INTRODUCTION

Fungi colonized land before plants, and plant-fungi symbiosis was a key step in the evolution of land plants (Delaux et al., 2015; Lutzoni et al., 2018; Wang et al., 2010). Thus, early lineages of terrestrial plants already had a molecular toolkit for fungal symbiosis, and over time these associations diversified to include a wide range of fungal and bacterial species.

Plant-rhizobia symbiosis is the best-studied example, as legume crops are important for human nutrition (reviewed in Dilworth, James, Spret, & Newton, 2008). Rhizobial host plants have evolved signaling pathways that allow the bacteria to enter and colonize the root without triggering a sustained defense response (Kouchi et al., 2004; Lohar et al., 2006; Maunoury et al., 2010). Once inside target root cells, rhizobia stimulate the development of specialized plant organs called nodules that facilitate the exchange of rhizobial nitrogen for plant host-derived carbon compounds.

Although less widely studied than rhizobial symbiosis, symbioses between plants and fungi are older, more diverse, and much more widespread (Wang et al., 2010; Delaux et al., 2015; Lutzoni et al., 2018; reviewed in Smith & Read, 2008). There are thousands of species of mycorrhizal fungi, and in their various forms they play an important yet still incompletely understood role in plant nutrition. Typically, the fungal hyphae form a continuum from the soil into the outer layers of the root, effectively expanding the soil volume explored by the root system and accumulating critical elements like phosphorus for use by the plant.

In this paper, we will focus on two categories of mycorrhizal associations: arbuscular mycorrhiza and ectomycorrhiza. The association between arbuscular mycorrhiza (AM) and plants is ubiquitous, with 80% of terrestrial plants competent to form these symbioses (reviewed in Wang & Qiu, 2006; Smith & Read, 2008). Morphologically, AM are distinguishable from other mycorrhizal fungi by the presence of densely coiled or ramified hyphae located inside living cells of the root cortex.
AM are named after these ramified hyphae, which are called "arbuscles." Ectomycorrhizal (ECM) symbioses are more recently evolved and much less common among plant species (3%), but they are especially important for forest trees (Smith & Read, 2008). The hyphae of ECM fungi form a network that densely occupies the spaces between the cells of the outer plant root, but they do not penetrate living host cells.

An additional category of plants comprises those species that have lost their competence for mycorrhizal symbiosis (reviewed in Wang & Qiu, 2006; Brundrett, 2017). Nonmycorrhizal species (NM) have evolved multiple times from AM species. They are generally short-lived, non-rhizobial, and nonwoody. Notably, NM species include the model species Arabidopsis thaliana and other members of the Brassicaceae.

In this paper, we examine the possible connection between plant symbiotic competence and the innate immune repertoire.

Plants lack mobile defensive cells, so their resistance to microbial pathogens is mediated by an innate immune response (reviewed in Chisholm, Coaker, Day, & Staskawicz, 2006; Jones & Dangl, 2006; Bent & Mackey, 2007; Cook, Mesarich, & Thomma, 2015; Haque, Spaepen, Garrido-Oter, & Schulze-Lefert, 2017). Pathogen perception can take place outside the cell or in the cytoplasm. Outside the cell, transmembrane receptor kinases are sensitive to pathogen-associated or damage-associated molecular patterns (Brutus, Sicilia, Macone, Cervone, & De Lorenzo, 2010; Gomez-Gomez & Boller, 2003). There is also some evidence that the NBS family has a role in symbiosis. NBS genes have been shown to regulate host-rhizobia specificity in some legumes (Yang, Tang, Gao, Krishnan, & Zhu, 2010). These and other examples have prompted recent reviews (Cook et al., 2015; Haque et al., 2017) to describe the plant immune system as a "microbe management" or "surveillance" system, rather than a defense system per se.

In this paper, we initially focus on the NBS gene family. The NBS gene family is remarkable for both its overall size – often comprising several percent of the entire genome – and for the wide size variation between species. Counts of NBS range from ~40 in eelgrass (Zostera marina) to ~1,000 in apple (Malus domestica) (Olsen et al., 2016; Velasco et al., 2010). Beginning with the sequencing of the first tree genome, Populus trichocarpa (black cottonwood), several authors observed that woody perennials, especially trees, have relatively large NBS families (see Tuskan et al., 2006; Yang, Zhang, Yue, Tian, & Chen, 2008 and the opinion article Tobias & Guest, 2014). More recently, Plomion et al. (2018) compared the genomes of nine woody and seven nonwoody species and found several large clades of NBS genes that show significant expansions in woody species. They speculated that the expansion of defense-related gene families is related to the long life-span of woody perennials.

