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Research

Trait-based assembly of arbuscular mycorrhizal fungal communities determines soil carbon formation and retention

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Summary

- Fungi are crucial for soil organic carbon (SOC) formation, especially for the more persistent mineral-associated organic C (MAOC) pool. Yet, evidence for this often overlooks arbuscular mycorrhizal fungi (AMF) communities and how their composition and traits impact SOC accumulation.
- We grew sudangrass with AMF communities representing different traits conserved at the family level: competitors, from the Gigasporaceae family; ruderals, from the Glomeraceae family; or both families combined. We labeled sudangrass with ^{13}C-CO_2 to assess AMF contributions to SOC, impacts on SOC priming, and fungal biomass persistence in MAOC.
- Single-family AMF communities decreased total SOC by 13.8%, likely due to fungal priming. Despite net SOC losses, all AMF communities contributed fungal C to soil but only the Glomeraceae community initially contributed to MAOC. After a month of decomposition, both the Glomeraceae and mixed-family communities contributed to MAOC formation. Plant phosphorus uptake, but not hyphal chemistry, was positively related to AMF soil C and MAOC accumulation.
- Arbuscular mycorrhizal fungi contribution to MAOC is dependent on the specific traits of the AMF community and related to phosphorus uptake. These findings provide insight into how variations in AMF community composition and traits, and thus processes like environmental filtering of AMF, may impact SOC accumulation.

Introduction

Microbial community composition and function shape soil organic carbon (SOC) accumulation rates and its residence time (Domeignoz-Horta *et al.*, 2021). The plant symbionts arbuscular mycorrhizal fungi (AMF) are a potential source of newly fixed SOC, exchanging soil nutrients for plant photosynthetic C (Ricklefs *et al.*, 2014). While 4–20% of plant photosynthate C is allocated to AMF (Parniske, 2008), not all this C necessarily accumulates as SOC. In some instances, this photosynthate sent to AMF can stimulate microbial decomposition, resulting in SOC losses due to priming (Cheng *et al.*, 2012; Herman *et al.*, 2012; Paterson *et al.*, 2016). At the same time, SOC may increase if AMF enhance soil aggregation, protecting SOC from mineralization (Miller & Jastrow, 2000). AMF spores, hyphal exudates, and extraradical dead hyphae (i.e. necromass) may also contribute directly to SOC accumulation, but the potential for these direct inputs to offset SOC losses due to accelerated decomposition rates remains unresolved (Frey, 2019). This highlights the complex role of AMF in the cycling of C in soils, and the need for further research to fully understand the mechanisms involved.

If AMF are effective contributors to net SOC accumulation, their traits associated with biomass turnover and C allocation to extraradical hyphae could directly impact new SOC formation. These traits primarily vary at the family level (Hart & Reader, 2002; Powell *et al.*, 2009; Koch *et al.*, 2017), such that manipulation of AMF communities through the promotion of certain families may lead to more (or less) effective C sequestration strategies. However, before exploring such strategies, we must first understand whether family-level communities differ in the amount and persistence of fungal inputs to SOC and whether this contribution is enough to counteract potential SOC priming.

Several microbial trait-based frameworks propose that functional or life-history traits can be effective predictors of SOC accumulation and SOC response to disturbances (Wallenstein & Hall, 2012; Cotrufo *et al.*, 2013; Malik *et al.*, 2020; Hicks *et al.*, 2022). Chagnon *et al.* (2013) suggested that the conserved traits within two of the largest AMF families, Gigasporaceae and

Glomeraceae, parallel Grime's C-S-R framework (Grime, 1977). Species in the Gigasporaceae family act as competitors in communities, as they invest heavily in the acquisition of growth-limiting resources by allocating proportionally more of their biomass to extraradical hyphae at the cost of delayed sporulation, and exhibit slower growth rates (Table 1; Hart & Reader, 2002, 2005; Staddon *et al.*, 2003; Maherali & Klironomos, 2007). This behavior is in contrast to species in the Glomeraceae family, which function as ruderals, prioritizing fast growth rates and frequent sporulation, leading to higher hyphal turnover and decreased investment in extraradical hyphae.

This family-level phylogenetic conservation of traits may impact how communities dominated by different AMF families influence SOC accumulation. For instance, AMF receive C from their hosts in accordance with the amount of phosphorus (P) they provide their plant host (Kafle *et al.*, 2019). Gigasporaceae species are thought to be better P scavengers than Glomeraceae, due to their relatively more extensive extraradical mycelium which may, in turn, lead to greater plant C allocation to Gigasporaceae (Johnson *et al.*, 2003; Bücking & Shachar-Hill, 2005; Maherali & Klironomos, 2007). Alternatively, Glomeraceae species' faster growth may promote more rapid nutrient absorption and thus faster photosynthetic C deposition and SOC accumulation.

Such trait-mediated variation in plant C allocation to AMF, nutrient uptake, and biomass growth may determine the amount of fungal C inputs to soil, but the accumulation of fungal C also depends on its persistence in the soil. The slower cycling mineral-associated organic C (MAOC) is an important pool for longer-term C storage (Torn *et al.*, 1997). MAOC often has a high abundance of compounds originating from microbial inputs or highly decomposed plant and fungal tissues (Gleixner *et al.*, 2002; Grandy & Neff, 2008; Angst *et al.*, 2021). The close interaction and sorption of these microbial and decomposed plant compounds with mineral surfaces helps to protect them from decay (Kögel-Knabner, 2002). Though clay content and mineralogy are important determinants of MAOC, fungal community composition may also mediate the proportion of fungal C that accumulates in MAOC (Sokol *et al.*, 2022). Recent studies suggested that functionally distinct fungal communities (ectomycorrhizal fungi, AMF, and saprotrophs) impact MOAC formation in different ways (Hicks Pries *et al.*, 2022; See *et al.*, 2022). However, whether variation exclusively within the ubiquitous AMF guild results in different outcomes for fungal MAOC accumulation and thus retention in the soil is still uncertain.

Arbuscular mycorrhizal fungi inputs to MAOC may result from the direct contact between mineral surfaces and hyphae, or when AMF inputs decompose to lower molecular weight compounds that have high mineral sorption potential (Smits *et al.*, 2009; See *et al.*, 2022). Moreover, in the same way that root exudates can stimulate MAOC mineralization (Keiluweit *et al.*, 2015; Jilling *et al.*, 2021), hyphal exudation may also promote processes that destabilize MAOC (Bonneville *et al.*, 2009; Andrino *et al.*, 2021). The chemical composition of AMF inputs, which may differ between families, could also affect fungal C decomposition and thus its transition to MAOC (Fernandez & Kennedy, 2018; Frey, 2019).

For plants, ectomycorrhizal fungi, and some bacteria, biomass chemical composition varies with life-history traits (Donaldson *et al.*, 2006; Franklin *et al.*, 2011; Fernandez & Koide, 2014; Manzella *et al.*, 2019). For example, phenolics and N concentrations, respectively, are often negatively and positively correlated with growth rate (Franklin *et al.*, 2011; Siletti *et al.*, 2017). Variation in Glomeraceae mycelium chemistry and between ectomycorrhizal fungi and AMF has previously been observed (Huang *et al.*, 2022). Hyphal chemistry might also differ between AMF families based on their conserved traits, further impacting AMF biomass transition into MAOC. While a community dominated by extensive, yet slow growing, extraradical hyphae (e.g. Gigasporaceae) may contribute more C inputs to soil, MAOC accumulation could be limited by a relatively slow microbial decay of hyphal necromass. Conversely, fungal C from a community that

Table 1 Arbuscular mycorrhizal fungal isolates used in the glasshouse experiment organized by family and the conserved traits associated with each family.

