Research

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Tracking arbuscular mycorrhizal fungi to their source: active inoculation and passive dispersal differentially affect community assembly in urban soils

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Summary

- Communities of arbuscular mycorrhizal (AM) fungi assemble passively over time via biotic and abiotic mechanisms. In degraded soils, AM fungal communities can assemble actively when humans manage mycorrhizas for ecosystem restoration.
- We investigated mechanisms of urban AM fungal community assembly in a 2-yr green roof experiment. We compared AM fungal communities in inoculated and uninoculated trays to samples from two potential sources: the inoculum and air.
- Active inoculation stimulated more distinct and diverse AM fungal communities, an effect that intensified over time. In the treatment trays, 45% of AM fungal taxa were detected in the inoculum, 2% were detected in aerial samples, 23% were detected in both inoculum and air, and 30% were not detected in either source.
- Passive dispersal of AM fungi likely resulted in the successful establishment of a small number of species, but active inoculation with native AM fungal species resulted in an immediate shift to a diverse and unique fungal community. When urban soils are constructed or modified by human activity, this is an opportunity for intervention with AM fungi that will persist and add diversity to that system.

Introduction

Diverse, productive, and resilient urban ecosystems provide ecosystem services such as carbon storage, nutrient cycling, reduced air pollution, stormwater capture, habitat and food provisions, and mitigation of the urban heat island (Robinson & Lundholm, 2012; Speak *et al.*, 2015; Potgieter *et al.*, 2017; Ksiazek-Mikenas *et al.*, 2023). But, these ecosystems face challenges due to soil disturbance and unique anthropogenic stressors in urban environments (Morel *et al.*, 2015; Nelson & Lajtha, 2017). Plants in urban ecosystems respond to these stressors in many ways, but often rely on key components of the soil microbiome such as arbuscular mycorrhizal (AM) fungi, which can help plants adapt to nutrient limitation, altered soil-water relations, altered temperature regimes, and exposure to pollutants (Chaudhary *et al.*, 2019).

As urban ecosystems expand world-wide, they are increasingly called upon to provide a myriad of ecosystem services to the majority of the world's population residing within them (Ziter, 2016). Diverse communities of AM fungi, either directly or indirectly, provide key ecosystem services such as disturbance regulation, climate regulation, water regulation, nutrient cycling, and erosion control (Markovchick *et al.*, 2023). Still, anthropogenic activities associated with urban areas and reduced proximity to wilderness can reduce AM fungal diversity and alter AM fungal community structure (Pärtel *et al.*, 2017). Restoring urban AM fungal communities in an effort to support urban plant biodiversity and function, as well as urban ecosystem services remains a key challenge in urban environmental management (Chaudhary *et al.*, 2019; Markovchick *et al.*, 2023). Because diverse communities of AM fungi likely support greater above- and belowground biodiversity and ecosystem function across spatial and temporal scales (Powell & Rillig, 2018), there is a need to better understand how urban AM fungal communities assemble as a result of both natural and human-assisted processes (Hart *et al.*, 2018).

Urban AM fungal communities likely assemble through a combination of both deterministic, or niche-based, and stochastic mechanisms (Lekberg *et al.*, 2007). External abiotic factors such as climate, soil pH, and soil texture, as well as external biotic factors like plant community structure and interspecific interactions all contribute to deterministic environmental filtering that impacts AM fungal community structure (Chaudhary *et al.*, 2008; Davison *et al.*, 2015). In cities, niche-based factors that drive community assembly are further complicated by anthropogenic impacts on ecosystems through physical, chemical, and thermal disturbances (Morel *et al.*, 2015; Nelson & Lajtha, 2017). Temporal dynamics also likely play a role as assembly of AM fungal communities over time may also depend on initial plant partners available at a site, forming early

symbiotic relationships and setting up a pattern of priority effects whereby early successional AM fungal species affect the future trajectory of the community (Hausmann & Hawkes, 2010). In disturbed environments like urban habitats, these priority effects may favor generalist species of both plants and fungi (Verbruggen *et al.*, 2013). Newly introduced taxa may have difficulty establishing in such disturbance-adapted local communities (Verbruggen *et al.*, 2013).

Despite being comparatively understudied, stochastic mechanisms also contribute to AM fungal community assembly (Caruso *et al.*, 2012). Often, dispersal is used synonymously with 'neutral' (Lowe & McPeek, 2014), but dispersal traits (e.g. spore morphology and dispersal syndromes) may predict AM fungal species occurrences and geographic range extent (Aguilar-Trigueros *et al.*, 2023). In AM fungi, biotic and abiotic vectors, such as migratory animals, wind, and water could disperse AM fungi over long distances (Harner *et al.*, 2011; Egan *et al.*, 2014; Correia *et al.*, 2019; Paz *et al.*, 2021; Chaudhary *et al.*, 2022). In urban ecosystems, spore traits determined which AM fungal species are more likely to disperse aerially (Chaudhary *et al.*, 2020) and dispersal is likely not limited between rooftops and ground-level sources (Droz *et al.*, 2022; Hénault *et al.*, 2022). Soil bioinoculants are often researched or sold as soil amendments (Hart *et al.*, 2018), with the assumption that AM fungi are dispersal-limited and will not reform these beneficial associations on their own as well or as quickly as needed (Markovchick *et al.*, 2023). In restoration, they have been used to overcome dispersal limitations and accelerate restoration and the recovery of disturbed systems (Harris, 2009; Koziol & Bever, 2017). Native inoculum, collected from local, late successional soils, can often lead to increased restoration success (Koziol *et al.*, 2018). In some environments, native AM fungi improve host growth, establishment, nutrient uptake, and water retention better than nonnative AM fungi (Requena *et al.*, 2001; Querejeta *et al.*, 2006). Furthermore, native AM fungal inoculum increases plant defense against herbivory more than nonnative inoculum (Sikes *et al.*, 2009; Middleton *et al.*, 2015). The beneficial effect of AM fungal inoculation can be particularly pronounced in the case of extreme soil disturbances such as topsoil removal (Molineux *et al.*, 2015; Wubs *et al.*, 2016). Additionally, the beneficial effects of bioinoculants can increase after multiple years (Requena *et al.*, 2001; Koziol *et al.*, 2022), suggesting the importance of examining the effects of AM fungal inoculation and passive establishment over time (John *et al.*, 2014; Yang & Davidson, 2021).

