Widespread co-occurrence of Sebacinales and arbuscular mycorrhizal fungi in switchgrass roots and soils has limited dependence on soil carbon or nutrients

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Societal Impact Statement
This work addresses a novel group of Sebacinales mycorrhizal fungi being considered for development as inoculants in managed ecosystems because of their potential access to nutrients in soil organic matter. By comparing the diversity, distributions, and abundances of the Sebacinales with the more common arbuscular mycorrhizal fungi in switchgrass, a biofuel crop, we demonstrate that current suggestions for a Sebacinales revolution in agriculture should be tempered by their ecology. In particular, Sebacinales are rare compared to arbuscular mycorrhizal fungi, and are only weakly associated with soil carbon, suggesting that ideas about improved soil carbon cycling associated with Sebacinales need to be sufficiently studied across a range of environmental conditions prior to their consideration for broad-spectrum soil inoculants.

Summary
• Arbuscular mycorrhizal (AM) fungi are widespread and important root symbionts, but recent work suggests that Sebacinales fungi may play an equally important role in both plant success and ecosystem carbon and nutrient cycling based on their worldwide occurrence and putative access to organic matter. However, the ecological impacts of Sebacinales will depend on their abundance and distribution relative to AM fungi and environmental soil carbon and nutrient patterns, which remain unexplored.
• We characterized Glomeromycota and Sebacinales fungi in switchgrass (Panicum virgatum L.) roots and soils across 14 sites with diverse soil conditions. We examined group richness differences, co-occurrence patterns, and how the relative abundance of these fungi related to soil carbon, nutrient stoichiometry, and host size.
• Sebacinales were widespread, but less diverse, common, and abundant than Glomeromycota. Moreover, co-occurrences were predominantly random, suggesting relatively few interactions between these groups. Sebacinales increased relative to Glomeromycota in soils with more carbon, but explanatory power was limited.
Based on our findings, we suggest that Sebacinales are likely complementary to AM fungi in roots. Expectations that Sebacinales have large effects on soil carbon and nutrient cycling may need to be reconsidered, at least based on their limited abundances relative to AM fungi in switchgrass. This is an important consideration as Sebacinales are candidates for use as inoculants in managed ecosystems.

**KEYWORDS**
biofuel crop, co-occurrence, Glomeromycota, grass, *Panicum virgatum*, *Sebacina*, *Serendipita*

## 1 | INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that associate with the vast majority of terrestrial plant species (Parrish & Fike, 2005; Wang & Qiu, 2006) and are well-known to support plant growth and nutrition (Schroeder-Moreno et al., 2011). However, AM fungi are not alone in roots, which are also occupied by diverse endophytic fungi that often have consequences for plant function (White et al., 2019). More recently, Sebacinales fungi have been recognized as common root associates with putative mycorrhizal abilities (Ray et al., 2017), but far less is known about their abiotic niche, ecological significance, or interactions with AM fungi. Recent evidence from genomic, phylogenetic, and morphological studies suggests that the Sebacinales form mycorrhizal associations with a wide range of plant hosts (Oberwinkler et al., 2013; Weiß et al., 2016) in diverse ecosystems worldwide (Selosse et al., 2009; Weiß et al., 2011). In addition, Sebacinales fungi can improve plant growth (Ghimire et al., 2009; Ghimire & Craven, 2011) and stress tolerance (Ghimire & Craven, 2011), and may have access to organic carbon and nutrient pools that are inaccessible to AM fungi (Craven & Ray, 2019). Thus, there is an expectation that Sebacinales can differentially benefit plants and substantially shift soil organic matter dynamics compared to AM fungi due to their potential to breakdown organic pools, with calls for their development as inoculants in managed systems (Craven & Ray, 2019). However, potential ecological differences between Sebacinales and AM fungi must first be addressed. To do so requires an understanding of how these fungi are distributed in the landscape with respect to one another and soil carbon, which remains unknown.

One possibility is that AM and Sebacinales fungi are sufficiently ecologically distinct to coexist in the same root systems. AM fungi generally specialize in uptake and transfer of inorganic phosphorus and nitrogen from soil to the host, with isotope studies demonstrating that 10%–20% of host photosynthetic nitrogen is transferred to the fungus (Smith & Read, 2008). In contrast, Sebacinales species exhibit diverse ecological strategies, including mycorrhizal, endophytic, and saprotrophic lifestyles (Weiß et al., 2016). Importantly, the Sebacinales have retained saprotrophic abilities (Weiß et al., 2016) based on genomic encoding of carbon degrading enzymes (Craven & Ray, 2019), in vitro organic nitrogen uptake (Ray et al., 2019), and formation of mycorrhizal structures (Oberwinkler et al., 2013).