Family	Isolates	Associated traits[1,2,3,4]
Gigasporaceae	*Cetraspora pellucida*	Lower hyphal turnover
	*Dentiscutata heterogama*	Lower growth rate
	Gigaspora margarita	Later sporulation
	Racocetra fulgida	Larger and fewer spores
	Scutellospora calospora	More extra-radical hyphae
Glomeraceae/Claroideoglomeraceae[5]	*Claroideoglomus etunicatum*	Higher hyphal turnover
	*Funneliformis mosseae*	Higher growth rates
	Rhizophagus clarus	Earlier sporulation
	Rhizophagus intraradices	Smaller and more spores
	Septoglomus deserticola	More root-internal hyphae and nutrient exchange structures

[1]Chaudhary *et al*. (2022).
[2]Treseder *et al*. (2018).
[3]Chagnon *et al*. (2013).
[4]Hart & Reader (2002).
[5]Claroideoglomeraceae represented by *Claroideoglomus etunicatum*.

exhibits traits for less extensive, yet fast-growing, and short-lived extraradical hyphae (e.g. Glomeraceae) may be more rapidly microbially processed and converted to MAOC.

The connections between distinct AMF trait-based families and their contributions to SOC must also consider that no single AMF family may be optimal for SOC accumulation under varying temporal scales and soil heterogeneity (Kallenbach *et al.*, 2019). For instance, because of greater complementarity, a mixed-family community can contribute to greater plant growth and P concentrations, and thus may increase AMF C acquisition and deposition (Crossay *et al.*, 2019; Horsch *et al.*, 2023). In addition, AMF communities composed of families with a different set of conserved traits may deposit C at different times and with different chemistries, building more SOC than single-family communities dominated by one set of life-history traits. While Glomeraceae could initially contribute relatively more fungal C to MAOC due to faster turnover rates, C from Gigasporaceae could also accumulate in MAOC, just at a slower rate. As such, diverse AMF communities composed of species across families may be most effective for SOC accumulation and retention.

Arbuscular mycorrhizal fungi are the most widespread mycorrhizal guild globally. Yet, how AMF communities, especially those where family-level trait conservation occurs, directly affect SOC and MAOC accumulation has not been well documented. This study addresses this knowledge gap by examining if and how AMF trait-based communities (i.e. assembled with species in the same family) impact AMF inputs to SOC and MAOC, and explores what factors may drive differential community impacts on SOC. We hypothesize that: AMF increase total SOC, especially in the MAOC pool, via direct fungal inputs that compensate for potential SOC losses due to stimulated decomposition, irrespective of life history; high P-supplying Gigasporaceae species contribute more to new total SOC than Glomeraceae; relatively faster to decompose Glomeraceae species contribute more to MAOC than Gigasporaceae species; and communities composed of species across the Gigasporaceae and Glomeraceae families contribute the most to both SOC and MAOC formation.

Materials and Methods

Experimental design

A glasshouse experiment was conducted to study the impact of phylogenetically distinct or mixed AMF communities on SOC formation and accumulation as more persistence MAOC. We grew *Sorghum drummondii* (Steud.) Millsp. & Chase (sudangrass) with four completely randomized AMF community treatments: Gigasporaceae, comprising five species (Giga); Glomeraceae, comprising four Glomeraceae and one Claroideoglomeraceae species (Glom); a mixture of all 10 AMF species (Mixed); or sterilized inoculum (Control). Each treatment was replicated nine times, for a total of 36 pots. The sudangrass in all treatments was exposed to ^{13}C-CO_2 to isotopically label AMF C inputs to soil. The study was conducted in tree-pots with separate root and hyphal compartments, allowing us to isolate hyphal C from root C (Fig. 1).

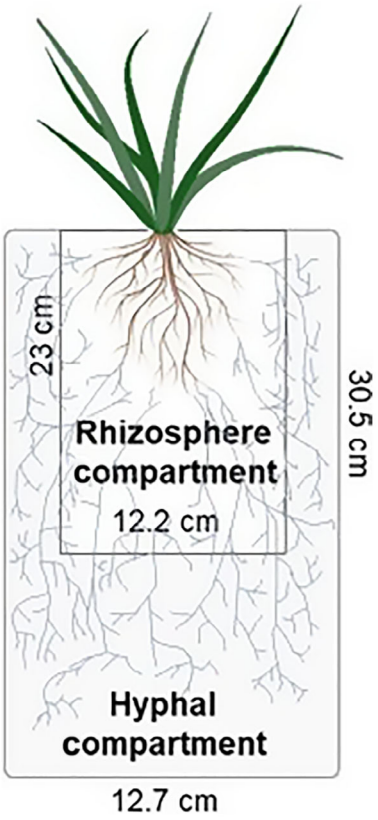

Fig. 1 Experimental pot layout with root and hyphal compartments. Tree-pots were 12.7 cm wide, 12.7 cm long, and 30.5 cm tall, and contained 5 l of soil. The hyphal compartment was made with a 30 μm-nylon mesh bag, *c.* 23 cm tall, and 12 cm wide. Sudangrass roots could not grow through the mesh bag which created a root-free hyphal compartment. The top edge of the mesh bag was placed 1 cm above the soil surface.

AMF inoculum

All AMF isolates, each representing one species, were obtained from the International Cultural Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM; University of Kansas). Gigasporaceae isolates were chosen for their relatively low fecundity and large spore size, while Glomeraceae and Claroideoglomeraceae isolates (henceforth, Glomeraceae community) were chosen for their high fecundity and relatively small spores (Table 1; Hart & Reader, 2002).

To increase the amount of AMF inoculum, we grew each of the 10 AMF isolates from INVAM with sudangrass for *c.* 4 months. These isolates were used to prepare the Gigasporaceae and Glomeraceae treatments. We made each community by combining 660 g from their five respective isolates (including root fragments, hyphae, spores, and substrate), for a total of 3300 g. The mixed treatment inoculum, composed of 10 isolates, contained equal parts of each mixture. We prepared the control inoculum by sterilizing the mixed treatment inoculum. At planting, each pot received 97.2 ± 0.2 g dry weight of their respective dry AMF inoculum. In addition, we added a 20-ml soil microbial community inoculum, free of AMF, to each pot to establish a saprotrophic microbial community and to standardize for

potential variability in non-AMF microbial communities among inoculum treatments. The microbial inoculum was prepared following Horsch *et al.* (2023) by mixing 600 g of the unsterilized soil used for planting (see later) and 150 g of each of the Gigasporaceae and Glomeraceae inocula in 2 l of deionized water and passing the resulting slurry through a 20-μm sieve that did not allow AMF spores or hyphae to pass through.

Planting

Sudangrass seeds were surface sterilized and germinated and then three seedlings were transplanted to 5 l tree-pots (Stuewe & Sons Inc., Tangent, OR, USA) containing an autoclaved 1 : 1, v/v, sand : soil mixture. We collected the soil from the McGill Morgan Arboretum in Québec, Canada (45°25′45.4″N; 73°56′32.5″W). The soil is a Sainte Rosalie clay loam Gleysol, is 6.31 pH, 1.36% SOC, 0.12% nitrogen, and has a soil isotopic signature of −26.4 $\delta^{13}C$. During transplant, we inoculated pots with their respective AMF inoculum and the AMF-free microbial inoculum. We also included three extra pots without seedlings to use as controls for $\delta^{13}C$ in the absence of plant photosynthesis.