In work preliminary to this paper, we saw hints of an alternative explanation for variations in NBS family size. We noted that species with small NBS families tend to be NM. We also noticed that tree species with especially large NBS families were typically ECM. We thus set out to establish the statistical significance of this result, to find other gene families with similar correlations, and to seek possible explanations in the ecology and evolution of flowering plants.

2 | METHODS

2.1 | Genomes

We downloaded the genomes of 39 species of angiosperms, including 9 monocots (2 NM and 7 AM) and 30 eudicots (5 NM, 20 AM, and 5 ECM). Of the 39 genomes, 38 are public. Permission to use the 39th, Salix purpurea, was generously granted prior to the end of the official embargo. A complete list of sources is provided in the Supporting Information Table S1. The majority were downloaded from the Phytozome archive (https://phytozome.jgi.doe.gov; Goodstein et al., 2012).

To provide consistency across genomes, we limited consideration to one transcript per locus, called the "primary transcript" in Phytozome annotations. This was to avoid statistical bias due to large differences in the completeness of various genome annotations. For example, Arabidopsis TAIR10 averages 1.29 transcripts per locus, while many species genomes are still in version 1.0 and are annotated with exactly one transcript per locus.

2.2 | Protein domains

Pfam domains were identified using PfamScan (Punta et al., 2012) and transmembrane domains were identified using Phobius (Kall, Krogh, & Sonnhammer, 2007).
2.3 Species data

We collected the following information for each species: mycorrhizal and rhizobial symbiotic competence, annual/perennial life cycle, and woodiness (i.e., capable of secondary growth and lignification). This information was collected from a variety of sources, as detailed in the Supporting Information. We were careful to include only species whose mycorrhizal status has been confirmed by published observation. Figure 1 summarizes some properties of the 39 species used, including a taxonomy after (APGIV 2016). The woody category includes trees and vines having woody secondary growth. It also includes Lotus japonicus, which has nonhardy stems but a perennial woody taproot, and Solanum lycopersicum, which, while typically grown as an annual crop, develops a woody stem and roots when grown as a perennial (Peralta, Spooner, & Knapp, 2008).

2.4 Family expansion

To test whether a gene family expands between two sets of species, we calculated a p-value using the exact, one-sided Wilcoxon-Man-Whitney rank sum test, implemented using the COIN package in R (COIN version 1.2-2, Hothorn, Hornik, van de Wiel, & Zeileis, 2006;
Note that all seven NM species in our list are nonwoody while the nonwoody AM-related gene lists

To assess whether a gene family is over-represented in lists related to AM competence, we used data from the following papers.

Delaux et al. (2014) compared genomic and transcriptomic data from ~40 plant species to identify a list of 174 Medicago truncatula genes conserved in AM-competent species but lost in NM species. Bravo, York, Pumplin, Mueller, and Harrison (2016) conducted a similar phylogenomic analysis to identify a list of 138 genes. Sugimura and Saito (2017) used RNA-seq to compare the root transcriptomes of 4-week-old S. lycopersicum plants grown with or without an inoculation of spores from the AM fungal species *Rhizophagus irregularis*. They found 744 genes up-regulated in the AM-treated plants. Recchia, Konzen, Cassieri, Caldas, and Tsai (2018) used RNA-seq to compare root transcriptomes of *Phaseolus vulgaris* plants grown with or without AM fungal inoculum. They found 714 genes up-regulated in the plants grown with AM. Vangelisti et al. (2018) used RNA-seq to determine the transcriptomes of *Helianthus annuus* seedling roots 16 days after inoculation with the AM fungal species *Rhizoglomus irregularis*. Compared to controls without the inoculation, they found 694 genes up-regulated in AM plants.

Over-representation was assessed using a hypergeometric test to find the p-value (Excel v.16, Microsoft), then further restricted to FDR < 5% (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | NBS family size depends on mycorrhizal competence

We determined the size of the NBS gene family in 39 species of angiosperms for which mycorrhizal status was also available (Figure 1, Supporting Information Tables S1, S2). Figure 2 shows the distribution of NBS family size in host species with different mycorrhizal competence. The median number of NBS loci in NM, AM, and ECM species is 116, 293, and 683, respectively. Comparing these groups using nonparametric statistics, we find that NM plant species have significantly smaller NBS families than AM species (p = 3e-4) and ECM plant species have significantly larger NBS families than either NM or AM species (p = 1e-3 for both comparisons).

Note that all seven NM species in our list are nonwoody while the five ECM species are woody perennials (Figure 1). Thus, we chose to examine the relative importance of woodiness and mycorrhizal competence for NBS family size using a simultaneous linear regression (results in Supporting Information Table S3). The regression coefficient associated with woodiness was positive, but not significantly different from 0 (p = 0.10 using a t-test). The regression coefficient associated with mycorrhizal competence was about 5× larger, and was significantly different from 0 (p = 6e-4 using a t-test).

