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Contributions of environmental and maternal transmission to the assembly of leaf fungal endophyte communities

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Abstract

Leaf fungal endophytes (LFEs) contribute to plant growth and responses to stress. Fungi colonize leaves through maternal transmission, e.g., via the seed, and through environmental transmission, e.g., via aerial dispersal. The relative importance of these two pathways in assembly and function of the LFE community is poorly understood. We used amplicon sequencing to track switchgrass (*Panicum virgatum*) LFEs in a greenhouse and field experiment as communities assembled from seed endophytes and rain fungi (integration of wet and dry aerial dispersal) in germinating seeds, seedlings, and adult plants. Rain fungi varied temporally and hosted a greater portion of switchgrass LFE richness (>65%) than were found in seed endophytes (>25%). Exposure of germinating seeds to rain inoculum increased dissimilarity between LFE communities and seed endophytes, increasing the abundance of rain-derived taxa, but did not change diversity. In the field, seedling LFE composition changed more over time, with a decline in seed-derived taxa and an increase in richness, in response to environmental transmission than LFE of adult plants. We show that environmental transmission is an important driver of LFE assembly, and likely plant growth, but its influence depends on both the conditions at the time of colonization and plant life stage.

Keywords: leaf fungal endophytes, community assembly, environmental transmission, maternal transmission, perennial grass, *Panicum virgatum*

1. Introduction

Globally the leaf is one of the largest terrestrial biotic habitats for microbial communities, representing $6.4x10^8$ km^2 of global surface area [1]. Within this habitat, leaf fungal endophyte (LFE) taxa are found in all plant species surveyed to date and contribute to plant host growth and survival. Leaf fungal endophytes are taxa living asymptomatically within, or between, cells of host leaves for the majority of the fungus' life cycle [2]. These taxa can take on many roles in relation to their plant host, including mutualistic (e.g. increasing drought tolerance [3]), neutral (e.g. latent saprotrophs [4]), or pathogenic, both weak and latent [5]. Thus, LFEs are an important factor in determining plant community composition and productivity [6, 7]

Despite the importance of LFEs to large-scale processes, the factors that determine the composition of these communities are thus far unresolved. Microbial community assembly is strongly shaped by selection, or biotic filtering [8, 9]. Selection can be observed when different plant species host different LFE communities, even at the same sites [10]. However, many studies now show that selection by the host plays a relatively minor part in assembly, compared to other processes, as indicated by LFE communities showing strong signatures of site [11, 12]. This importance of site could be due to environmental selection (e.g., site's climate) or spatial dynamics (e.g., dispersal limitation within and between sites) outweighing host selection [13]. Historical and current climatic factors may filter regional pools of LFEs [14, 15] (e.g., those in soil or air) affecting the kind of taxa that are available to colonize the leaf. Thus, while host selection no doubt plays a role in LFE assembly, predicting its assembly will require that we understand dispersal, transmission, and colonization.

Leaf fungal endophyte transmission can be broadly split into maternal, i.e., taxa transmitted directly or indirectly from maternal plants, and environmental, i.e., taxa transmitted

from surrounding environment. Our understanding of maternal transmission comes from studying systemic LFE, i.e., those distributed throughout the host plant, while localized LFEs with restricted distributions within plants make up a higher proportion of global LFE diversity [2, 16]. While environmental transmission may come from many sources (e.g., soil and other plants), here we focus on aerial transmission since it may be especially important to LFEs, as leaf surfaces have high exposure to atmospheric deposition. Colonization of aerially transmitted fungi may be particularly successful during rain events, when fungal communities become more active and release more spores and hyphae than during dry periods [17, 18]. However, the importance of environmental transmission, relative to maternal transmission, is unknown for LFEs.

The contributions of maternal versus environmental transmission to LFE communities may alter the direction and intensity of interactions between host plants and LFEs. For instance, fungi originating from maternal transmission are predicted to form strong plant-fungal interactions because of LFE dependence on the host for survival and growth [19] giving rise to cross-generational mutualistic and/or parasitic interactions. Although less is known about the functional implications of environmental transmission, this mode is the dominant mode of transmission of pathogenic taxa [20], but also may be important in the spread of some mutualistic LFEs (e.g., [21]) and saprotrophs [4]. With few characterizations of aerial dispersal, and even fewer that contextualize its impact in combination with maternal transmission, it has been impossible to assess the relative importance of transmission mode, and the outcome on microbe-host interactions [2].

Plant life stage is also likely to interact with modes of transmission in the assembly of LFEs. Seedling LFEs are likely more variable and have higher beta diversity (i.e. more differences between individuals) than mature leaves due to the lesser selection by physical and

chemical defenses. Additionally, mature leaves have experienced longer exposer to the propagules coming from the environment likely stabilizing the community [22, 23]. Leaf fungal endophyte communities increase in alpha diversity and abundance as leaves age [21] . Furthermore, the seedling LFE community is strongly affected by seed endophytes (due to the proximity in time) and soil fungal community (due to its proximity in space) than the mature LFE community. These differences in the contribution of transmission modes between LFE of seedling and mature leaves is of particular importance for perennial species since LFE must either overwinter with their host or recolonize each growing season.