After 6 d, seedlings were thinned to one plant per pot. The sudangrass was grown in the glasshouse for 89 d at 25°C during the day and 19°C at night. Plants received natural sunlight with an average photoperiod of 11 h. Plants were watered by hand as needed, occurring, on average, 2–3 d wk^{-1}.

Glasshouse ^{13}C isotope labeling

To determine the amount of new AMF C in each treatment, we exposed sudangrass to ^{13}C-labeled CO_2 using a pulse label method similar to Bromand *et al.* (2001). Pots were sealed in an air-tight chamber and exposed to *c.* 33 atom percent ^{13}C-CO_2 for a 4–6-h period weekly for 12 wk. A 4–6-h labeling period was considered appropriate to obtain a targeted ^{13}C-enrichment of *c.* 5 atom percent in AMF. Chamber CO_2 concentrations were monitored with a LI-830 (Li-Cor, Lincoln, NE, USA). When photosynthesis caused chamber CO_2 concentrations to fall to 340–370 ppm, ^{13}C-CO_2 was added to the chamber, raising CO_2 concentrations back to 460 ppm.

Sample collection and biomass analyses

Soil and plants were sampled 89 d after transplanting. This was based on when we first detected spores (following Brundrett, 1994) in the hyphal compartment of all AMF treatments, indicating that the isolates matured outside of the root compartment. We used extra pots from each AMF treatment prepared for the purpose of verifying spore presence. We homogenized soil from the hyphal compartment and subsampled for C analyses, hyphal chemistry, and for the subsequent 1-month incubation described later. Soil samples for the C analyses were oven-dried at 105°C and soils sampled for hyphal chemistry were frozen at −20°C.

We subsampled roots for AMF colonization and DNA sequencing, and oven-dried (60°C) aboveground biomass samples were sent to the Stable Isotope Facility for Ecosystem Research (University of Alberta, Canada) for biomass $\delta^{13}C$ analysis on an elemental analyzer coupled to an isotope ratio mass spectrometer (Thermo Delta V Advantage; Thermo Scientific Inc., Bremen, Germany) and for nutrient analyses (Thermo iCAP6300 Duo; Thermo Fisher, Cambridge, UK).

Soil incubation

To allow for hyphal decomposition, we incubated homogenized soil collected from each hyphal compartment, 9 d after sudangrass biomass harvest. For the incubation, 100 g of soil was placed in a 1-quart mason jar and covered with parafilm. We incubated soils in the dark at 20°C at 50% water holding capacity for 34 d. Immediately after the incubation, all soils were homogenized and oven-dried at 105°C for C analyses.

Soil carbon and $\delta^{13}C$ analyses

We determined soil MAOC concentrations from the oven-dried hyphal compartment soil 2–4 d after sudangrass harvest and after 1 month of incubation. To isolate the MAOC fraction, we used the particle size fraction method (Bradford *et al.*, 2008). Briefly, 20 g of dry soil was added to 80 ml of 0.008 M sodium hexametaphosphate solution and mixed on an end-to-end shaker for *c.* 18 h to disperse aggregates. The resulting slurry was washed through a 53-μm sieve to collect the clay and fine silt fraction, and oven-dried at 105°C.

Pre-incubation MAOC, MAOC after incubation, and bulk soil from the hyphal compartments were homogenized with mortar and pestle and analyzed for total C and $\delta^{13}C$ at the University of Ottawa, Canada on an elemental analyzer (VarioEL Cube; Elementar, Langenselbold, Germany) paired with an isotope ratio mass spectrometer (DeltaPlus Advantage; Thermo Fisher Scientific, Waltham, MA, USA).

Hyphal biomass chemistry

Hyphae and spores were extracted using an adaptation from Jakobsen *et al.* (1992). Briefly, a soil slurry (100 g wet soil : 1000 ml of deionized water) was passed through a 38-μm mesh. We combined the rinsed material > 38 μm with a 70% sucrose solution (5 : 1, v/v, sucrose : slurry), centrifuged it at 950 ***g*** for 2 m, and then passed it through a 150-μm mesh. We then rinsed the > 150-μm material with 60% sucrose solution to de-aggregate the hyphae and remove particulate organic matter. The extracted hyphae were oven-dried at 60°C and analyzed for molecular compound chemistry by pyrolysis gas chromatography–mass spectrometry (Py-GC/MS) at the University of New Hampshire, following Kallenbach & Grandy (2015). Samples were pyrolyzed on a CDS Pyroprobe 5150 in-line with a ThermoTrace GC Ultra gas chromatograph and a ITQ 900 mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA). Compound peaks were identified with AMDIS (chemdata.nist.gov; v.2.65), the NIST compound library, and published literature. Compounds were expressed as the % relative abundance of total sample peak area and classified based on origin (lipids, lignin derivatives,

polysaccharides, proteins, non-protein N-bearing, and phenolics). Compounds that could not be specifically attributed to a particular origin were classified as 'unknown origin' to ensure accuracy in identifying their sources.

Isotopic calculations

An isotopic mixing model was used to determine the fraction of C in soil that originated from fungal hyphae (Eqn 1):

$$F\ \text{AMF C in soil} = \frac{(\text{atom\% soil fraction} - \text{atom\% unplanted control})}{(\text{atom\% plant} - \text{atom\% unplanted control})} \quad \text{Eqn 1}$$

where F is the fraction of C from AMF in the soil fraction of interest (e.g. MAOC); atom% soil fraction is the ^{13}C atom% for the soil fraction of interest; atom% unplanted control is the average ^{13}C atom% soil not seeded with sudangrass; and atom% plant is the ^{13}C atom% of the sample's respective plant aboveground tissue. The unplanted control allowed us to account for potential soil autotrophic ^{13}C enrichment (e.g. cyanobacteria). Each C pool of interest was multiplied by its respective $F\,^{13}$C value to determine the fraction of that pool that originated from the AMF hyphae (Eqn 2):

$$\text{Pool AMF C} = F^{13}\text{C} \times \text{Pool C} \quad \text{Eqn 2}$$

where Pool AMF C is the amount of C in the pool of interest originating from AMF, $F\,^{13}$C is the fraction of ^{13}C from AMF in the soil fraction of interest, and Pool C is the amount of C found in the pool of interest.

Since there is potential for some ^{13}C contribution in the hyphal compartment from root exudates, we included a planted non-mycorrhizal control to account for ^{13}C contributions not associated with AMF C. We used Eqn 2 to calculate the pool of non-AMF C from the non-mycorrhizal planted control, which would contain only plant inputs (e.g. root exudates). We then subtracted the average of this value from the AMF treatments to determine a final AMF community's contribution to any given C pool.

AMF root colonization

To quantify AMF colonization at harvest, segments of *c.* 1 cm from each root system were stored in 50% ethanol until staining the roots using the ink-vinegar method (Vierheilig *et al.*, 1998). AMF colonization was determined through observation of 100 intersections per sample at ×400 magnification (McGonigle *et al.*, 1990). Percent colonization was calculated as the ratio of intersections where AMF colonization was visible to the total number of intersections observed, multiplied by 100.