We also tested for a dependence on woodiness using the subset of 27 AM species. The AM-competent species were divided into two cohorts of 18 nonwoody species and 9 woody species, respectively (Figure 1). The distributions of NBS family sizes are compared in Figure 2. Median NBS counts of the nonwoody and woody AM-competent species are 298 and 276, respectively, and nonparametric statistics find no significant difference between the two groups.
finds no significant difference between the two sets \( (p = 0.29 \text{ using an exact, one-sided Wilcoxon-Mann-Whitney rank sum test}).

While the distributions shown in Figure 2 suggest a small NBS expansion in the rhizobia-competent species, nonparametric statistics find some MycEx genes, but they are not over-represented in any set (quaking aspen) leaves to a fungal pathogen shows a phase at 15 days after inoculation (dai) where all MycEx families except PGG-ankyrins are over-represented, and at 4 dai only the RLP are over-represented. The response takes time to develop. At 1 dai, none of the MycEx families are over-represented, and at 4 dai only the RLP are over-represented.

A search among gene lists related to arbuscular mycorrhization finds some MycEx genes, but they are not over-represented in any set we examined (Bravo et al., 2016; Delaux et al., 2014; Recchia et al., 2018; Sugimura & Saito, 2017; Vangelisti et al., 2018) (Supporting Information Table S8).

For each gene category, we used nonparametric statistics to calculate a \( p \)-value characterizing gene loss in NM vs AM species, and gene gains in AM vs ECM species. In both comparisons, we limited the false discovery rate to 5% using the technique of Benjamini and Hochberg (1995). There are 22 gene categories that show significant differences in both comparisons (category 1 in Supporting Information Table S4). We next required the category size differences to be large, with at least a 50% gain from NM to AM, and again from AM to ECM. The final list is readily curated into just six families (the three Pfam domains PF01453, PF00954, and PF08276 occur together in members of the Bulb-type lectin receptor kinase family). These include the NBS family, two families of receptor-like kinases, the glutamate receptor-like family (GLR), a large clade of ankyrins (PGG-ankyrins), and the receptor-like proteins (RLP). We call these the Mycorrhizal-Expanded (MycEx) gene families (Figure 3, Supporting Information Tables S5 and S6).

Starting with the same list of 331 gene categories, we also looked for gene family expansions in woody vs nonwoody AM species, and for families that expand in the rhizobial legumes as compared with other AM species. In both cases, no significant family expansions were found.

3.3 | Properties of the MycEx gene families

The MycEx gene families are prominent in several immune response transcriptomes. In A. thaliana, a nonmycorrhizal model species, all six families were over-represented in the response to \( P. syringae \) infection, and the majority were over-represented in response to the immune elicitors flagellin, chitin, and oligogalacturonides (Bernsdorff et al., 2016; Denoux et al., 2008; Wan et al., 2008) (Supporting Information Table S7, FDR < 5%). The transcriptional response of \( P. tremuloides \) (quaking aspen) leaves to a fungal pathogen shows a phase at 15 days after inoculation (dai) where all MycEx families except PGG-ankyrins are over-represented (Foster et al., 2015). It is notable that this response takes time to develop. At 1 dai, none of the MycEx families are over-represented, and at 4 dai only the RLP are over-represented.

A search among gene lists related to arbuscular mycorrhization finds some MycEx genes, but they are not over-represented in any set we examined (Bravo et al., 2016; Delaux et al., 2014; Recchia et al., 2018; Sugimura & Saito, 2017; Vangelisti et al., 2018) (Supporting Information Table S8).

4 | DISCUSSION

4.1 | MycEx gene families have a role in defense signaling

After demonstrating that the NBS gene family shows significant expansions in comparison of AM with NM species, and ECM with AM species, we conducted a search for other large gene families with receptor-like proteins (RLPs), and soluble kinases. The resulting list of large gene families contains 331 entries (Supporting Information Table S2).