Green roofs are vegetated rooftops installed to improve building energy efficiency and provide environmental and aesthetic benefits that promote urban ecosystem resiliency and sustainable development (Cristiano *et al.*, 2021). In cities, green roof benefits include the reduction of the urban heat island effect, improved stormwater management (Wang *et al.*, 2017; Droz *et al.*, 2021), and the potential to enhance urban biodiversity (Oberndorfer *et al.*, 2007). Green roofs are a model system to study urban AM fungal community assembly because of their similar design and construction across sites, initial homogeneity of soils, and relative isolation from other ecosystems. In North America, most green roof plants form AM associations, yet green roof soils comprise a rocky, porous, constructed medium that is initially devoid of AM fungal propagules (John *et al.*, 2014; Chaudhary *et al.*, 2019). The relative sterility and isolation of green roofs not only make them great candidates for mycorrhizal inoculation but also make them useful models to study fungal dispersal and community assembly. The addition of AM fungi may improve green roof function and ecosystem services by increasing plant drought tolerance, promoting plant access to limited nutrients, supporting overall plant diversity, and enhancing green roof carbon sequestration capabilities (John *et al.*, 2017; Fulthorpe *et al.*, 2018). On green roofs, AM fungal inoculum increases microbial biomass (Molineux *et al.*, 2014) and inoculation leads to increased AM fungal colonization and higher tissue phosphorus (Young *et al.*, 2015). The effects of AM fungal inoculum on plant growth and vigor is often context-dependent, varying according to the presence of other soil amendments (Sutton, 2008) and plant identity (Busch & Lelley, 1997). Despite the potential benefits that AM fungi could provide to urban green roof biodiversity and function, little is known about how inoculation affects the trajectory of AM fungal community structure in these systems.

To untangle active (i.e. human-mediated) and passive mechanisms of AM fungal community assembly in urban green roofs, we conducted a 2-yr green roof inoculation experiment using locally collected and species-rich native inoculum. We tracked the establishment and persistence of AM fungal inoculum and its effect on green roof AM fungal communities and compared this to aerial collections of passively dispersing AM fungi. We also examined how AM fungal community structure changed depending on varying green roof plant communities (i.e. habitat analog vs commercial monoculture). Because AM fungal spore abundance and diversity are relatively low in passively dispersing urban aerial samples (Chaudhary *et al.*, 2020), we predict that active inoculation will lead to more diverse and distinct AM fungal communities compared with uninoculated controls (Hypothesis 1). Prior research has documented the accumulation of AM fungal propagule density in urban green roofs over time (Chaudhary *et al.*, 2019; Yang & Davidson, 2021). As such, we predict that AM fungal species from both the aerial samples and native inoculum will persist and accumulate in green roof soils along with species from other unmeasured sources (Hypothesis 2). Finally, as greater plant diversity supports greater primary productivity and ability to support obligate symbionts (Antoninka *et al.*, 2011) we predict that experimental green roof trays planted with a higher diversity of plants would lead to a higher diversity of AM fungal species (Hypothesis 3). Tracking the fate of AM fungal inoculum and urban AM fungal community trajectories under differing management approaches supports the need for human intervention in urban ecosystem restoration.

Materials and Methods

Study design

To examine the difference between passive dispersal and active inoculation on AM fungal community assembly, we initiated a green roof inoculation experiment in Chicago, IL, USA, in the

spring of 2014. The experiment consisted of 40 modular 30 × 60 cm green roof trays (EcoRoofs LLC, Berrien Springs, MI, USA) each filled 10 cm deep with engineered commercial green roof soil. Trays were used to mimic the shallow substrate depth, high soil porosity, and low maintenance nature of extensive green roof systems that are commercially available in North America (Oberndorfer *et al.*, 2007). The trays were set up adjacent to each other in a 10 × 4 grid with a border of unplanted trays filled with soil surrounding the grid to control for any edge effects such as moisture loss or temperature.