We quantified the importance of maternal and environmental transmission of fungal communities to the LFEs of seedlings and adult plants, testing three hypotheses. First, we hypothesize that (1) rain community shapes LFEs and reduces the relative contribution of seed endophytes. We predict exposure to the rain inoculum will alter the LFE composition and increase LFE diversity. We test this by manipulating seed exposure to live/sterile rain inoculum in petri dishes (Fig. 1a). Second, we hypothesize that (2) the importance of maternal vs. environmental transmission in LFE assembly depends on plant life stage. We predict that seed endophytes will be abundant in the seedling LFEs, but replaced as seedlings are exposed to environmental transmission, and test this using seedling and adult plants in the field (Fig. 1b). Finally, we hypothesize (3) that the mode of transmission will alter the putative function of LFE communities. Maternal contributions may result in more mutualistic LFE communities, while a high environmental contribution could increase pathogens. We infer putative functions using previously-published effects of these taxa on host growth [3, 24, 25] and on the possible sources of LFEs [26].

2. Material and methods

(a) Site and focal host

We focused on the assembly of the LFE community of switchgrass (*Panicum virgatum*) because it is a perennial bioenergy crop of economic importance that hosts potentially-beneficial LFEs [3, 27, 28], but little is known about the sources of these LFEs (but see [26]). All field samples were collected, and experiments were conducted, in a mature switchgrass monoculture established in 2009 at the Marshall Farms site of the Great Lakes Bioenergy Research Center Scale-up experiment (42.4475522 N, 85.3109636 W). For site and management descriptions, see [29].

(b) Seed endophyte and rain fungal collection

Seeds used for both the petri and field experiments were Cave-in-Rock switchgrass variety from 2007 lot SFD-07-F11 (USDA Elsberry Plant Materials Center). Seeds were surface sterilized and stratified at 4°C in petri dishes with autoclave filter paper soaked with nanopure water for ~2 months. On July 10, 2018, five random groups of 3-5 seeds were frozen at -80°C for sequence-based characterization of the maternal community (hereafter, 'seed endophytes') with this characterization used for both experiments.

At four field blocks, near trays of seedlings (see 'Field experiment'), we set out rain collectors to capture the aerial dispersed, both dry and wet, fungal community that the seedlings and adult plants were exposed to over the course of the experiment (hereafter, 'rain fungi'). Rain collectors were left in the field for the full 51 days of the experiment with samples collected within 6 hours of each rain event (15 events and 60 total samples). In this way, we captured a realistic view of what the leaf sees, all air/wind deposition up to, and in, a rain event. Rain from two rain events were used to inoculate germinating seeds for the petri experiment (hereafter,

'rain inoculum') and used in the characterization of rain fungi for both experiments. Rain was brought back to the lab, vacuum filtered, and stored at -80°C prior to characterization of rain fungi for each sample separately. For a full description of rain collectors and collection, see Supplementary Methods.

(c) Petri dish experiment

To test hypothesis 1, we directly manipulated the presence/absence of rain inoculum on the LFE community of germinating seeds in petri dishes (hereafter 'Petri experiment'; Fig. 1a). In each 100 mm x 15 mm petri dish 20 seeds from the stratified batch described above were place on autoclaved Whatman no. 5 filter paper. Petri dishes received 5mL of either autoclave sterilized rainwater or live rainwater (hereafter, 'sterilized rain' and 'live rain', respectively). Sterilized rain was autoclaved using a 30min liquid cycle then cooled at 4°C for at least 2 hrs. Petri dishes were sealed with parafilm and placed in the greenhouse. This experiment was conducted twice with rainwater collected (see 'Rain collection') on July 21, 2018 (Round 1) and August 21, 2018 (Round 2), seeds were allowed to germinate and grow for 24 and 28 days, respectively. For each rain event, two petri dishes were inoculated from collections from three field blocks (total 24 petri dishes). At harvest, fungal colonization was visually estimated by number of seeds with fungal growth, germination was recorded, and germinated seedlings were bulk by petri dish then stored at -80°C.

(d) Field experiment

To test hypothesis 2, we sowed 10 seeds per pot into autoclaved 50:50 sand and vermiculite in 107mL conetainers (SC7 Stewe and Sons, Tangent, Oregon) that were eventually placed in the

147 field (hereafter, 'Field experiment'; Fig. 1b). The 48 pots were blocked into four groups of 12 by
148 tray to control for greenhouse effects and watered daily with nanopure water in an empty
149 greenhouse for 6 days whereupon seedlings began to emerge from the soil (July 16, 2018) and
150 seedlings were transported to the field. Eight of the pots (two per block) transported to the field
151 were haphazardly chosen for harvest. Five of these pots, with emerged seedlings, were used to
152 characterize initial LFE community and colonists from the greenhouse (hereafter, 'start
153 seedlings'). Of the remaining pots, 10 pots were randomly distributed in 98 cell trays at each of
154 four locations along the southern and western edge of the field (hereafter, 'field blocks')
155 surrounding the mature stand of switchgrass. Plants and pots were not allowed to touch the soil
156 or adult plants; therefore, any environmental transmission of fungi occurred through aerial
157 spread. Seedlings were fertilized at the beginning of the experiment and every week with 10 mL
158 of 0.2 μm filtered half strength Miracle-Gro All Purpose Liquid Plant Food. After 52 days in the
159 field (September 9, 2018), leaves from the 17 emerged seedlings that survived (4-5 seedlings per
160 field block; hereafter, 'end seedlings'). At establishment and end of experiment, leaves from
161 three randomly chosen adult plants were harvested at each field block (four adult plant replicates;
162 hereafter, 'start adults' and 'end adults', respectively). All plant material was stored at -80°C
163 prior to sequencing.
164
165 **(e) Fungal community characterization**
166 For full description of community characterization, see Supplemental Methods. Plant
167 samples, seeds and leaves, were surface sterilized then DNA was extracted using Plant DNeasy
168 kits. Rain DNA was extracted from filters using PowerWater kits (Qiagen, Hilden, Germany).
169 Communities were characterized using 250-bp paired-end MiSeq sequencing (MSU Genomics

