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Successional adaptive strategies revealed by correlating arbuscular mycorrhizal fungal abundance with host plant gene expression

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Abstract

The shifts in adaptive strategies revealed by ecological succession and the mechanisms that facilitate these shifts are fundamental to ecology. These adaptive strategies could be particularly important in communities of arbuscular mycorrhizal fungi (AMF) mutualistic with sorghum, where strong AMF succession replaces initially ruderal species with competitive ones and where the strongest plant response to drought is to manage these AMF. Although most studies of agriculturally important fungi focus on parasites, the mutualistic symbionts, AMF, constitute a research system of human-associated fungi whose relative simplicity and synchrony are conducive to experimental ecology. First, we hypothesize that, when irrigation is stopped to mimic drought, competitive AMF species should be replaced by AMF species tolerant to drought stress. We then, for the first time, correlate AMF abundance and host plant transcription to test two novel hypotheses about the mechanisms behind the shift from ruderal to competitive AMF. Surprisingly, despite imposing drought stress, we found no stress-tolerant AMF, probably due to our agricultural system having been irrigated for nearly six decades. Remarkably, we found strong and differential correlation between the successional shift from ruderal to competitive AMF and sorghum genes whose products (i) produce and release strigolactone signals, (ii) perceive

mycorrhizal-lipochitinoligosaccharide (Myc-LCO) signals, (iii) provide plant lipid and sugar to AMF, and (iv) import minerals and water provided by AMF. These novel insights frame new hypotheses about AMF adaptive evolution and suggest a rationale for selecting AMF to reduce inputs and maximize yields in commercial agriculture.

KEYWORDS
adaptive strategy, arbuscular mycorrhizal fungi, community ecology, host plant genes

1 | INTRODUCTION

The changes in adaptive strategies that accompany microbial succession and the mechanisms behind the changes are fundamental aspects of microbial ecology that are now being studied owing to advances in environmental molecular biology (Datta et al., 2016; Green et al., 2008; Guittar et al., 2019; Kearns & Shade, 2018; Kim et al., 2017; Martiny et al., 2015; Nemergut et al., 2016; Ortiz-Alvarez et al., 2018; Prest et al., 2018) (Table S1). The prolonged succession seen in natural plant communities (Bruelheide et al., 2011) is compressed by the rapid development of microbial communities that follows colonization of a new environment (Fierer et al., 2010), for example a field newly planted to sorghum (Gao et al., 2019), thus providing a more tractable system to investigate the patterns and mechanisms underlying community assembly (Datta et al., 2016). The system is made even more tractable by focusing on crops, which have been domesticated consciously, and the arbuscular mycorrhizal fungi (AMF) mutualists, which have been domesticated unconsciously, because individual crop plants are, typically, all of one genotype and develop, along with their AMF, in synchrony. A key ecological phenomenon studied with fungi, succession or temporal dynamics, has been documented for fungi in general (Guo et al., 2018; Han et al., 2017; Voriskova et al., 2014), as well as for fungal functional guilds, which include saprotrophic fungi (Harper & Webster, 1964; Macauley & Thrower, 1966), endophytic and pathogenic fungi (Tadych et al., 2012), ectomycorrhizal fungi (Gao et al., 2015; Twieg et al., 2007) and, the focus of our report, AMF (Bahram et al., 2015; Bainard et al., 2014). Using the annual crop system employed here, sorghum, exceptionally strong succession has been reported for AMF (Gao et al., 2019) as has a remarkably strong drought response led by the strong and coordinated down-regulation of transcription of hundreds of sorghum genes known to manage AMF (Varoquaux et al., 2019).

The value to civilization of AMF is not limited to their experimental tractability and history of unwitting domestication. AMF are an essential ingredient for natural ecosystem productivity, diversity and stability, as evidenced by the fact that this earliest evolving (400–600 million years ago) and most prevalent type of mycorrhiza continues to form mutualisms with 85% of vascular plant species, including most crops (van der Heijden et al., 1998; Martin et al., 2018; Redecker et al., 2000; Smith & Read, 2008; Taylor et al., 1995). AMF also present ample opportunity to examine fundamental biological questions because, owing to their recalcitrance to cultivation away from their plant symbiont, features as basic as sexuality and intra-individual genetic homogeneity remain controversial (Bruns et al., 2018; Ropars et al., 2016; Thiery et al., 2016). As noted above, AMF are obligate symbionts living in and feeding from the roots of their host plant (Smith & Read, 2008). Therefore, annual plant development over a growing season can be expected to play a dominant role in the succession of AMF, particularly in relatively homogeneous agricultural systems with predictable environmental parameters, as we have recently documented (Gao et al., 2019, 2020). Of course, one cannot exclude smaller contributions to succession by environmental perturbation, dispersal limitation, stochastic rearrangements and priority effects (Chagnon et al., 2012; Dumbrell et al., 2010; Werner & Kiers, 2015a). As with any group of organisms, AMF embrace a variety of ecological strategies and these can be recognized using Grime's three strategies (C, competitors; S, stress-tolerators; and R, ruderals), which are based on the response of species to stress and disturbance (Grime, 1974). With AMF, Chagnon et al. (2013) investigated the C–S–R framework by using previously published, trait-based studies to propose definitions of the three strategies: (i) ruderal AMF that quickly re-establish symbiosis after disturbance (e.g., the cultivation associated with replanting fields) and quickly complete their life history by generating large amounts of spores; (ii) competitive AMF that enjoy low stress and low disturbance while providing plants with large amounts of soil-derived resources in exchange for high levels of host-derived carbon; and (iii) stress-tolerant AMF that withstand the stress of harsh environments (e.g., drought) (Hart & Reader, 2002; Helgason et al., 1998; Maherali & Klironomos, 2007; Staddon et al., 2003). Chagnon et al. (2013), however, did not match specific AMF species with Grime's categories, and currently there is no consensus as to which AMF species are competitive, ruderal or stress-tolerant. Here, we investigate the C–S–R framework in light of our study of AMF succession with sorghum (Gao et al., 2019), which employed abundant sampling of root, rhizosphere and soil, and used internal transcribed spacer 2 (ITS2) metabarcoding to document strong succession in AMF communities over a summer field season (Gao et al., 2019).