Half of the trays were inoculated with a native AM fungal inoculum before planting. To make the inoculum, we collected rhizosphere soil from numerous native prairie plants at the 32-yr-old Dixon Prairie restoration at the Chicago Botanic Garden in Glencoe, IL, USA. To collect AM fungal species with a greater likelihood of persisting in green roof soils, we preferentially collected rhizosphere soil from plants growing in dry, shallow, and rocky prairie outcroppings, homogenizing all samples into a single inoculum. Treatments were set up by mixing 250 ml of inoculum with engineered green roof soil per tray for 20 experimental trays. To control for potential abiotic effects of inoculum (e.g. fertilization effect), we dry sterilized half of the inoculum in an autoclave at 120°C for 1 h and mixed 250 ml of the sterilized inoculum with the engineered roof soil for 20 noninoculated control trays. To verify the presence of viable AM fungal propagules in the live inoculum and the absence of propagules in both the sterile inoculum and background tray soil, we conducted a mycorrhizal infection potential bioassay using *Zea mays* L. (Ksiazek-Mikenas *et al.*, 2021).

Each tray was planted with one of three plant species pools or left bare as an unplanted control. Two of the species pools were made up of local native forbs and grasses from analogous natural habitats and the third species pool was made up of a mix of commonly used nonnative succulent *Sedum* L. species. Species selection for the pools is described in greater detail in Ksiazek-Mikenas *et al.* (2021), but briefly, native species were chosen based on two natural community types corresponding to rock prairies and sand prairies. Native prairie species were grown from seed in sterile soil in a glasshouse for *c.* 8 wk before planting. *Sedum* individuals were grown outdoors from cuttings for at least a year and arrived as fully grown mature individuals. We planted the native forb seedlings as plugs with potting soil left intact. For the *Sedum*, we dug up individuals from the original soil, and gently brushed off any large pieces of soil before planting. At this stage, *Sedum* individuals likely transferred over some of their existing soil microbiome, which was acceptable to this study as this is a much more realistic horticultural application of *Sedum* than plants grown in a sterile glasshouse. The planted trays were placed in an indoor glasshouse, where they were watered to support establishment for 6 wk, and then transported outdoors to the 4th-floor terrace of the Quinlan Life Science Building on the campus of Loyola University, Chicago, USA.

To examine AM fungal community structure across experimental treatments and time, we collected soil samples from each green roof tray after a 4-month period of establishment (fall of 2014) and then again after 2 yr (fall 2016). In order to not damage the structural integrity of the green roof trays, one soil sample per tray per year was taken by first gently loosening the soil with a 2.5 cm hand corer and then collecting *c.* 200 cm^3. This soil sample was homogenized and 0.25 g was used for DNA extraction. Soil samples were stored at −20°C until the time of DNA extraction. To examine AM fungal communities from active inoculation, a portion of live AM fungal inoculum was stored at −20°C until the time of DNA extraction. To examine passive dispersal of AM fungal propagules for green roofs, we include AM fungal community structure data from aerial samples collected for a study conducted nearby (Chaudhary *et al.*, 2020). Briefly, aerial dispersal of AM fungi was assessed using passive dust collectors that were placed on a 4th-floor Chicago rooftop, from January to December 2017. These aerial samples were collected in the year following our green roof tray soil sampling but were analyzed on the same sequencing run and are used here to offer a qualitative comparison between soil and potential aerially dispersed species.

DNA extraction, processing, and analysis

DNA was extracted from soil using the DNeasy PowerSoil Kit (Qiagen). DNA concentration was quantified using a fluorometer (Qubit; Invitrogen) and sent to Argonne National Laboratory (https://www.anl.gov/bio/environmental-sample-preparation-and-sequencing-facility) for sequencing, using a protocol (Morgan & Egerton-Warburton, 2017), which targets a segment of Glomeromycotinan small subunit ribosomal RNA (SSU rRNA) using the NS31 and AML2 primers (Simon *et al.*, 1992; Lee *et al.*, 2008). There remains no consensus regarding the best amplicon rRNA gene region for the species-level identification of AM fungi (see Delavaux *et al.*, 2021); we utilized SSU because it provides better species-level resolution than the internal transcribed spacer and has a curated, accessible public database (i.e. MaarjAM) for taxon assignment (Egan *et al.*, 2018). Raw demultiplexed paired-end reads were processed using QIIME2 CORE 2020.2 (Bolyen *et al.*, 2018). Denoising was carried out using the DADA2 plugin according to the QIIME2 tutorials (Callahan *et al.*, 2016). Forward and reverse reads were truncated at 300 bp or earlier when expected error was ≥ 2, merged with a minimum overlap of 15 bp, and chimeras were identified and removed. This resulted in a frequency table of 643 amplicon sequence variants (ASVs) used for further analysis.

Community similarity, species richness, and taxonomic assignment

Patterns in AM fungal community structure were visualized using ASVs in a nonmetric multidimensional scaling (NMDS) ordination, with dissimilarity based on Bray–Curtis distance and $k = 2$ axes, using the VEGAN package (Oksanen *et al.*, 2022) in R, v.4.3.0 (R Core Team, 2023). Although a stress value of 0.2 is often used as a cutoff for biological interpretation of NMDS, this value is known to increase with sample size and decrease with dimensionality (Kruskal, 1964; Clarke, 1993). For larger sample sizes ($n = 30$–40), like those used in this study, we applied the

recommendations of Dexter *et al.* (2018) to compare our stress to a permutational null model of random species association to help interpret ordination fit.