Core, East Lansing, MI) of the ITS2 region [30]. Sequences were merged, quality checked, and clustered into zero-radius operational taxonomic units (hereafter, approximate sequence variants or ASVs) using unoise3 [31]. We used the level of 100% similarity to be conservative in our estimate of overlap between rain fungi and plant communities. We classified representative sequences against the UNITEv8.2 database [32] using CONSTAX [33]. We identified and removed possible contaminant taxa based on blank controls using microDecon [34]. Finally, we rarified the community to a depth of 1,000 reads resulting in 2,586 ASVs and 117,000 reads. In total, four plant samples were filtered out due to poor amplification and sequencing (Table S1).

To determine possible functional roles of LFEs, addressing hypothesis 3, we matched ASVs to previously published switchgrass LFEs at ≥97% sequence similarity. Leaf fungal endophytes were classified into pathogens, mutualists, or context mutualist based on published effects of LFE on switchgrass [14, 15, 24]. Putative sources of LFEs were classified based on significant plant community indicator taxa from [26]. For a full description of the functional classifications, see Supplemental Methods.

(f) Statistical analysis

We used indicator value index [35], the product of taxon's specificity (i.e., uniqueness to a given habitat) and fidelity (i.e., frequency of occurrence in a given habitat), to classify the likely sources of LFE, either rain fungi or seed endophytes. We weighted this value by taxon abundances to calculate the contribution of sources to the LFE community. We also calculated the abundance of significant indicator taxa ($p<0.05$). We created PERMANOVA and mixed effects models to test the dissimilarity between, and diversity of, LFE communities, seed endophyte, and rain fungi (see Supplemental Methods). Additionally, we tested whether

community change was more driven by nestedness (i.e., loss of taxa with no replacement) or turnover (i.e., loss of taxa with replacement) [36] by calculating the ratio (nestedness:turnover; higher values indicate a larger role for nestedness). All PERMANOVA [37] and mixed effects models [38] included field block as a random grouping variable.

(3) Results

Rain fungi showed tremendous taxonomic and functional variability over the course of the experiment (Fig. S1 and S2). The richness and diversity of rain fungi was also consistently higher than the LFE community (Fig. S3 and S4). Basidiomycota dominated rain fungi until the end of the experiment, when Ascomycota reached equal abundance (Fig. S2a). Overall, these rain fungi appear to be a significant source of LFE taxa; rain fungi made up >65% of the richness and ~90% of the reads found in adult and seedling LFE communities.

(a) Hyp 1: Rain inoculum alter LFE

We found that live rain inoculum altered the LFE of germinating seeds (Table S2, Fig S5); however, the strength of these effects differed across our two experimental rounds. Specifically, live rain increased similarity between LFE communities and rain fungi in round 2, but significantly increased dissimilarity between LFEs and seeds in round 1 (Table S3; Fig. 2a), and only when taking account abundance (i.e., Bray-Curtis distance). In both rounds, live rain increased rain-indicator taxa in LFE communities, without increasing LFE diversity (Table S4; Fig. S3) or replacing seed-indicator taxa (Table S5; Fig. 3a and S6a). Though turnover explained much of the difference between LFE communities and each source (rain or seeds), nestedness

explained more differences between LFE communities and rain fungi, presumably because LFEs were a subset of the highly diverse rain fungal community (Table S3; Fig. 2c and S7a).

In general, rain fungi were highly distinct from both LFEs and seed endophytes, (Fig. S5), tended to have lower variance in terms of taxa presence/absence (i.e., Jaccard distance; Table S4; Fig. S8b), and higher diversity (Fig. S3). There were also significant differences in rain fungi used in round 1 and round 2 (pairwise PERMANOVA $p<0.03$). Rain fungi in round 1, collected earlier in the summer, was more dominated by Basidiomycota, specifically Agaricomycetes, Exobasiomycetes, and Tremellomycetes, while round 2 was dominated by Ascomycota, specifically Dothideomycetes (Fig. S9). Still, across both rounds, live rain exposure consistently increased the abundance of Dothideomycetes and Sordariomycetes while decreasing abundance of Tremellomycetes, which dominated seeds and LFEs receiving sterilized rain (Fig. S9b). Finally, inoculation with live rain did not significantly alter seed germination rate (Fig. S10a) or visible fungal colonization (Table S6; Fig. S10b).