Two controversial aspects of AMF ecology deserve consideration at the outset. First, should AMF species be recognized in mycobiome studies by rDNA regions that are variable (ITS) or conservative (18S) (Bruns et al., 2018; Bruns & Taylor, 2016)? We have shown that metabarcoding by the more variable ITS2 (i) recognizes AMF species-level operational taxonomic units (OTUs) that are comparable to those recognized for other fungi, (ii) is robust to possible individual and intraspecific ITS variation, and (iii) reveals succession

whether the threshold for OTU recognition is based on fast evolving ITS or lowered to mimic the slow evolving 18S rDNA (Gao et al., 2019). The second controversy concerns succession, both its definition and its modelling. A definition of microbial succession has been proposed by Fierer and colleagues (2010), "… the somewhat orderly and predictable manner by which communities change over time following the colonization of a new environment …" These authors go on to consider three types of primary succession, based on whether the organisms are autotrophic or heterotrophic and, if heterotrophic, whether the substrate is finite and consumed by the microbes (as in a newly fallen leaf) or continually renewed and not consumed by the microbes (as in a sewage treatment reactor or mammalian gut). Fierer and colleagues focus on primary succession and avoid "… temporal changes in microbial communities that occur following disturbances …" Although Fierer et al. (2010) formulated their definition for bacteria that consume biological carbon in an asymbiotic manner, their definition can be applied to AMF, which are obligately symbiotic with plants and live by trading minerals and water for products of photosynthesis. As obligate symbionts with plants, AMF are clearly heterotrophic, and the supply of biological carbon is exogenous. Therefore, AMF fit the general category of exogenous–heterotrophic succession proposed by Fierer et al. (2010). Less clear is the distinction between primary and secondary succession. Recognizing that some might consider emerging root tips to constitute a new environment whereas others might consider the sowing of seeds and emergence of plants to constitute a disturbance in an existing environment, our use of the term succession does not specify either primary or secondary succession. Turning to models, the classic autogenic succession model postulates that early arriving species can either tolerate, promote or inhibit later arriving species (Connell & Slatyer, 1977). However, because AMF are obligately symbiotic to host plants, this type of autogenic succession is not expected; rather any succession would be due to significant changes in the external biotic environment (i.e., host plant growth, which is the key biotic factor for heterotrophic AMF) or the abiotic environment (i.e., drought) (Connell & Slatyer, 1977).

In our previous study of sorghum AMF succession (Gao et al., 2019), we identified two, initially dominant AMF species-level OTUs (OTU51 [*Rhizophagus*], OTU70 [*Claroideoglomus*]), and 13 initially rare AMF species-level OTUs (OTUs 150, 132, 166, 251, 118, 213, 161 [*Rhizophagus*] and OTUs 133, 126, 323, 229, 476, 400 [*Glomus*]) (Gao et al., 2019). Based on their rapid establishment of arbuscular mycorrhizae with sorghum roots and their even distribution in root and soil compartments, we consider the two initially dominant AMFs to have a ruderal lifestyle. Because the 13 initially rare AMFs rose in abundance to displace the two ruderal AMF and dominate arbuscular mycorrhiza in the sorghum roots, we consider them to have a competitive lifestyle. Analyses and data supporting our definition of ruderal and competitive in terms of abundance of AMF species, competition for the same niche and coexistence of competitive species can be found in Gao et al. (2019). However, lacking a stressful environment in our prior research (Gao et al., 2019), we were unable to identify species whose abundance depended on stress. In a subsequent study of the same AMF–sorghum system we included drought stress and discovered that AM fungal abundance (together with the abundance of transcripts from sorghum genes induced by AMF) was drastically decreased by both pre- and postflowering drought (Varoquaux et al., 2019). This result indicated that the AM fungal community, as a whole, is not tolerant to drought stress, but we could not say that no individual AMF species could maintain its abundance or even increase it during drought. Here, to look for drought-tolerant AMF, we add drought stress to our study in framing the first of three hypotheses, H_1: Competitive AMF species will be replaced by drought-stress-tolerant AMF species when irrigation is withheld to induce pre- or postflowering drought.

For our second and third hypotheses, we use expression of host genes involved in key physiological AMF traits to investigate the mechanism of the transition of AMF species from ruderal to competitive. H_2: The transition from ruderal to competitive AMF will correlate with shifts in transcription of host genes responsible for producing and receiving signalling molecules. H_3: The transition in abundance of AMF from ruderal to competitive species will correlate with shifts in transcription of host genes responsible for resource transfer between fungi and host. Testing these hypotheses involves combining data from our study of AMF succession (Gao et al., 2019) with those of our study of the effect of drought on the expression of plant genes activated when forming arbuscular mycorrhizae (Varoquaux et al., 2019). Knowing that key plant and AMF responses to the arbuscular mycorrhizal symbiosis involve signalling and nutrient transfer (Akiyama et al., 2005; Besserer et al., 2006; Jiang et al., 2017; Luginbuehl et al., 2017; Olah et al., 2005; Rausch et al., 2001), we correlate the abundance of AMF species with the expression of host plant genes involved in these two areas. Exchanges of signals and resources between plants and AMF are reciprocally regulated to enable efficient partner selection (Bonfante & Genre, 2010; Werner & Kiers, 2015b), which might be central to the transition from ruderal to competitive AMF. Communication between plant and AMF can be monitored from the transcription of host genes involved in detecting the AMF-produced signal molecules, mycorrhizal-lipochitinoligosaccharides (Myc-LCOs), or producing the plant signal molecules, strigolactones (Akiyama et al., 2005; Besserer et al., 2006; Olah et al., 2005). Resource exchange between AMF and plants can similarly be detected from the transcription of host genes involved in the exchange of host-produced sugars and lipids for minerals and water provided by the fungus (Jiang et al., 2017; Luginbuehl et al., 2017; Rausch et al., 2001). Results by Kiers et al. (2011) from inoculation experiments demonstrated that outcomes of mycorrhizal symbiosis are affected by manipulation of host-produced or AMF-transported resources (Table S3). However, the "culturable" AMF used in these artificial inoculation experiments are mostly ruderal (Ohsowski et al., 2014), and leave open the question of whether efficient partner selection exists in nature (van der Heijden & Walder, 2016; Kiers et al., 2016; Walder & van der Heijden, 2015).

The system that we use to address hypotheses about the occurrence of stress-tolerant AMF (H_1) and host selection of AMF

partners through signalling (H_2) and potential resource exchange (H_3) consists of an open, agricultural field with two cultivars of one host species, *Sorghum bicolor* (L.) Moench, and three irrigation regimes, continual irrigation, preflowering drought and postflowering drought, with three replicate plots (Xu et al., 2018). Our discovery that the effect of host genotype is negligible allowed us to use six replicate plots in most of our analyses (Gao et al., 2020; Xu et al., 2018). Samples of soil, rhizosphere and root were taken weekly over the 17 weeks from seedling emergence to grain maturation. DNA was isolated to characterize fungal, species-level, OTUs by ITS2 sequence and to estimate fungal biomass by quantitative polymerase chain reaction (qPCR) of 18S rDNA (Gao et al., 2020). RNA was isolated to characterize the plant transcriptome as a whole (Varoquaux et al., 2019). The analyses presented here integrate data published on both the AMF mycobiome and the sorghum transcriptome (Gao et al., 2019, 2020; Varoquaux et al., 2019). By combining these two data sets and focusing on specific plant genes involved in the AMF symbiosis, we test our hypotheses 2 and 3. We focused on plant genes involved in the exchange of resources and signal communications during mycorrhizal symbiosis, to determine if their expression is related to AMF succession.