Sebacinales also have non-resource benefits to the host, such as improving plant drought tolerance (Ghimire & Craven, 2011; Sherameti et al., 2008) and pathogen defense (Harrach et al., 2013; Waller et al., 2005). Many plant species can and do associate simultaneously with multiple groups of fungi (Teste et al., 2019) and co-occurring taxa may synergistically benefit the plant (Chen et al., 2000). Coexistence of AM and Sebacinales would potentially maximize host plant access to diverse sources of soil nutrients as well as non-resource benefits.

Alternatively, AM fungi and Sebacinales may compete for space and photosynthetic resources in the root, which could result in exclusion depending on resource availability or stoichiometry (Averill et al., 2014; Phillips et al., 2013). Given the potential for Sebacinales to augment access to organic resources, we might expect a pattern similar to what has been found for AM and ectomycorrhizal (EM) fungi. EM fungi specialize in acquisition of organic nitrogen and tend to become more dominant relative to AM fungi in conditions with ample organic carbon, slow mineralization, and limited inorganic nutrients (Phillips et al., 2013). Conversely, AM fungi can replace EM and ericoid fungi when inorganic nitrogen deposition creates phosphorus-limited conditions in soils that were previously nitrogen-limited and high in organic matter (Read & Perez-Moreno, 2003; Wurzburger et al., 2011). Shifts between AM and EM fungi are typically confounded with turnover in plant community composition, but the assumption is that these effects are mediated by fungal competition for nutrients (Read & Perez-Moreno, 2003). It is possible that AM fungi and Sebacinales exhibit similar trade-offs in host occupancy based on resource availability.

Here, we addressed the co-occurrence, drivers, and putative interactions of AM fungi (phylum Glomeromycota) and Sebacinales fungi in switchgrass (*Panicum virgatum* L.) roots across 14 sites ranging from the coastal plain to the lower mountains of North Carolina, USA. Switchgrass was chosen because existing work has shown that some Sebacinales isolates can readily colonize its roots and confer growth-promoting benefits (Ghimire et al., 2009; Ghimire & Craven, 2011; Ray et al., 2015). In addition, switchgrass is widely planted in North Carolina given its potential as a cellulosic bioenergy crop, and is cultivated on a large number of sites with varying soil conditions. By holding the host constant and sampling across sites, we can elucidate potential competition versus coexistence of these mycorrhizal groups and their relationships to soil carbon and
nutrients. Given the known ubiquity of AM fungi in grasses, we expected that (1) Sebacinales would be less common and less abundant than Glomeromycota in both roots and soils. (2) Based on their apparent niche differences, we further predicted that Sebacinales and Glomeromycota would not competitively exclude one another and might even routinely co-exist, perhaps due to complementary lifestyles, supported by random or positive co-occurrences, respectively. (3) Finally, we anticipated that niche differences would be reflected in how the relative abundances of Sebacinales and Glomeromycota vary with soil organic carbon or resource stoichiometry given their putative differential access to soil organic nutrients. Specifically, we hypothesized that Sebacinales would be relatively more common and abundant in high soil carbon environments, particularly as carbon increases relative to nitrogen and phosphorus. In contrast, we expected Glomeromycota to be more closely correlated with plant host size, as the host represents their sole source of carbon.

2 | MATERIALS AND METHODS

2.1 | Experimental design and sample collection

We selected 14 stands of switchgrass (Panicum virgatum L.) located across a 462-km span in North Carolina, including the lower mountains, piedmont, and coastal plain (Table S1). Between late September and early October 2018, we sampled roots and soils of eight plants per site in a stratified random design. Roots and soils were collected directly adjacent the plant base on its north and south sides, using a shovel to 15-cm depth. All samples were stored on ice for transport to the laboratory. In the laboratory, fine roots (<2 mm) were separated from the soil, washed to remove soil residue, and frozen at −80°C for DNA extractions, along with an aliquot of soil. Fresh soils were used in biogeochemical extractions and the remainder was air-dried for analysis of total soil carbon.

2.2 | Plant and soil characteristics

For each plant, we measured mean maximum height of the three tallest, fully extended tillers (m), maximum basal diameter (m), and the basal diameter perpendicular to the maximum (m). Basal measurements were used to estimate basal area (m²) as an ellipse.