(b) Hyp 2: Importance of seed endophytes and rain fungi across two life stages

Leaf fungal endophyte communities of both life stages, end seedling and adult plants, were significantly different than the starting LFE communities (Table S7; Fig. S11) and gained rain indicators (Tukey HSD: $p=0.016$; Table S8; Fig. 3b) by the end of the experiment. Seedling LFE communities shifted more from start to end compared to adult plants (Jaccard-based composition; Table S9; Fig. 4b) and experienced a significant loss of seed indicator taxa (Fig. 3b). In addition, the richness of seedling LFEs more than doubled from start to end while there was no change in adult LFEs (Table S10; Fig. S4a). Still, some patterns were similar across life stage. Seedling LFEs were no more similar to rain fungi than adults (Fig. 4ab) supported by the fact that LFEs had similar contributions from rain fungi overall (Fig. 3b). While turnover

dominated changes in fungal communities, when comparing relative importance between life stages, nestedness contributed more to the distance between rain fungi and end adult LFEs (Fig. 4c and S12a).

The endophyte communities of the seeds, start seedlings, and start adults were dominated by likely yeast from Basidiomycota, specifically Tremellomycetes, but, by the end of the experiment, both adult plants and seedlings were dominated by Ascomycota, specifically Dothideomycetes (Fig. S13). There was no difference in beta-dispersion across endophyte communities (Table S10; Fig. S14).

(c) Hyp 3: Mode of transmission alters function of LFE communities

Switchgrass LFEs were common in rain fungi (~18% of reads; Fig. 5a and S1a). Pathogens made up the largest portion of the putative LFEs found in rain fungi (~11% of reads; Fig. 5b and S1b). In the field experiment, the relative proportion of pathogens in seedling LFE increased from start to end (Fig. 5b) further supporting rain as the dominant pathway for pathogens. This was corroborated by the petri experiment, in which LFEs originating from live rain were primarily pathogens (Fig. S15b). We found no recorded mutualists in the seed endophyte community (Fig. 5cd) instead likely pathogens made up ~16% of the putative LFE found in seed endophytes (Fig. 5b). On the other hand, mutualists and context mutualists were found in rain fungi (Fig. 5cd and S1cd). In general, functional attributes of LFEs changed more in seedlings than adults, consistent with the compositional data (Fig. 5).

(4) Discussion

We show how maternal and environmental transmission contribute to short-term (post germination) and long-term (adult leaves) assembly of leaf fungal endophyte (LFE) communities. We found that rain (representing wet and dry aerial dispersal) is comprised of a rich community of fungi, many of which are found in LFE communities, and exposure to this environmental transmission changes LFE composition. Together this suggests rain is an important driver of LFE assembly, supporting our first hypothesis. This first look at the relative influence of seed vs. rain communities in LFE assembly revealed three roles for environmental transmission. First, rain affects LFE assembly, but these effects are likely temporally dependent and not necessarily predictable. Depending on characteristics of the rain event, rain inoculum seems to shift the germinating LFE community compositionally away from seed endophytes by enrichment of taxa and not by displacing seed endophytes. As LFEs continue to assemble under environmental transmission, and increase in richness, seed indicators are lost from the LFE community. Second, LFE communities of early life stages (seedlings) are most responsive to environmental transmission. We observed large shifts in LFE composition when seedlings were exposed to environmental transmission, and relatively little change in adult LFE. Finally, wet and dry aerial dispersed fungi, integrated through rain, hosts a large temporally variable community of putative LFEs, with fungi able to colonize contributing unique functions to the LFE community.

(a) Rain fungi shifts LFE community away from the maternal endophytes via enrichment

We show that environmentally transmitted taxa can alter the communities of germinating seedlings, reducing similarity to maternal communities, but not through displacement of seed endophytes by novel rain fungi. Rather, LFEs became more dissimilar to seed endophyte

communities under the first round of inoculation (Fig. 2a) without a loss of seed endophytes (Fig. 3a). This lack of displacement of seed endophytes may be driven by rain inoculum enriching taxa that have overlapping presence in seed endophytes and rain fungi (>55% of seed endophytes are found in rain fungi) which may be the result of historical environmental transmission. Even though all seeds were from the same USDA grown population, gamma diversity across seeds was high (>150 ASVs; Table S1). This rich pool of maternal taxa may have originated from aerial dispersed microbes colonizing florets during fertilization and seed development (reviewed in [39]). Seed endophytes possibly originating from 'historical' environmental transmission makes separating environmental and maternal transmission challenging, but our study allows the separation of maternal contributions from contemporary environmental transmission. We primarily found that rain inoculum increased the abundance of Ascomycota and pathogens (Fig. S9a and S15b), but effects of environmental transmission appeared temporally dependent.

High diversity and temporal variability in the rain fungi seems to have important implications for the assembly of LFEs. Despite the rich rain fungal community, inoculation with rain inoculum had no effect on LFE diversity (Fig. S3). Many rain fungi may not have been able to colonize the plant due to strong leaf selection, making increasing abundance of extant endophytes the primary effect of rain inoculum, not introduction of new taxa. The increased similarity between LFE and rain fungi only in round 2 (Fig. 2a) may be partially a result of the variability of the rain fungi over time. Specifically, rain inoculum used for round 2 had a higher portion of Ascomycota (Fig. S9a), a high portion of previously documented LFEs, mostly putative pathogens (Fig. S15ab; [15, 24]), and higher concentrations of fungal hyphae and spores (data not included). The higher concentration of putative LFEs, and fungi in general, may have