2 | MATERIALS AND METHODS

Both fungal mycobiome and sorghum transcriptome data were published earlier in separate papers (Gao et al., 2019, 2020; Varoquaux et al., 2019). The results reported here provide new analyses that integrate the two different data sets, one about AMF succession and the other about expression of host plant genes known to be important for arbuscular mycorrhizal symbiosis. The experimental design, sampling, and DNA and RNA analyses described here are summarized from our previous publications on research conducted at the same study site (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018).

2.1 | Experiment design and sampling

Our experiment is a random block design using three replicate plots (16 × 8 m) for each of three treatments (control, preflowering drought and postflowering drought) and two sorghum (*Sorghum bicolor* (L.) Moench) cultivars (the preflowering, drought-tolerant sorghum cultivar RTx430, and the postflowering, drought-tolerant [or "stay green"] cultivar BTx642) (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018). During the course of our experiment (May 27 to September 28, 2016), no precipitation occurred; the daily minimum temperature ranged from 7.8 to 22.8°C, and the daily maximum temperature ranged from 22.8 to 40.5°C (Gao et al., 2019). The trial was planted on May 27, 2016 and plant emergence was recorded on June 1, 2016 (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018). From the 3rd week until the 17th week of growth, the plots were either: (i) regularly watered in the control treatment, or (ii) were not watered until the ninth week in the preflowering drought treatment at which time regular watering was initiated, or (iii) were regularly watered until the 10th week in the postflowering drought treatment at which time watering ceased (Gao et al., 2020). Weekly samples of leaf, root, rhizosphere and soil were taken in 2016 for control plots on June 8, 15, 22, 29; July 6, 13, 20, 27; August 3, 10, 17, 24, 31; and September 7, 14, 21, 28 (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018). To avoid redundancy of control conditions, preflowering treatment sampling began on June 22 (TP03) and postflowering sampling began on July 27 (TP08) (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018). In each plot on every sampling date, at least 10 individual sorghum plants were collected and pooled to generate one combined sample each of leaf, root and rhizosphere, and 10 soil cores were collected and pooled to generate one combined soil sample, as described in our previous publications (Gao et al., 2019, 2020; Varoquaux et al., 2019; Xu et al., 2018).

2.2 | DNA-based analysis

Detailed description of DNA extraction, fungal 18S qPCR, fungal ITS2 amplification and MiSeq sequencing can be found in our publication of the sorghum mycobiome (Gao et al., 2020). DNA was extracted from 0.2 g of root, rhizosphere or soil samples using the MoBio PowerSoil DNA kit (MoBio) (Gao et al., 2020). Fungal biomass was estimated by qPCR of the fungal small subunit rRNA (SSU or 18S) using the FF2 and FR1 primers (Gao et al., 2020; Zhou et al., 2000). Standard curves were developed using a series of 10-fold dilutions of plasmids containing an inserted fragment of the 18S gene of *Penicillium purpurogenum* (Adams et al., 2013; Gao et al., 2020). We recognize that no current method of estimating fungal biomass in field conditions is ideal (Baumgartner et al., 2010; Song et al., 2014; Tellenbach et al., 2010). All methods of estimating biomass have their drawbacks; for example, the use of the fungal cell wall polymer, chitin, is confounded by chitin found in dead hyphae and insects, the use of the fungal membrane lipid, ergosterol is confounded by varying ratios of lipid to biomass and the use of DNA by qPCR can be confounded by variation in target copy number (Song et al., 2014). Of the three methods, the one that we chose, qPCR, is increasing in popularity because the same DNA used to identify fungal OTUs can be used for quantification and because qPCR is economical in terms of supplies and labour (Adams et al., 2013; Gao et al., 2020; Varoquaux et al., 2019). Fungal ITS2 was PCR-amplified from DNAs diluted to 5 ng μl^{-1} with ddH_2O, using dual-barcoded 5.8SFun and ITS4Fun primers (Gao et al., 2019, 2020; Taylor et al., 2016). The yields of PCR products were quantified using a Qubit dsDNA HS kit (Life Technologies) and 200 ng of DNA from each sample was randomly assigned to four different pools, purified using AMPure magnetic beads (Beckman Coulter), checked for concentration and amplicon

size using an Agilent 2100 Bioanalyzer (Agilent Technologies), and sequenced (four runs) on the Illumina MiSeq PE300 sequencing platform (Illumina) at the Vincent J. Coates Genomics Sequencing Laboratory (GSL, University of California, Berkeley, CA, USA) (Gao et al., 2020). Raw fastq sequences were subjected to quality evaluation using FASTQC version 0.11.5 (Andrews, 2010), removal of primers using CUTADAPTER version 1.9.1 (Martin, 2011), merging of forward and reverse reads, control of quality, clustering of OTUs, global search using USEARCH version 8.0 (Edgar, 2013), and BLASTN search for identification of OTUs, to generate a table of 776 samples ×65 AMF OTUs (Gao et al., 2020).

2.3 | RNA-based analysis

Detailed description of RNA extraction and sequencing can be found in our publication of the sorghum transcriptome (Varoquaux et al., 2019). RNA was extracted from root samples using the Qiagen miRNeasy Mini Kit (Cat. no. AM217004) with modifications (Varoquaux et al., 2019). DNA contamination was removed using the TURBO DNA-free kit (Cat. no. AM1907; Invitrogen) (Varoquaux et al., 2019). Stranded cDNA libraries were generated using the Illumina Truseq Stranded RNA LT kit (Varoquaux et al., 2019). The fragmented cDNA was treated with end-pair, A-tailing, adapter ligation and eight cycles of PCR (Varoquaux et al., 2019). qPCR was used to determine the concentration of the libraries. Libraries were sequenced on the Illumina HiSeq (Varoquaux et al., 2019). Raw fastq reads were filtered and trimmed using the JGI QC pipeline (Varoquaux et al., 2019). Filtered reads from each library were aligned to the reference genome (phytozome version 3.1, supplemented with RTx430 and BTx642 single nucleotide polymorphism [SNP] information) using HISAT version 2.1.0 (Kim et al., 2015). FEATURECOUNTS (Liao et al., 2014) was used to generate the raw gene counts (counts.txt) file using gff3 annotations.

