

INCREASING PLANT AND MICROBE DIVERSITY ON GREEN ROOFS AS METHODS TO HELP CONSERVE NATIVE FORBS IN ILLINOIS

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ABSTRACT

Anthropogenic activities continue to alter habitats, imperiling native plant communities throughout Illinois and around the globe. Green roofs, which are rooftop habitats engineered specifically to incorporate habitat for plants, have the potential to help conserve native plant species, including those that are threatened or endangered due to habitat loss. However, green roofs are harsh habitats and successful establishment and persistence of plant populations may depend on facilitation within communities. Plant growth and survival may be supported by increasing the diversity of the plants which are selected as well and increasing the diversity of below-ground symbiotic microbes, such as arbuscular mycorrhizal fungi (AMF). Understanding the complex community interactions that promote facilitation may help predict which native species could benefit most from conservation-based green roof design. In this investigation we tested the hypotheses that increased plant and microbial diversity would increase the survival, growth, and reproduction of native forbs on green roofs. Our experiment included eight native Illinois forb species grown at three levels of diversity both with and without added AMF. By measuring survival, growth, reproduction, and AMF infection over one initial growing season,

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we found that facilitative and symbiotic effects are largely species-specific. Several species had increased survival when grown in diverse communities compared to monocultures and when grown in AMF-inoculated soil, but these results were inconsistent across species and may have been due to AMF species other than the one used for our experiment. Future understanding of the factors that contribute to population establishments and persistence will aid in designing green roofs to maximize their conservation potential for native Illinois plant species.

INTRODUCTION

Anthropogenic activity threatens native plant populations and communities, both around the world and in Illinois (Aronson et al. 2014; Hallfors et al. 2020). Some plant species with advantageous traits may flourish in the Midwest despite these pressures (Swanston et al. 2018), but between 0.17% and 42.5% of plant species are predicted to become extinct within the next century (Zettlemoyer et al. 2019). Biodiversity at all levels will be impacted, with a high risk of homogenization in affected areas (Cao and Natuhara 2020). The reduction or extirpation of native plant populations increases homogenization, which further threatens biodiversity and can result in extinction cascades where many species are lost in a very short period (Cao and Natuhara 2020). To prevent extinction cascades, reconciliation ecology helps promote local biodiversity by informing the design of novel ecosystems in urban areas (Hobbs et al. 2009; Pouso et al. 2020).

One system that is increasingly contributing to restoration ecology in urban environments is shallow-soil extensive green roofs. Extensive green roofs (hereafter “green roofs”) are typically installed to provide ecosystem services, such as trapping stormwater, insulating buildings to reduce energy costs, and counteracting the urban heat island effect, among many

others (Oberndorfer et al. 2007; Francis and Lorimer 2011; Kowarik 2011; Xie et al. 2018).

Green roofs can also potentially provide habitat to local native plant species that have lost habitat elsewhere (Armstrong 2009; Aloisio et al. 2019). Although not the typical motivation behind installing green roofs, they can be planted with local native forbs and grasses to promote biodiversity (Aguilar et al. 2019), for example, by providing food and habitat for important pollinators (Williams et al. 2014; Ksiazek-Mikenas et al. 2018). Finding ways to support native plants on green roofs is therefore important to promoting overall urban biodiversity (Cook-Patton and Bauerle 2012).

Despite the theoretical potential of urban green roofs to support native plants, ensuring their survival is difficult in practice. Plants on extensive green roofs are exposed to harsh environmental factors such as high drought caused by shallow soils and intense solar radiation caused by all-day sunlight (Dunnett and Kingsbury 2004; Oberndorfer et al. 2007). While non-native *Sedum* species are typically used in green roof designs due to their drought-resistant properties, requests for native plants have increased (Butler et al. 2012). This trend has been fueled by the increasing knowledge that native plants can support biodiversity by facilitating improved habitats for other native plants (Butler and Orians 2011; Kramer et al. 2019) and provide food and shelter for a wide range of wildlife, including specialist invertebrates dependent upon certain native plant species (Parsons et al. 2020). In the prairie-based Midwest and Great Plains regions of the United States specifically, previous work has shown that it can be difficult to establish native forbs without additional inputs of water and possibly continuous beds of deeper soil (Monterusso et al. 2005; Armstrong 2009; Sutton et al. 2012; Sutton 2015; Ksiazek-Mikenas et al. 2021). To support more complex urban ecosystems that include native prairie species, new insight into the design and performance of green roofs in the region is needed.

Specific communities may positively impact the survival of individual native species on green roofs and promote stable coexistence (Butler and Orians 2011). Species selection and community composition are known to affect overall ecosystem functioning (Hautier et al. 2018), with direct implications for the survival of individuals (Ksiazek-Mikenas and Köhler 2018). Specifically, previous work has found that greater functional and phylogenetic diversity of plant species on green roofs leads to greater levels of nitrogen and phosphorous in the soil, which results in higher overall plant biomass (Xie et al. 2018). Niche complementarity, or the ability of species with different physiological needs to maximize resource use in a community, may help explain why increased diversity helps promote biodiversity and contribute to greater biomass and carbon sequestration (Cadotte 2013; Barber et al. 2017). Greater diversity of functional traits in an ecosystem can improve defense against foreign invasion (Barber et al. 2017) and therefore support native plant establishment. Increasing diversity beyond the typical array of one or two plant families characteristically included on green roofs may therefore support a wider diversity of both native flora and fauna.

In addition to facilitative support between different plant species, many forbs benefit from below-ground interactions with mutualistic fungi and bacteria (Hoch et al. 2019). In natural areas, soil with robust microbial communities may support increased plant establishment by aiding germination and early survival (Burns and Strauss 2011; Guzman et al. 2021). Plant-microbial interactions have been linked to improved drought tolerance, pathogen protection, nutrient availability, and soil stabilization on green roofs (Fulthorpe et al. 2018). However, green roofs are almost always constructed with sterile, engineered soil that lacks microbial communities; a condition which may have negative consequences for plant health and survival (Fulthorpe et al. 2018). Plant establishment and persistence on green roofs may be enhanced if

beneficial arbuscular mycorrhizal fungi (AMF) are added to the soil early in a plant's life cycle (Al-Yahya'ei et al. 2022). If effective, adding AMF during the planting stage could be an important step to supporting more native plants in urban green roofs. Further research is necessary to support AMF inoculation as a conservation strategy because the ability of AMF to enhance survival and growth of native forbs on green roofs is not yet well established.