A permutational multivariate analysis of variance (PERMANOVA) of the ASV Bray–Curtis distance matrix, calculated using the 'adonis' function, was used to assess the community difference between inoculation treatment, plant community, and year (blocked by tray). Due to disagreement in the literature about interpreting relative abundance from sequencing data (McMurdie & Holmes, 2014; Schloss, 2023), we repeated this analysis with rarefied ASV tables rarefied to the lowest number (626) of reads per treatment (Cameron *et al.*, 2021) using the 'avgdist' function in the VEGAN package (Oksanen *et al.*, 2022) and with a presence–absence distance matrix based on Jaccard distances (McCune *et al.*, 2002). Based on a visual interpretation of the NMDS, we also tested for homogeneity of variance of the distances using the 'betadisper' function and pairwise analysis of variance on a dummy variable that combined year and inoculation.

To identify important environmental predictors of AM fungal ASV richness, the 'dredge' function of the MUMIN package (Burnham & Anderson, 2002) was used to compare linear mixed-effects models using the LME4 package v.1.1-33 (Bates *et al.*, 2015) that included plant cover type, inoculation treatment, year, their two-way interaction terms, and the random variable: tray ID. Only informative models with Δ_i values less than four were used to identify important predictor variables (Burnham & Anderson, 2002).

Taxonomy of ASVs was assigned by BLAST+ searching against the MaarjAM database (Öpik *et al.*, 2010). The taxon with the highest query coverage and max identity (minimum 97%, if ambiguous, highest max score), was assigned to the sequence, limiting the results to taxa with MaarjAM virtual taxa (VT) numbers. In a single case, one 524-bp sequence search resulted in two VT matches with identical query coverage (100%) and identity match (99%) (*Diversispora eburnea* (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüßler VTX00060 and *Diversispora spurca* (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüßler VTX00263). This likely occurred due to limitations on next-generation sequencing of the SSU region to resolve some taxa at the species level, so this VT is listed as VTX00060. Note that this approach to DNA based identification of AM fungi relies on VT that may be over or underestimating actual or morphological species depending on rates of inter- and intraspecific variation in this region. Likely, VT's provide a conservative estimation of true species diversity (Bruns & Taylor, 2016). For example, VT00193 contains a closely related (Błaszkowski *et al.*, 2022) species complex of *Claroideoglomus claroideum* C. Walker & A. Schüssler, *C. etunicatum* C. Walker & A. Schüssler, *C. lamellosum* C. Walker & A. Schüssler, *C. luteum* C. Walker & A. Schüssler. Taxon names and VTX numbers were assigned using the most recent MaarjAM database version, which was updated in 2019 and does not reflect more recent changes to the taxonomy of the Glomeromycotinan fungi (Błaszkowski *et al.*, 2022). Temporal shifts in AM fungal VTs and potential sources of species pools were visualized using the alluvial package in R (Bojanowski & Edwards, 2016).

Results

Across the entire study, including inoculum, aerial samples, and experimental trays, 643 ASVs corresponding to 63 AM fungal VTs were detected across seven genera from seven families: *Paraglomus* J.B. Morton & D. Redecker, *Claroideoglomus* C. Walker & A. Schüssler, *Glomus* Tul. & C. Tul., *Diversispora* C. Walker & A. Schüssler, *Archaeospora* J.B. Morton & D. Redecker, *Acaulospora* Gerd. & Trappe, and *Scutellospora* C. Walker & F.E. Sanders (Supporting Information Table S1). After 4 months (2014), 59% (19 VTs) of the 32 VTs unique to the inoculum were detected in the experimental green roof trays and most of these (15 VTs) persisted for 2 yr (2016). Of the unique inoculum VTs, only two additional VTs were detected in 2016, making a total of 66% of inoculum VTs being detected in the experimental trays at some point during the experiment; 44% never established to a detectable level. The vast majority of these established VTs (all but three) were detected in trays treated with the inoculum or, if detected in control trays, were also detected in inoculated trays.

Of the 11 VTs found in both inoculation sources, 'inoculum' and 'air', 100% of the VTs were detected in the experimental trays in 2016, all except one were persistently detected since 2014. Of these 11 VTs, three shifted from only being detected in inoculated treatment trays in 2014 to also being detected in control trays in 2016, suggesting movement between treatments. Of the six VT uniquely found in the 'air' source (Fig. 1), nearly all were never detected in the experimental trays aside from one (17%), *Paraglomus Pa 1*, which was detected in inoculated treatment trays in 2014 but did not persist to 2016. Fourteen VTs were not detected in either source but were detected broadly across all experimental trays and are labeled as 'Unknown Source' in Fig. 1. Half of these were detected in 2014 and half were newly detected in 2016.

The experimental trays showed distinct community structure based on treatments (Fig. 2, $k = 2$, stress = 0.24). Random permutations of the community data based on a null model of community associations produced a stress value of 0.30, which was significantly different than our observed stress value ($Z = -13.96$, $P < 0.01$), which supports a biological interpretation of this NMDS despite a relatively high-stress value (Fig. S1). Visually, the NMDS ordination is structured by year and by inoculation. With regard to plant communities, bare ground (circles, Fig. 2) tended to cluster differently than planted communities. An 'adonis' PERMANOVA of treatments resulted in a significant effect of sampling year ($P < 0.001$, $R^2 = 0.16$), inoculation ($P < 0.001$, $R^2 = 0.06$), and plant community ($P < 0.001$, $R^2 = 0.1$) on community similarity, as well as the interaction between inoculation and plant community ($P < 0.001$, $R^2 = 0.05$). The results for the PERMANOVA repeated on the community dissimilarity metrics computed from the presence–absence matrices were similar but had additional significant two-way interactions (Fig. S2). Visually, inoculated trays in 2016 seem to have more tightly clustered and more similar