DNA extraction and sequencing

We extracted root genomic DNA to test inoculation success. The DNA was extracted from root subsamples (100 mg) stored at −20°C using the Qiagen DNeasy Plant Pro extraction kit (69204; Qiagen), following the manufacturer's protocol. The SSU rRNA was amplified for each sample using the WANDA-AML2 primer set. The primers were modified for the Illumina platform by fusing CS1 and CS2 linker primers for forward and reverse primers, respectively. Initial polymerase chain reactions (PCRs) were conducted with duplicate 25 µl assays with 12.5 µl KAPA HiFi MasterMix, 1 µl of each primer (10 µM), 4.5 µl of PCR-grade water, and 6 µl of a genomic DNA template (*c.* 10 ng µl^{-1}) were mixed in a 200-µl PCR tube for each sample. The following thermal profile was used for the PCR: an initial denaturation and enzyme activation step of 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 5 min. PCR product qualities were evaluated by agarose gel electrophoresis. Duplicate PCRs were pooled by sample. Additional rounds of PCR were performed to fuse CS1/CS2 linker primers to the indices and adapters (an initial denaturation and enzyme activation at 95°C for 10 min, followed by 15 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 60 s with a final extension at 72°C for 10 min) before Illumina MiSeq PE300 sequencing at Génome Québec (Québec, QC, Canada).

Illumina adapters and primer complements were trimmed using CUTADAPT (Martin, 2011). Illumina sequences were then processed with the DADA2 pipeline to remove low-quality reads and reads < 450 bp, filter chimeras, and to resolve amplicon sequence variants (ASVs) (Callahan *et al.*, 2016; Pauvert *et al.*, 2019). Taxonomy was assigned to each ASV against the MAARJAM database with custom outgroups using the DADA2 *AssignTaxonomy* algorithm (Öpik *et al.*, 2010). Sequences that were not identified as Glomeromycota (66%) at the phylum level were removed. Because no AMF colonization was observed in the control treatment, ASVs that occurred in the control samples (eight ASVs) were considered contaminant DNA and removed for further analysis. The AMF data were rarefied to an even depth of 3497 sequences per sample.

Data analyses

We conducted two-way ANOVA's to determine AMF treatment effects on sudangrass biomass, soil C pools before and after the incubation, and on plant P uptake. We used AMF family as two separate factors (Gigasporaceae or Glomeraceae) with two levels: presence or absence. If the ANOVA model was significant, we used Tukey's tests to make pairwise comparisons both between AMF communities and relative to the sterilized, planted control. We used the Shapiro–Wilk's test for normality and Levene's test for homogeneity of residual variance to assure the ANOVA assumptions were met. For AMF root colonization, we used the nonparametric Kruskal–Wallis due to 0 values in the control, followed by Dunn's tests for pairwise comparison testing. Treatments were considered significant at $\alpha = 0.05$.

Potential differences among the AMF community hyphal chemistry were visualized using principal coordinate analysis on all individual hyphal compounds with a relative abundance > 1% and on grouped compound classes. We visualized differences in AMF community ASVs with multidimensional scaling

ordination using Bray–Curtis distances. Significant differences among AMF communities in hyphal chemistry and in AMF ASV relative abundances were tested using permutational multivariate analysis (PERMANOVA). Lastly, we conducted correlation analyses on aboveground P uptake and AMF C using Spearman's rank correlation coefficient ($P < 0.05$). Statistical analyses were conducted in R v.4.1.0.

Results

AMF community root colonization

Arbuscular mycorrhizal fungi root colonization was observed in all AMF treatments and there was no evidence of contamination in the sterile control (Supporting Information Fig. S1). AMF root colonization rates were different for all treatments, with plants in the mixed community being the most colonized (75%), followed by the Glomeraceae (68% colonization) and Gigasporaceae (61% colonization) communities.

Based on our DNA sequence results, root colonization largely represented the communities we constructed (Fig. 2). For the Glomeraceae community, 100% of sequences were in the Glomeraceae family, and for the Gigasporaceae community, on average, 78% of sequences were in the Gigasporaceae family (Fig. 2a). The mixed community was, on average, 82% Glomeraceae but their proportion varied across replicates (48–100%). When looking at the ASVs that best represented the isolates we added, some isolates had higher relative abundances than others (Fig. 2b). In the Glomeraceae community, the dominant species was *Rhizophagus intraradices* (41%), followed by *Claroideoglomus etunicatum* (16%). In the Gigasporaceae community, *Gigaspora margarita* represented, on average, 66% of the sequences. *Dentiscutata heterogama* was the second most dominant of the Gigasporaceae isolates but was always lower than 12% in relative abundance. The dominant Glomeraceae ASVs that we detected in the Gigasporaceae community was *Rhizophagus clarus* (13%), which in the Glomeraceae and mixed community had low relative abundances. In the mixed community, both dominant species from the single-family treatments were present, *R. intraradices* (35%) and *G. margarita* (16%). The AMF community composition was significantly different among all treatments (Fig. S2, $P = 0.001$) and the richness of virtual taxa in the mixed

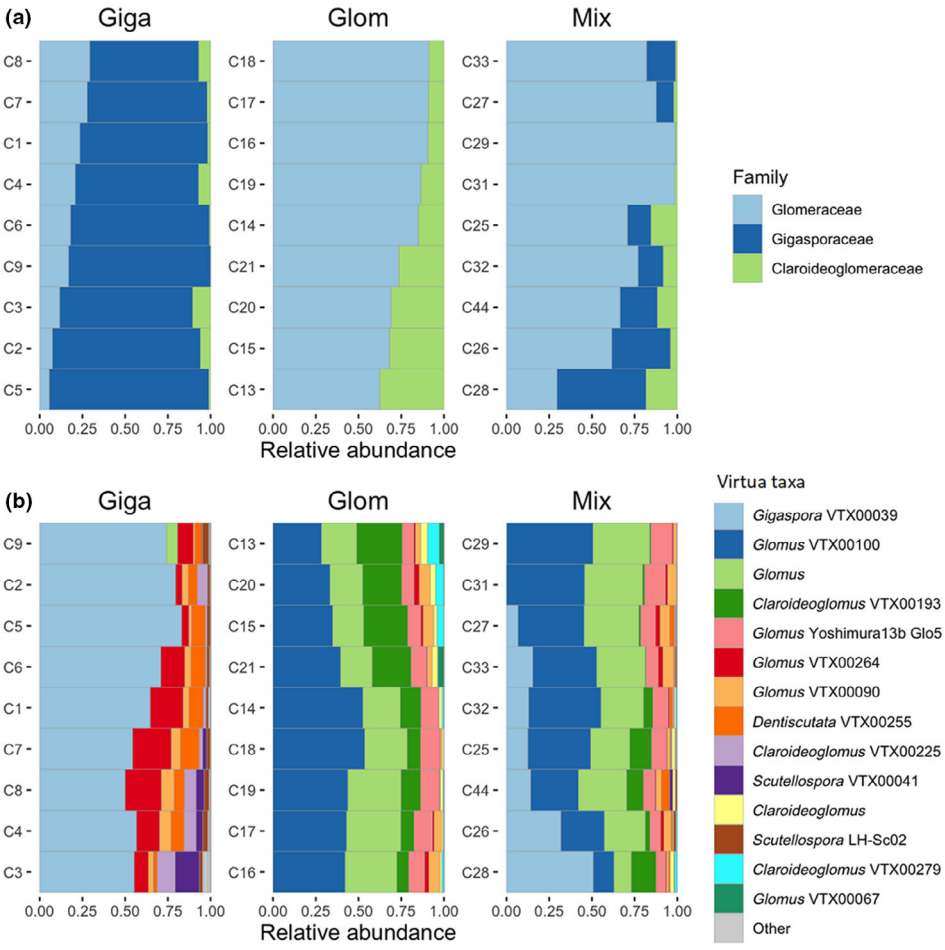

Fig. 2 Arbuscular mycorrhizal fungal (AMF) root colonization for three AMF communities. The relative abundances of amplicon sequence variants (ASVs) in Gigasporaceae (Giga) or the Glomeraceae (Glom)/Claroidioglomeraceae family (a) and ASVs identified to the virtual taxa level (b) for each of the three AMF communities *Giga*, *Glom*, or *Mixed* (Giga plus Glom) grown with sudangrass. Each replicate within a community treatment is shown along the vertical axis (C followed by a number).

community was higher than in the single-family communities ($P < 0.001$).