Both short-term and long-term survival is important to establish sustainable populations of native plants in urban habitats. Long-term survival of a population depends on the ability of native plants to reproduce (Rondina et al. 2014). Plant reproduction can be low in stressful environments with low nutrient availability and high drought, such as that found on green roofs (Aragón et al. 2008). Reduced reproduction can therefore impact long-term community persistence and resilience to changes in the environment. Previous research has found that reproduction of native plants on green roofs may be affected by pollen limitation due to a difference of the pollinator community between urban roofs and other ground-level habitats (Ksiazek et al. 2012). Reproductive strategies might also be altered given tradeoffs between present and future reproductive abilities in a stressful environment (Aragón et al. 2008).

Previous studies suggest that increasing diversity, both in terms of plant species and below-ground symbionts, will aid in the establishment, growth, and persistence of native plants on green roofs (e.g. Vasal et al. 2017; Ksiazek-Mikenas et al. 2018; Xie et al. 2018; Aguiar et al. 2019; Hoch et al. 2019; Droz et al. 2022). In this experiment, we hypothesized that native forb species grown on green roofs would experience increased rates of survival, growth, and reproduction when (1) they were grown in communities with greater plant diversity and (2) they were grown in soil inoculated with symbiotic AMF.

METHODS

Green Roof Construction

We set up an experimental green roof at Elmhurst University in Elmhurst, Illinois on top of the two-story Frick Center in the center of campus. The green roof was constructed from modular green roof trays (Columbia Green Technologies) measuring 61 cm x 61 cm (2 ft x 2 ft) and 10 cm (4 in) deep. We used 28 experimental trays placed in four rows with room to move around each tray to collect samples and data (Figure 1). The roof was not accessible to the public and was located in full sun. We filled each experimental tray first with a single layer of light-weight lava rock (Vigoro), followed by approximately 6 cm of CM63 Grower's Mix with Pine Fines (Midwest Trading). To test our second hypothesis, we then inoculated half of the trays with *Rhizophagus intraradices* (formerly known as *Globus intraradices*) by mixing 150 ml of Mykos Pure Mycorrhizal Inoculant (Extreme Gardening) with the CM63 Grower's Mix in each tray. We then covered the soil in all the trays with another single layer of mixed lava rocks and wood chips (Vigoro) to prevent loss of the soil through wind scouring.

Species Selection, Preparation and Planting

We selected eight species of forbs native to Illinois based on their typical natural habitat, phylogenetic relationships, and commercial availability (Table 1). Key to our selection process was to include a variety of species, from those that were known to grow successfully on green roofs in the region (i.e. *Allium stellatum* and *Opuntia humifusa* [Armstrong 2009]) to some that, to our knowledge, had not been tried on green roofs before, and even those that came from habitats which would most likely benefit from the water-absorption assistance provided by AMF (i.e. *Lobelia* spp.). Most individuals used in the experiment were purchased as mature seedlings

from a regional nursery (Prairie Moon Nursery) while *Sedum pulchellum* and *Liatris ligulistylis* were started from seed in the greenhouse at Elmhurst University. All plants were kept in a greenhouse for at least a week prior to planting on the green roof. Half of the *Lobelia cardinalis* seedlings available from the grower were small seedlings about three months old and the other half were about six months old and slightly larger so both maturity levels were mixed and evenly planted in the experimental treatments.

Each tray was divided into a 16-cell grid and seedlings from each species were planted, one individual per cell, into three diversity treatments: monocultures containing a single species, polycultures containing four species, or polycultures containing eight species. In the monoculture treatments, 12 individuals per species were planted. We created two lower diversity polyculture treatments that included four species each (P4A and P4B) where four individuals per species were planted. In the highest diversity treatment with a polyculture of eight species (P8), two individuals per species were planted (Figure 1). For each of the eight species, this setup resulted in a total of 24 individuals in monoculture, 16 in a four-species polyculture, and 8 in an eight-species polyculture. Placement of species into the grid on the trays as well as placement of the trays on the roof was determined by a random number generator. Placement of each individual into each treatment and placement within a tray was also randomized so that any effects of the tray were minimized. We watered all trays until saturation immediately following the initial planting as well as twice a week for the first two weeks (Figure 2). No supplemental water was provided after that time, which would be typical of many extensive green roof installations.

Vegetation Assessment

Once per month during the growing season (June – September), we measured survival, height, cover, and reproduction of all individuals. To measure survival, we counted the number of individuals per species in each treatment type that had living above-ground tissues. Any individual that had additional shoots growing from the same root or shoot system was counted as one individual. The percent species survival was simply calculated by dividing the number of individuals that survived to the end of the growing season (September) by the number of individuals planted in May. We measured above-ground growth as the height of the plant from the soil to the highest living tissue for all individuals. We visually estimated the percentage of vegetation cover where the total area of the tray represented 100% cover. All cover measurements were rounded to the nearest 5%. To measure reproduction, we recorded the presence or absence of a reproductive structure (flowers and/or fruits) for each individual during the growing season.

Root Collection, Staining, and Visualization

At the end of the growing season (September), we collected roots from four individuals in each monoculture tray, and two individuals in each of the polyculture trays to measure the rate of AMF colonization for each plant species in the different treatments. We cut primary and lateral roots from the base of the plant and temporarily stored them in Whirl-pak bags for transport to the laboratory. We removed soil and other debris by rinsing the roots in tap water. Following the protocol established by Vierheilig et al. (1998), we cleared the roots by boiling them in 10% KOH and rinsing them with tap water. We then boiled the cleared roots in a solution of 2% acetic acid and black ink (Sheaffer) for three minutes, let them cool, and then rinsed them with tap water. Finally, we placed the stained roots in tap water mixed with a few

drops of 2% acetic acid for 20 minutes and then rinsed and stored them in tap water until visualization was performed.

Sedum pulchellum and *Oxalis violacea* did not have above-ground living tissues due to their early senescent phenology so we were unable to collect live roots from these species in September to visualize AMF. Instead, we collected and bulked soil from each tray where the species had been growing in September, filled small pots with the soil, and planted seeds of each species after appropriate recommended pretreatments. The *S. pulchellum* and *O. violacea* plants were then grown in a greenhouse for 16 weeks. We harvested the roots and stained them following the same procedure described above.