In each soil, we measured dissolved organic carbon (DOC), microbial biomass carbon (MBC), soil organic matter (SOM), total carbon (TC), total nitrogen (N), and total phosphorus (P). For DOC, soils were extracted in 0.5M K₂SO₄ in a 1:4 ratio; for MBC, soils were fumigated with chloroform and then extracted in K₂SO₄ (Scott-Denton et al., 2006; Vance et al., 1987). MBC was calculated as the difference between fumigated and unfumigated extractions divided by 0.45 to convert from salt extractable to total carbon (Vance et al., 1987). Both DOC and MBC were quantified colorimetrically (Bartlett & Ross, 1988) using a microplate reader (BioTEK Synergy H1 Hybrid Plate Reader, Winooski, Vermont, USA). TC and N (%) were measured via combustion by the Environmental and Agricultural Testing Service at North Carolina State University (https://eats.wordpress.ncsu.edu). Soil organic matter and total soil P were measured by the NC Department of Agriculture and Consumer Services (https://www.ncagr.gov/agronomi/uyrst.htm).

2.3 | Amplicon metagenomics

DNA was extracted from 40 to 50 mg root material using the Synergy 2.0 Plant DNA Extraction Kit (OPS Diagnostics, Lebanon, New Jersey, USA) and from 250 mg soil material using the DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA). Amplicons to identify fungal taxa were generated using the primer set 5.8S_FUN (5'-AACCTYRRCAAAYGATCWCCT-3') and ITS4_FUN (5'-AGCCCTCCGCTTATAGATGCTTAART-3') (Taylor et al 2016) with Illumina adapters. We included a custom peptide nucleic acid (PNA) sequence (5'-CAGAATCCCGCGAACC-3') to block non-target host amplification (Lee & Hawkes, unpublished). We also included three synthetic mock sequences from Palmer et al. (2018) (Data S1). The following PCR conditions were used to generate fungal amplicons: 12.5 µl of 2X KAPA Taq Ready Mix (Roche, Pleasanton, CA, USA), 0.625 µl of 20 mg/ml bovine serum albumin, 1 µl of 5mM forward primer, 1 µl of 5 mM reverse primer, 2.5 µl of 15 µM PNA, 25 ng of DNA template, and 2.375 µl water. For soils, PNA was replaced with an equal volume of water. Amplicons were purified of fragments < 50 bp using the Agencourt AMPure XP system at sample-to-bead ratio of 1:1.8 (Beckman Coulter, Indianapolis, Indiana, USA) and genomic libraries were prepared using the Nextera XT Index Kit (Illumina, San Diego, CA, USA). Paired-end (2 x 250 bases) sequencing was performed on the Illumina MiSeq v2 platform in the Genomic Sciences Laboratory at North Carolina State University (Raleigh, NC, USA). All sequences were deposited in the NCBI Short Read Archive under BioProject PRJNA627480.

2.4 | Bioinformatics

To identify amplicon sequence variants (ASVs), we removed primers with the software cutadapt (Martin, 2011), processed sequences with the dada2 bioinformatic pipeline with default settings (R package dada2, version 1.10.1, Callahan et al., 2016), and performed post-clustering curation of ASVs with lulu using default settings (R package lulu, version 0.1.0, Frøslev et al., 2017). The minimum number of reads necessary to count an ASV as present was chosen based on the maximum number of non-biological reads, 7, that were detected in biological samples due to index switching or contamination from synthetic mock samples (Palmer et al., 2018). Enforcing a detection threshold is one way of improving confidence in the presence of rare taxa in a sample (Jia et al., 2018). Thus, we removed...
ASVs with fewer than 8 reads. One sample (R115) was removed from the dataset because it had only 18 reads in total after the dada2 processing; for context, the next smallest sample library included 4,726 reads.

Initial ASV taxonomic assignments were made with the dada2 function “assignTaxonomy” (Wang et al., 2016) using the UNITE database (Abarenkov et al., 2020) followed by the RDP classifier with the Warcup fungal ITS database (Deshpande et al., 2017; Wang et al., 2016) for any sequences not identified at the phylum level. In addition, because ASVs in the phylum Glomeromycota and in the order Sebacinales were the focus of this study, we attempted to obtain more detailed classification for these ASVs using pairwise sequence alignment in MycoBank (Robert et al., 2013). Specifically, the most specific taxonomic classification with the highest MycoBank confidence ranking was selected for ASVs previously assigned as unknown Glomeromycota (n = 151) and all Sebacinales ASVs (n = 35). Ultimately, taxonomic identities could be assigned to (i) all Glomeromycota ASVs at the class level, 97% at order, 87% at family, and 42% at genus, and to (ii) all Sebacinales ASVs at the family level and 88% at genus. In total, there were 1,081 Glomeromycota ASVs and 34 Sebacinales ASVs identified. Sequencing depth provided adequate coverage of fungal communities in roots and soils with sample libraries ranging from 2,576 to 65,544 reads (Figure S1). Species accumulation curves using sample-based rarefaction (Colwell et al., 2012) suggested that ASV richness within Glomeromycota and Sebacinales were well sampled (Figure S1).