Pre-incubation total SOC and total mineral-associated C

At the time of harvest, AMF reduced total soil C, with Glomeraceae and Gigasporaceae communities decreasing soil C by 15% and 14% compared to the control, respectively (Fig. 3; Table S1). However, there was only weak evidence that the mixed community decreased soil C ($P = 0.057$) compared to the control. Despite total soil C losses, AMF neither affected the proportion of total MAOC content within the soil nor within soil C (Fig. 3b; Table S1).

Pre-incubation arbuscular mycorrhizal fungal soil C

Immediately following sudangrass harvest, we detected ^{13}C enrichment in the hyphal compartment soil across all AMF communities, compared with the planted non-mycorrhizal control soil. The mean ^{13}C enrichment ($\delta^{13}C$) was −21.0 with AMF and −25.1 in the control (slightly higher than the starting soil enrichment of −25.8).

All AMF communities added detectable AMF C to total soil C, collectively contributing, on average, 2.5 mg g^{-1} of AMF C (0.25% of SOC) (Fig. 4; Table S1; $P < 0.05$). There was no evidence for differences in soil C contributions among AMF communities. However, a trend emerged where Glomeraceae contributed the most fungal C to total C and Gigasporaceae the least, contributing 4.8 and 3.0 times more C, respectively, compared to the planted non-mycorrhizal control. As AMF root colonization led to a decline in total C concentrations, this C input from AMF suggests that total soil C loss is associated with non-AMF C.

Arbuscular mycorrhizal fungal mineral-associated C

We examined the amount of fungal C that accumulated as MAOC following plant harvest and after 1 month of a soil incubation providing time for saprotrophic decomposition. At harvest, there was overall more fungal-derived MAOC with AMF root colonization, relative to the control, when calculated both as a fraction of soil and as a fraction of total soil C ($P < 0.05$; Table S1). However, this effect was driven by the Glomeraceae community, which was the only AMF community to add detectable fungal-derived MAOC (1.5 mg MAOC g^{-1} soil C) at harvest compared to the planted non-mycorrhizal control (Fig. 5a; Table S1).

After 1 month of incubation, the Glomeraceae community still contributed the highest amount of fungal-derived MAOC to total soil C but, unlike the pre-incubation soil, we also detected fungal MAOC from the mixed community (Fig. 5b; Table S1). After 1 month, the mixed community increased fungal MAOC in soil C by 2.9 times compared to the planted non-mycorrhizal control and contributed *c.* 1.2 mg fungal MAOC g^{-1} soil C. In the Glomeraceae community, fungal-derived MAOC in soil C was 3.4 times higher compared to the planted control, with an average 1.4 mg fungal MAOC g^{-1} soil C.

When we compared fungal-derived MAOC relative to total MAOC instead of relative to soil C, the proportion was higher when relativized to total MAOC (Fig. 5c). For instance, at pre-incubation, fungal biomass across communities contributed 0.86 mg fungal-derived MAOC g^{-1} total MAOC, whereas the average fungal MAOC in total soil C was 0.71 mg g^{-1} (Table S1).

Across all AMF communities, in both pre- and post-incubation soils, mean fungal MAOC in total MAOC was higher by 110% and 140%, respectively, compared to the planted non-mycorrhizal control (Fig. 5c; Table S1). Similar to

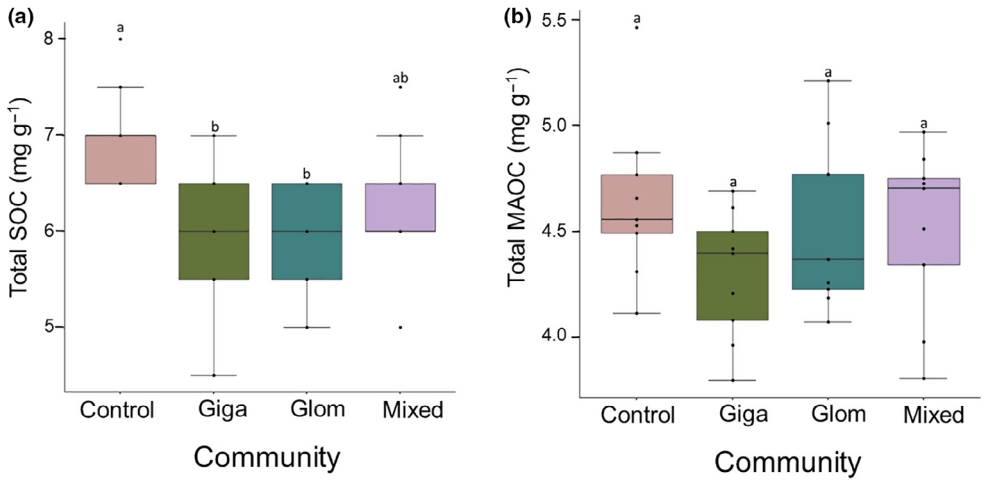

Fig. 3 Effect of arbuscular mycorrhizal fungal (AMF) communities on total soil carbon at the time of sudangrass harvest. Total soil organic carbon (SOC) in hyphal compartment by community (mg total SOC g^{-1} of soil) before a decomposition incubation (a). Total soil mineral-associated organic carbon (MAOC) in hyphal compartment by AMF community (mg total MAOC g^{-1} of soil) before a decomposition incubation (b). Giga (Gigasporaceae); Glom (Glomeraceae); Mixed (Gigasporaceae plus Glomeraceae); Control (planted with sterile soil). Boxes show median and the 25% and 75% quartiles, and whiskers are data points within 1.5 times the interquartile range. The same letter above boxes indicates no pairwise difference between treatments (Tukey's $P < 0.05$; df = 3; $n = 9$).