307 increased colonization success and thus environmental transmission [40, 41]. Variation in rain
308 chemistry between rounds also could have driven the rain inoculum effect on LFE, nitrate levels
309 were higher the week that round 2 inoculant was collected (~2X higher, Station MI26
310 http://nadp.slh.wisc.edu/ntn/). Regardless of the mechanism, our study highlights LFE assembly
311 may be sensitive to the changes in the composition, and colonization ability, of rain fungi, which
312 appear highly temporally variable (Fig. S1a). Additionally, this temporal dependency of
313 colonization appears to continue into the adult life stage since rain indicators increased in the
314 LFE community over time (Fig. 3b). Our results, as well as a recent study of switchgrass fungal
315 epiphytes in our region [42] show seasonal succession in the leaf microbiome which may be
316 driven by an active exchange between the leaf microbiome and the rain community.
317
318 **(b) Seedlings are more responsive to environmental transmission than adults**
319 Persistence of seed endophytes and magnitude of temporal change in the LFE community
320 differed by life stage, confirming our second hypothesis. The change in the presence and absence
321 of taxa best captured the greater change in LFE communities, and increased dissimilarity from
322 seed endophytes (Fig. 4b), in seedlings compared to adults, suggesting changes in rare taxa drove
323 shifts in community composition. Our findings are consistent with other studies that have found
324 greater temporal change in seedling than adult LFE communities [22, 43]. These rapid changes
325 in seedling LFE may be driven by a lack of physical and chemical defenses making the leaves of
326 seedlings more susceptible to colonization from external sources. Early life stages may be
327 especially important in shaping the final LFE community, and we show they are susceptible to
328 environmental transmission, including of pathogens (Fig. 5b).

Though their importance declined with exposure to rain inoculum, seed endophytes had a surprisingly long-lasting presence in the switchgrass LFE community. Our finding that seed indicators persisted into seedling and even adult stages (making up ~14% of adult reads; Fig S6b) suggests that even though colonization of rain fungi reduces its importance, the seed community is still important for long-term LFE dynamics. To better understand the long-term effects of seed endophytes on LFE communities requires experimental manipulation such as knocking out the current seed endophyte community and monitoring LFE assembly. Importantly, though seed endophytes are a part of the long-term LFE community, two-month-old seedling LFE communities already greatly diverge from the seed endophyte community (Fig. S11).

Though the drivers of the shift differed between the two life stages, both the seedling and adult LFE communities tended to show increased relative abundances of rain indicators (Fig. 3b). In contrast to the petri experiment, the colonization of novel rain fungi played a role in seedling LFE community change and increased LFE richness (Fig. S4a). Interestingly, the increase in richness with exposure to rain inoculum did not occur in the LFE of seedlings in the petri experiment (Fig. S3a). It is possible that the relatively short duration, or the single inoculation under controlled conditions, of the petri experiment reduced our ability to observe the effects of rain inoculum on LFE richness. Though turnover dominated the changes in the LFE communities, compared to seedlings, the adult LFE community was more of a taxonomic subset of the rain fungi (i.e., higher nestedness Fig. 4c), suggesting that adult plants may exert higher selection on colonists from the diverse rain community.

(c) Environmentally transmitted taxa contribute unique functions to LFEs

Our data also suggests that transmission pathway influences LFE functional diversity and LFE community impact on plant host, partially supporting our third hypothesis. Rain fungi hosted many taxa that have been identified as switchgrass LFEs from previous culture-based surveys (Fig. S1a; [15, 24, 26]), allowing us to infer putative functions. The dominance of putative pathogens in rain fungi, and the fact that these groups became abundant in the seedling LFE by the end of the experiment (Fig. 5b), suggests that rain is a significant source for pathogen dispersal [20, 44, 45]. Contrary to our predictions, seed endophytes hosted no recorded mutualists and instead hosted putative LFE composed of possible pathogens (Fig. 5b). Furthermore, we found that rain hosted a small portion of LFE mutualists and context mutualists, beneficial under drought but antagonistic under other conditions [14]. These mutualists became somewhat enriched in both the adult and seedling LFE communities by the end of the experiment (Fig. 5cd) highlighting that aerial transmission can introduce both beneficial as well as harmful taxa.

Rain also appears to be a major pathway for the transmission of taxa from surrounding plant communities. Leaf fungal endophytes that have been found to be indicative of prairie ecosystems [26] were present at low frequency, but consistent levels across rain events. These same taxa became relatively dominant in the final seedling community compared to all other LFE communities (Fig. 5e). Since our field experiment was conducted near an experimental prairie restoration [29], these taxa may also be colonists from the prairie plant community in our experiment, highlighting the potential for spillover between cultivated and non-cultivated lands via aerial dispersal [46]. On the other hand, taxa indicative of the specific population from which our target switchgrass plants were derived (i.e., Cave-in-Rock variety) [26] were relatively rare in the rain fungal and LFE communities (Fig. 5f). This suggests that selection of regional pools