For each species in each treatment, we haphazardly selected five stained root sections and visualized them using a light microscope. At 100x total magnification, we haphazardly chose 10 separate viewing fields. In each field, we counted the number of visible AMF structures including arbuscules, spores and vesicles, and recorded the presence or absence of fungal hyphae. Data for all types of AMF structures were pooled and for each species by treatment combination we calculated a total proportion of viewing fields that contained any evidence of AMF.

Statistical Analyses

We used generalized linear mixed-effects models with a binomial response distribution and tray as a random effect to test for effects of inoculation, plant diversity, and their interaction on survival and reproduction for each species. Using “tray” as the random effect in the mixed effects model allowed us to ensure that we were able to consider the effects of the manipulated variables on the plants individually. We used repeated-measures ANOVA of linear models to test

for the effects of inoculation, plant diversity, and their interaction on height and the effects of inoculation on cover for each species. We used linear mixed effects models to test for significant relationships between the inoculum treatment and the detected proportion of roots that had been colonized by AMF, using the community diversity as a random effect. All statistical tests were conducted in R, version 4.0.2 (R Core Team 2021), with package lme4 to create the random effects models (Bates et al. 2015).

RESULTS

Survival

There was an observable trend for some species when survival was visualized by community diversity and AMF inoculation treatment (Figure 3). We found no statistically significant effect of the interaction between inoculation and diversity treatment on survival for any species. However, in the monocultures there was a tendency toward slightly greater survival in the AMF treatment for *Lobelia cardinalis* (+15%). *Liatrix ligulistylis* (-15%) and *Lobelia siphilitica* (-8%) tended toward slightly lower survival in the AMF treatment. All *Echinacea purpurea* plants died in the AMF treatment but 85% survived in the non-AMF treatment and all *A. stellatum* and *O. humifusa* survived in both the non-AMF and AMF treatments. In the P4A diversity treatments, there was a tendency toward lower survival in the AMF treatment for both *L. ligulistylis* (-12%) and *E. purpurea* (-12%). In P4B, *L. cardinalis* tended toward greater survival (+50%) in the AMF treatment while *L. siphilitica* survived slightly worse in the AMF treatment (-12%). Finally, in the P8 polyculture treatments, half of the species had a tendency toward greater survival in the AMF treatment (*L. siphilitica* and *L. cardinalis* both at +25%, *L.*

ligulistylis +30% and *E. purpurea* +50%). The other four species either had 100% or 0% survival over the study period in both soil treatments.

Because we found no statistical effect of the interaction between community diversity and AMF inoculation on survival, we pooled the data and looked at the effects of diversity and inoculation separately. We found that diversity had a significant effect on survival for three species: *L. ligulistylis* ($p = 0.008$), *L. cardinalis* ($p = 0.018$) and *L. siphilitica* ($p = 0.005$). When looking at the effect of inoculation on survival, we found a significant relationship for half of the species: *E. purpurea* had approximately 18.7% lower survival in the AMF-inoculated treatments ($p = 0.004$), while *L. ligulistylis* (6.4%, $p < 0.001$), *L. cardinalis* (15.7%, $p = 0.002$), and *L. siphilitica* (6.3%, $p < 0.001$) had significantly greater survival in the AMF-inoculated treatments (Figure 4). The other four species either had 100% or 0% survival in both inoculation conditions.

Growth

Overall, we found very few effects of plant diversity or inoculation on plant height. However, we did find a significant interactive effect of diversity and inoculation on height in *L. siphilitica* ($p = 0.005$, $F = 6.11$). Diversity alone had a significant effect on height for *L. ligulistylis* ($p < 0.001$, $F = 12.82$ on 2 and 45 DF) and inoculation alone had a significant effect on height for *E. purpurea* ($p = 0.009$, $F = 7.020$ on 1 and 190 DF).

We found no significant effect of inoculation on cover for any of the species. In fact, the cover of most of the species decreased over the growing season except for *A. stellatum* and *O. humifusa* and a slight increase in *L. ligulistylis* in the AMF treatments (Figure 5). Notably, the cover of many species decreased in August due to a drought. However, some species (*E.*

purpurea, *L. cardinalis*, *L. siphilitica*) experienced a subsequent increase in cover between August and September as basal rosettes regenerated from the roots.

Reproduction

We found that the interaction between inoculation and diversity treatment had a significant effect on the presence of reproductive structures for three species: *A. stellatum* ($p < 0.001$), *E. purpurea* ($p < 0.001$), and *S. pulchellum* ($p = 0.011$). Inoculation alone also had a significant effect on reproduction for two additional species: *L. cardinalis* ($p = 0.004$) and *O. violacea* ($p = 0.002$). The diversity treatment alone had a significant effect on reproduction for *L. siphilitica* ($p = 0.004$).

AMF Infection

We found that all six species remaining at the end of the growing season were colonized by various AM fungal structures (arbuscules, hyphae, and vesicles). The proportions of root sections that were colonized was significantly greater in the non-inoculated condition for all species (Figure 6).

DISCUSSION

During a single growing season, it appears that neither increasing species diversity nor inoculating the soil with the AMF *Rhizophagus intraradices* significantly impacts above-ground growth of native forbs on a green roof. However, using these two methods does impact survival and reproduction for some species and may therefore offer potential strategies for increasing native plant establishment on green roofs in the Midwest USA.

Effects of Plant Diversity

Our study supports the likelihood that the effect of species diversity on growth and survival is highly species-dependent; some species had 100% survival, some had 0% (due to their phenologies) and some saw a significant effect of diversity treatment on survival. There are several reasons for these differences. Each species can vary in its physiological adaptations to competition for space, nutrients, and water, depending on its surrounding conspecifics (Montesinos-Navarro 2017). Within species, a combination of trait plasticity and genetic differences can also lead to individual variation in the degree to which interspecific and intraspecific competition or facilitation may affect survival (Munzbergova et al. 2020). High species diversity in plant communities can improve plant survival and coexistence for many species under drought conditions, particularly when the plant species are from a variety of plant families and the phylogenetic diversity is high (Chaves 2021). When increased assemblages of species are grown together in a community under drought conditions, the stress gradient hypothesis suggests that facilitation becomes a more important factor than competition (Chaves 2021). Some species can act as nurse-plants, improving the environment for other species to survive (Navarro-Cano et al. 2016), which has been shown to be beneficial on green roofs (Bulter and Orians 2011). This implies that species having higher survival in our experimental polycultures compared to monocultures may be aided by other species. This finding therefore supports increasing species diversity on green roofs to increase survival although even our most diverse treatment only had only eight species; far fewer than the hundreds of native species that make up Illinois' natural prairies (Chadde 2019). Further studies that include long-term monitoring and an increased species pallet could help to further develop this theory.