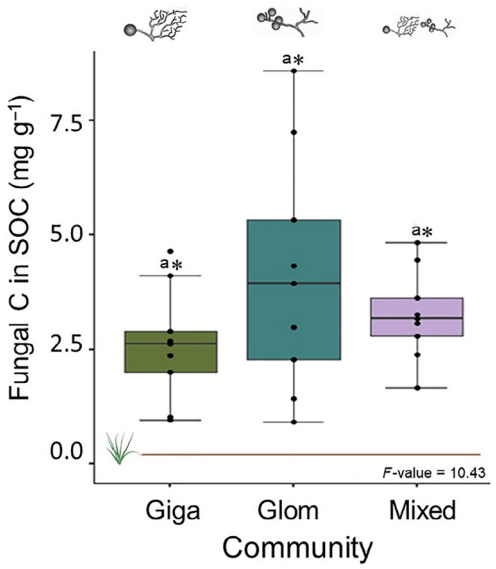

Fig. 4 Soil organic carbon (SOC) derived from arbuscular mycorrhizal fungal (AMF) communities (mg fungal C g^{-1} of soil carbon) at sudangrass harvest. The brown line is the planted non-mycorrhizal control's mean value of new C ($n = 9$), representing likely background enrichment from root exudates. AMF communities are as follows: Giga, Gigasporaceae; Glom, Glomeraceae; Mixed, Gigasporaceae plus Glomeraceae. Boxes show median and the 25% and 75% quartiles, and whiskers are data points within 1.5 times the interquartile range. Asterisks indicate a significant difference between a community's new carbon and the planted non-mycorrhizal control new carbon and the same letter above boxes indicates no pairwise difference between treatments (Tukey's $P < 0.05$). *F*-value is the ANOVA model; df = 3; $n = 9$.

pre-incubation fungal MAOC in total C, Glomeraceae was the primary contributor to MAOC and the only community significantly different from the planted control in both pre- and post-incubation soils. Glomeraceae contributed *c.* 1.2 mg g^{-1} before incubation and 1.0 mg g^{-1} of fungal MAOC to total MAOC following incubation.

No change in fungal mineral-associated C during incubation

The incubation had no effect on the amount nor the proportion of fungal-derived MAOC relative to total C or total MAOC when looking within each of the AMF communities (Table S1). Yet, the incubation showed non-significant opposing trends between the two comparisons, with fungal MAOC in total MAOC trending toward a decline after 1 month, while fungal MAOC in total C trended toward an increase.

Hyphal biomass chemistry

We examined hyphal biomass chemistry from each of the three AMF communities to explore if hyphal chemistry might explain the variation we saw in the community-dependent effect on MAOC. We did not observe any clustering of communities based on hyphal compound chemistry (e.g. lipids, polysaccharides) using PCoA visualization and Bray–Curtis distances ($P = 0.29$; Fig. S3; Tables S2, S3).

When we compared the hyphae by community for a specific compound class, only lipid relative abundances differed by

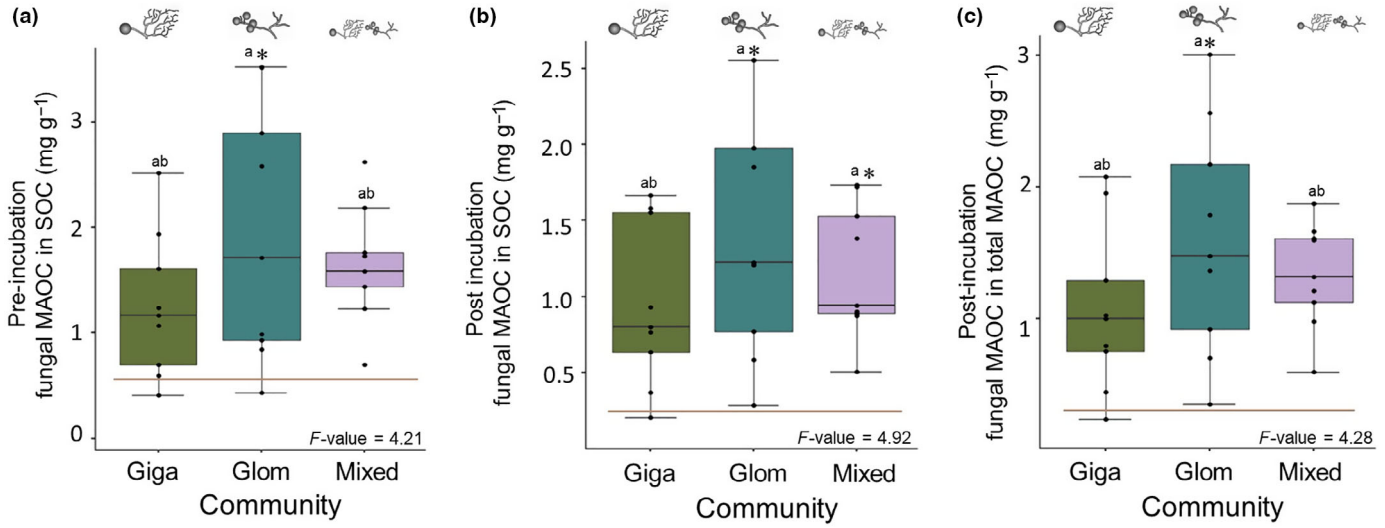

Fig. 5 The proportion of mineral-associated organic carbon (MAOC) derived from arbuscular mycorrhizal fungi (AMF) by each community. The amount of fungal-derived MAOC in soil organic carbon (SOC) by AMF community at sudangrass harvest (mg fungal MAOC g^{-1} SOC) (a); the amount of fungal-derived MAOC in SOC by AMF community after 1 month of a decomposition incubation (b), and the amount of fungal MAOC in total MAOC (mg fungal-derived MAOC g^{-1} MAOC) by AMF community after 1 month of incubation (c). The brown line is the planted non-mycorrhizal control's mean new carbon input, representing likely background enrichment from root exudates. AMF communities are as follows: Giga, Gigasporaceae; Glom, Glomeraceae; Mixed, Gigasporaceae plus Glomeraceae. Boxes show median and the 25% and 75% quartiles, and whiskers are data points within 1.5 times the interquartile range. Asterisks indicate a significant difference between a AMF community's new carbon and the planted non-mycorrhizal control new carbon and the same letter above boxes indicates no pairwise difference between treatments (Tukey's $P < 0.05$). *F*-value is the ANOVA model; df = 3; $n = 9$.

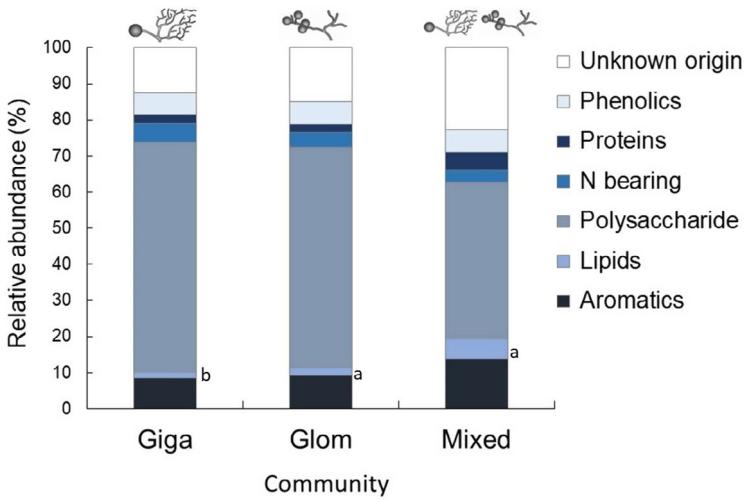

Fig. 6 Arbuscular mycorrhizal fungal (AMF) hyphal chemical composition. Mean relative abundances of compound classes (%) using pyrolysis-GC/MS of extracted hyphae of Giga (Gigasporaceae), Glom (Glomeraceae), and Mixed (Gigasporaceae plus Glomeraceae) AMF communities. Different letters indicate significant difference within a class of compounds among communities (ANOVA $P < 0.05$), $n = 5$.

community (Fig. 6). The mixed community (5.8% lipids) and Glomeraceae community (2.3% lipids) had higher lipid abundances compared to the Gigasporaceae community (1.7% lipids) ($P < 0.05$). Across the communities, polysaccharides and proteins accounted for 43–64% and 2.1–4.9%, respectively, but high variability within replicates led to non-significant community effects (Table S4).