2.5 | Statistical analyses

To characterize the diversity, abundance, and distribution of Glomeromycota and Sebacinales, we calculated ASV richness, relative abundances, and incidences at the sample level. Paired non-parametric t-tests were used to examine differences between Glomeromycota and Sebacinales richness and abundance across samples using the base R function wilcox.test in R (version 4.0.2, R Core Team, 2020). A two-proportion z-test was used to determine whether incidences differed using the base R function prop.test also in R (version 4.0.2, R Core Team, 2020). To confirm that our sampling captured variation across sites needed for distributional comparisons, we used ANOVA to test ASV richness as a function of site and plant-associated habitats (i.e., roots and soils); details can be found in the supporting information (Data S2, Table S2, Figure S2).

To understand co-occurrences and potential interactions between Glomeromycota and Sebacinales, we performed a probabilistic species co-occurrence analysis (Veech, 2012) using the R package cooccur (version 1.3; Griffith et al., 2016) in R (version 4.0.2, R Core Team, 2020). The model uses combinatorics rather than permutations to determine the probability that the rates of observed co-occurrences are either random, more likely (positive), or less likely (negative) than expected by chance. To classify taxa associations, we used α = 0.05, meaning that non-random pairs had greater than a 95% probability of co-occurring more or less frequently than expected by chance. The analysis was conducted separately on root and soil samples and after aggregating all Sebacinales incidences. Consequently, the dependent variable represented the probability that a given Glomeromycota ASV would be observed in the same root or soil sample as any Sebacinales ASV. Species pairs with expected co-occurrences of less than 1 were excluded because they are not informative. Although the analysis included all pairs of Glomeromycota ASVs and Sebacinales, pairs that included Glomeromycota and Sebacinales were the focus of this study and hence, are presented and discussed in more detail.

To determine whether high soil carbon environments, high carbon to nutrient ratios, and smaller plant hosts favored Sebacinales relative to Glomeromycota, we calculated the log-transformed ratio of Sebacinales reads divided by Glomeromycota reads. We took the approach of examining the ratio of Sebacinales-to-Glomeromycota reads rather than the reads directly because it accounts for bias in taxon detectability (McLaren et al., 2019) and variation in input microbial loads among samples (Morton et al., 2019). Log-ratio calculations, samples without any detectable Sebacinales reads (i.e., zeros) were assigned a value of 7 based on the detection threshold determined using non-biological sequences in the sequencing run (Nelson et al., 2014). Then, we used stepwise best subsets regression (R packageolsrr, version 0.5.3, Hebbali, 2020) in R (version 3.6.3, R Core Team, 2020) on the log-ratio with source (root, soil), soil carbon pools (DOC, MBC, SOM), carbon-to-nutrient ratios (C:N, C:P), and plant size metrics (height, basal area) as predictors. Because source was a significant predictor, we re-ran the regressions separately for root and soil. To address variance inflation and heteroscedasticity, SOM was log + 1 transformed and C:P and plant height were log transformed. We did not include TC in the model because it was highly correlated with several other carbon pools (Table S4). All data are reported in Table S3.

3 | RESULTS

3.1 | Richness, incidence, and abundance of Glomeromycota and Sebacinales

Glomeromycota were far more diverse, abundant, and widely distributed than Sebacinales in switchgrass roots and soils. Glomeromycota ASVs were primarily comprised of Glomerales (66%), followed by Diversisporales (11%), Gigasporales (7%), Paraglomerales (6%), and Archaeosporales (6%). Most Sebacinales ASVs belonged to the family Serendipitaceae (62%), about half of which could be identified to the genus Serendipita (50%). The remaining Sebacinales ASVs (38%) were identified as Sebacinaeaceae in the genus Sebacina.

The total number of ASVs detected in roots and soils was more than an order of magnitude greater for Glomeromycota than Sebacinales (Figure 1a,b) and on average, 23 Glomeromycota ASVs as compared to 0.7 Sebacinales ASVs were observed per sample
For both groups of fungi, the majority of ASVs were found in both root and soil samples, although a larger fraction of Sebacinales diversity was found exclusively in soils (32%) as compared to Glomeromycota (13%; Figure 1a,b).