FIGURE 2  Size distribution of the NBS gene family. Left panel: NBS family size in nonmycorrhizal (NM), arbuscular mycorrhizal (AM), and ectomycorrhizal (ECM) plant species \((n = 7, 27, \text{ and } 5, \text{ respectively})\). Center panel: NBS family size in nonwoody AM species \((-w)\) and woody AM species \((+w)\) \((n = 18 \text{ and } 9, \text{ respectively})\). Right panel: NBS family size in non-rhizobial AM species \((-L)\) and rhizobial AM species \((+L)\) \((n = 21 \text{ and } 6, \text{ respectively})\). Letters at top of panel indicate significantly different cohorts (Wilcoxon-Mann-Whitney rank sum test, \(p < 0.05\); within-panel comparisons only)
similar properties. We found five additional families, which together with NBS we call the MycEx families. The five additional families are:

4.1.1 | Bulb-type lectin receptor kinases (B-type RKs, also called G-type RKs)

This family includes the S-locus receptor kinases that play a role in self-incompatibility during fertilization (Takasaki et al., 2000), and the LORE receptor kinase that recognizes lipopolysaccharides from Gram-negative bacteria (Ranf et al., 2015). A functional role in symbiosis has yet to be demonstrated, although Favre et al. (2014) identified three B-type RKs in the conserved genetic module common to AM species.

4.1.2 | Glutamate Receptor-Like (GLR)

These are the homologs of ionotropic glutamate receptors in mammals. Plant GLRs respond to a range of amino acids, and in most cases their functional roles remain to be clarified (Forde & Roberts, 2014). Some members of this family participate in long-distance signaling and wound responses (Mousavi, Chauvin, Pascaud, Kellenberger, & Farmer, 2013), but a role in symbiotic competence has not been demonstrated. Notably, AM symbiosis can up-regulate glutamate synthase in rice (Perez-Tienda, Correa, Azcon-Aguilar, & Ferrol, 2014), which suggests the possibility that glutamate participates in a long-distance signal of mycorrhizal status to the rest of the plant.

4.1.3 | PGG-Ankyrins

PGG is a domain of unknown function common to a large clade of transmembrane ankyrins. This family includes the gene ACCELERATED CELL DEATH 6 (ACD6), which plays a role in immunity and systemic acquired resistance (Rate, Cuenca, Bowman, Guttman, & Greenberg, 1999), and INEFFECTIVE GREENISH NODULES (IGN1), which regulates symbiosis with nitrogen-fixing bacteria (Kumagai et al., 2007).

4.1.4 | Receptor-Like Proteins (RLP)

Receptor-like proteins are transmembrane proteins with an LRR domain that extends into the extracellular space, but they lack a kinase domain inside the cell. Their role in signaling is thus mediated by binding to other proteins on the cell surface. Some RLPs are known to play important roles in plant development, and others in immunity (Wang et al., 2008). Notably, many RLPs interact with SOBIR1/EVR to establish an immune response against fungal pathogens (Liebrand et al., 2013).

4.1.5 | Wall-associated receptor kinases (WAK/WAKL)

Many WAKs have an extracellular domain that binds oligogalacturonides (OGs), products of cell wall damage. Consistent with this, WAKs have a role in both cell growth and pathogen response (Kohorn & Kohorn, 2012).

We found additional evidence for a connection between the MycEx families and innate immunity from immune response transcriptomes. While we did not attempt a systematic survey, we found five transcriptomes – four in A. thaliana and one in P. tremuloides - where the majority of MycEx gene families were over-represented in lists of genes up-regulated in response to pathogen infection or immune elicitors. One notable feature of the P. tremuloides data is the MycEx gene response is detectable at 15 days after infection (dai), but not earlier (RLP being the exception, with over-representation detectable at 4 dai).

The six MycEx gene families together comprise hundreds of loci per species. The median number of MycEx genes in NM, AM, and ECM plant species is 322, 636, and 1,657, respectively. Since NBS proteins are largely cytoplasmic while the others are mostly localized at the plasma membrane, we conclude that large segments of
the plant defense repertoire expand in parallel with mycorrhizal competence.

4.2 | MycEx gene families do not expand significantly with woodiness

It is notable that the MycEx gene family has substantial overlap with the list of gene families that expand in a comparison of woody vs non-woody plant species previously published by Plomion et al. (2018) (see their Supplementary Dataset 7). Of the clades highlighted in their analysis, 5/15 are in the NBS family, 4/15 are B-type receptor kinases, 2/15 are RLPs, and one clade is in the WAK family. The only large family highlighted by their analysis but absent in ours is the family of Leucine-rich repeat receptor-like kinases (LRR-RLKs; 3/15 clades). Looking back to our own analysis, we find that LRR-RLKs do expand significantly with mycorrhizal competence (category 1 in Supporting Information Table S4), but the size of the expansions falls short of our 50% threshold for inclusion in the MycEx list (46% from NM to AM and 48% from AM to ECM). Similarly, only two of the six MycEx families – GLR and PGG-ankyrins – are absent from the Plomion et al. (2018) list.