2.4 | Statistical methods

To visualize the relative abundances of AMF OTUs, bar plots were constructed using the GGPLOT2 package (Wickham, 2009) in R version 3.5.1 (R Development Core Team, 2018). Bray–Curtis dissimilarities were calculated to construct distance matrices of the fungal community (Hellinger-transformed) using the vegdist command in the vegan package (Oksanen et al., 2013), which were subjected to principal coordinate analysis using the pcoa command in the ape package (Paradis et al., 2004) in R. The most important age-discriminant AMF OTUs (those OTUs that most effectively predict community age) were identified from a random subset of 50% control samples using the random forest (RF) model in the RANDOMFOREST package (Liaw & Wiener, 2002) in R. The sparse RF model of these age-discriminant fungal OTUs was used to predict the ages of drought samples and another subset of 50% control samples; and the discrepancies between predicted ages of control and drought samples were used to assess the extent of the effect of drought on AMF succession. Permutational analysis of variance (PERMANOVA) was carried out to assess the effect of compartment (soil, rhizosphere or root), sample time (weeks 1–17), predicted time, cultivar (BTx642, RTx430) and drought treatment (control, preflowering drought or postflowering drought) on the nestedness (members in the control community, due to the loss of drought-tolerant species, is a subset of that of drought community) and turnover (some members in the control community are replaced by drought-tolerant species in the drought community, such that the drought community is not simply a subset of the control community) components of AMF community variation using the vegan package in R. Abundances of initially ruderal AMF and later competitive AMF were visualized by locally weighted scatterplot smoothing (LOWESS) fitting curves in the GGPLOT2 package in R.

From the sorghum transcriptome data set, we focused on the subset of sorghum genes known to be involved in signalling and nutrient transfer between AMF and their hosts (Akiyama et al., 2005; Besserer et al., 2006; Jiang et al., 2017; Luginbuehl et al., 2017; Olah et al., 2005; Rausch et al., 2001). Pearson correlations of AMF and sorghum genes coding importers of soil nutrient and water were visualized by a heatmap in the PHEATMAP package (Kolde, 2012) in R. The *p* values were adjusted using the Bonferroni method (Bonferroni, 1935). For each of the targeted sorghum genes, the correlations were carried out for relative abundance (RA, log-transformed) of each of the OTUs in the either ruderal (two OTUs) or competitive AMF (13 OTUs), and thus *R* values represent a range of two or 13 values. Transcription of sorghum genes coding for the protein processing signal of Myc-LCOs (signalling molecules produced by AMF), and coding for strigolactones (signalling molecules produced by sorghum) were visualized by LOWESS fitting curves in the GGPLOT2 package in R, and were placed in the Myc-LCOs signal molecules pathway as documented in Recorbet et al. (2013), and the strigolactone synthesis pathway as documented in Seto and Yamaguchi (2014). Transcriptions of sorghum genes coding for the synthesis and export of 2-MAG (C16:0 2-monoacylglycerol) lipid were visualized by LOWESS fitting curves, and placed in the lipid pathway as documented in Wang et al. (2017).

3 | RESULTS AND DISCUSSION

3.1 | Testing H_1: Competitive AMF species will be replaced by drought-stress-tolerant AMF species when irrigation is withheld to induce pre- or postflowering drought

Having documented ruderal and competitive OTUs during AMF succession in an irrigated system (Gao et al., 2019), we expected to see the appearance of drought-stress-tolerant species when we imposed stress in the form of preflowering drought (which we then alleviated at the time of flowering), or imposed stress

in the form of postflowering drought, having provided water for the first half of the sorghum growth cycle (Gao et al., 2020). We imagined three possible sources of drought-tolerant AMF. First, drought-tolerant AMF could simply be present in the soil species pool and be recruited to the root in drought. Second, drought-tolerant AMF could be absent from our plots but disperse to our site from nearby sites to be recruited by roots. Third, AMF could evolve drought tolerance, although an unlikely event over one growing season. We found previously that drought drastically decreased total AMF abundance (Varoquaux et al., 2019), but to

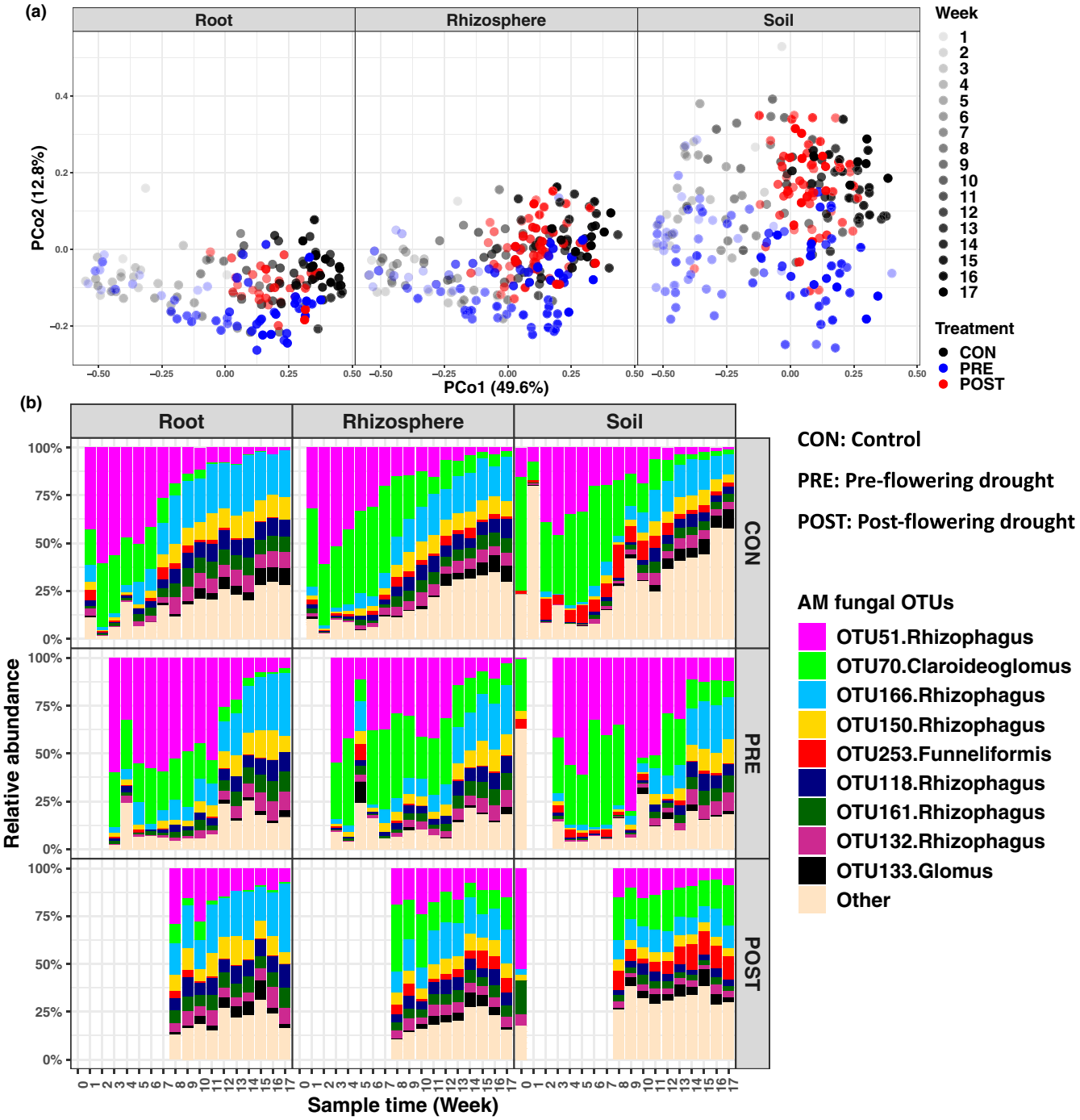
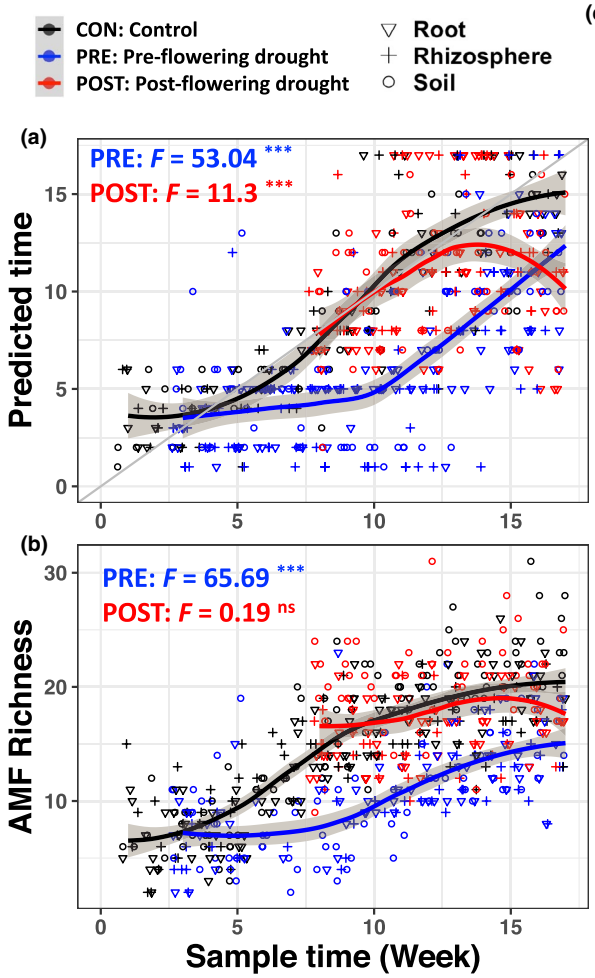