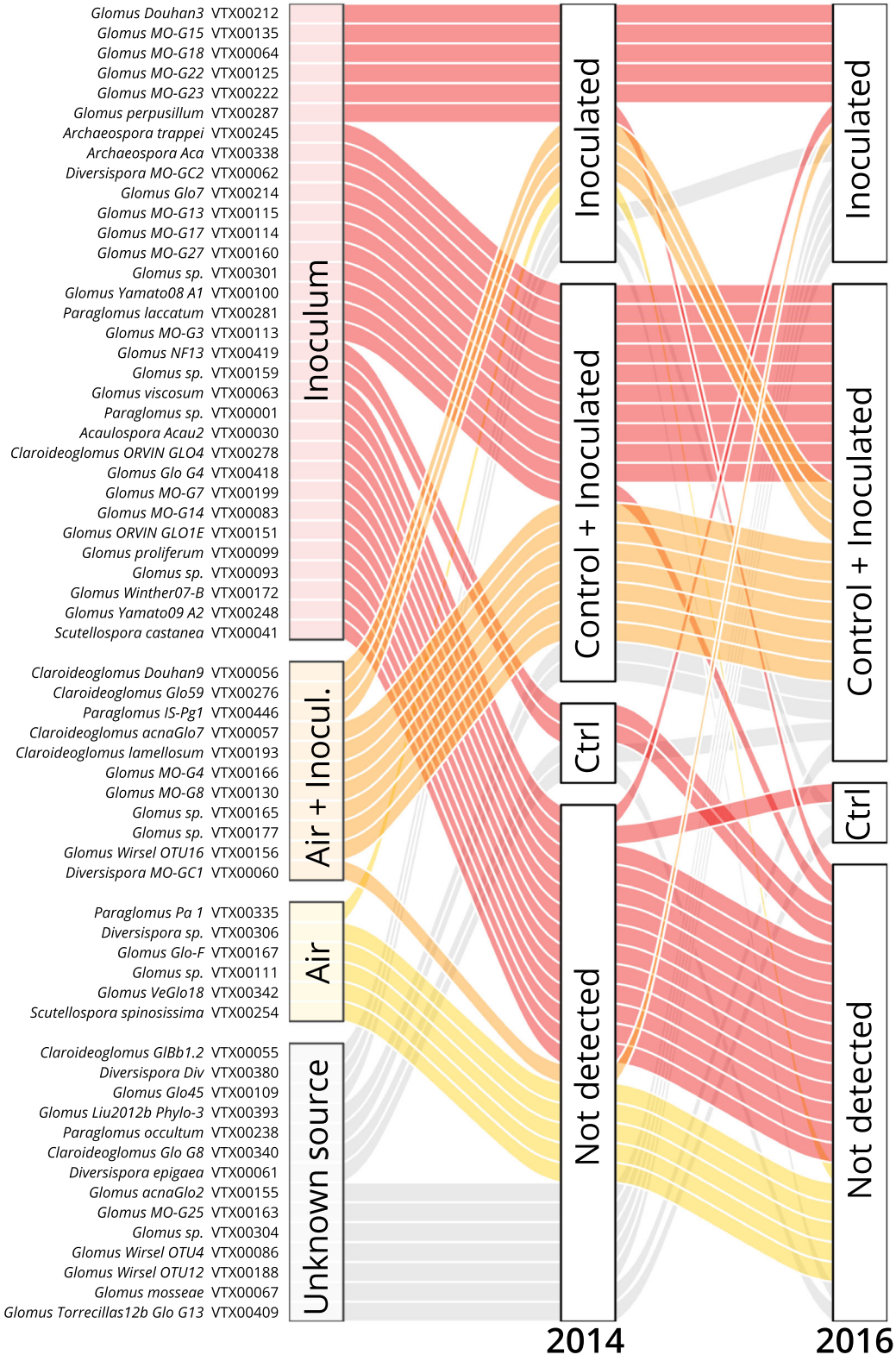

Fig. 1 Alluvial plot of all arbuscular mycorrhizal (AM) fungal virtual taxa (VT) identified in this study across potential sources and treatments. Lines indicate VT present in potential propagule sources (left) and in treatment trays in 2014 (middle) and 2016 (right). Red lines indicate VT that was detected in the inoculum but not air. Orange lines indicate VT detected in both sources (air and inoculum). Yellow represents VT only detected in the air. Gray represents VT detected in the treatment plots that were not detected in the species pool of either potential propagule source. Treatment years are separated by which species were found in the control plot, the inoculated plot, both, or not detected.

communities than any other group. This is confirmed with the test for homogeneity of dispersions where the average distance to centroids in control 2014, control 2016, inoculated 2014, and inoculated 2016 treatments were 0.6550, 0.6239, 0.6308, and 0.5197, respectively. A pairwise test determined that this group had a smaller distance to the centroid than any other pairwise comparison of year and inoculation ($P < 0.001$), and rarefied results were similar to reported results (Table S2).

