure 2P, Table 6), composition (Figure 3C, Table 7) or dispersion of compounds (Figure 3C, Table 7) between droughted and well-watered treatments.

## Discussion

This research suggests that AMF can mitigate the negative effects of warming and drought on forbs. *Achillea millefolium* and *L. lewisii* differed in their responses to combinations of AMF and climate treatments, having variously positive, neutral, and negative effects on survival, biomass, phenology, Fv/Fm, and floral traits (Figure 4). While previous research has shown that AMF can have positive effects on climate-stressed crop species (e.g. Mathur et al., 2018; Wu et al., 2013), this study provides evidence that AMF can ameliorate the negative effects of temperature and drought stress on native forb traits, but the magnitude of effect is specific to the forb species, climate treatment, and AMF inoculant. *Rhizophagus clarus* best mitigated the negative effects of increased temperature, while *C. etunicatum* best mitigated the negative effects of drought on host plants. The magnitude of these effects varied between forb species, consistent with previous literature (Al-Karaki and Al-Raddad, 1997, Kivlin et al., 2013, Worchel et al., 2013). *Rhizophagus clarus* and *C. etunicatum* also positively and negatively altered forb phenology, floral VOCs, and flowering success and duration, which have broad implications for plant-pollinator interactions and the links between belowground and aboveground symbioses. There was limited evidence of *G. rosea* colonization of either host plant, so no conclusions could be made as to its ability to mitigate warming or drought for host plants. *Gigaspora rosea* provided evidence that experimental inoculation of soil with AMF does not guarantee fungal colonization of plant roots and hence, providing confirmation of (or lack thereof) AMF colonization is fundamental to investigations of AMF treatment effects. Overall, broadening our understanding of the relationships between plants and climate change to include

the impacts of mycorrhizal association will allow us to better predict the responses of native plant communities in a warmer and drier future.

The ability of AMF to mitigate the negative effects of warming was specific to the AMF inoculant and the degree of temperature stress. Generally, AMF improved survival, flowering success, Fv/Fm, and vegetative growth of forbs under warming, consistent with existing literature on crop species (Matsubara et al., 2004; Wu et al., 2013; Mathur et al., 2018). For both forbs, *R. clarus* had the most positive effect at the hottest temperature and *C. etunicatum* had the most positive effect at the low and mid-level temperatures. These findings suggest a spectrum of heat-stress mitigation dependent on the AMF species and severity of warming, which has not been previously found in the literature to our knowledge. For example, *R. clarus* consistently increased forb survival, flowering, and vegetative growth across all three temperatures, but had the most pronounced effect in the hottest temperature compared to the uninoculated control. *Claroideoglomus etunicatum* had an overall positive impact on survival, vegetative growth, flowering, and phenology compared to the uninoculated control, but this effect was reduced as temperature increased. These data give us a more nuanced understanding of the relationships between warming, AMF, and plant hosts, as prior research has used multi-species AMF inoculations (3+ species) and fluctuating greenhouse temperatures as treatments to test plant response; therefore, AMF species-specific effects in plants' responses to specific degrees of warming could not discerned (Mathur et al., 2018; Matsubara et al., 2004; Wu et al., 2013). If the ability of AMF to mitigate the negative effects of heat-stress is species-specific, we may expect forbs to shift their carbon allocation to certain AMF partners as temperatures increase. By doing so, forbs may be able to withstand more extreme heat stress without dramatic reductions in survival and growth.

For drought-treated plants, the ability of AMF to mitigate drought stress was again specific to the AMF species, but also highly specific to the forb species. AMF amelioration of the negative effects of drought stress varied between the two forb species studied. Most *L. lewisii* plants died during the drought treatment, but inoculation with *C. etunicatum* successfully mitigated this stress, increasing survival, flowering, biomass, and Fv/Fm in both the droughted and well-watered treatments compared to the uninoculated control and other AMF treatments. Furthermore, *C. etunicatum*-inoculated *L. lewisii* that were droughted had similar survival, flowering success, Fv/Fm, and phenology to well-watered individuals. Counterintuitively, *C. etunicatum*-inoculated *L. lewisii* had significantly greater biomass when droughted than when well-watered. This suggests that AMF did not just mitigate the negative effects of drought but may have aided plants in vegetative growth to an even greater degree when stressed, without reductions in other measured plant traits. These results indicate that certain AMF species are extremely successful at ameliorating the negative effects of drought on host plants, while other AMF species have little to no effect. This could be due to greater production of extraradical hyphae by *C. etunicatum*, which can provide greater access to soil moisture, cause morphological changes in plant structure, and improve nutrient status for host plants and thereby drastically improve their survival during drought. If this species-specific AMF amelioration of drought-stress can increase host plant survival, vegetative growth, and reproductive traits, then AMF have the potential to indirectly alter the structure and function of plant communities. However, this effect was specific to only one forb, *L. lewisii*, so more research on the mitigation of drought stress by AMF species (especially *C. etunicatum*) in a variety of native plant species is warranted.