Some native species may be more likely to survive and reproduce on green roofs if they possess specific advantageous traits or if their traits complement others growing in the community. If the species that are planted have a variety of functional traits, they may be more likely to work together in facultative relationships. It has been shown that increased functional diversity in a community can improve coexistence by reducing competition between species that share similar functional traits (Montesinos-Navarro 2017). Specifically, functional diversity can govern response to environmental changes by affecting growth, reproduction, and survival (Caruso 2006; Bu et al. 2019). When plant communities are intentionally designed to harbor species with a greater variety of traits, this can increase niche partitioning and promote overall improved resource use by the community (Goberna et al. 2016). For example, Goberna et al. (2016) found that increased plant community diversity resulted in greater uptake of various resources from the soil. This idea is supported in the current experiment by the trend toward an increasing survival rate for *E. purpurea* from the monoculture to the P4 and then to the P8 treatment. While these results should be interpreted with caution due to the low number of replicates in the current investigation (two monocultures per species and four each of the polycultures), they suggest that functional trait complementarity within the community may be especially important for some species. In our experiment, the two species in the P4A treatment that did not survive the entire growing season (*O. violacea* and *S. pulchellum*) are known to be ephemeral species and likely strategized for increased carbon storage below ground rather than production of above-ground biomass after the spring and early summer. This strategy would be supported for species that invest energy in roots, tubers, and other storage structures rather than investing energy in strategies to grow leaves, stems, and flowers under increased abiotic and biotic stress (Bu et al. 2019). *Oxalis violacea* is especially known to use bulbs as a method of

underground storage (Nesom 2009). While both *S. pulchellum* and *O. violaceae* were absent by September in our study, their expected early arrival in the early spring may support other species in future years, with increased shading and transpiration to cool the soil when the seedlings and new leaves of conspecifics are just starting to emerge. Continued monitoring of species over several generations and with additional research sites is needed for a more complete understanding of how competition and facilitation contribute to community structure on green roofs.

Effects of Below-Ground Diversity

Although supported by studies in natural and agricultural environments, the true impact of AMF on the survival and growth of native forbs on green roofs in general remains undetermined. The interactive effects of inoculation and diversity on survival and reproduction compound the understanding of which facultative relationships plant species may depend on in a green roof habitat. Research has shown that symbiotic AMF can increase nutrient acquisition especially when nutrients are low, in addition to improving protection against microbial pathogens and parasites (Rekret and Maherali 2019). AMF can increase drought tolerance, soil moisture and nutrient uptake during times of stress (Davidson et al. 2016; Qiao 2016), conditions that are typical of green roof habitats in the Midwest U.S.A. Inoculating soil with AMF can also improve plant growth by acting as a biofertilizer, suppressing pathogenic diseases, and improving water retention in the soil (Samuel and Veeramani 2020), which would show promise for green roofs as well. Adding symbiotic AMF to green roof soil should therefore contribute to growth and survival of plants in these drought-prone, low-nutrient environments. In our study, four species (*E. purpurea*, *L. ligulistylis*, *L. cardinalis* and *L. siphilitica*) out of eight had

significantly greater survival when the commercially-available AMF *R. intraradices* was added to the soil. These results suggest that if such species are planted on green roofs, their survival may be enhanced by the ability of *R. intraradices* to increase nutrient uptake and water retention in the soil (Varga 2015).

On the other hand, the surprisingly lower colonization rate of AMF in the roots of all species that we observed in the treatments that had *R. intraradices* added suggests that other factors in addition to inoculation with this one species may also be important. Specifically, these findings suggest that *Rhizophagus intraradices* may not be a symbiotic AMF species for the Illinois natives in our experiment but may instead be competing with other AMF in the environment that more readily colonize the roots of these particular species (Loján et al. 2017). Competition between AMF species is still not well-understood but does occur and can reduce the overall fungal abundance in the soil (Engelmoer et al. 2014) and effect nutrient uptake and growth of plants (Thonar 2014). While beyond the scope of the current study, the countless species of AMF found in soil will no doubt have various species-specific effects on plants in constructed habitats like green roofs. For example, many plant species considered generalists may not benefit much from AMF inoculation, no matter which species are used (Rondina et al. 2014).

Furthermore, some species may not need AMF to tolerate the harsh abiotic conditions of the green roof so the rate of root colonization may be irrelevant. For example, we found that *A. stellatum* and *O. humifusa* did not need AMF to survive, although *A. stellatum* had greater reproduction in the AMF treatment. This is not surprising given the high degree of drought tolerance of these species. At least for *A. stellatum*, it did not appear that the green roof microhabitat was negatively affecting the survival and reproduction of this species. AMF might

not improve nutrient and water uptake if it is not needed for certain aspects of the plant's life cycle (Young et al. 2015). For example, previous research in a related cactus species, *Opuntia ficus-indicas*, found no impact of AMF on growth and survival when low but adequate moisture was provided (Neffar and Chenchouni 2015). *Opuntia* and many other species in Cactaceae generally have low AMF colonization (Dhillion and Friese 1994) although *O. humifusa* can be found with high rates of AMF colonization in the wild (Deotare et al. 2014). The *O. humifusa*, as well as the other species used in our experiment, were planted as seedlings that had been growing in greenhouses for a few weeks to several months. Without AMF collection prior to planting, it is difficult to know whether previous microbial infection occurred within each individual plant.

It is likely that seedlings bought from nurseries are already infected with microbes (Halleen et al. 2003) and that AMF may colonize green roof soils when spores are brought in by wind or animals (Chaudhary et al. 2019; Metzler et al. 2024), although this was not directly measured in this experiment. Late-colonizing AMF species may confer an advantage to plants regardless of previous intentional inoculation. Furthermore, plants can develop specific local adaptations related to the soil microbial community (Rekret and Maherali 2019). Symbiosis with the local microbiota may help explain why we found a dramatically higher survival rate in *E. purpurea* in the non-inoculated monoculture treatment than in the treatment inoculated with *R. intraradices*. Our root staining and visualization confirmed the presence of AMF in both the inoculated and non-inoculated treatments of all six species that were still growing at the conclusion of the first growing season and neither of those grown as fresh seedlings in the greenhouse after the conclusion of the growing season (*O. violaceae* and *S. pulchellum*),

suggesting that a single-species inoculum can have a very limited or no effect on plant survival and growth.