FIGURE 1 Structure of AMF community by time point, compartment, treatment, and cultivar. (a) Principal coordinate (PCo) analysis of AMF community Bray–Curtis dissimilarity with permutational analysis of variance (PERMANOVA) showing significant association of AMF community composition with, in order of importance, time point ($R^2 = 0.346$, $p < 0.001$), compartment ($R^2 = 0.075$, $p < 0.001$), drought treatment ($R^2 = 0.061$, $p < 0.001$) and sorghum cultivar ($R^2 = 0.003$, $p = 0.008$). (b) Temporal change in relative abundance of AMF operational taxonomic units (OTUs) at each time point in the three compartments, and three treatments. To avoid redundancy of control conditions (CON), preflowering (PRE) treatment sampling began at the 3rd week and postflowering (POST) sampling began at the 8th week. A similar barplot of control samples was reported by Gao et al. (2019)

identify drought-tolerant species, we would need to find species that increased in relative abundance with drought. Although in our study none of the AMF species showed an obvious increase in their relative abundance with drought (Figure 1), we did find, by PERMANOVA of all root, rhizosphere and soil samples, that drought does have a small but significant effect on AMF community composition ($R^2 = 0.061$, $p < 0.001$). However, the effect is far weaker than that of sampling time (17 weeks, $R^2 = 0.346$, $p < 0.001$) and similar to that of compartment (soil, rhizosphere or root) ($R^2 = 0.075$, $p < 0.001$) (Figure 1a). This small but significant drought effect on AMF community composition does not necessarily stem from recruitment of drought-tolerant species, because the effect also could be attributed to (i) a delay in the plant-driven succession of the AMF community in root, rhizosphere and soil, or to (ii) the promotion of AMF sporulation in drought-stressed compartments of rhizosphere and soil, which would increase the DNA of sporulating species in these compartments but not in root. To investigate the first of these two alternatives to selection of drought-tolerant AMF, we turned to RF analysis to detect and estimate any delay imposed by drought on succession of community composition (Figure 2a). To investigate the second alternative, we analysed components of community composition, separately, in each of the three compartments, root, rhizosphere and soil. For the first alternative, our RF results showed that both pre- and



(c) **Effect of drought treatment on nestedness and turnover components of arbuscular mycorrhizal fungal communities, as determined by permutational analysis of variance controlling sample time and predicted time in each compartment and each drought treatment scenario**

Compartment	Variable	Nestedness *F*	Turnover *F*
Root	Predicted time	13.353***	53.253***
	Sample time	1.770	3.363*
	PRE	3.841	2.369
	Predicted time	<0	4.131**
	Sample time	1.762	0.558
	POST	4.915	1.375
Rhizosphere	Predicted time	125.022***	0.991
	Sample time	5.939*	2.031
	PRE	12.31**	1.876
	Predicted time	38.52***	3.810**
	Sample time	1.808	1.374
	POST	0.000	4.682***
Soil	Predicted time	110.287***	6.066***
	Sample time	<0	8.393***
	PRE	12.448***	4.192**
	Predicted time	11.146**	4.929***
	Sample time	9.1506**	1.734
	POST	2.894	2.291*

PRE: pre-flowering drought; POST: post-flowering drought;
*** P<0.001; ** P <0.01; *P<0.05; ns: not significant