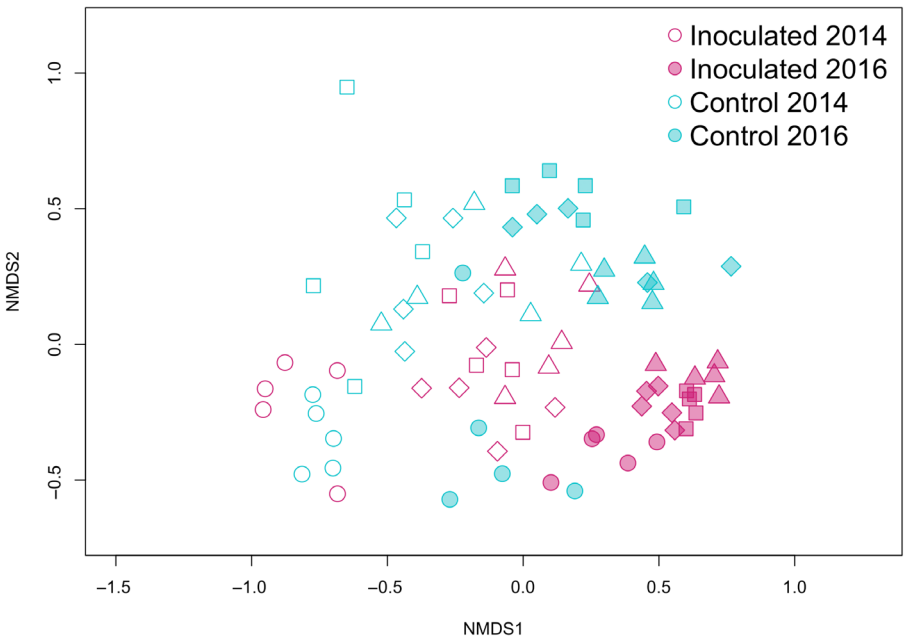

Fig. 2 Nonmetric multidimensional scaling ordination of arbuscular mycorrhizal (AM) fungal amplicon sequence variant (ASV) community similarity across experimental green roof trays. Control and inoculation treatments are represented as blue and pink, respectively. Sample years, 2014 and 2016, are represented as unfilled and filled respectively. Plant community is represented by shapes. Circle, square, diamond, and triangle represent bare, rock prairie, sand prairie, and *Sedum* plant cover, respectively ($k = 2$, stress = 0.24). An 'adonis' PERMANOVA of treatments resulted in a significant effect of sampling year ($P < 0.001$, $R^2 = 0.16$), inoculation ($P < 0.001$, $R^2 = 0.06$), and plant community ($P < 0.001$, $R^2 = 0.1$) on community similarity, as well as the interaction between inoculation and plant community ($P < 0.001$, $R^2 = 0.05$).

The highest AM fungal species richness was observed in inoculated trays from the later (2016) treatment and in the *Sedum* L. plant cover type (Fig. 3). Upward trends in richness are seen from bare to *Sedum* plant cover, from 2014 to 2016, and from inoculated to control treatments (Fig. 3). The linear mixed-effects model showing the best fit includes inoculation, plant cover type, sampling year, and all two-way interactions (AICc = 544.2). Only the global model containing all predictors had a Δ_i value less than four indicating substantial empirical support for the model ($\Delta_i = 0.00$). ASV richness was significantly greater in trays containing the sedum plant cover type, and ASV richness was greater in inoculated plots containing the rock prairie plant cover type (Table S3).

Discussion

Native inoculum alters AM fungal community trajectory

Inoculation with AM fungi likely provides a benefit to green roof function (John *et al.*, 2017). These changes could be direct, mediated by the mutualism, or indirect, by impacting the microbial communities in the soil (Rumble & Gange, 2017; Rumble *et al.*, 2018, 2022). In support of Hypothesis 1, inoculated green roof trays contained more distinct and species-rich AM fungal communities. The most unique and diverse AM fungal communities occurred in the inoculated trays in 2016, suggesting an effect of inoculation which is enhanced 2 yr later, likely accumulating over time (Chaudhary *et al.*, 2019). Over half of the VTs detected in the inoculum were detected again and retained in inoculated trays, suggesting that active management of belowground communities results in a persistent change. Roughly half of the VTs in the inoculum failed to establish at detectable levels. It is possible that those species were not suited to the abiotic environment, plant hosts, or persisted at a low, undetectable abundance. Creating inoculum from later successional soils may have preferentially selected for species that take longer to establish and could be more effectively incorporated at a later successional stage.

Relying on passive aerial dispersal to restore or rewild AM fungal communities is slower and may be more susceptible to the establishment of AM fungal species with traits or life history strategies that resemble weedy or ruderal species (Chaudhary *et al.*, 2020). This could be the case with *Paraglomus* Pa 1 (VTX00335), which was present in the aerial samples and established on the green roof in 2014. *Paraglomus* J.B. Morton & D. Redecker species typically have small diameter spores (Aguilar-Trigueros *et al.*, 2019), supporting evidence that AM fungal spore traits can predict aerial dispersal capabilities (Chaudhary *et al.*, 2020). Most species found exclusively in aerial samples were not detected in treatment trays, which could be due to competition, low biomass of aerial spores, stress from aerial movement in the atmosphere (Branco *et al.*, 2022), or potential host preference (Ramana *et al.*, 2023). Our experiment was not designed to detect aerial dispersal *per se*. Instead, we aimed to consider alternate and passive sources of propagules and measure community shifts over time, taking into account numerous potential species pools.