It is highly likely that complex and community-wide below-ground dynamics are impacting plants' growth and survival. For example, many AMF species rely on bacteria to colonize plant roots. A recent study in Finland found that inoculation with the AMF species *Rhizophagus irregularis* alone did not significantly increase biomass of forbs on green roofs unless also combined with the bacterium *Bacillus amyloliquefaciens* (Xie G et al. 2018). The plants themselves also contribute to soil biota. For example, vegetation monocultures have been found to have lower AMF diversity in the soil while polycultures support a greater diversity of microbes (Dietrich et al. 2020). In a study conducted by Guzman et al. (2021), *Rhizophagus* was found to dominate plant monocultures, while *Glomus* dominated polycultures, indicating that fungal communities are impacted by plant communities. It is possible, therefore, that the effects of the *R. intraradices* treatment seen on our green roof trays was impacted by both the other plant species and other microspecies present and further investigations would be needed to tease apart these complex ecological interactions.

Additional time will be needed to determine the comprehensive effect that AMF has on herbaceous plants on green roofs. Some AMF species only colonize roots during certain portions of a plant's lifecycle, which can vary considerably by species (Smilauer et al. 2021). Individuals included in the current study were all at the early stages of their life cycles when planted in the inoculated soil. Life cycle differences within a species can further alter AMF symbiosis and nutrient uptake, which may increase intraspecific competition for AMF between plants in different life stages (Merrild et al. 2013). We have yet to examine the effects of AMF on multiple stages of the plants' life cycles such as seed germination. However, in our experiment,

half of the species growing in the inoculated soil (*E. purpurea*, *L. ligulistylis*, *L. cardinalis* and *L. siphilitica*) demonstrated a tendency toward greater vegetation regeneration after a drought in August compared to the non-AMF treatment. In other early successional communities, vegetation regeneration is more likely to occur in soils where AMF communities are present (Neuenkamp et al. 2018), suggesting that AMF inoculation may have contributed to successful regeneration after drought for at least some species in the current study. Long-term monitoring throughout the entire lifecycle of the species would be an ideal next step to developing a more complete understanding of the impacts of AMF.

CONCLUSION

In all habitats, above-ground and below-ground community diversity can be important biotic factors that can alter plant community structure, abundance, growth, and survival. Increasing soil or plant community diversity may contribute to successfully growing native plants in novel urban habitats like green roofs, but more work is needed in this important area of research. Over a single growing season, our results demonstrate that AMF and species diversity should be considered when determining how to best support native plant populations in constructed urban ecosystems, as their effects are complex. Except for one species grown in monoculture (*E. pulchellum*), it seems that commercial inoculum containing *R. intraradices* decreases root colonization of native Illinois wildflower species but not to a degree that negatively affects plant growth and reproduction. Community diversity was only found to significantly improve the survival of some species but our experiment was limited by a short time frame. Continued research is needed to determine if the observed trends persist into the future, especially in the case of ephemeral species. Application of these results to support future

projects will depend upon the outcome sought by a plant enthusiast, building manager, conservationist, or urban developer in that location. Overall, extensive green roofs remain a very harsh microhabitat for native Illinois forbs, specifically those that lack traits for prolonged drought tolerance.

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Table 1: Native Illinois prairie species used in the experiment. All species were included in a monoculture treatment (M; 1 species), polyculture treatment with four species in one of two different combinations (P4A or P4B), and a polyculture treatment with all eight species (P8). All species diversity treatments were also included in both AMF-inoculated and non-inoculated soil treatments. Species names are according to USDA (2024).

Polyculture Group	Family	Species	Common Name
P4A	Asteraceae	<i>Echinacea purpurea</i> (L.) Moench	Eastern purple coneflower
	Asteraceae	<i>Liatris ligulistylis</i> (A. Nelson) K. Schum.	Meadow blazing star
	Crassulaceae	<i>Sedum pulchellum</i> Michx.	Widow's cross
	Oxalidaceae	<i>Oxalis violacea</i> L.	Violet wood-sorrel
P4B	Alliaceae	<i>Allium stellatum</i> Nutt. Ex Ker Gawl.	Prairie onion
	Cactaceae	<i>Opuntia humifusa</i> (Raf.) Raf.	Eastern prickly pear
	Campanulaceae	<i>Lobelia cardinalis</i> L.	Cardinal flower
	Campanulaceae	<i>Lobelia siphilitica</i> L.	Blue cardinal flower