The overlap between the MycEx family list and the Woody expansion found by Plomion et al. (2018) is not surprising. The NM species on our list are nonwoody and the ECM species are woody, so the two characteristics are not independent. We conducted a simultaneous linear regression to quantify the relative importance of woodiness and mycorrhizal competence for the observed expansions in the MycEx families. This regression finds a larger correlation with mycorrhizal competence than with woodiness for all six families. More importantly, the regression coefficients for woodiness are not statistically different from 0, while those for mycorrhizal competence are significant. As an additional check, we tested for a dependence on woodiness just among the 27 AM species on our list. None was found.

As mentioned in the Introduction, there has been repeated speculation that plant defense gene families may expand in woody perennials as an adaptation for a long life-span (see Tuskan et al., 2006; Yang et al., 2008; Plomion et al., 2018 and the opinion article Tobias & Guest, 2014). By contrast, our results suggest that the observed correlations of gene family size with woodiness may be an artifact of a stronger correlation with mycorrhizal competence. It should be emphasized, however, that our analysis relies on Pfam domain assignments (Punta et al., 2012), rather than the more highly resolved sub-families used by Plomion et al. (2018), so it is possible that their clades have a stronger dependence on woodiness than found by us.

4.3 | A hypothesis to explain MycEx family expansion

Our results suggest that the diversity of immune signaling components expands with mycorrhizal competence, but we have not yet considered possible explanations. In this section, we consider the hypothesis that this correlation is related to the species diversity of microbes in each kind of symbiotic association.

Arbuscular mycorrhiza fungi are monophyletic, and comprise ~150 species (Schüßler, Schwarzott, & Walker, 2001). Field observation and transplant experiments suggest that most AM fungi are promiscuous, able to colonize any AM-competent host plant (reviewed in Smith & Read, 2008). ECM fungal species are much more diverse than AM fungi. The ECM character has evolved independently in dozens of distinct fungal lineages, and includes thousands of species (Tedersoo & Brundrett, 2017; Tedersoo & Smith, 2013). ECM fungi typically form assemblages of 10 or more species that co-occur on the roots of a single host plant. The composition of these assemblages varies across the geographic range of the host, so that a single ECM plant species may be competent to form symbioses with hundreds, or even thousands of distinct fungal species (Trappe, 1977; van der Linde et al., 2018; Van Geel et al., 2018). In addition, the ECM plant species we consider retain their AM competence (Figure 1: Supporting Information Table S1). Thus, nonmycorrhizal, arbuscular mycorrhizal, and ectomycorrhizal plant species lie on a continuum of symbiote diversity, NM < AM < ECM.

As discussed in the Introduction, there is a growing body of evidence that the plant immune system regulates interactions with both pathogenic and nonpathogenic microbes (see, e.g. Madsen et al., 2003; Yang et al., 2010; Miyata et al., 2014; and the reviews Cook et al., 2015; Hacquard et al., 2017). Consistent with this, our results suggest that the diversity of nonpathogenic microbes is a major driver of immune repertoire diversification. A host plant competent to form symbiotic associations with a greater diversity of symbiotic fungal species will need to rely on a greater diversity of signals to distinguish pathogenic from nonpathogenic fungi, especially in those cases where the symbiotic species is closely related to a pathogenic one. Therefore, the expansion of MycEx families may be an adaptation for symbiotic promiscuity.

4.4 | Caveats

The symbiote diversity hypothesis outlined above offers a possible explanation for our results, but there are several caveats or potential objections we wish to consider.

First, genomic and transcriptomic approaches have mostly failed to find a significant relationship between the MycEx families and mycorrhizal symbiosis. Lists of genes conserved across AM plant species have relatively few MycEx genes (Delaux et al., 2014; Bravo et al., 2016; and Supporting Information Table S8). The only statistically significant over-representation we found is six RLPs in the list compiled by Delaux et al. (2014). Similarly, transcriptomic studies of genes up-regulated in response to AM colonization find some MycEx genes, but no family is over-represented (Sugimura & Saito, 2017; Recchia et al., 2018; Vangelisti et al., 2018; and Supporting Information Table S8). Transcriptomic studies do report up-regulation of defense-related genes following AM colonization, but these genes are mostly downstream of the signaling pathways represented by the MycEx families (Calabrese et al., 2017; Hohnjec,
One possible explanation for this result comes from studies of legume-rhizobia symbioses, which share many signaling pathways in common with mycorrhiza and have been more thoroughly studied (reviewed in Parniske, 2008). Transcriptomic studies of rhizobial symbiosis show an up-regulation of plant defenses in response to the initial infection, followed by a suppression of those pathways after the successful establishment of symbiosis (Kouchi et al., 2004; Lohar et al., 2006; Maunoury et al., 2010). If the role of the innate immune system during AM colonization is similar to nodule formation, then any increase in MycEx family expression may be localized and transient, and therefore difficult to detect.