AMF treatments had much weaker effects on droughted *A. millefolium* plants than droughted *L. lewisii*. Survival of *A. millefolium* was high in all treatment groups and unaffected by AMF or drought treatments. Despite survival being high, the likelihood of *A. millefolium* flowering during the course of our experiment was very low and unaffected by AMF or drought treatments. One explanation for these results is that *A. millefolium* is a very drought-tolerant and hardy forb (Ijaz *et al.*, 2020; Rowe *et al.*, 2018), so it does not benefit as much from AMF mitigation of drought, compared to a less drought-tolerant species like *L. lewisii*. Previous research has found a similar pattern in wheat, where a drought-sensitive genotype was more dependent on and benefited more from AMF symbiosis than a drought-resistant genotype (Al-Karaki and Al-Raddad, 1997; Worchel et al., 2013). Additionally, plant species that are more sensitive to stressful environmental conditions may put more resources into reproductive traits and flowering than stress-tolerant plant species in order to ensure reproduction prior to mortality (Shavrukov et al., 2017). To better understand this pattern, future studies could select forb species known to be either drought-tolerant or drought-susceptible and test whether AMF species often ameliorate drought-stress to a greater degree in drought-susceptible host species.

Although AMF treatments did not affect *A. millefolium* survival or flowering success, one AMF species did have a positive effect on *A. millefolium* biomass and phenology. *Claroideoglomus etunicatum* increased *A. millefolium* biomass and led to earlier flowering compared to the uninoculated control and other AMF inoculants. As this AMF treatment also had extremely positive effects on droughted *L. lewisii*, there is evidence for *C. etunicatum* being a beneficial symbiont across two droughted forb species. Furthermore, given the ability of *C. etunicatum* to mitigate low to mid-level heat stress, this AMF species has potential to also aid host plants under combined climate stressors. As plant communities will continue to be

threatened by both rising temperatures and drought conditions (Kivlin et al., 2013), associating with AMF species that can mitigate the effects of multiple stressors could be extremely beneficial to plants.

In drought-treated *A. millefolium*, the composition of floral volatile compounds in uninoculated control plants was significantly different from all AMF inoculated and well-watered control plants. Total volatiles emitted were also significantly higher in droughted, uninoculated control plants than in well-watered control and all AMF inoculated *A. millefolium*. As AMF and drought had no significant effects on *A. millefolium* survival or likelihood of flowering, an interactive effect of these treatments on the composition and total emission of floral volatiles is noteworthy. Previous literature has shown that drought treatment can increase total VOC emissions in native, perennial forbs (Burkle and Runyon, 2016; Glenny et al., 2018). Additionally, a handful of studies have shown that AMF can modify the composition of floral volatile compounds in native forbs, including St. John's-wort and snapdragon, but these studies have not tested the combined effects of climate stress and AMF (Besmer and Koide, 1999; Lazzara et al., 2017). Given AMF's ability to modify plant water availability and nutrient uptake, there is strong potential for AMF association to indirectly modify floral VOC composition. These data show interesting potential trends in the effect of AMF association on droughted plants' VOCs, despite sample sizes being very small. There were insufficient replicates in temperature-treated *A. millefolium* to test the effects on floral VOCs, but the limited data also suggest differences in floral volatile composition and decrease in total compounds released with AMF inoculation. As *L. lewisii* saw no effect of AMF, warming, or drought on plants' VOCs, we can speculate that the species of forb plays a large role in the response of VOCs to AMF and climate treatments. As flower visitation preference and the community of pollinators visiting a

plant can shift based on the composition and quantity of floral VOCs emitted (Burkle and Runyon, 2016; Glenny et al., 2018; Muhlemann et al., 2014), there are broad implications for the indirect influence of fungal symbionts on plant pollinator interactions.

## Concluding Remarks

This research builds on previous work by demonstrating that AMF can ameliorate the negative effects of heat and drought stress on native forbs, in addition to crop species. AMF generally increased the survival, vegetative growth, and flowering success of climate-stressed forbs. Total emissions and composition of floral VOCs were modified by AMF and drought treatments in *Achillea millefolium*. These results indicate that AMF may indirectly alter the structure and function of plant communities and modify aboveground species interactions, such as with pollinators. Future studies could explore the relationships between AMF and drought-tolerant or drought-susceptible host species, the influence of AMF and climate-stress on floral VOCs, and the effects of combined climate stressors on plant-AMF symbiosis. A more complete understanding of the response of native plant communities to climate change will better inform their management and protection as warming and drought are projected to worsen.

Tables

Table 1. GLM results testing for differences in survival and proportion flowering for *A. millefolium* and *L. lewisii* in response to AMF inoculation, temperature, and drought treatments. Bolded text illustrates a significant difference in means ($P < 0.05$). Greyed areas indicate where an interaction could not be included in the model or run.

| | Survival | | | Flowered | | |
|---|---|---|---|---|---|---|
| | df | Chi-sq | P | df | Chi-sq | P |
| TEMPERATURE | | | | | | |
| *A. millefolium* | | | | | | |
| AMF | 3 | 29.18 | **<0.0001** | 3 | 13.30 | **0.004** |
| temp | 1 | 29.11 | **<0.0001** | 1 | 0.00 | 1.00 |
| AMF*temp | 3 | 6.75 | 0.08 | 3 | 6.99 | 0.07 |
| *L. lewisii* | | | | | | |
| AMF | 3 | 28.33 | **<0.0001** | 3 | 28.51 | **<0.0001** |
| temp | 1 | 12.23 | **0.0005** | 1 | 13.75 | **0.0002** |
| AMF*temp | 3 | 7.52 | 0.06 | 3 | 5.53 | 0.14 |
| DROUGHT | | | | | | |
| *A. millefolium* | | | | | | |
| AMF | 3 | 0.00 | 1.00 | 3 | 5.89 | 0.12 |
| drought | 1 | 0.002 | 0.96 | 1 | 0.43 | 0.51 |
| AMF*drought | 3 | 0.00 | 1.00 | 3 | 2.30 | 0.51 |
| *L. lewisii* | | | | | | |
| AMF | 3 | 75.54 | **<0.0001** | 3 | 162.7 | **<0.0001** |
| drought | 1 | 23.46 | **<0.0001** | 1 | 0.00 | 1.00 |
| AMF*drought | | | | | | |