FIGURE 2 Effect of drought on successional AMF community. (a) Delay in the succession of AMF community composition due to pre- and postflowering drought shown by comparing predicted AMF community composition for all compartments (based on Random Forest modelling of well-watered sorghum) with observed AMF community composition for well-watered and droughted sorghum. Note that preflowering drought delays succession until irrigation is initiated at week 9, at which time succession begins to parallel that seen for well-watered sorghum. Postflowering drought delays and then disrupts succession. (b) Preflowering drought prevented AMF richness from increasing for all compartments until irrigation was initiated. With postflowering drought, richness appears to decrease following withholding water, but the decrease is not significant. (c) Succession of AMF community composition in all compartments involves both nestedness (members in the control community, due to the loss of drought-tolerant species, are a subset of that of the drought community) and turnover (some members in the control community are replaced by drought-tolerant species in the drought community, such that the drought community is not simply a subset of the control community). Drought does not affect the nestedness and turnover components of AMF community succession in roots, but it does cause effects in rhizosphere and soil. In this figure and throughout this paper, the shadows represent the 95% confidence interval for the fitting of LOWESS curves. F represents the results of Fisher's test

postflowering drought does delay the development of the AMF community (Figure 2a), and that AMF richness is significantly decreased by preflowering drought but not by postflowering drought (Figure 2b). Thus, the weak effect of drought on total AMF community composition can be explained by a delay in its development. The second alternative involves changes in particular species, which can be evaluated in terms of two components of community composition (Baselga, 2010), nestedness (a situation in which species in the control community, due to the loss of drought-tolerant species, would be a subset of those in the drought community) and turnover (where some members in the control community would be replaced by drought-tolerant species in the drought community, such that the drought community would not simply be a subset of the control community). To evaluate these scenarios, we carried out PERMANOVA using the nestedness and turnover (Gao et al., 2019) components of AMF community composition as response variables, and sample time, predicted time and drought treatment (pre- or postflowering) as independent variables. The within-compartment analysis showed that, in roots, neither pre- nor postflowering drought exerted a significant influence on nestedness or turnover (Figure 2c), implying neither recruitment nor loss of drought-stress-tolerant AMF in roots. In contrast to roots, in rhizosphere and soil a significant drought effect on nestedness or turnover was observed that could be explained by sporulation (Figure 2c). Therefore, although we found changes in AMF richness and community composition, we can reject H_1 because we found no evidence of the recruitment to roots of drought-stress-tolerant AMF from comparison with well-irrigated, control plants, making it unnecessary to speculate, as we did above, about possible sources for drought-resistant AMF in roots.

It could be argued that we saw no recruitment of drought-tolerant AMF because, in the sorghum system, all of the AMF were already drought-adapted. After all, our study site was chosen for its absence of precipitation throughout the growing season to avoid summer rain that could ruin field experiments (Gao et al., 2020). However, as noted above, our study site has been irrigated with ground water over six decades, time enough to lose drought-adapted AMF, as seen by the drastic decrease in AMF abundance when we imposed drought (Varoquaux et al., 2019). Our inability to find drought-adapted AMF in sorghum roots at our study site does not mean that none exist. They may exist in unirrigated soils near our fields and have been reported in other studies from, for example, semi-arid grasslands (mean annual precipitation [MAP] 300–800 mm) where several studies have reported significant

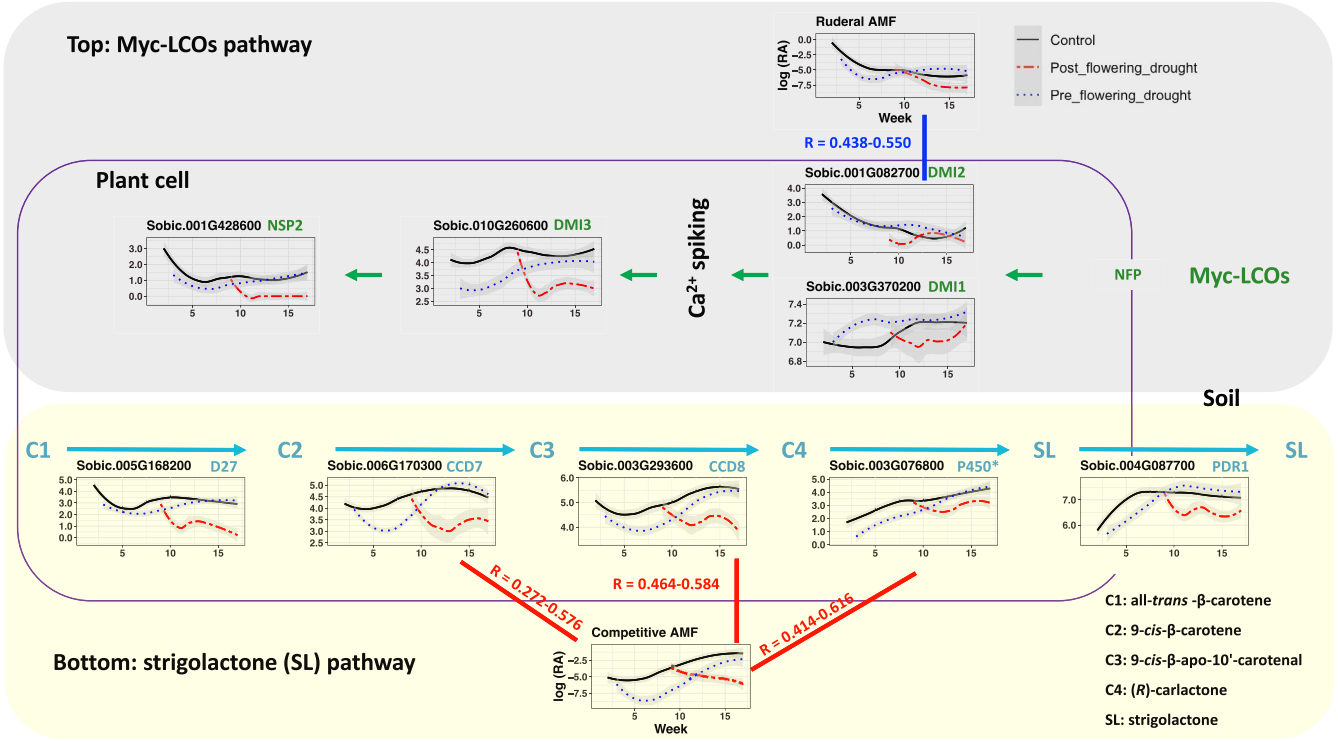


FIGURE 3 Ruderal and competitive AMF groups and signal communication. Top, green pathway: perception of Myc-LCO signal molecules. Transcription over a season of sorghum genes (*DMI1*, *DMI2*, *DMI3* and *NSP2*) known (Recorbet et al., 2013) to perceive Myc-LCOs signal molecules produced by AM fungi. Blue line: ruderal AMF abundance is correlated with the transcription of *DMI2*. Bottom, yellow pathway: synthesis of strigolactones. Transcription of sorghum genes (*D27*, *CCD7*, *CCD8*, *P450* and *PDR1*) known (Seto & Yamaguchi, 2014) to synthesize the plant signalling molecule strigolactone (SL). Red lines: competitive AMF abundance is correlated with the transcription of *CCD7*, *CCD8* and *P450*. *Sobic.003G076800 is a representative of eight Cytochrome *P450* genes that are significantly correlated with competitive AMF, as detailed in Figure S1. The relative abundances (RA, log-transformed) of AMF represent the summed RA of two ruderal AMF, or summed RA of 13 competitive AMFs. For each of the targeted sorghum genes, the correlations were carried out for each of the OTUs in the either ruderal (two OTUs) or competitive AMFs (13 OTUs), and thus *R* values represent a range of two or 13 values

effects of drought on AMF community composition (Chen et al., 2017; Deveautour et al., 2018, 2019; Gao et al., 2016; Li et al., 2015) (Table S2). Where water is abundant and reliable, as in wet subtropical forests (MAP 1440–1750 mm), studies of drought have, like our sorghum system, failed to find a significant effect of drought on AMF communities (Cao et al., 2020; Maitra et al., 2019) (Table S2).