Figures

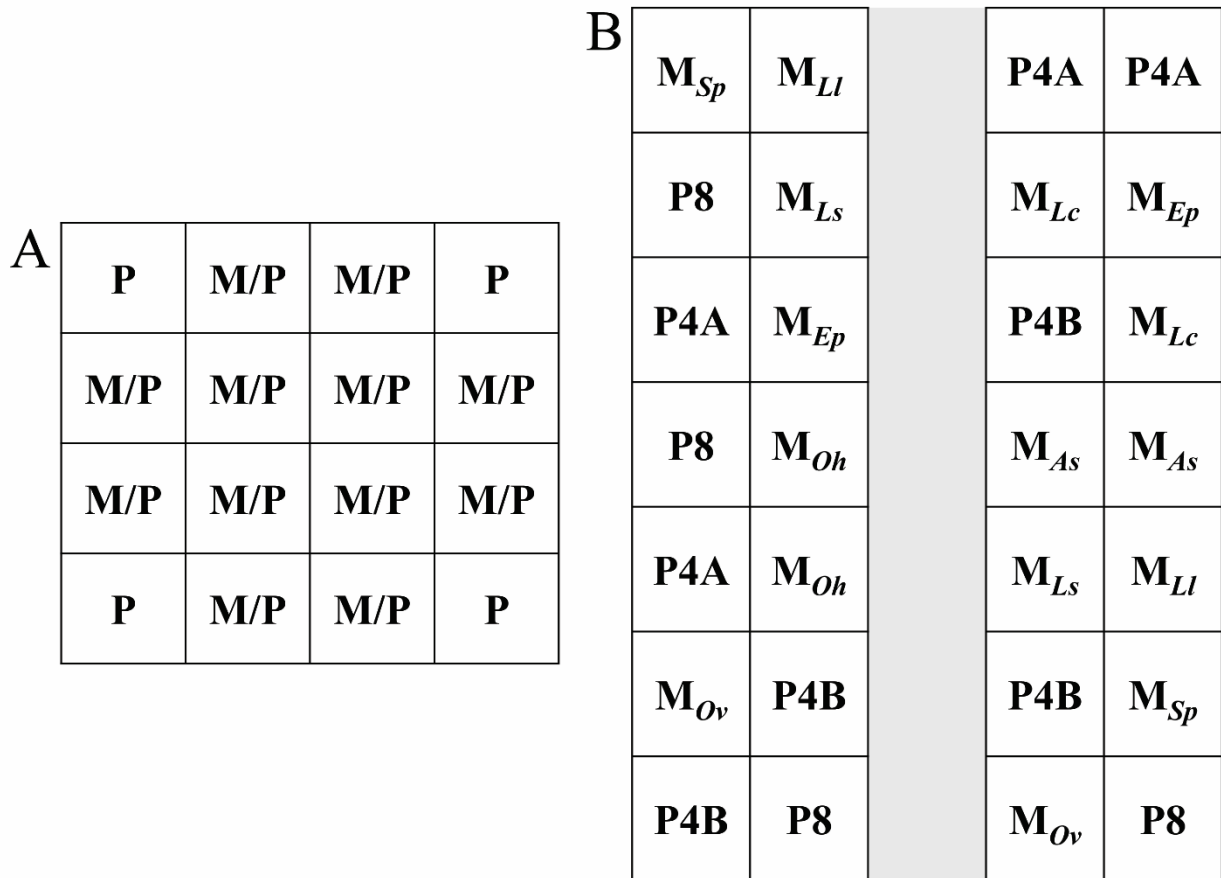


Figure 1. Layout of the planted seedlings in the experimental trays. Part A shows where the seedlings were planted within the tray monoculture treatments (M) and all of the polyculture treatments (P). Part B shows the location of the treatment trays with the top row at the north and bottom row at the south. The species in the monocultures are indicated by the subscripts representing the first letter of the genus and species in the scientific Latin name (e.g., *Ep* = *Echinacea purpurea*). The gray shaded area between columns 2 and 3 indicates a walking path that was kept open for data collection. Placement of the seedlings within the trays as well as the placement of the trays on the roof were determined by a random number generator.



Figure 2. Seedlings of native Illinois forbs were planted in either monocultures or one of two polyculture mixtures (P4A/B and P8) in experimental trays on the green roof. This photograph is from the start of the experiment just after planting in May, 2021.

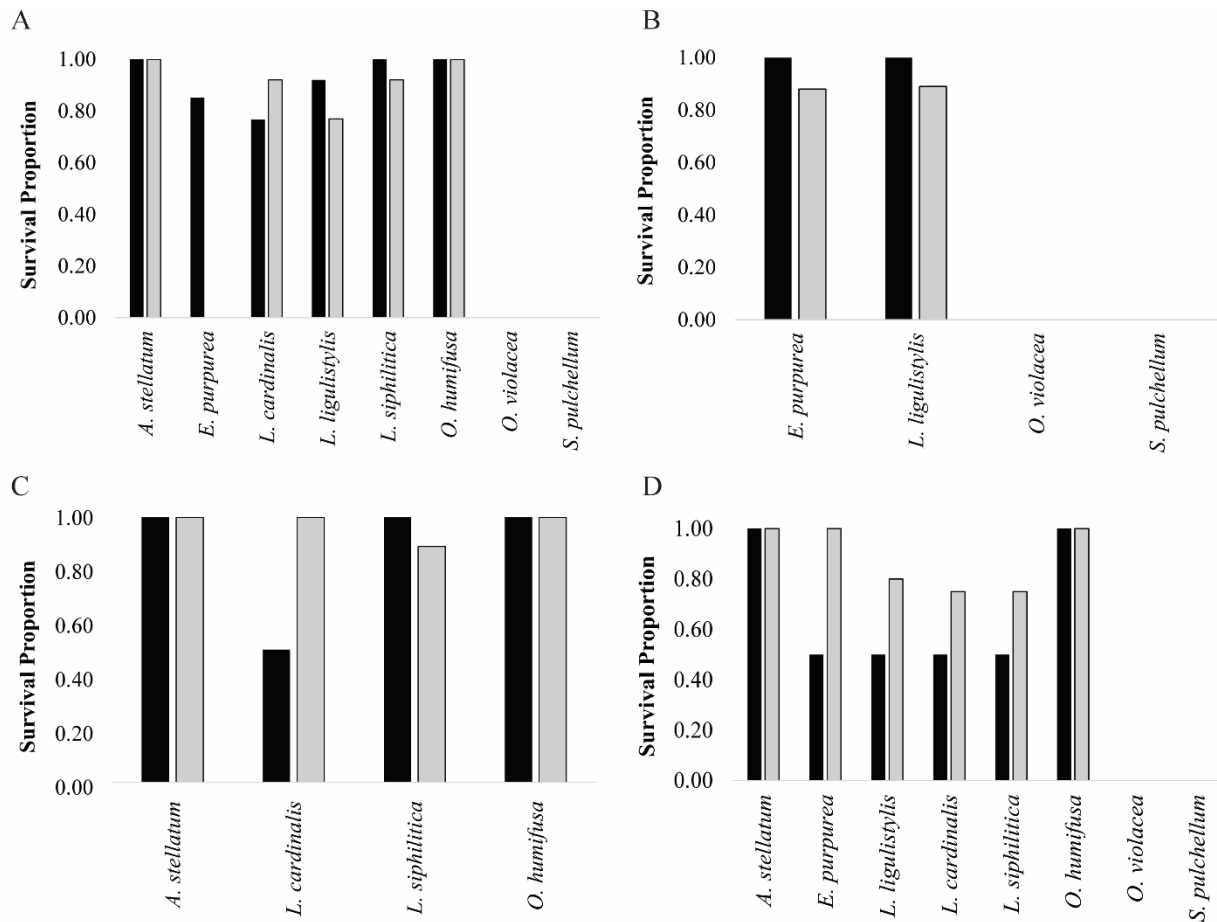


Figure 3. Survival proportion for eight native plant species on a green roof, broken down by both diversity and AMF treatment. Black bars are treatments without AMF, gray bars are treatments with AMF. Species were grown in monocultures (panel A, upper left), polyculture mixes with four species (P4A in panel B, upper right; P4B in panel C, lower left), and a polyculture mix with eight species (panel D, lower right). Lack of data for *O. violacea* and *S. pulchellum* is due to 0% survival of those species.