AMF community association with carbon and plant phosphorus uptake

All communities increased plant P uptake relative to the sterile control but the amount varied by community (Fig. S4). The mixed and Glomeraceae communities increased plant P uptake the most relative to the control by 9.2 and 8.7 times, respectively. Similarly, aboveground biomass was *c.* 1.5 higher with colonization, but only for the mixed and Glomeraceae communities (Table S5). We observed positive correlations with plant P uptake and all the AMF C pools ($P < 0.05$). The strongest correlation was between plant P uptake and fungal C in soil ($P = 8.1 \times 10^{-5}$), followed by fungal MAOC in soil after harvest ($P = 1.5 \times 10^{-4}$) (Fig. 7). The relationship of P uptake with soil C and with MAOC appears to cluster by AMF community, with the Glomeraceae and mixed community positioned at the higher end of the correlation.

Discussion

We examined whether AMF communities with divergent life-history strategies differentially affected SOC cycling. Specifically, we tested whether single-family AMF communities composed of competitors (Gigasporaceae), ruderals (Glomeraceae), or mixed-family communities containing both ruderals and competitors varied in their effect on SOC and MAOC accumulation and retention. All AMF communities contributed to new SOC accumulation. Nonetheless, net SOC concentrations decreased compared to the non-mycorrhizal control. Initially, only C from the Glomeraceae community accumulated in the MAOC pool, yet after the soil incubation, both the Glomeraceae and the mixed communities contributed to MAOC accumulation indicating a community-dependent effect on the rate of fungal C movement into more persistent C pools. Plant P uptake correlated with SOC and MAOC accumulation, suggesting plant C deposition in proportion to P uptake may be impacting new SOC formation.

AMF decrease total SOC but not mineral-associated C

We expected that AMF C inputs would increase total SOC, but we found that AMF communities generally decreased total SOC (Fig. 3a; Table S1). This total SOC loss can only be explained by priming of existing SOC mineralization, since the SOC loss in AMF treatments is relative to a control with no new AMF inputs. Although AMF are not decomposers, they can indirectly accelerate SOC mineralization (Tisserant *et al.*, 2013; Huang *et al.*, 2021). This can occur when AMF deposit hyphal or exudate C, stimulating saprotrophic activity (Toljander *et al.*, 2007; Zak *et al.*, 2019; Chowdhury *et al.*, 2022). AMF hyphae also provide a network of moist surfaces through the soil matrix that can increase saprotrophs' access to organic resources and SOC mineralization (Dechesne *et al.*, 2010; Gorka *et al.*, 2019). Notably, the decrease in SOC was observed immediately after harvest, indicating that some SOC loss occurred while AMF were associated with their host. Active AMF may benefit from increased saprotrophic organic matter mineralization, which would increase AMF access to nutrients, and thus improve their ability to 'trade' nutrients with their host for more C (Talbot *et al.*, 2008; Kaiser *et al.*, 2015).

Unlike total SOC, we did not detect a decrease in the amount of total MAOC (Fig. 3b; Table S1). This suggests that AMF preferentially promote the decomposition of faster-cycling C pools, consistent with the understanding that MAOC is mineralized at a slower rate than other C pools (Balesdent & Mariotti, 1996). However, a rapid exchange between new fungal inputs and extant MAOC may be occurring. For instance, AMF hyphae may cause previously mineral-sorbed C to enter solution via exudate-driven

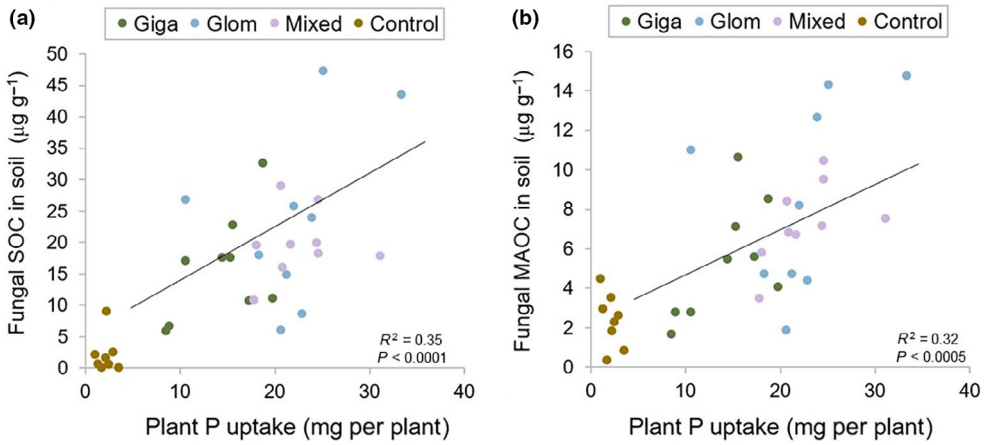

Fig. 7 Correlation between sudangrass phosphorus uptake and arbuscular mycorrhizal fungal (AMF)-derived carbon. Sudangrass phosphorus uptake and (a) AMF soil organic carbon (μg fungal SOC g^{-1} soil) and (b) AMF mineral-associated organic carbon (μg fungal MAOC g^{-1} soil) including all AMF communities and the control, $n = 36$. AMF community treatments are indicated by different colors: Giga, Gigasporaceae; Glom, Glomeraceae; Mixed, Gigasporaceae plus Glomeraceae; and planted non-AMF control. The R^2 is the adjusted R-squared; P-value is based on Pearson's correlation.

dissolution reactions, while hyphal exudates themselves are added to MAOC. Thus, we may observe no net change in the amount MAOC if mineralized extant MAOC is being replaced by new inputs.

AMF communities similarly contribute to new SOC formation

We anticipated that the mixed-family community would contribute the largest amount of fungal C to SOC, followed by the Gigasporaceae community. However, all AMF communities contributed similarly to fungal SOC formation. This indicates that regardless of phylogenetic diversity, AMF hyphae are a notable contributor to SOC. Following the relatively short 3-month glasshouse experiment, AMF hyphae accounted for 0.25% of SOC on average (Fig. 4). The number of times hyphal C turned over during this period, depositing new C, was likely low, as AMF hyphae turnover is typically 12 d (Allen, 2007). Thus, over time, the proportion of fungal SOC is expected to increase as hyphae continue to grow and turnover, and as the fungi mature and hyphae expand.

It is possible that community differences in fungal SOC inputs could emerge with more time. Plants provide C to AMF in proportion to the amount of P that AMF give in return, such that more C should be allocated to AMF with more P uptake (Kiers *et al.*, 2011). Both the Glomeraceae and mixed community had higher P uptake compared to Gigasporaceae, similar to other short-term studies (Thonar *et al.*, 2014; Gosling *et al.*, 2016), and this was strongly associated with AMF MAOC and SOC (Figs 7, S4). Thus, it is possible that with more time, plants may provide Glomeraceae containing communities with more photosynthetic C, resulting in more total fungal SOC accumulation.

Conversely, if the slower growing Gigasporaceae species had more time to fully mature, they could eventually contribute relatively more to fungal SOC. Not only would the total biomass of the Gigasporaceae community increase, but the plant may change how it allocates C as P-depleted zones expand. This could create a positive feedback effect where plant C allocation to Gigasporaceae increases relative to Glomeraceae if Gigasporaceae are more capable of supplying P over time (Hart & Reader, 2002; Staddon *et al.*, 2003). This would further increase growth and hyphal expansion of Gigasporaceae species and, in turn, more C would be deposited further from the plant while increasing P acquisition by Gigasporaceae. Further research would be needed to examine if AMF community growth rates and C inputs change over time within a single root system and if Glomeraceae continued growth, and more frequent hyphal inputs would outpace inputs from Gigasporaceae.