3.1.1 | Testing H_2: The transition from ruderal to competitive AMF will correlate with shifts in transcription of host genes responsible for producing and receiving signalling molecules

A key aspect of the arbuscular mycorrhizal symbiosis is the chemical communication between the symbionts via host-derived strigolactones and AMF-derived Myc-LCOs (Akiyama et al., 2005; Besserer et al., 2006; Olah et al., 2005). Host-derived strigolactones are recognized by AMF, leading to AMF hyphal branching and increased metabolism (Gough & Bécard, 2016). Strigolactone is produced from carotene within plant cells by the action of enzymes that include carotenoid isomerase encoded by *DWARF 27* (*D27*), two carotenoid cleavage dioxygenases encoded by *CCD7* and *CCD8*, and a cytochrome *P450*. Strigolactones are then exported through the action of proteins encoded by *pleiotrophic drug resistance 1* (*PDR1*) (Gough & Bécard, 2016). Conversely, Myc-LCOs are produced by AMF and these signalling molecules are then recognized by host proteins encoded by *nod factor perception* (*NFP*) and processed via proteins encoded by *does-not-make-infections* (*DMI1*/*DMI2*, *DMI3*) and nodulation-specific transcription factor (*NSP2*) (Gough & Bécard, 2016).

To address H_2, we compared transcription of these plant signalling-genes with abundance of AMF species. We found that the abundance of ruderal species positively correlated with expression of a plant gene that processes fungally produced Myc-LCOs (*DMI2*) (Figure 3). We also found that the abundance of competitive AMF positively correlated with expression of plant genes involved in producing strigolactones (*D27*, *CCD7*, *CCD8*, *P450* and *PDR1*) (Figure 3). Therefore, we are unable to reject H_2 that the transition from ruderal to competitive AMF will correlate with shifts in transcription of host genes responsible for producing and receiving signalling molecules. Our results are consistent with a scenario where sorghum seedlings produce enough strigolactone to stimulate the germination of dormant ruderal AMF spores but not dormant competitive AMF spores. As the host develops, increased export of strigolactones promotes the germination of competitive AMF species, and the abundance of ruderal AMF species decreases along with the decrease in transcription of host genes involved in recognizing Myc-LCOs signals. The abundance of competitive AMF is also positively correlated with transcription of a sorghum phosphate transporter (Sobic.001G234800, Figure 4). Consistent with our finding, previous work has found that the strigolactone synthesis pathway is stimulated to recruit AMF by phosphorus starvation, and suppressed by an increase in phosphorus supply (Recorbet et al., 2013).

3.1.2 | Testing H_3: The transition in abundance of AMF from ruderal to competitive species will correlate with shifts in transcription of host genes responsible for resource transfer between fungi and host

Mycorrhizal symbiosis involves resource exchange of host-produced sugars and lipids for soil-derived and AMF-transported minerals and water. Plants generate and transfer to AMF the lipid, $C_{16:0}$ 2-monoacylglycerol (2-MAG), via the activities of acyl-ACP thioesterases (FaTM), glycerol-3-phosphate acyltransferase (*reduced arbuscular mycorrhiza 2* gene, *RAM2*) and ABCG transporters (stunted arbuscle, STR/STR2) (Wang et al., 2017). Plants also export sugar to AMF via sugars-will-eventually-be-exported-transporter (SWEET), sucrose transporters (SUT) and monosaccharide transporters (MST), and plants import soil-derived resources via transporters for phosphate (PT), ammonium (AMT), potassium (KT), sulphate (ST), etc. (Wang et al., 2017).

To address H_3, we retrieved transcripts of these genes from the sorghum transcriptome (Varoquaux et al., 2019), and correlated them with AMF abundance (Gao et al., 2019). Our results showed that the shift from ruderal to competitive AMF involves shifts in transcription of genes involved with both plant-derived resources and soil-derived, AMF-transported resources. For plant-derived resources, ruderal AMF OTU abundance correlates with transcription of *FaTM* in the lipid synthesis pathway and two *SWEET* genes coding for sucrose exporter proteins. For competitive AMF, their OTU abundance is positively correlated with the transcription of a *SWEET* gene coding for a different sucrose exporter protein (Figure 4). For AMF-provided resources, the abundance of ruderal AMF OTUs correlated positively with expression from a higher number and proportion of aquaporin genes, whereas the abundance of competitive AMF OTUs correlated positively with expression from a higher number and proportion of transporters of soil phosphorus, nitrogen, potassium, sulphur and metals (Figure 4). From these results, we infer that ruderal AMF are less beneficial than competitive AMF in terms of providing the host plant with soil phosphorus, nitrogen, potassium, sulphur and metals, and the developing host plant selects against ruderal AMF by limiting the provision of lipid and sucrose. Using plants of the same age would be necessary for testing whether ruderal taxa are less beneficial compared to competitive taxa. However, the ruderal and competitive AMFs are differentially distributed over the growth season of plant, and thus the maturing sorghum plants would not have ruderal AMF. Certainly, the different benefits of ruderal and competitive AMFs could be tested by manipulating inoculation in the laboratory or glasshouse, but those studies are beyond the scope of the research project presented here. Therefore, we are unable to falsify H_3, that the transition in abundance of AMF from ruderal to competitive species correlates with shifts in transcription of host genes responsible for resource transfer between fungi and host.