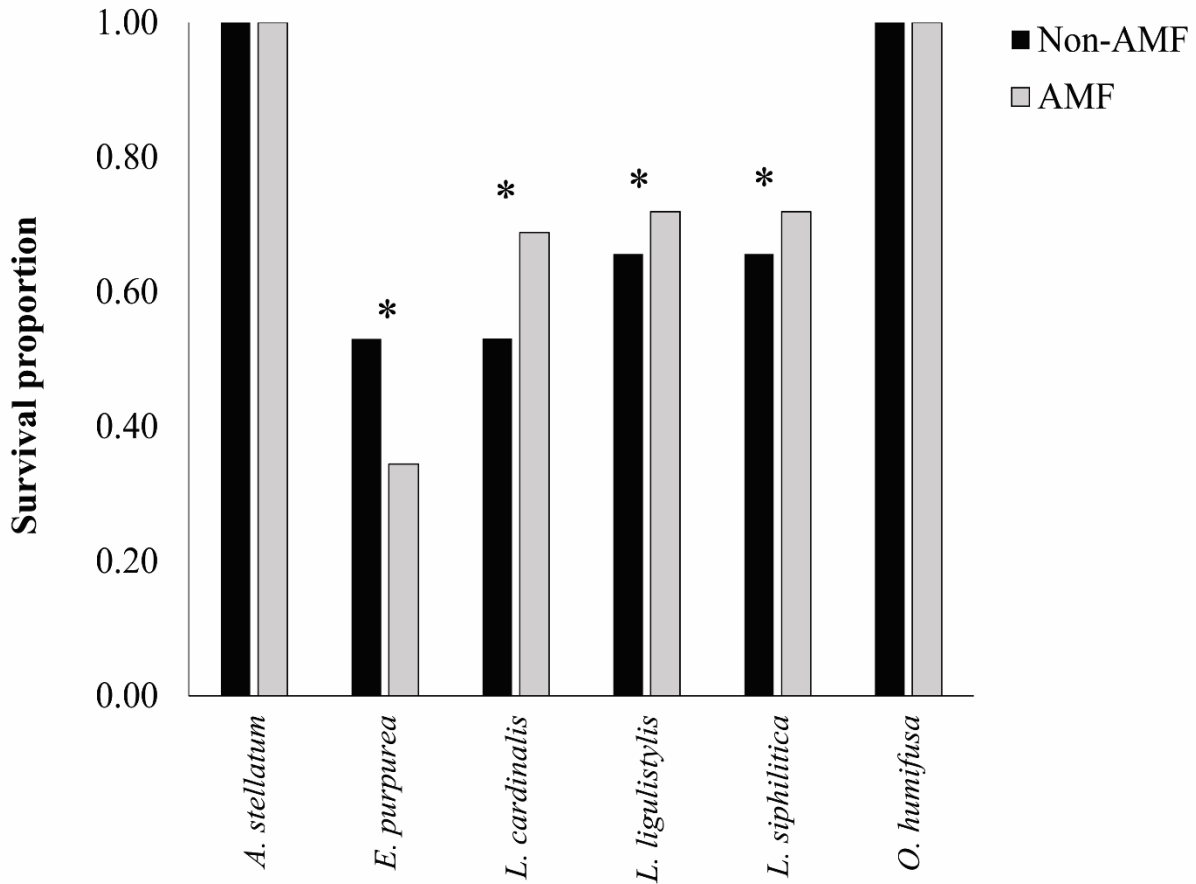


Figure 4. Survival proportion of six species of native plants on a green roof in soil that were either inoculated (AMF) or not inoculated (Non-AMF) with *Rhizophagus intraradices*. An asterisk (*) indicates a statistically significant difference ($p < 0.05$) between the soil treatments. Data for *O. violacea* and *S. pulchellum* are not included because these species had 0% aboveground biomass in both inoculum treatments by the end of the growing season due to their phenologies.