A second caveat to consider is that the AM and ECM fungi do not constitute the entire plant microbiome. Flowering plants also host large communities of bacterial and fungal endophytes - species that live within the plant without causing disease symptoms (reviewed in Porras-Alfaro & Bayman, 2011; Reinhold-Hurek, Burger, Burbano, Sabale, & Hurek, 2015). The diversity of microbial endophytes is a focus of much current research, with studies finding about ~100 endophyte species per plant host (Lundberg et al., 2012). There is also a complex and poorly understood network of interactions between plant roots and the microbes that live at the root-soil interface. The fact that AM and ECM symbioses stand out against this background is somewhat surprising.

Lastly, if the observed expansion of the MycEx gene families is principally to detect signals derived from invading microbes, that implies a much higher diversity of microbe-originated signals than is currently known. One line of evidence consistent with the existence of additional signals comes from recent work sequencing the genomes of dozens of fungal species, both pathogenic and non-pathogenic (Kohler et al., 2015; Lo Presti et al., 2015). This reveals the presence of hundreds of short (<300 aa) proteins with secretion peptide signals and little similarity to known gene families. The extent to which these secreted proteins play a role in microbe-host signaling remains to be explored. Thus, while the observed correlation between mycorrhizal competence and immune repertoire size is strong, our proposed explanation in terms of symbiote species diversity remains speculative.

4.5 | Comparisons with legume-rhizobia symbiosis

We find that the NBS and other MycEx gene families do not expand significantly in the rhizobial legumes. This is consistent with our hypothesis about symbiote species diversity, since many legume species are competent to associate with fewer than ~10 species of rhizobia (reviewed in Dilworth et al., 2008). However, there are several confounding factors that need to be considered. First, since all the rhizobial species in our data set fall in a single family (Fabaceae, the legumes), we must be cautious about generalizing to rhizobia-competent species outside the legumes. Second, the genomes of rhizobia display a high degree of genetic variation, and include evidence for the horizontal transfer of genes related to the establishment and maintenance of symbiosis (Epstein et al., 2012; Sugawara et al., 2013). Thus, the genetic diversity of rhizobia may not be well-estimated by simple species counts. Third, there are important differences in the cell biology of rhizobial and mycorrhizal symbioses. Rhizobia occupy specialized root nodules that isolate them from the soil environment (reviewed in Dilworth et al., 2008; Guinel, 2009). This contrasts with fungal symbiosis, where the mycorrhizal filament network remains in direct contact with both the soil and the cells of the host plant root (reviewed in Smith & Read, 2008). Thus, the maintenance of mycorrhizal symbiosis may present a greater ongoing challenge to the plant immune system than rhizobial symbiosis.

5 | CONCLUSION

In this paper, we have shown that the plant immune repertoire expands with mycorrhizal competence, and argued that symbiote diversity is a plausible explanation for this expansion. This idea has interesting parallels with the proposal that the evolution of the adaptive immune system in vertebrates facilitated the competence for a diverse and stable gut microbiota (McFall-Ngai, 2007). The role of symbiote diversity as a driver of immune system expansion may be common to both kingdoms.

Some related work was recently published by Munch et al. (2018). They identified a clade of NBS genes with relatively few members in the Brassicaceae, and speculated that NM plants may have evolved immune signaling pathways distinct from the NBS system to compensate. We cannot rule out this explanation, but our observation that NM species have deficits in multiple defense pathways, not just NBS, makes this explanation less likely.

There are several ways to expand on the current paper that would improve confidence in our hypothesis. To better assess the significance of rhizobial symbiosis, it would be helpful to have multiple genomes from nonleguminous rhizobial species, and also from leguminous species that do not support rhizobial symbiosis. The hypothesis may be further tested using the genomes of plant species with distinct types of mycorrhizal association, such as orchids and the Ericaceae (reviewed in Smith & Read, 2008). To clarify the relative importance of pathogens, endophytes, and mycorrhizas for immune expansions, it would helpful to have a more complete survey of microbial diversity in all three classes. Another topic of interest is the search for sub-families within the MycEx gene families that show especially strong dependence on mycorrhizal competence, or sub-families that expanded during the evolutionary development of mycorrhiza (e.g. Yue, Meyers, Chen, Tian, & Yang, 2012). These projects may point the way to an improved functional understanding of the relationship between the immune repertoire and fungal symbiosis.

CONFLICT OF INTEREST

The authors declare no competing interests.
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AUTHOR CONTRIBUTIONS

E.M.K. planned the research, conducted the statistical tests, and wrote the manuscript. S.A.S., H.J.Y. and J.W.C. contributed to the genome analysis. D.H.R.M. discussed the research and contributed to the manuscript.