AM fungal species from passive dispersal and active inoculation persist and accumulate in green roof soils

Engineered green roof soils contain very few microbes when they are first delivered to a building site (Rumble *et al.*, 2018), but once established, postinoculation, AM fungal species in green roofs tend to persist. In support of Hypothesis 2, we found that many species from both the aerial samples and the inoculum persisted and accumulated along with other species from unmeasured human-mediated (e.g. soil medium, plantings, and seeds)

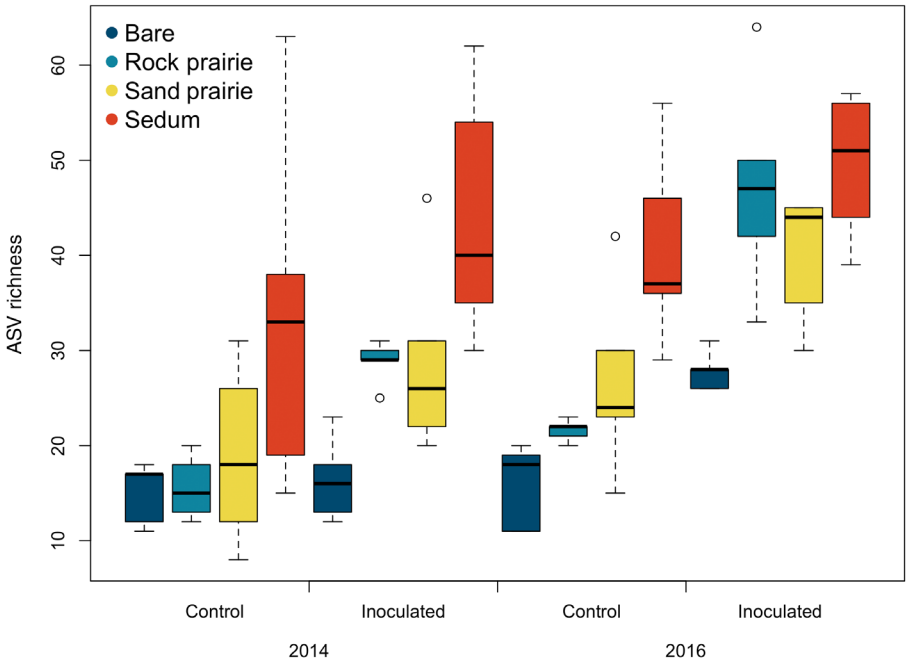

Fig. 3 Boxplots of the number of arbuscular mycorrhizal fungal amplicon sequence variants (ASVs) in controlled and inoculated experimental green roof trays in 2014 and 2016, broken up by plant cover type. Boxes represent the interquartile range of the data with horizontal lines indicating the median. Dotted vertical lines represent the data between the lower quartiles and the minimum or maximum with outlier represented as unfilled dots. The linear mixed-effects model showing the best fit includes inoculation, plant cover type, sampling year, and all two-way interactions (AICc = 544.2).

and environment-mediated (e.g. birds, insects, and precipitation) sources. This is consistent with other studies where AM fungi have been found to colonize green roof soil from sources other than transplanted plugs (John *et al.*, 2014; Rumble *et al.*, 2018). In microbial soil communities, even transient invaders can induce shifts in stable states, especially where communities are isolated from natural soils (Amor *et al.*, 2020). Priority effects may also introduce barriers that prevent newly introduced tax from establishing (Verbruggen *et al.*, 2013). In AM fungal communities, it is unclear whether unmanaged sites simply take longer to catch up to actively managed sites, or if they will lead to a separate mature community (Harris, 2009; Koziol & Bever, 2017). Few field inoculation studies track AM fungal community trajectories over time, data that are sorely needed to better inform the management of urban AM fungal biodiversity and function.

Interestingly, we found that VTs present in both inoculum and aerial samples were always found in treatment trays, suggesting that this group is common and potentially represents early successional or weedy species. For example, three of these taxa, *Glomus* MO-G8 (VTX00130), *C. lamellosum* (Dalpé, Koske & Tews) C. Walker & A. Schüßler (VTX00193), and *Glomus* sp. (VTX00165), were commonly detected in urban aerial samples during all seasons (Chaudhary *et al.*, 2020). Another of these common species was *Paraglomus* IS-Pg1 (VTX00446), belonging to a genus that forms small-diameter spores (Aguilar-Trigueros *et al.*, 2019), which may enable a comparatively large geographic range. We also saw that some VTs detected in inoculated trays in 2014 were detected later in control trays in 2016, possibly due to short-distance dispersal throughout the experimental trays. Half of the species from unknown sources were detected only in 2016 which could be because they were introduced unintentionally through a management decision and took time to increase to detectable levels, or because they moved into the system through an unmeasured dispersal mechanism. Overall, species pools grew from 2014 to 2016 in support of Hypothesis 2.