Glomeromycota were present in all but one sample and had over 20 times more reads per sample on average than Sebacinales (p < .001, Table S5). Sebacinales were found in 33% of root samples and 51% of soil samples (Figure 1c). Sebacinales were equally or more abundant than Glomeromycota in only 5% of the samples, the majority of which were comprised of ASVs exclusively from the Sebacinaeae, that is, members of the Seredipitaceae were typically absent.

3.2 Co-occurrences of Glomeromycota and Sebacinales

Of the 1,388 root and 1,373 soil taxa pairs among Glomeromycota ASVs, the vast majority co-occurred at random. Non-random co-occurrences among Glomeromycota ASVs accounted for 13.2% of root pairs and 14.1% of soil pairs (Figure 2). Most non-random co-occurrences were significantly more frequent than expected by chance, that is, positive co-occurrences were the norm (root = 179 positive versus 5 negative pairs; soil = 192 positive versus 2 negative pairs).

![Figure 1](image1.png)

**Figure 1** Number of (a) Glomeromycota and (b) Sebacinales ASVs in switchgrass roots and soils and (c) their relative abundances across 14 sites (rows) and 8 plants per site (columns). “NA” indicates one root sample had to be removed from the dataset due to too few reads. See Table S1 for site names and locations.

![Figure 2](image2.png)

**Figure 2** Relationship between observed and expected co-occurrences between Glomeromycota ASV pairs and Glomeromycota and Sebacinales in roots and soils. Each point represents a pairing between (a) Glomeromycota ASV pairs or (b) a Glomeromycota ASV and Sebacinales in roots (circles) and soils (triangles). Colors indicate pairs that co-occur significantly more (blue) or less (orange) than expected, as well as pairs for which co-occurrence is not significantly different from random (gray). Co-occurrences are integer values, however, these data were jittered slightly to minimize fully overlapping points.
Of the 236 root and 516 soil taxa pairs between Glomeromycota ASVs and Sebacinales, most pairs also co-occurred at random. Of these, non-random co-occurrences were detected for 17 Glomeromycota ASVs (2.2%) with negative co-occurrences represented in 6/10 pairs in roots and 2/7 pairs in soil (Table 1). All but three Glomeromycota ASVs found in non-random associations belonged to the family Glomeraceae, many in the genera *Rhizophagus* and *Glomus*. The remaining ASVs belonged to the order Diversisporales. Each Glomeromycota ASV that was significantly correlated with Sebacinales was found in 5 to 17 samples, which is greater than or equal to the average rate of Glomeromycota ASV incidence (mean ± SE 4.8 ± 0.2, median 3, Figure S3).

### 3.3 Carbon, nutrients, and the relative abundance of Sebacinales and Glomeromycota

In soils, the best model to explain variation in the log-transformed ratio of Sebacinales to Glomeromycota included DOC and SOM ($R^2 = 0.28$, Table 2). Soil samples with higher DOC and SOM had an increasing abundance of Sebacinales, whereas Glomeromycota only declined with SOM (Figure 3). For SOM, this pattern was driven by eight soil samples with more than 4% SOM, all from a single site; when this site was excluded, the effect of SOM disappeared. Other top-five models included plant height, plant basal area, and C:P, but these did not significantly improve model fit and collectively added only ~ 1.2% to the variance explained (Table 2).

In roots, variation in the log-ratio of Sebacinales to Glomeromycota was best explained by DOC and plant height ($R^2 = 0.08$, Table 2). Although the explanatory value of this model was limited, Sebacinales were more abundant in roots where soil DOC was higher and declined in taller plants, whereas Glomeromycota were relatively stable. Other, more complex models included plant basal area, soil C:P, and MBC, but these did not significantly improve model fit and provided a maximum of ~ 1.5% additional variance explained (Table 2).

### 4 DISCUSSION

Sebacinales were both less common and less abundant than Glomeromycota in switchgrass roots but were only completely lacking from 1 of 14 sites. While consistent with other studies that have found Sebacinales in plant roots worldwide (Selosse et al., 2009; Weiß et al., 2011), this extends our understanding of Sebacinales distributions relative to AM fungi and soil carbon and provides additional clues regarding their ecology. We detected very few significant negative co-occurrences between Glomeromycota and Sebacinales, as expected with limited evidence of competitive exclusion. In addition, soil carbon pools (DOC and SOM) helped explain variation in the abundance of Sebacinales relative to Glomeromycota, but the effect was relatively weak. Together, these findings support widespread associations of Sebacinales with switchgrass in the presence of AM fungi and across most habitats encountered in the region, while questioning their putative functional roles in the ecosystem.