374 of potential LFEs by nearby plant communities may explain the 'site signal' found in many LFE
375 studies [11, 12].
376
377 **(5) Conclusion**
378 Rain, as an integrator of wet and dry aerial dispersal, hosts a functionally diverse
379 community of putative LFEs and alters community assembly and function. We found that seed
380 endophytes remained in the LFE communities of seedlings and adult plants, but exposure to
381 environmental transmission made LFE communities less similar to seed endophytes and
382 increased the contribution of aerial transmitted taxa to the LFE community, demonstrating that
383 environmental transmission is an important driver of LFE community assembly. This interaction
384 is dynamic over time, with plant ontogeny and seasonal shifts in rain community composition
385 and chemistry likely affecting assembly outcomes. Future work should test the seasonal LFE-
386 rain interchange and its importance for long-term dynamics of the plant microbiome.
387
388 **Data accessibility.** Sequence data are available at NCBI SRA under Bio-Project no.
389 PRJNA709151, and datasets, bioinformatics scripts and metadata used in the current study are
390 available at https://github.com/ldereske/Bell-Dereske_Evans_Fugal_Rain and archived at DOI:
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540

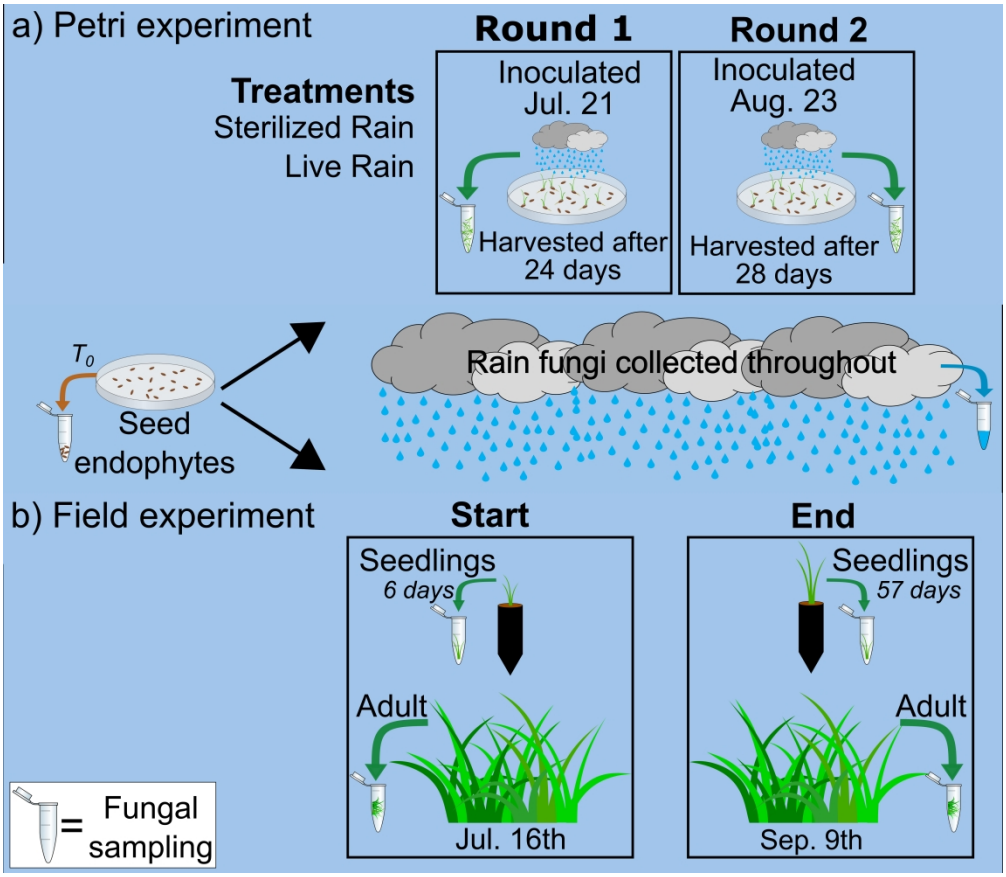

Figure 1. Two experiments were established to test the importance of maternal and environmental transmission in leaf fungal endophyte (LFE) community assembly. a) The petri experiment tested the effects of rain inoculum from two rain events (rounds) on germinating seedlings by inoculating seeds with autoclaved sterilized rain or live rain. b) The field experiment tested the correlation between seedling and adult LFE communities to rain fungi by placing greenhouse germinated seedlings in a field monoculture of adult switchgrass. We collected seedling and adult samples at the start and end of the experiment to characterize the change in LFEs. Seed endophytes were used to characterize the maternally transmitted community while rain fungi were collected throughout the experiment, with events characterized separately, to capture the environmentally transmitted community. For full numbers of replicates, see Table S1).