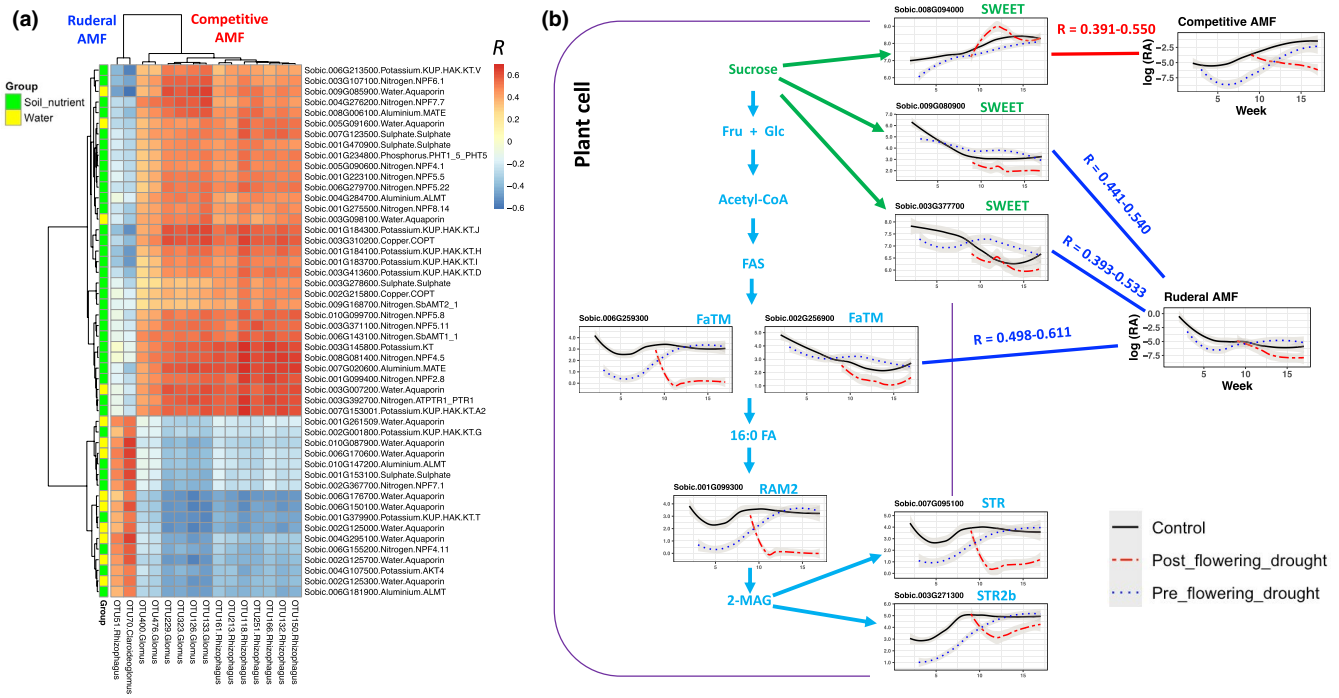


FIGURE 4 Ruderal–competitive transition involves resource exchange. (a) Different benefits of ruderal and competitive AMF to host plants. Heatmap showing co-occurrence (positive correlations in red hues) and co-avoidance (negative correlations in blue hues) between AMF OTUs and transcription of sorghum genes. Correlation analysis showed that the 13 competitive AMF are coupled with a higher proportion of transporters (green squares) of soil nitrogen (N), phosphorus (P), potassium (K), sulphur (S) and metal in contrast to the two ruderal AMF, which are coupled with a higher proportion of aquaporins (yellow squares). (b) Successional AMF groups and host-derived resources of sucrose and lipid. Ruderal AMF abundance is coupled with the transcription of FaTM in the lipid synthesis pathway and two SWEET genes coding for sucrose exporter proteins. Competitive AMF abundance is correlated with the transcription of a SWEET gene coding for a different sucrose exporter protein. The relative abundances (RA, log-transformed) of AMF represent the summed RA of two ruderal AMF, or summed RA of 13 competitive AMFs. The pathways are referenced from Wang et al. (2017). STR, stunted arbuscle; RAM, reduced arbuscular mycorrhiza; SWEET, sugars-will eventually-be-exported-transporter; 2-MAG, $C_{16:0}$ 2-monoacylglycerol; FAS, fatty acid synthase system; FaTM, fat required for arbuscular mycorrhizal symbiosis

4 | CONCLUSIONS

Our introduction of severe drought stress to the AMF–sorghum symbiosis in agricultural fields revealed that the community composition of AMF remains stable to change by stress (Figure 1), although the abundance of AMF declines in drought (Varoquaux et al., 2019). Together, these results indicate the absence of drought-tolerant AMFs in our agricultural system. Knowledge of the levels of transcription of plant genes for the entire sorghum genome allowed us to correlate the expression of plant genes with AMF abundance, particularly during the transition from early, ruderal AMF species to later, competitive AMF species. We found that abundances of ruderal and competitive AMF species were differentially correlated with transcription of sorghum genes coding for the processing of Myc-LCOs (signalling molecules produced by AMF), and coding for strigolactones (signalling molecules produced by sorghum). We also found differences in abundance between ruderal and competitive AMF in correlations with plant genes involved in providing sugar and lipid to the fungus. More broadly, plant genes involved with water transport (i.e., aquaporins) are more strongly associated with the early, ruderal AMF, while those involved in mineral nutrient uptake, principally phosphorus, are more strongly associated with the later, competitive AMF. The novel insights from the correlations between the type of AMF and plant gene expression allow us to infer adaptive strategies employed by AMF and their host plants. The replacement of ruderal AMF with competitive AMF correlates with: (i) a shift from aquaporins to mineral transporters, indicative of a shift in plant host needs from water to phosphorous; (ii) a shift from fatty acid transporters and one set of sugar transporters to a second set of sugar transporters, indicative of a shift in nutritional needs from those specific to ruderal to those specific to competitive AMF; and (iii) a shift in signalling from Myco-LCOs to strigolactones, suggesting that the heterotrophic AMF, wholly dependent on host plant resources, initiate communication, but that the needs of the growing, autotrophic host plant come to dominate signalling.

We hope that our research will stimulate studies testing the specifics of our hypotheses through the use of host plants that have been genetically modified to disrupt signalling or nutrient transfer. We also hope to test the generality of our hypotheses through the exploration of additional plant genes as well as fungal genes that underlie the adaptive strategies that accompany microbial succession in nature. We look forward to studies aimed at discovering

stress-tolerant AMF in environments where irrigation is not practised. Although our results pertain to basic aspects of biology, they could also be applied to improving commercial sorghum cultivation. Treating sorghum seed with ruderal species of AMF could, for example, improve water availability in agricultural areas where large-scale irrigation has either not been developed or is being abandoned due to ground water depletion and global change. Similarly, application of competitive AMF might accelerate the development of sorghum plants, which could then result in fewer inputs or improved yield or both.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

C.G. and J.W.T. conceived of and wrote the manuscript for the fungal portion of a larger project conceived by P.G.L., D.C.D., E.P., J.V., J.A.D. and J.W.T. with P.G.L. as director. P.G.L., R.B.H. and J.A.D. coordinated the field work and sample storage. C.G., L.M. and L.X. performed the molecular analysis. N.V. and V.S. performed the sorghum transcriptome data analysis. P.C. provided the list of sorghum genes involved in resource and signal communications in mycorrhizal symbiosis. All authors contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT

No new data were generated in this study. All data have been deposited in NCBI (i) GenBank (representative AM fungal read set) with accession codes MG008508–MG008559, (ii) Sequence Read Archive (fungal raw data) with accession codes Bioproject PRJNA412410 and PRJNA494573, and (ii) Gene Expression Omnibus database (GEO) with accession code GSE128441.

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