Co-occurrence between Glomeromycota and Sebacinales rarely deviated from chance encounters. When co-occurrences were non-random, Glomeromycota and Sebacinales were more likely to co-occur in soils and about evenly split between more and less expected to co-occur in roots. These results are consistent with the hypothesis that Sebacinales and Glomeromycota do not directly compete, although co-occurrence data alone are insufficient to demonstrate this conclusively. While random co-occurrences may not be surprising given that Glomeromycota were observed in all but one sample, it is important to note that co-occurrence rates were calculated for the Glomeromycota on a per-ASV basis, which allows for identification of potential specialists. However, only 4% of Glomeromycota ASVs were identified as significantly correlated with Sebacinales compared to 14% of Glomeromycota ASV pairs, suggesting that the average Glomeromycota ASV does not have strong niche overlap or biotic interactions with Sebacinales. Yet, many Glomeromycota may have simply been too scarce to evaluate since significant relationships were identified primarily with ASVs that had high prevalence overall (e.g., 75 *Rhizophagus* and 139 *Glomus* ASVs). It is also impossible to form genus-level predictions because other ASVs in these genera were found to have both positive and negative associations with Sebacinales in roots and soils. Although co-occurrence patterns are not evidence of ecological interactions (Blanchet et al., 2020), the non-specific, random associations and putative lack of competitive exclusion can be used to direct future studies.

The observed co-occurrence between Glomeromycota and Sebacinales is consistent with many studies that have found varying degrees of coexistence among AM fungi and fungal endophytes in roots. For example, niche differentiation between AM and endophytic fungi is supported by colonization dependence on environmental factors such as soil nutrients and pH (Göransson et al., 2008; de Mesquita et al., 2018; Postma et al., 2007). Nevertheless, random co-occurrence does not translate to positive interactions. Although random co-occurrences are often interpreted as a lack of competition, it is possible that the dominant Glomeromycota limit abundance of Sebacinales in roots and soils. Field studies have shown that dark septate fungi frequently parasitize AM hyphal coils when they are in high abundance, which can generate more co-occurrences among AM and endophyte fungi than expected by chance (Mandam & Jumpponen, 2008). Beyond parasitism, AM and endophyte fungi can engage in complex interactions (Scervino et al., 2009) that result in a range of net positive to negative consequences for plant function (Hashem et al., 2016; Zhou et al., 2016). Because the majority of co-occurrences were random, it is also possible that AM fungi and Sebacinales do not directly interact, or that the interactions are not ASV specific.

Beyond co-occurrence, we found that abundances of Sebacinales relative to Glomeromycota increased with increasing soil carbon across root and soil samples. The role of soil carbon was more important for the ratio of fungi in soils than in roots, but...
| ASV      | Co-occurrence relationship | ASV taxonomy       | ASV taxonomy       | Order | Family | Genus | Species |
|----------|-----------------------------|--------------------|--------------------|-------|--------|-------|---------|
|          | Direction*                  | Prob. less         | Prob. greater      | ASV   | Incidence | Co-occurrences | Diversisporales | NA | NA | NA |
| Root     |                            | than               | than               |       |         |                   | Glomerales       | Glomeraceae     | Glomus | NA |
| ASV_451  | (-)                         | 1.000              | 0.037              | 8     | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | NA |
| ASV_1305 | (-)                         | 1.000              | 0.015              | 10    | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | NA |
| ASV_679  | (-)                         | 1.000              | 0.024              | 9     | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | NA |
| ASV_1200 | (-)                         | 1.000              | 0.037              | 8     | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | NA |
| ASV_213  | (-)                         | 0.999              | 0.007              | 17    | 1       |                   | Glomerales       | Glomeraceae     | Rhizophagus | intraradices | |
| ASV_395  | (-)                         | 1.000              | 0.004              | 13    | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | intraradices | |
| ASV_1242 | (+)                         | 0.003              | 1.000              | 5     | 5       |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_933  | (+)                         | 0.033              | 0.995              | 9     | 6       |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_610  | (+)                         | 0.037              | 0.995              | 7     | 5       |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_379  | (+)                         | 0.039              | 0.997              | 5     | 4       |                   | Glomerales       | Glomeraceae     | Rhizophagus | NA | |
| Soil     |                            |                    |                    |       |         |                   | Diversisporales | NA | NA | NA |
| ASV_812  | (-)                         | 1.000              | 0.001              | 9     | 0       |                   | Glomerales       | Glomeraceae     | Rhizophagus | irregularis | |
| ASV_319  | (-)                         | 0.997              | 0.027              | 8     | 1       |                   | Glomerales       | Glomeraceae     | Rhizophagus | irregularis | |
| ASV_926  | (+)                         | 0.005              | 1.000              | 11    | 10      |                   | Diversisporales | NA | NA | NA |
| ASV_886  | (+)                         | 0.030              | 0.995              | 11    | 9       |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_834  | (+)                         | 0.030              | 0.995              | 11    | 9       |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_314  | (+)                         | 0.005              | 1.000              | 11    | 10      |                   | Glomerales       | Glomeraceae     | NA | NA | |
| ASV_528  | (+)                         | 0.034              | 0.997              | 8     | 7       |                   | Glomerales       | Glomeraceae     | Glomus         | NA | |