Implications of AM community change on plants and urban soils

Because AM fungi are obligate biotrophs and display some degree of host preference (Bever, 2002; Klironomos, 2003), the management of urban plant communities is likely to have a strong effect on urban AM fungal assembly processes. Indeed, our study found differential impacts of plant communities on AM fungal diversity and community structure. Contrary to Hypothesis 3, we found that a monoculture of *Sedum* sp. supported a higher diversity of AM fungi compared with the mixed plant communities of local prairie species (Ksiazek-Mikenas *et al.*, 2021, 2023). *Sedum* L. monocultures held consistently higher plant cover (80–90%) compared with native plant communities that significantly decreased in cover and plant survival over time (Ksiazek-Mikenas *et al.*, 2023). Our expectation that *Sedum* and the bare control would support few AM species was due to previous work indicating that *Sedum* sp. are poor AM hosts (Wang & Qiu, 2006). We did not directly measure AM fungal root colonization in experimental trays, but prior green roof research has demonstrated an increase in AM fungal viable propagule density with increasing *Sedum* sp. cover (Chaudhary *et al.*, 2019) and AM fungi readily colonize *Sedum* sp. (John *et al.*, 2014) though their function remains unclear (Olsson & Tyler, 2004; Rumble *et al.*, 2018). The predominant surviving plants in the prairie tray communities, prickly pear cactus *Opuntia humifusa* (Raf.) Raf. and nodding wild onion *Allium cernuum* Roth (Ksiazek-Mikenas *et al.*, 2021, 2023), typically host AM fungi (Eason *et al.*, 1999; Jansa *et al.*, 2008; Lahbouki *et al.*, 2022) and are aided by them

during periods of drought (Cantrell & Linderman, 2001; Bolandnazar *et al.*, 2007; Lahbouki *et al.*, 2022), which is experienced frequently on green roofs. However, inoculation with AM fungi had no effect on overall plant survival, plant community structure, or other ecosystem services of interest to green roofs (Ksiazek-Mikenas *et al.*, 2021, 2023). The current results are likely attributable to the planting method and possible priority effects: The *Sedum* plant community had high levels of AM fungal diversity to start with, likely because they were planted as fully mature individuals rather than seedlings (Ksiazek-Mikenas *et al.*, 2021, 2023). Colonization of green roof soil by AM fungi can occur quickly after transplantation (Rumble *et al.*, 2018). Previous studies have shown that AM fungi can be transplanted along with seedlings and can then contribute to increased water-use efficiency and drought tolerance of the symbiotic plant species (Urgiles *et al.*, 2014; Davidson *et al.*, 2016). Together, these findings suggest that the planting method is an important consideration in establishing AM fungal communities in urban habitats.

The AM fungal community shifts observed across our study did not correspond to observable benefits to green roof plant growth or survival (Ksiazek-Mikenas *et al.*, 2021). Beneficial plant growth effects of inoculation have not been observed in prior green roof inoculation research (Young *et al.*, 2015; Rumble & Gange, 2017). It is possible that a plant growth response to AM fungal inoculation was not observed due to the relatively short duration of the experiment or because in field experiments, positive plant growth effects of inoculation are more likely observed over time (Requena *et al.*, 2001; Koziol *et al.*, 2022). Belowground AM fungal community shifts may preempt aboveground shifts or plant benefits may manifest in ways that were not measured (e.g. increased drought tolerance, pathogen protection, see Eck *et al.*, 2022; Kakouridis *et al.*, 2022). It is also unclear whether the robust AM fungal community created by inoculation confers any advantage or disadvantage to the green roof plants as inoculation is not always associated with higher growth or survival for some plants (Jin *et al.*, 2017; Durr & Ksiazek-Mikenas, 2023). Finally, we used a whole soil inoculum that may have included other bacterial and fungal soil microorganisms that may have affected green roof plants, resulting in a net neutral plant growth effect (Hoch *et al.*, 2019).

While we used green roofs as a model for urban soil, it should be noted that green roof soils may vary from other urban soils which cover a wide range in their construction, depth, and other physical and biotic properties. Ground-level green spaces and green roofs support different assemblies of fungal communities (McGuire *et al.*, 2013; Droz *et al.*, 2022), though it is expected that dispersal between adjacent sites does happen (Droz *et al.*, 2022), likely by fungi with traits conducive to movement through the air (Chaudhary *et al.*, 2020). This differs from the bacterial microbiome where spatial isolation did not lead to lower levels of diversity on green roofs (Hénault *et al.*, 2022). Rumble *et al.* (2018) suggest that dispersal limitation could be ameliorated by transplanting seedlings that have been growing at ground level onto a green roof, but they caution that the soil community would need to be adapted to the harsh abiotic conditions of green roofs.

In this study, we observe a distinct change in the AM fungal community of urban soils after inoculation, but it remains unclear whether inoculation with AM fungi can affect the function of urban soils. Studies on longer time scales may be able to give us a clearer understanding of the effects of inoculation on the aboveground plant community and of passive AM community assembly, as we suspect from these results that passive assembly takes longer to establish diverse communities. We still do not know whether these communities will come to alternative stable states or whether they will eventually converge (Barber *et al.*, 2017), which could impact a decision to intervene with active management. We propose that multiple vectors of inoculation of newly engineered urban soils are possible, including long-distance dispersal, but that inoculation with late-successional urban soil can have a strong, immediate effect on the AM fungal community.

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Competing interests

None declared.

Author contributions

VBC and KK-M conceived of the research question and led the experimental work. PM led the figure creation, analysis, and writing. All authors contributed to interpreting results, editing, writing, and providing comments on the manuscript.

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Data availability

Sequencing data are available through the NCBI SRA BioProject ID PRJNA989570.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Histogram of simulated stress values of NMDS.

Fig. S2 NMDS ordinations using rarefied data with Bray–Curtis distances and using Jaccard distances.

Table S1 List of VTX found in this study includes family and which source or treatment plot the species was found in.

Table S2 ANOVA of the beta dispersion test for homogeneity of variance.

Table S3 Terms used in our global mixed effect model that includes inoculation, plant community, sampling year, and their two-way interactions as well as model selection criteria.

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