Table 2. Effects of AMF and temperature treatments and their interaction on *A. millefolium* traits. P-values < 0.05 are boldface. Greyed areas indicate where interactions could not be included in the model or tested.

| | Biomass | | | First Day of Flowering | | | Flowering Duration | | | Max Floral Area | | | Fv/Fm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P | df | F | P | df | F | P |
| *A. millefolium* | | | | | | | | | | | | | | | |
| AMF | 3,38 | 3.25 | **0.03** | 2,5 | 0.16 | 0.86 | 2,5 | 0.16 | **<0.0001** | 2,5 | 0.18 | 0.84 | 3,39 | 9.96 | **<0.0001** |
| temp | 2,38 | 0.77 | 0.47 | 2,5 | 0.49 | 0.64 | 2,5 | 0.49 | 0.64 | 2,5 | 0.62 | 0.57 | 3,39 | 1.85 | 0.17 |
| AMF*temp | 4,38 | 0.67 | 0.62 | | | | | | | | | | 4,39 | 0.71 | 0.59 |

Table 3. Effects of AMF and temperature treatments and their interaction on *L. lewisii* traits. P-values < 0.05 are boldface. Total volatile organic compounds are labeled as Total VOCs.

| | Max Height | | | First Day of Flowering | | | Total VOCs | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P |
| *L. lewisii* | | | | | | | | | |
| AMF | 3,66 | 6.20 | **<0.0001** | 3,50 | 4.69 | **<0.01** | 3,10 | 1.08 | 0.40 |
| temp | 2,66 | 1.05 | 0.36 | 2,50 | 6.43 | **<0.01** | 2,10 | 1.32 | 0.31 |
| AMF*temp | 6,66 | 1.40 | 0.23 | 5,50 | 2.51 | **0.04** | 4,10 | 2.00 | 0.17 |

Table 4. Effects of AMF and drought treatments and their interaction on *A. millefolium* traits. P-values < 0.05 are boldface. Total volatile organic compounds are labeled as Total VOCs.

| | Biomass | | | First Day of Flowering | | | Total VOCs | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P |
| *A. millefolium* | | | | | | | | | |
| AMF | 3,143 | 10.59 | **<0.0001** | 3,16 | 4.74 | **0.02** | 3,13 | 6.25 | **0.007** |
| drought | 1,143 | 2.20 | 0.14 | 1,16 | 4.31 | 0.054 | 1,13 | 2.18 | 0.16 |
| AMF*drought | 3,143 | 0.96 | 0.41 | 3,16 | 3.04 | 0.06 | 2,13 | 7.47 | **0.007** |

Table 5. Effects of AMF on well-watered *L. lewisii* traits. P-values < 0.05 are boldface. Greyed areas indicate where AMF could not be tested or included in the model. Effects of AMF on droughted *L. lewisii* could not be tested due to limited replicates in the *G. rosea, R. clarus*, or uninoculated control treatments.

| *L. lewisii* (well-watered) | | Biomass | | | First Day of Flowering | | | Floral Abundance | | | Average Floral Area | | | Fv/Fm | | | Total VOCs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | df | F | P | df | F | P | df | F | P | df | F | P | df | F | P | df | F | P |
| | AMF | 3,87 | 184.29 | **<0.0001** | | | | | | | | | | 3,49 | 5.08 | **0.004** | | | |

Table 6. Effects of drought on *C. etunicatum* inoculated *L. lewisii* traits. P-values < 0.05 are boldface. Effects of drought on *G. rosea*, *R. clarus*, or uninoculated control treatments could not be tested due to limited replicates.

| | | Biomass | | | First Day of Flowering | | | Floral Abundance | | | Average Floral Area | | | Fv/Fm | | | Total VOCs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. lewisii* (*C. etunicatum* treatment) | | df | F | P | df | F | P | df | F | P | df | F | P | df | F | P | df | F | P |
| | drought | 1,32 | 3.93 | 0.056 | 1,35 | 1.43 | 0.24 | 1,35 | 0.01 | 0.94 | 1,35 | 0.05 | 0.83 | 1,37 | 2.35 | 0.13 | 1,21 | 0.05 | 0.83 |

Table 7. Effects of AMF and climate treatments on the relative composition and dispersion of floral volatile organic compounds. Greyed areas indicate where AMF could not be tested or included in the model. Temperature-treated *A. millefolium* could not be tested due to limited replicates.

| | | Composition | | | Dispersion | | |
|---|---|---|---|---|---|---|---|
| | | df | F | P | df | F | P |
| TEMPERATURE | | | | | | | |
| *L. lewisii* | | | | | | | |
| | AMF | 3,19 | 0.95 | 0.51 | 3,16 | 1.03 | 0.41 |
| | temp | 2,19 | 1.16 | 0.32 | 2,17 | 0.98 | 0.40 |
| DROUGHT | | | | | | | |
| *A. millefolium* | | | | | | | |
| | AMF | 3,19 | 2.14 | **0.02** | 3,16 | 1.57 | 0.24 |
| | drought | 1,19 | 1.65 | 0.12 | 1,18 | 2.21 | 0.15 |
| *L. lewisii* | | | | | | | |
| | AMF | | | | | | |
| | drought | 1,21 | 0.77 | 0.56 | 1,20 | 0.24 | 0.63 |

## Figures

Figure 1. Treatment effects of temperature (left panels) or drought (right panels) and AMF inoculants, *Claroideoglomus etunicatum* (yellow), *Rhizophagus clarus* (blue), *Gigaspora rosea* (green), and uninoculated control (red), on survival (A-D) and proportion flowering (E-H) for *Achillea millefolium* and *Linum lewisii*.