*Positive or negative direction indicates a greater than 95% probability of observing co-occurrences more than or less than expected by chance.
overall this was a relatively small effect, suggesting that a major role for Sebacinales in soil carbon may not be realistic at their current abundances. Nevertheless, this relationship supports our expectation that Sebacinales are more successful in high soil carbon conditions where they can putatively access organic carbon pools (Ray et al., 2019; Zuccaro et al., 2011). Sebacinales genomes also encode CAZymes that can liberate more recalcitrant forms of soil carbon (Kohler et al., 2015; Zuccaro et al., 2011). If this is a common Sebacinales attribute, perhaps higher soil carbon concentrations not only promote Sebacinales relative to Glomeromycota but also are a consequence of Sebacinales presence and abundance through their decay activities. Alternatively, indirect facilitative interactions between Sebacinales and Glomeromycota could arise if Sebacinales scavenge carbon delivered to the rhizosphere by AM fungi, since AM fungal mycelium has been found to release more photosynthate carbon to soils than roots (Kaiser et al., 2014; Zhou et al., 2020).

Relative to the Glomeromycota, Sebacinales in soils increased with SOM, but this pattern was driven by fewer than 10% of the samples with greater than 4% SOM and even that contributed very little to the variance explained. It is possible that ecological relationships among switchgrass, Sebacinales, and Glomeromycota categorically differ in high and low SOM conditions, but to parse this would require additional sampling and consideration of potentially confounding factors. In this study, we captured the range of SOM conditions that are typically observed in agricultural to forested terrestrial soils (0.2%–8.2%, Fisher & Binkley, 2012) by including stands under a variety of management regimes and stand ages (1–26 years old). However, there was a lack of sites with intermediate SOM (e.g., 1%–4%), which can make regression inferences difficult. In addition, the highest SOM was found at one site where switchgrass was intercropped with pine trees. The combination of unique biotic and abiotic conditions at this site supported 5 of the 17 unique Sebacinales ASVs detected, all of which were identified as Sebacina and 41 of the 380 unique Glomeromycota ASVs which were dominated by Glomerales but also included Archaeosporales and Diversisporales. Because these ASVs were not found at the two other sites with pine trees but lower SOM, and because there were no ASVs exclusive to all three pine sites, we can infer that the high SOM is more likely than the presence of trees to explain the diversity and dominance of Sebacinales at this site. Finally, Sebacinales were also more abundant than Glomeromycota in a small number of low SOM samples, suggesting that other factors can drive this ratio. Studies of additional intermediate and high SOM sites in more diverse settings will be needed to confirm these insights. Furthermore, molecular scale analysis of SOM may be more relevant and informative, since SOM consists of a mixture of organic compounds in various states of decay (Tfaily et al., 2015) with the accessibility of those compounds dependent upon the relative abundance of labile nutrients and substrates (Nicolás et al., 2019).

The ratio of Sebacinales to Glomeromycota in roots was also affected by plant size, specifically plant height and, in more complex models, by basal area. Contrary to our expectations, Glomeromycota were largely unaffected by plant size and the shift in the ratio was primarily caused by loss of Sebacinales in larger plants. We used aboveground plant size as a proxy for potential photosynthate supply to root fungi, but the weak relationships observed suggest either that size is not a good metric for plant carbon supply or that plant carbon supply does not have a large impact on the relative abundances of these fungi. Although other studies have used aboveground plant size successfully as an indicator of photosynthate supplied to fungal symbionts (Zheng et al., 2015), direct measurement of carbon and nutrient exchange between the plant and fungus is likely to be more relevant (Ji & Bever, 2016), particularly when discriminating between co-occurring mycorrhizas. For instance, fungal attraction to the root may result from

| Model | Predictors | $R^2$ | adj. $R^2$ | AIC | SBC | $C_p$ |
|-------|------------|-------|------------|-----|-----|-------|
| Root  | 1          | 0.066 | 0.057      | 291.92 | -20.09 | -0.444 |
|       | 2*         | 0.081 | 0.064      | 292.06 | -19.76 | -0.211 |
|       | 3          | 0.086 | 0.060      | 293.55 | -18.11 | 1.308 |
|       | 4          | 0.097 | 0.062      | 294.22 | -17.18 | 2.072 |
|       | 5          | 0.097 | 0.053      | 296.18 | -15.06 | 4.029 |
| Soil  | 1          | 0.226 | 0.219      | 256.87 | -61.02 | 5.506 |
|       | 2*         | 0.276 | 0.263      | 251.46 | -66.06 | 0.237 |
|       | 3          | 0.281 | 0.261      | 252.65 | -64.71 | 1.470 |
|       | 4          | 0.283 | 0.256      | 254.37 | -62.83 | 3.211 |
|       | 5          | 0.289 | 0.255      | 255.51 | -61.47 | 4.403 |