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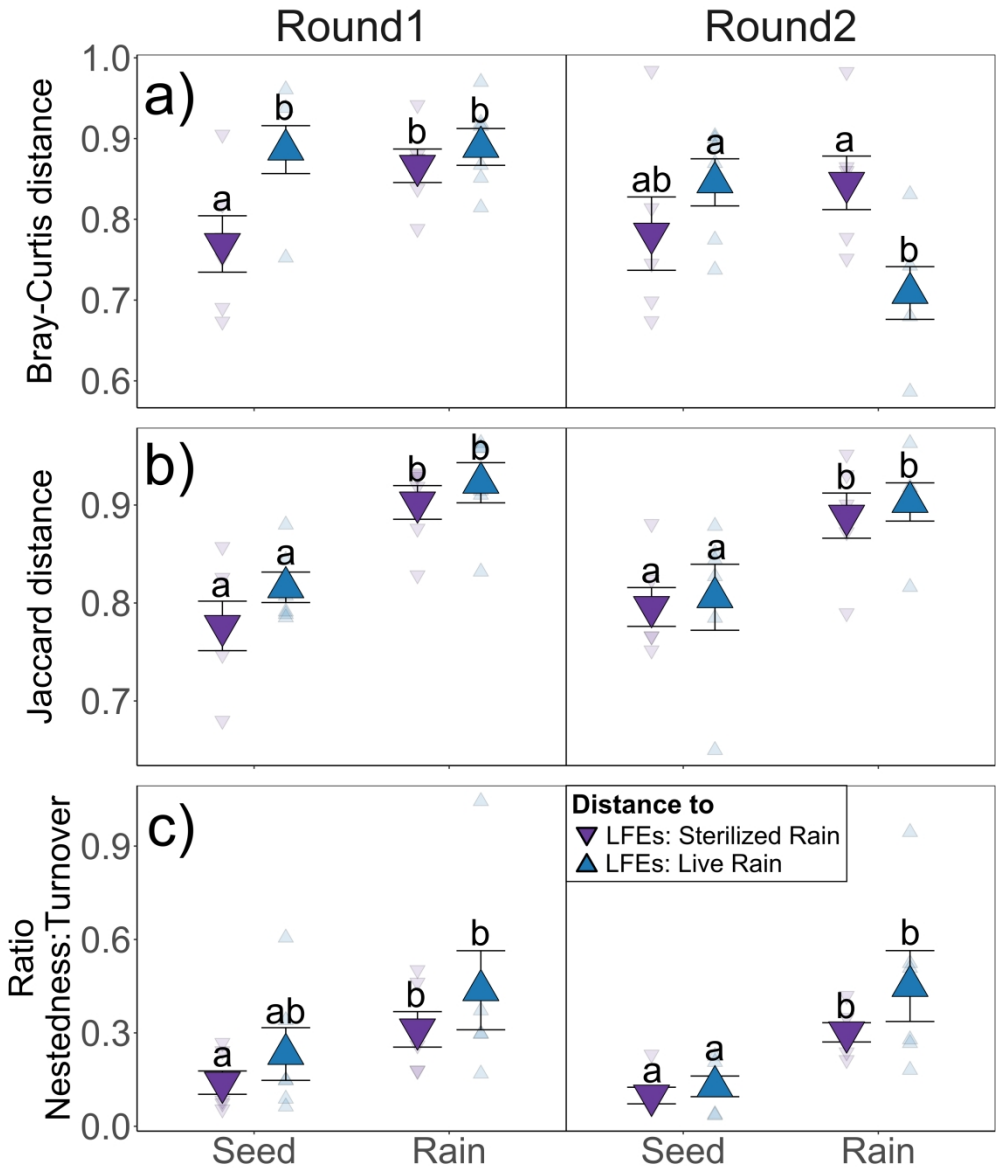

Figure 2. Pairwise community distance of leaf fungal endophyte (LFE) communities to seed endophytes (Seed) or rain fungal (Rain) communities using a) Bray-Curtis, b) Jaccard distance, and c) ratio of nestedness to turnover of seedlings receiving autoclave sterilized rain (dark purple triangle pointing down) or live rain (dark blue triangle pointing up). Round of inoculations are Round 1 (July 21) and Round 2 (August 21). Points represent means with SE. Raw data is represented by transparent points. Within round posthoc pairwise significances are FDR adjusted represented by letters.

738x874mm (118 x 118 DPI)

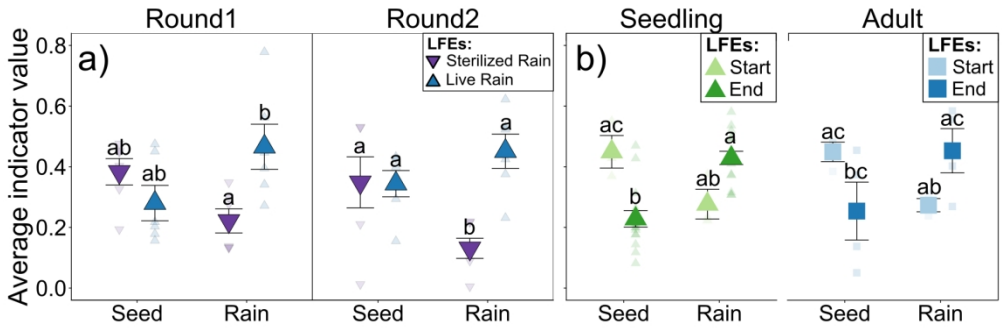

Figure 3. Average seed endophyte and rain fungal indicator values in leaf fungal endophyte (LFE) communities of the a) petri experiment and b) field experiment. a) Germinating seedling LFE communities were inoculated in Round 1 (July 21) and Round 2 (August 21) with autoclave sterilized rain (dark purple triangle pointing down) or live rain (dark blue triangle pointing up). b) Seedling LFEs were characterized at the start (July 16: light green triangle) or end (September 5: dark green triangle), and adult LFEs were characterized at the start (July 16: light blue square) or end (September 5: dark blue square). Points represent means with SE. Raw data is represented by transparent points. FDR adjusted posthoc pairwise significances for a) within round comparisons for petri experiment and b) all sample comparisons for field experiment are represented by letters.