DATA AVAILABILITY

All data are available in the main text or the Supporting information Materials.

REFERENCES

APGIV (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society, 181, 1–20.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B, 57, 289–300.

Bent, A. F., & Mackey, D. (2007). Elicitors, effectors, and R genes: The new paradigm and a lifetime supply of questions. Annual Review of Phytopathology, 45, 399–436. https://doi.org/10.1146/annurev.phyto.45.062806.094427

Berndstorf, F., Doring, A. C., Gruner, K., Schuck, S., Brautigam, A., & Zeier, J. (2016). Piperocid acid orchestrates plant systemic acquired resistance and defense priming via salicylic acid-dependent and -independent pathways. The Plant Cell, 28(1), 102–129.

Botella, M. A., Parker, J. E., Frost, L. N., Bittner-Eddy, P. D., Beynon, J. L., Daniels, M. J., ... Jones, J. D. (1998). Three genes of the Arabidopsis RPP1 complex resistance locus recognize distinct Peronospora parasitica avirulence determinants. The Plant Cell, 10(11), 1847-1860. https://doi.org/10.1105/tpc.10.11.1847

Bravo, A. York, T., Pumplin, N., Mueller, L. A., & Harrison, M. J. (2016). Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. Nature Plants, 2, 15208. https://doi.org/10.1038/nplants.2015.208

Brundrett, M. C. (2017). Global Diversity and Importance of Mycorrhizal and Nonmycorrhizal Plants. Biogeography of Mycorrhizal Symbiosis. L. Tedeross. Switzerland. Springer. 230, 533–556.

Brutus, A., Sicilia, F., Macone, A., Cerbone, F., & De Lorenzo, G. (2010). A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proceedings of the National Academy of Sciences of the United States of America, 107(20), 9452–9457. https://doi.org/10.1073/pnas.100075107

Cai, Q., Qiao, L., Wang, M., He, B., Lin, F. M., Palmquist, J., ... Jin, H. (2018). Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science, 360(6393), 1126–1129. https://doi.org/10.1126/science.aar4142

Calabrese, S., Kohler, A., Niehl, A., Veneault-Fourrey, C., Boller, T., & Courty, P. E. (2017). Transcriptome analysis of the Populus trichocarpa-Rhizobagus irregularis Mycorrhizal Symbiosis: Regulation of Plant and Fungal Transportomes under Nitrogen Starvation. Plant and Cell Physiology, 58(6), 1003–1017. https://doi.org/10.1093/pcp/pcx044

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. Cell, 124(4), 803–814. https://doi.org/10.1016/j.cell.2006.02.008

Collins, N., Drake, J., Ayliffe, M., Sun, Q., Ellis, J., Hulbert, S., & Pryor, T. (1999). Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants. Plant Cell, 11(7), 1365–1376. https://doi.org/10.1105/tpc.11.7.1365

Cook, D. E., Mesarich, C. H., & Thomma, B. P. (2015). Understanding plant immunity as a surveillance system to detect invasion. Annual review of Phytopathology, 53, 541–563. https://doi.org/10.1146/annurev-phoyo-080614-120114

Delaux, P. M., Radhakrishnan, G. V., Jayaraman, D., Cheema, J., Malbreil, M., Volkening, J. D., ... Ané, J. M. (2015). Algal ancestor of land plants was preadapted for symbiosis. Proceedings of the National Academy of Sciences of the United States of America, 112(43), 13390–13395. https://doi.org/10.1073/pnas.1514526112

Delaux, P. M., Varala, K., Edger, P. P., Coruzzi, G. M., Pires, J. C., & Ane, J. M. (2014). Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. PLoS Genetics, 10(7), e1004487. https://doi.org/10.1371/journal.pgen.1004487

Denoux, C., Galletti, R., Mammarella, N., Gopalan, S., Werck, D., De Lorenzo, G., ... Dewdney, J. (2008). Activation of defense response pathways by OGs and Flg22 elicitors in Arabidopsis seedlings. Molecular Plant, 1(3), 423–445. https://doi.org/10.1093/mp/ssn019

Dilworth, M. J., James, E. K., Sprent, J. I., & Newton, W. E. (2008). Nitrogen-fixing leguminous symbioses. Dordrecht, The Netherlands: Springer.