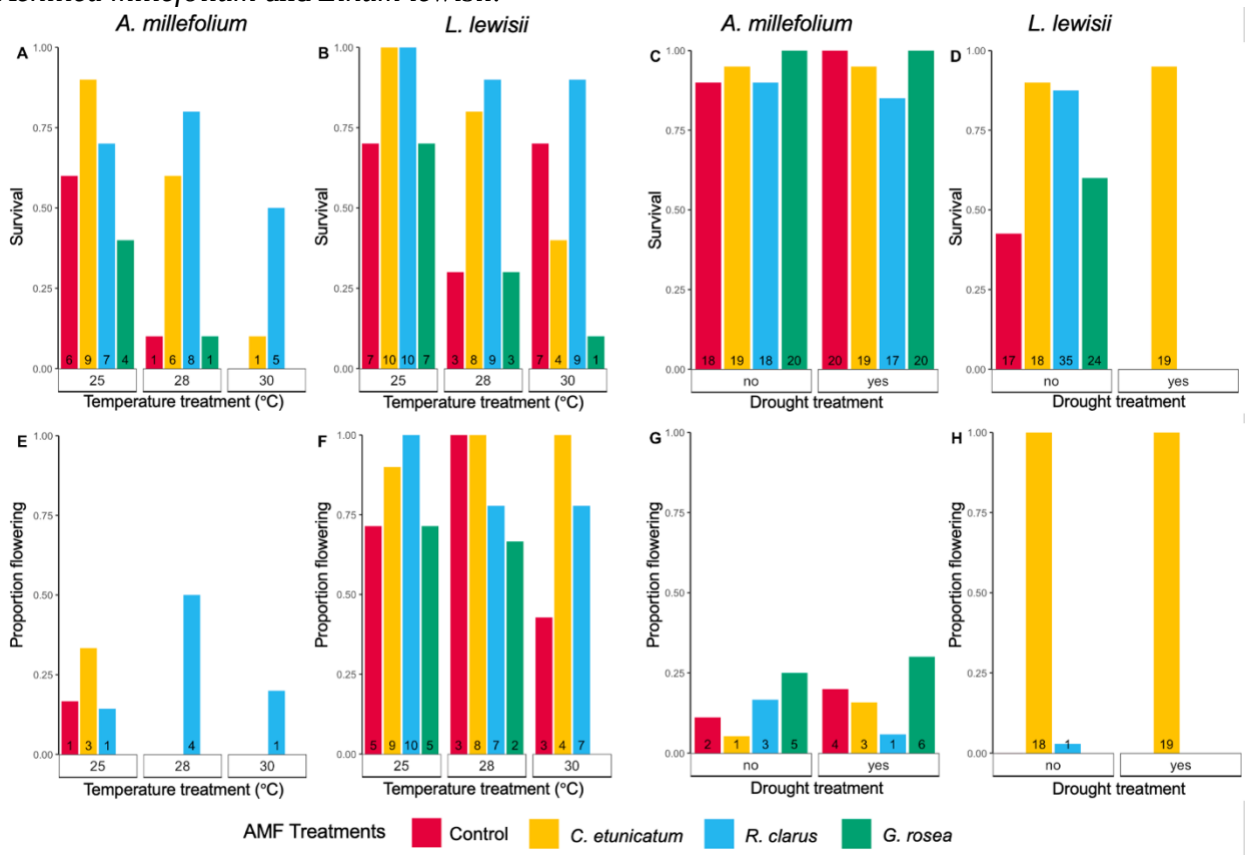


Figure 2. Treatment of temperature (left panels) or drought (right panels) and AMF inoculants, *C. etunicatum* (yellow), *R. clarus* (blue), *G. rosea* (green), and control (red), on plant traits (I-X) for *A. millefolium* and *L. lewisii*. Bars are means (±1 SE). Different letters indicate significant differences between treatment groups within a plant species (P>0.05). Different numbers at the bottom of bars indicate replicates per treatment.