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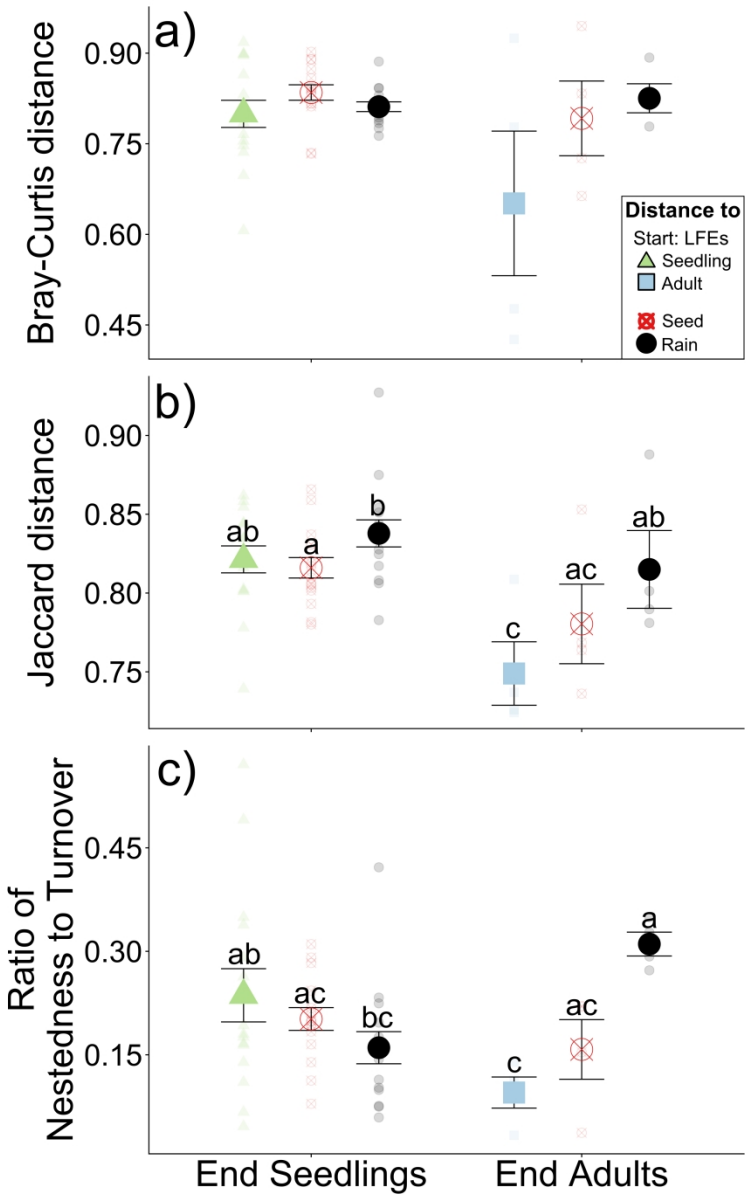

Figure 4. Pairwise community distance using a) Bray-Curtis, b) Jaccard distance, and c) ratio of nestedness to turnover from end (September 5) seedling and adult leaf fungal endophyte (LFE) communities to start LFE (July 16; seedlings: green triangle or adults: blue square), seed endophytes (red circled x), or rain fungi (black circles). Points represent means with SE. Raw data is represented by transparent points. Posthoc pairwise significances are FDR adjusted represented by letters, and no letters indicate no significant difference.

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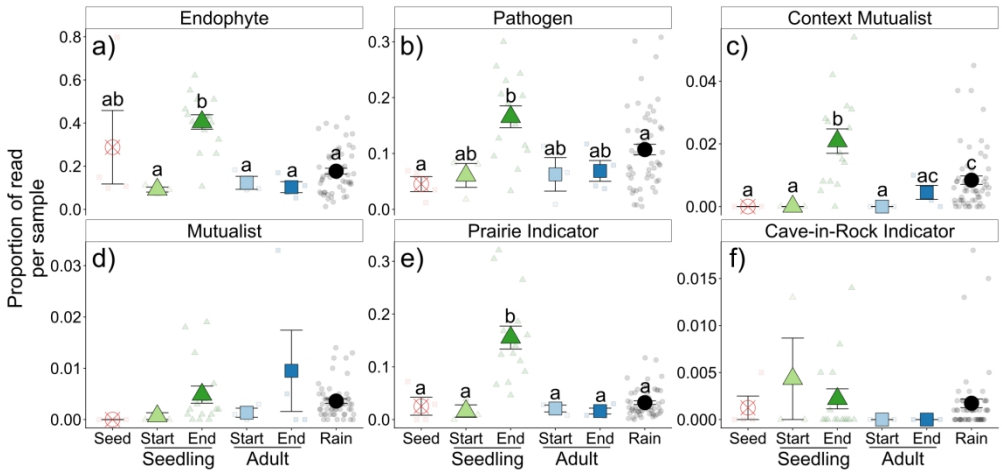

Figure 5. Proportion of fungal reads matching previously published endophytes. Points are seed endophytes (red circled x), rain fungi (black circles), seedling leaf fungal endophyte (LFE) collected at the start (July 16: light green triangle) or end (September 5: dark green triangle), adult LFE collected at the start (July 16: light blue square) or end (September 5: dark blue square). Points represent means with SE. Raw data is represented by transparent points. FDR adjusted posthoc pairwise significances are represented by letters, and no letters indicate no significant difference.

1355x638mm (39 x 39 DPI)