*Top models based on variance explained (adj. $R^2$) and model fit criteria: Akaike Information Criteria (AIC), Schwarz Bayesian Criteria (SBC), and Mallow’s $C_p$. Models are listed in order of complexity.
specific root exudates, which have been shown to influence root colonization by mycorrhizas and the assembly of rhizosphere microbes (Buée et al., 2000; Hugoni et al., 2018; Zhalnina et al., 2018).

Although non-mechanistic, these initial observations of root-associated Sebacinales suggest that they may be responding to different environmental cues compared to Glomeromycota.
Shifts from AM fungi to Sebacinales in the landscape differ from AM to EM fungal transitions. Sebacinales, like EM fungi, may outperform AM fungi in high soil carbon conditions, but both were still present and often in nearly equal abundances. EM shifts often reflect changes in soil nitrogen rather than soil carbon per se (Zhu et al., 2018), but we found that resource stoichiometry was not an important driver. In addition, transitions from AM to EM fungal dominance correspond to biogeochemical shifts from mineral to organic nutrient cycling (Lin et al., 2016) concomitant with changes in the plant community and associated plant nutrient-use traits (Averill et al., 2019). In contrast, our findings demonstrate that Sebacinales and Glomeromycota routinely co-occur in switchgrass stands, although there may be shifts in the type of Sebacinales fungi based on environmental conditions. Serendipitaceae were largely absent from the high SOM site, suggesting the need to consider how high SOM conditions may select for particular Sebacinales taxa and functions. If future work confirms that Sebacinales are less host specific and less monodominant than EM fungi, but functionally similar, then we might expect parallel biogeochemical effects to occur on smaller scales, that is, between roots or between individual plants based on relative occupancy of Sebacinales and AM fungi. Certain Glomeromycota and Sebacinales can be notoriously difficult to detect with general fungal primers (Oberwinkler et al., 2013). However, ecological patterns are similar in recent comparisons of general ITS fungal primers and specific Glomeromycota primers (Berruti et al., 2017; Lekberg et al., 2018). We did not find evidence that our data were biased because of under-sequencing or sampling. Sample ASV richness was weakly and non-significantly correlated with sequencing effort (i.e., library size) for both fungal groups (Glomeromycota = 8.3%, Sebacinales = 6.8%), indicating that the primers we used adequately captured these taxa (Vasar et al., 2017). In addition, we identified members of all known Glomeromycota orders and both Sebacinales families, despite using a conservative detection threshold based on synthetic mock sequences (Palmer et al., 2018). We also used the approach of estimating the relative abundance of Sebacinales to Glomeromycota as a ratio in order to control for variation among samples in the amount of genetic material that was amplified (McLaren et al., 2019; Morton et al., 2019). Nevertheless, future studies can improve detection and taxonomic specificity by using group-specific primers or multiple gene targets (Krüger et al., 2009; Weiß et al., 2011).

In demonstrating that Sebacinales have widespread associations with switchgrass in the presence of AM fungi, these findings further support the concept of Sebacinales as ubiquitous generalists and point to the next important question of the mechanisms by which these fungi affect plant function and soil carbon and nutrient cycling. A growing body of research demonstrates that a few Sebacinales strains in controlled conditions can promote plant growth in myriad ways with the potential to improve crop productivity (Craven & Ray, 2019). However, the development of these fungi as tools in managed systems requires an understanding of their real-world ecology. This, together with our observation that Sebacinales are diverse and abundant in switchgrass stands suggests there is still much more to learn about Sebacinales and plant associations. Future work will benefit from benchmarking Sebacinales function relative to AM fungi in a hostlike switchgrass that harbors both types of fungi and can survive in a wide range of environmental conditions.

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AUTHOR CONTRIBUTIONS

The experiment was designed by CVH. MRL collected the data. MRL and CVH analyzed the data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Sequence data were deposited in the NCBI Short Read Archive under BioProject PRJNA627480. All other sample data are provided in Table S3.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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