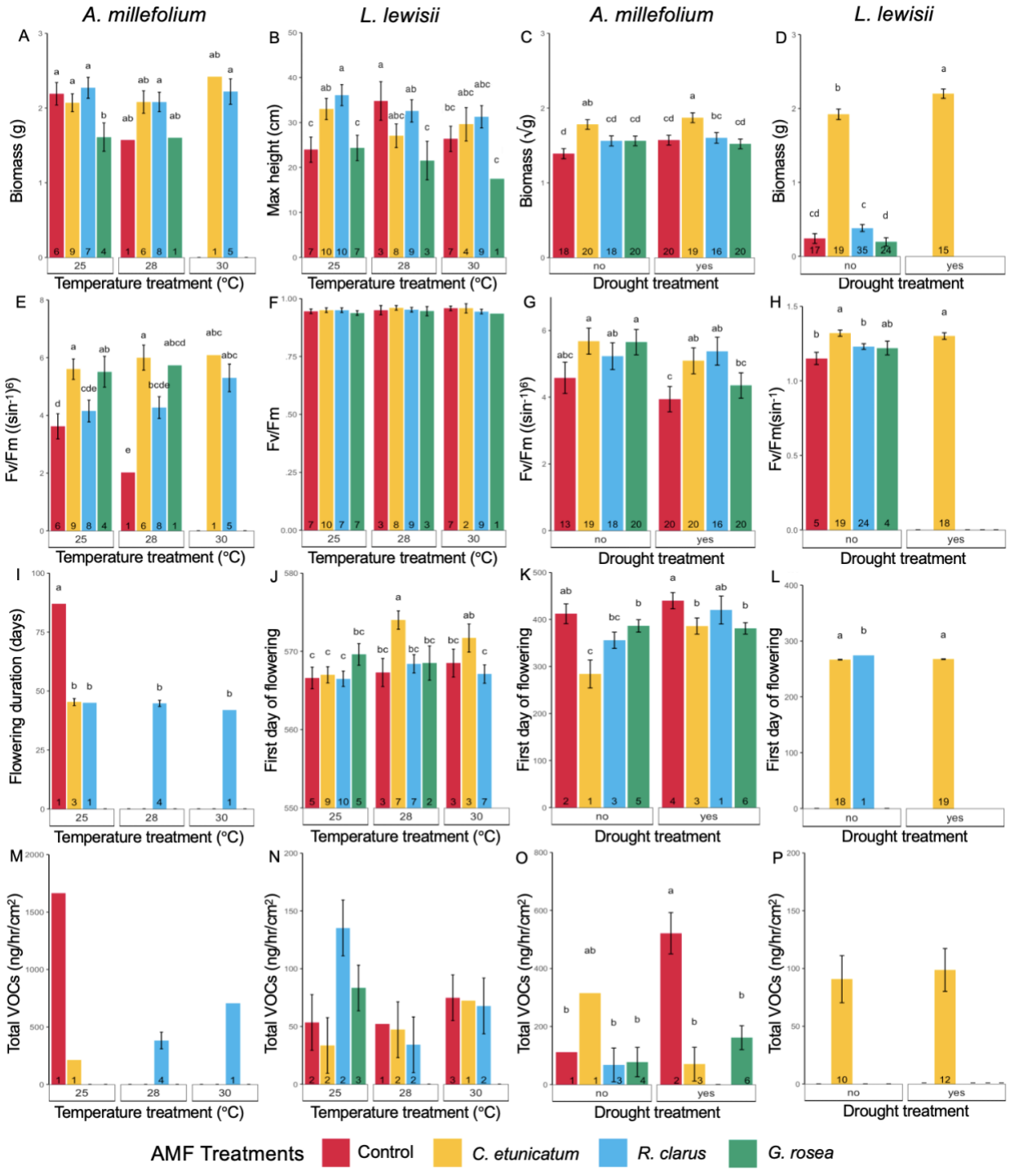


Figure 3. NMDS visualization of treatment effects on floral volatile composition. **A)** There were no differences in volatile composition between temperature treatments of 25 °C (circles), 28 °C (squares), 30 °C (triangles) and AMF treatments of *C. etunicatum* (yellow), *R. clarus* (blue), *G. rosea* (green), and control (red) for *L. lewisii*. **B)** The interaction between drought (triangles) and AMF, *C. etunicatum* (yellow), *R. clarus* (blue), *G. rosea* (green), significantly influenced volatile composition compared to well-watered, uninoculated controls (red circles) for *A. millefolium.* **C)** There was no difference in volatile composition between droughted *C. etunicatum* (yellow triangles) and well-watered *C. etunicatum* (yellow circles) for *L. lewisii.*