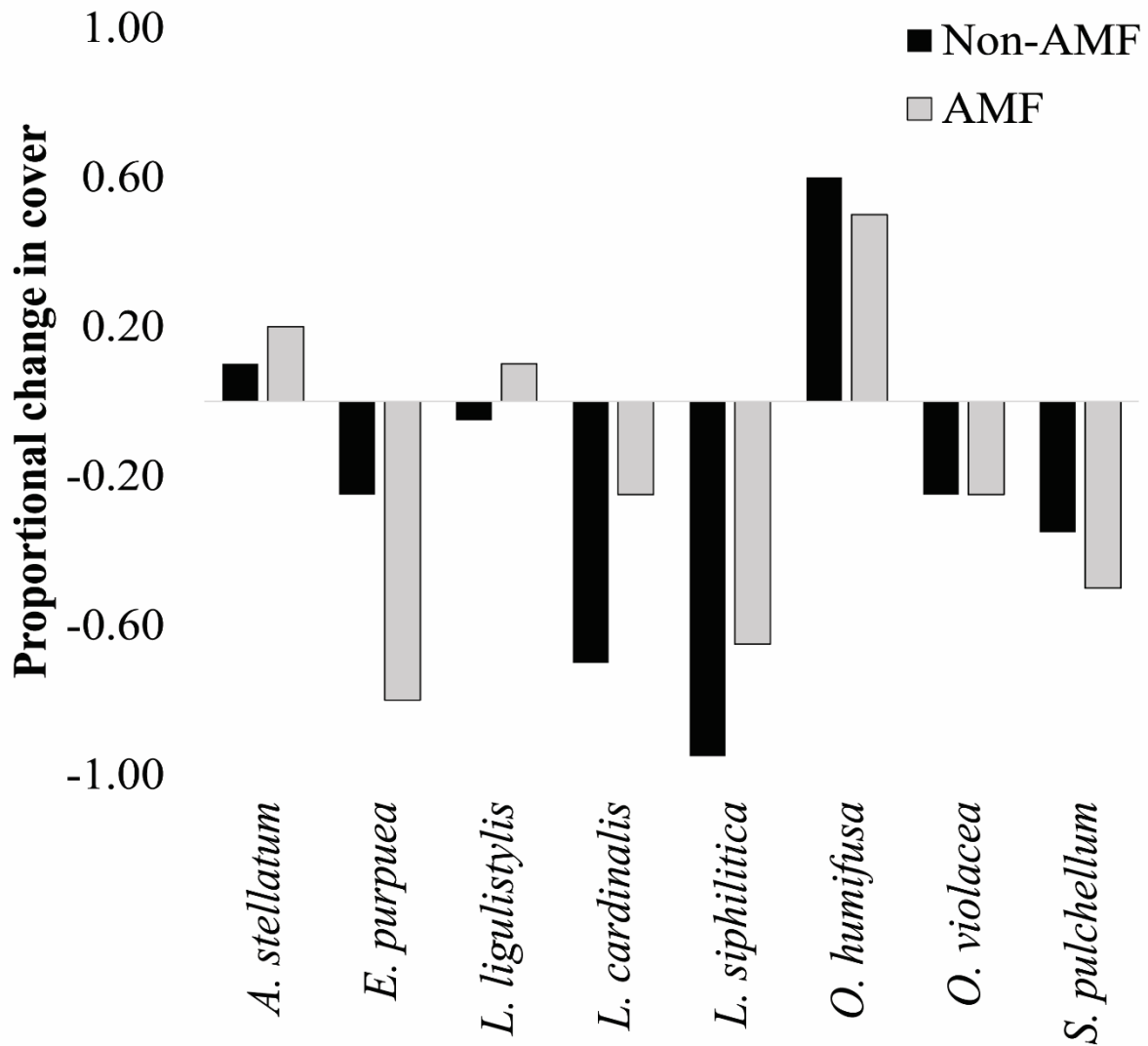


Figure 5. Change in vegetation cover over one growing season across all diversity treatments for eight species on a green roof. Increase in cover is indicated as bars above the zero line while decrease in cover is indicated as bars below the line.

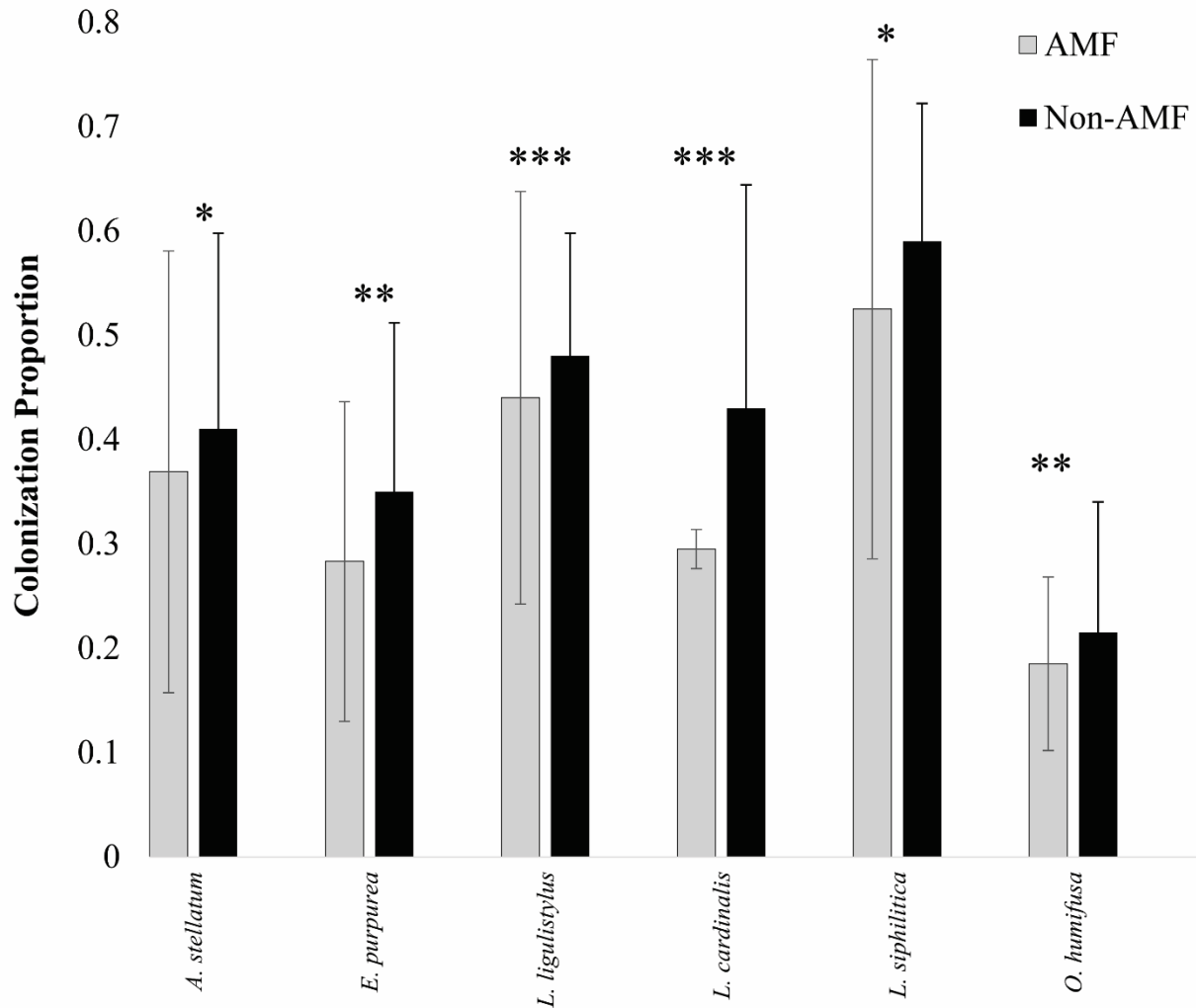


Figure 6. Effect of inoculation with *Rhizophagus intraradices* on the proportion of root sections colonized by AMF structures, including arbuscules, intraradical hyphae, extraradical hyphae, and vesicles. Asterisks indicate degree of certainty for significant effects: * $p = 0.01 - 0.05$; ** $p = 0.001 - 0.01$; *** $p < 0.001$. Error bars indicate standard deviation.