We demonstrate that, even though AMF SOC did not vary by AMF community, their hyphae are important for new SOC formation, and possibly more so than root exudates (Godbold *et al.*, 2006; See *et al.*, 2022). Root exudates rapidly contribute to newly fixed soil C, especially in the MAOC pool (Austin *et al.*, 2017; Sokol *et al.*, 2019), but outside of the rhizosphere, hyphae may be a larger contributor to newly fixed soil C (Kaiser *et al.*, 2015; Huang *et al.*, 2020). This is supported by our $\delta^{13}C$ data if we consider the $\delta^{13}C$ recovery from our sterilized planted control as a proxy for root exudates. Within the hyphal compartments that excluded, but were surrounded by, roots, the $\delta^{13}C$ in the planted non-mycorrhizal control was well below the $\delta^{13}C$ recovery with AMF. While keeping in mind potential treatment effects on exudation rates, hyphal contributions to SOC were thus likely greater than those from root exudates immediately outside the rhizosphere.

AMF mineral-associated C is mediated by trait-based AMF communities

As we hypothesized, we found that only communities containing Glomeraceae species contributed to fungal MAOC accumulation (Fig. 5). In many soils, the majority of MAOC is composed of compounds previously assimilated by microbes; thus, more

microbial-available hyphal compounds may be more likely to enter the MAOC pool (Angst *et al.*, 2021). Like with plants and some microbial species, Glomeraceae species' faster growth and turnover may be associated with hyphal chemistry that is relatively more decomposable compared to Gigasporaceae species, leading to more rapid transformation of their inputs to the MAOC pool. However, aside from lipids, we found little evidence that hyphal chemical composition varied by community. Glomeraceae necromass chemistry, *per se*, was thus not a driving factor explaining its accumulation in MAOC. In addition, AMF hyphae may be a broadly microbially accessible substrate, where production of more resistant compounds like melanin occur primarily under stressed conditions (Deveautour *et al.*, 2020).

If AMF hyphal decomposability is relatively invariable as our results suggest, net C flux becomes a critical factor regulating AMF community effects on MAOC. It is possible that the greater MAOC production of the Glomeraceae community was partly due to its higher growth rates and turnover times. Hyphal biomass quantification would help confirm this but would still not necessarily represent gross biomass production. Glomeraceae spores usually colonize roots within 1–4 wk of planting while Gigasporaceae spores can take up to 8 wk (Hart & Reader, 2002). Hart & Reader (2005) found that 7 wk after planting, Glomeraceae species had four times more runner hyphae and 3.5 times more absorptive hyphae than Gigasporaceae species, demonstrating the faster growth of Glomeraceae. The higher growth rates and turnover times of Glomeraceae could regularly produce more total fungal necromass, and at an earlier time than Gigasporaceae species, increasing the chances of Glomeraceae necromass to enter the MAOC pool. Indeed, fungal C in the MAOC pool was detected immediately upon harvest, suggesting that Glomeraceae contribution to MAOC was already occurring while the species were actively associated with its host.

While we expect Glomeraceae species to have relatively fast intrinsic growth and turnover given that these traits are conserved at the family level, the higher P uptake we observed for Glomeraceae may have further increased their growth if they, in turn, received more plant C. Our soils are generally P-limited, but autoclaving may have increase plant available P (Serrasolses *et al.*, 2008; Horsch *et al.*, 2023). Glomeraceae may be better at taking advantage of this spike in available P due to their rapid colonization and hyphal extension, allowing them to provide more of an immediate benefit to their host. In support of this, we saw a strong positive relationship between plant P uptake and fungal MAOC accumulation, with the high end of the relationship dominated by the Glomeraceae community (Fig. 7). Thus, rather than differences in hyphal biomass chemistry, the most likely explanation for AMF community-dependent effects on fungal MAOC was the faster growth and P uptake of Glomeraceae, increasing their C inputs and probability of fungal MAOC production.

Notably, only after the 1 month of decomposition did the mixed-family community contribute significantly to MAOC production ($P < 0.05$; Fig. 5). This indicates that further decomposition during the incubation led to the movement of pre-incubation fungal C into MAOC. Why further decomposition would be required to detect fungal MAOC with the mixed-family community is unclear since it was dominated by Glomeraceae species (Fig. 2) and the Glomeraceae community had immediate fungal MAOC formation. Perhaps less Glomeraceae biomass in the mixed community limited early detection in MAOC or interactions between the two families reduced hyphal exudation (that contributes to immediate fungal MAOC formation) or changed the decomposer community but this is only speculative.

Conclusion

We investigated the differential impacts of trait-based AMF assembled communities on SOC cycling. While AMF hyphal C rapidly contributed to fungal SOC formation, it was insufficient to counterbalance the loss in total SOC due to the AMF priming effect. However, the more persistent MAOC pool was unaffected by AMF priming. Fungal-derived MAOC accumulation was community dependent, initially involving only the ruderal Glomeraceae community and, after a decomposition period, the mixed-family community. This community-dependent effect on fungal MAOC accumulation was strongly correlated with plant P uptake, which we hypothesize enhanced the phylogenetically conserved faster growth of Glomeraceae species, consequently contributing to MAOC formation. The variability in AMF community contributions to MAOC suggests that AMF community traits can differentially alter SOC cycling and retention. Factors affecting AMF P acquisition, such as plant root development, soil P availability, or niche complementarity between families, may further modify MAOC formation associated with AMF inputs. Given that environmental selection pressures (e.g. disturbance, plant communities, nutrient availability) can impact AMF family abundances, this study provides insights into how selection for traits at the family-level influence SOC accumulation and loss.

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Competing interests

None declared.

Author contributions

CCAH, PMA and CMK conceived of and designed the study and CCAH carried out the experiments, and collected and analyzed the experimental data, except for the DNA sequencing data. ASG analyzed hyphal chemistry and carried out compound identification. CF prepared DNA for sequencing and conducted bioinformatics. CCAH wrote the first draft of the manuscript with contributions from PMA and CMK. All authors contributed to the interpretation of the data, writing, and revising manuscript drafts and are accountable for the accuracy and integrity of all aspects of the work.

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Data availability

The sequence reads generated in this study can be accessed from NCBI database under the BioProject accession no. PRJNA948813.

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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 Mean percent of arbuscular mycorrhizal fungal total root colonization on sudangrass roots.

Fig. S2 Multidimensional scaling ordination of arbuscular mycorrhizal fungal communities of inoculated sudangrass.

Fig. S3 Principal coordinate ordination of chemical compound classes from extracted hyphae for each arbuscular mycorrhizal fungal community.

Fig. S4 Sudangrass aboveground plant phosphorus uptake (mg per plant) by arbuscular mycorrhizal fungal community.

Table S1 Mean, SDs, and ANOVA *P*-values for arbuscular mycorrhizal fungal soil C contribution to different C pools.

Table S2 Compound relative abundance for chitin and arbuscular mycorrhizal fungal hyphae.

Table S3 Compound relative abundance for melanin and arbuscular mycorrhizal fungal hyphae.

Table S4 Class of chemical compounds of arbuscular mycorrhizal fungal hyphae by community.

Table S5 Mean and ANOVA *P*-values for sudangrass biomass by arbuscular mycorrhizal fungal community.

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