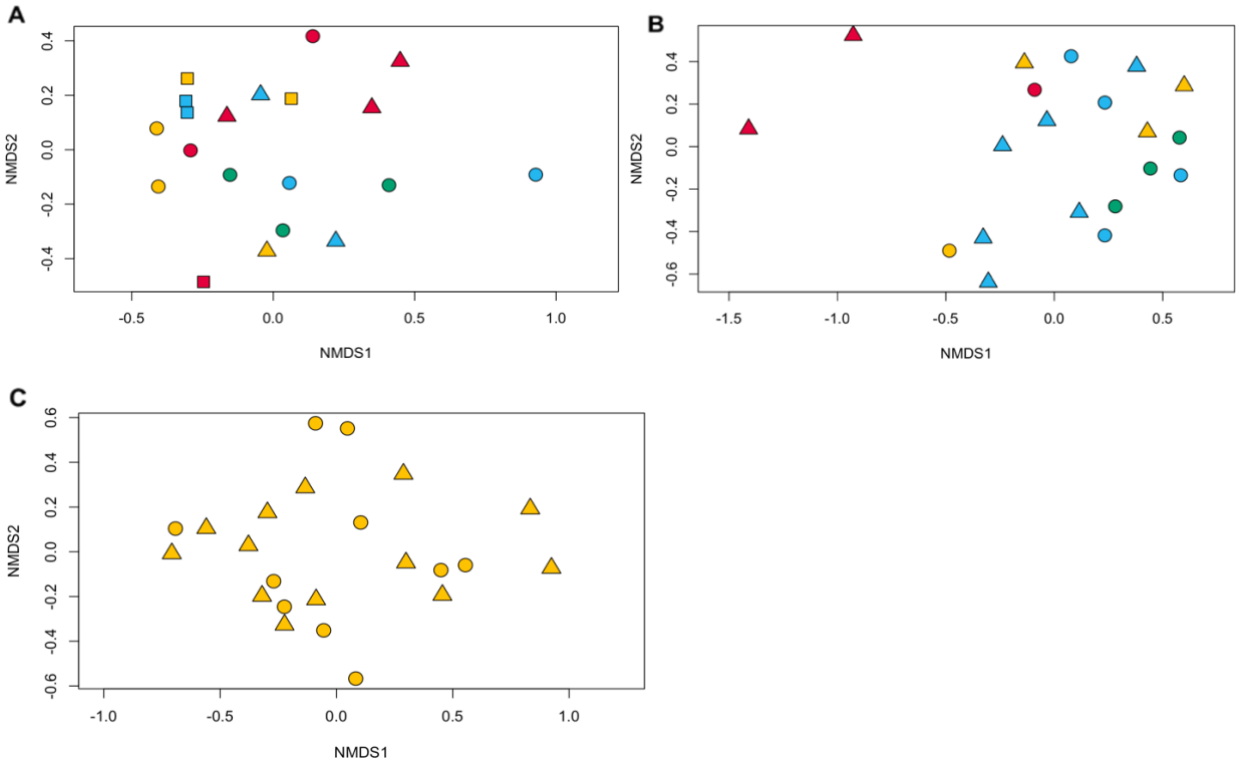
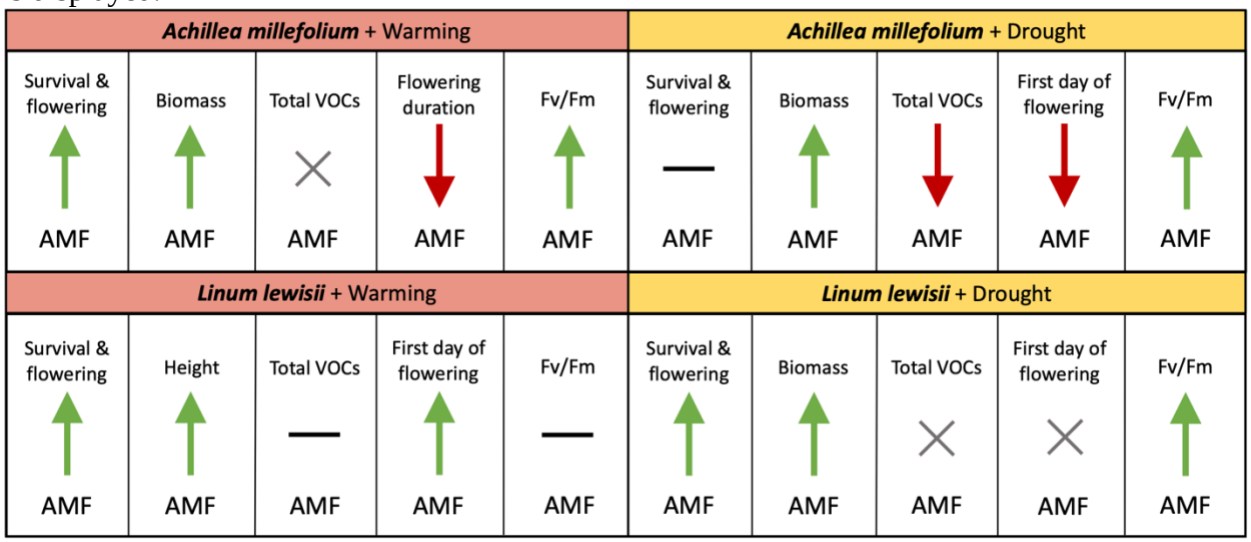


Figure 4. The positive (green up arrow), negative (red down arrow), and neutral (black dash) effects of AMF on drought (red boxes) and temperature (yellow boxes) treated *Achillea millefolium* and *Linum lewisii*. Where effects could not be tested due to limited replicates, an "X" is displayed.



References Cited

Al-Karaki, G. and A. Al-Raddad. 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7: 83-88.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

Augé, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84: 373-381.

Barber, N. A. and N. L. Soper Gorden. 2015. How do belowground organisms influence plant–pollinator interactions? Journal of Plant Ecology 8: 1-11.

Basu, S., R. C. Rabara and S. Negi. 2018. AMF: The future prospect for sustainable agriculture. Physiological and Molecular Plant Pathology 102: 36-45.

Bennett, A. E. and H. C. Meek. 2020. The influence of arbuscular mycorrhizal fungi on plant reproduction. Journal of Chemical Ecology 46: 707-721.

Ceccarelli, N., M. Curadi, L. Martelloni, C. Sbrana, P. Picciarelli and M. Giovannetti. 2010. Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. Plant and Soil 335: 311-323.

Compant, S., M. G. Van Der Heijden and A. Sessitsch. 2010. Climate change effects on beneficial plant–microorganism interactions. FEMS microbiology ecology 73: 197-214.

Cook, B. I., T. R. Ault and J. E. Smerdon. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science advances 1: e1400082.

Curtis, P. S. and X. Wang. 1998. A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113: 299-313.

Drew, E., R. Murray, S. Smith and I. Jakobsen. 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant and Soil 251: 105-114.

Duan, X., D. S. Neuman, J. M. Reiber, C. D. Green, A. M. Saxton and R. M. Augé. 1996. Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought. Journal of Experimental Botany 47: 1541-1550.

Ganade, G. and V. Brown. 1997. Effects of below-ground insects, mycorrhizal fungi and soil fertility on the establishment of Vicia in grassland communities. Oecologia 109: 374-381.

Gange, A. C. and A. K. Smith. 2005. Arbuscular mycorrhizal fungi influence visitation rates of pollinating insects. Ecological Entomology 30: 600-606.

Hawkes, C. V., I. P. Hartley, P. Ineson and A. H. Fitter. 2008. Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. Global Change Biology 14: 1181-1190.

Hostetler S, Whitlock C, Shuman B, Liefert D, Drimal C, Bischke S. 2021. Greater Yellowstone climate assessment: past, present, and future climate change in greater Yellowstone watersheds. Bozeman MT: Montana State University, Institute on Ecosystems. 260 p.

Jackson, S. T. and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26: 194-220.

Keeler, A. M., A. Rose-Person and N. E. Rafferty. 2021. From the ground up: Building predictions for how climate change will affect belowground mutualisms, floral traits, and bee behavior. Climate Change Ecology 1: 100013.

Kivlin, S. N., S. M. Emery and J. A. Rudgers. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany 100: 1445-1457.

Lazzara, S., M. Militello, A. Carrubba, E. Napoli and S. Saia. 2017. Arbuscular mycorrhizal fungi altered the hypericin, pseudohypericin, and hyperforin content in flowers of Hypericum perforatum grown under contrasting P availability in a highly organic substrate. Mycorrhiza 27: 345-354.

Mathur, S., M. P. Sharma and A. Jajoo. 2018. Improved photosynthetic efficacy of maize (Zea mays) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. Journal of Photochemistry and Photobiology B: Biology 180: 149-154.

Matsubara, Y., I. Hirano, D. Sassa and K. Koshikawa. 2004. Alleviation of high temperature stress in strawberry plants infected with arbuscular mycorrhizal fungi. Environment Control in Biology 42: 105-111.

Murchie, E. H. and T. Lawson. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany 64: 3983-3998.

Nievola, C., C. Carvalho, V. Carvalho and E. Rodrigues. 2017. Rapid responses of plants to temperature changes. Temperature, 4: 371–405.

Öpik, M., M. Moora, J. Liira and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. Journal of Ecology 94: 778-790.

Parmesan, C. and M. E. Hanley. 2015. Plants and climate change: complexities and surprises. Annals of Botany 116: 849-864.

Pendleton, R. L. 2000. Pre-inoculation by an arbuscular mycorrhizal fungus enhances male reproductive output of Cucurbita foetidissima. International Journal of Plant Sciences 161: 683-689.

Piao, S., Q. Liu, A. Chen, I. A. Janssens, Y. Fu, J. Dai, L. Liu, et al. 2019. Plant phenology and global climate change: Current progresses and challenges. Global Change Biology 25: 1922-1940.

Pischl, P. H. and N. A. Barber. 2017. Plant responses to arbuscular mycorrhizae under elevated temperature and drought. Journal of Plant Ecology 10: 692-701.

Poulton, J., R. Koide and A. Stephenson. 2001. Effects of mycorrhizal infection, soil phosphorus availability and fruit production on the male function in two cultivars of Lycopersicon esculentum. Plant, cell & environment 24: 841-849.

Poulton, J. L., D. Bryla, R. T. Koide and A. G. Stephenson. 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. New Phytologist 154: 255-264.

Pugnaire, F. I., J. A. Morillo, J. Peñuelas, P. B. Reich, R. D. Bardgett, A. Gaxiola, D. A. Wardle and W. H. Van Der Putten. 2019. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. Science advances 5: eaaz1834.

Richardson, A. D., T. F. Keenan, M. Migliavacca, Y. Ryu, O. Sonnentag and M. Toomey. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology 169: 156-173.

Rudgers, J. A., S. N. Kivlin, K. D. Whitney, M. V. Price, N. M. Waser and J. Harte. 2014. Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. Ecology 95: 1918-1928.

Schäfer, H., M. Dannoura, M. Ataka and A. Osawa. 2019. Decomposition rate of extraradical hyphae of arbuscular mycorrhizal fungi decreases rapidly over time and varies by hyphal diameter and season. Soil Biology and Biochemistry 136: 107533.

Solomon, S., M. Manning, M. Marquis and D. Qin. 2007. Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC. Cambridge university press.

Varga, S. and M. M. Kytöviita. 2010. Gender dimorphism and mycorrhizal symbiosis affect floral visitors and reproductive output in Geranium sylvaticum. Functional ecology 24: 750-758.

Whitlock C, Cross W, Maxwell B, Silverman N, Wade AA. 2017. 2017 Montana Climate Assessment. Bozeman and Missoula MT: Montana State University and University of Montana, Montana Institute on Ecosystems. 318 p.

Wolfe, B. E., B. C. Husband and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8: 218-223.

Worchel, E. R., H. E. Giauque and S. N. Kivlin. 2013. Fungal symbionts alter plant drought response. Microbial Ecology 65: 671-678.

Wu, Q.-S., A. K. Srivastava and Y.-N. Zou. 2013. AMF-induced tolerance to drought stress in citrus: a review. Scientia Horticulturae 164: 77-87.

# CONCLUSION

Terrestrial plant communities will be challenged by both the direct and indirect repercussions of anthropogenic climate change. This research provides evidence that AMF can mitigate the negative effects of warming and drought stress on native forbs. *Achillea millefolium* and *L. lewisii* had varied responses to combinations of AMF and climate treatments, having variously positive, neutral, and negative effects on survival, biomass, phenology, Fv/Fm, and floral traits. This study has demonstrated that AMF can ameliorate the negative effects of temperature and drought stress on forb traits, but the magnitude of effect is specific to the forb species, climate treatment, and AMF inoculant. *Rhizophagus clarus* best mitigated the negative effects of increased temperature, while *C. etunicatum* best mitigated the negative effects of drought on host plants. *Rhizophagus clarus* and *C. etunicatum* treatments also altered forb phenology, floral VOCs, and flowering success and duration, having broad implications for plant-pollinator interactions and the links between belowground and aboveground symbioses. There was limited evidence of *G. rosea* colonization of either host plant, so no conclusions could be made as to its ability to mitigate warming or drought for host plants. *Gigaspora rosea* provided evidence that experimental inoculation of soil with AMF does not guarantee fungal colonization of plant roots and hence, providing confirmation of (or lack thereof) AMF colonization is fundamental to investigations of AMF treatment effects. In conclusion, expanding our knowledge on the relationships between plants and climate change to include the impacts of mycorrhizal association will allow us to better understand the responses of native plant communities in a warmer and drier future.

# REFERENCES CITED

Al-Karaki, G. and A. Al-Raddad. 1997. Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7: 83-88.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42.

Augé, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84: 373-381.

Barber, N. A. and N. L. Soper Gorden. 2015. How do belowground organisms influence plant–pollinator interactions? Journal of Plant Ecology 8: 1-11.

Basu, S., R. C. Rabara and S. Negi. 2018. AMF: The future prospect for sustainable agriculture. Physiological and Molecular Plant Pathology 102: 36-45.

Bennett, A. E. and H. C. Meek. 2020. The influence of arbuscular mycorrhizal fungi on plant reproduction. Journal of Chemical Ecology 46: 707-721.

Ceccarelli, N., M. Curadi, L. Martelloni, C. Sbrana, P. Picciarelli and M. Giovannetti. 2010. Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. Plant and Soil 335: 311-323.

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Cook, B. I., T. R. Ault and J. E. Smerdon. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science advances 1: e1400082.

Curtis, P. S. and X. Wang. 1998. A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113: 299-313.

Drew, E., R. Murray, S. Smith and I. Jakobsen. 2003. Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant and Soil 251: 105-114.

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Ganade, G. and V. Brown. 1997. Effects of below-ground insects, mycorrhizal fungi and soil fertility on the establishment of Vicia in grassland communities. Oecologia 109: 374-381.

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Jackson, S. T. and J. T. Overpeck. 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26: 194-220.

Keeler, A. M., A. Rose-Person and N. E. Rafferty. 2021. From the ground up: Building predictions for how climate change will affect belowground mutualisms, floral traits, and bee behavior. Climate Change Ecology 1: 100013.

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Mathur, S., M. P. Sharma and A. Jajoo. 2018. Improved photosynthetic efficacy of maize (Zea mays) plants with arbuscular mycorrhizal fungi (AMF) under high temperature stress. Journal of Photochemistry and Photobiology B: Biology 180: 149-154.

Matsubara, Y., I. Hirano, D. Sassa and K. Koshikawa. 2004. Alleviation of high temperature stress in strawberry plants infected with arbuscular mycorrhizal fungi. Environment Control in Biology 42: 105-111.

Murchie, E. H. and T. Lawson. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany 64: 3983-3998.

Nievola, C., C. Carvalho, V. Carvalho and E. Rodrigues. 2017. Rapid responses of plants to temperature changes. Temperature, 4: 371–405.

Öpik, M., M. Moora, J. Liira and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. Journal of Ecology 94: 778-790.

Parmesan, C. and M. E. Hanley. 2015. Plants and climate change: complexities and surprises. Annals of Botany 116: 849-864.

Pendleton, R. L. 2000. Pre-inoculation by an arbuscular mycorrhizal fungus enhances male reproductive output of Cucurbita foetidissima. International Journal of Plant Sciences 161: 683-689.

Piao, S., Q. Liu, A. Chen, I. A. Janssens, Y. Fu, J. Dai, L. Liu, et al. 2019. Plant phenology and global climate change: Current progresses and challenges. Global Change Biology 25: 1922-1940.

Pischl, P. H. and N. A. Barber. 2017. Plant responses to arbuscular mycorrhizae under elevated temperature and drought. Journal of Plant Ecology 10: 692-701.

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Poulton, J. L., D. Bryla, R. T. Koide and A. G. Stephenson. 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. New Phytologist 154: 255-264.

Pugnaire, F. I., J. A. Morillo, J. Peñuelas, P. B. Reich, R. D. Bardgett, A. Gaxiola, D. A. Wardle and W. H. Van Der Putten. 2019. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. Science advances 5: eaaz1834.

Richardson, A. D., T. F. Keenan, M. Migliavacca, Y. Ryu, O. Sonnentag and M. Toomey. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology 169: 156-173.

Rudgers, J. A., S. N. Kivlin, K. D. Whitney, M. V. Price, N. M. Waser and J. Harte. 2014. Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. Ecology 95: 1918-1928.

Schäfer, H., M. Dannoura, M. Ataka and A. Osawa. 2019. Decomposition rate of extraradical hyphae of arbuscular mycorrhizal fungi decreases rapidly over time and varies by hyphal diameter and season. Soil Biology and Biochemistry 136: 107533.

Solomon, S., M. Manning, M. Marquis and D. Qin. 2007. Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC. Cambridge university press.

Varga, S. and M. M. Kytöviita. 2010. Gender dimorphism and mycorrhizal symbiosis affect floral visitors and reproductive output in Geranium sylvaticum. Functional ecology 24: 750-758.

Whitlock C, Cross W, Maxwell B, Silverman N, Wade AA. 2017. 2017 Montana Climate Assessment. Bozeman and Missoula MT: Montana State University and University of Montana, Montana Institute on Ecosystems. 318 p.

Wolfe, B. E., B. C. Husband and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecology Letters 8: 218-223.

Worchel, E. R., H. E. Giauque and S. N. Kivlin. 2013. Fungal symbionts alter plant drought response. Microbial Ecology 65: 671-678.

Wu, Q.-S., A. K. Srivastava and Y.-N. Zou. 2013. AMF-induced tolerance to drought stress in citrus: a review. Scientia Horticulturae 164: 77-87.