Epstein, B., Branca, A., Mudge, J., Bharti, A. K., Brikisne, R., Farmer, A. D., ... Tiffin, P. (2012). Population genomics of the facultatively mutualistic bacteria Sinorhizobium melloti and S. medicae. PLoS Genetics, 8(8), e1002868. https://doi.org/10.1371/journal.pgen.1002868

Favre, P., Bapaume, L., Bossolini, E., Delorenzi, M., Falquet, L., & Reinhardt, D. (2014). A novel bioinformatics pipeline to discover genes related to arbuscular mycorrhizal symbiosis based on their evolutionary conservation pattern among higher plants. BMC Plant Biology, 14, 333. https://doi.org/10.1186/s12870-014-0333-0

Forde, B. G., & Roberts, M. R. (2014). Glutamate receptor-like channels in plants: A role as amino acid sensors in plant defence? F1000Prime Reports, 6, 37.

Foster, A. J., Pelletier, G., Tanguay, P., & Seguin, A. (2015). Transcriptome analysis of poplar during leaf spot infection with Sphaerulina spp. PLoS ONE, 10(9), e0138162. https://doi.org/10.1371/journal.pone.0138162

Gomez-Gomez, L., & Boller, T. (2000). FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Molecular Cell, 5(6), 1003–1011. https://doi.org/10.1016/S1097-2765(00)80265-8

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., ... Rokhsar, D. S. (2012). Phytozome: A comparative platform for green plant genomics. Nucleic Acids Research, 40, D1178–D1186. https://doi.org/10.1093/nar/gkr944

Guinel, F. C. (2009). Getting around the legume nodule: I. The structure of the peripheral zone in four nodule types. Botany-Botanique, 87, 1117–1138. https://doi.org/10.1139/B09-074

Hacquard, S., Spaepen, S., Garriodo-Oter, R., & Schulze-Lefert, P. (2017). Interplay between innate immunity and the plant microbiota. Annual review of Phytopathology, 55, 565–589. https://doi.org/10.1146/annurev-phoyo-080516-035623

Hohnjec, N., Vieweg, M. F., Puhler, A., Becker, A., & Kuster, H. (2005). Overlaps in the transcriptional profiles of Medicago truncatula roots inoculated with two different Glomus fungi provide insights...
into the genetic program activated during arbuscular mycorrhiza. Plant Physiology, 137(4), 1283–1301. https://doi.org/10.1104/pp.104.056572

Hothorn, T., Hornik, K., van de, Wiel, M. A., & Zeileis, A. (2006). coin: A Computational Framework for Conditional Inference, R package version 0.4-5, http://CRAN.R-project.org/

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. Nature, 444(7117), 323–329. https://doi.org/10.1038/nature05286

Kall, L., Krogh, A., & Sonnhammer, E. L. (2007). Advantages of combined transmembrane topology and signal peptide prediction—the Phobius web server. Nucleic Acids Research, 35 (Web Server issue), W429–W432. https://doi.org/10.1093/nar/gkm256

Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., … Martin, F. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nature Genetics, 47(4), 410–415. https://doi.org/10.1038/ng.3223

Kohorn, B. D., & Kohorn, S. L. (2012). The cell wall-associated kinases, WAKs, as pectin receptors. Frontiers in Plant Science, 3. 88.

Kouchi, H., Shimomura, K., Hata, S., Hirota, A., Wu, G. J., Kumagai, H., … Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for cells and endosymbionts in B., Laporte, P., … Mergaert, P. (2010). Differentiation of symbiotic events in Medicago truncatula. Annual review of Phytopathology, 48, 291–315. https://doi.org/10.1146/annurev-phyto-080508-081831

Parniske, M. (2008). Arbuscular mycorrhiza: The mother of plant root endosymbioses. Nature Reviews Microbiology, 6(10), 763–775. https://doi.org/10.1038/nrmicro1987

Peralta, I. E., Spooner, D. M., & Knapp, S. (2008). Taxonomy of wild tomatoes and their relatives (Solanum sect. Lycopersicoides, sect. Juglandifolia, sect. Lycopersicon: Solanaceae).

Perez-Tienda, J., Correa, A., Azcon-Aguilar, C., & Ferrol, N. (2014). Transcriptional regulation of host NH4(+) transporters and GS/GOGAT pathway in arbuscular mycorrhizal root rice. Plant Physiology and Biochemistry, 75, 1–8. https://doi.org/10.1016/j.plaphy.2013.11.029

Plomion, C., Aury, J. M., Amselem, J., Leroy, T., Murat, F., Duplessis, S., … Salse, J. (2018). Oak genome reveals facets of long lifespan. Nature Plants, 4(7), 440–452. https://doi.org/10.1038/s41477-018-0172-3

Porras-Alfaro, A., & Bayman, P. (2011). Hidden fungi, emergent properties: Endophytes and microbiomes. Annual review of Phytopathology, 49, 291–315. https://doi.org/10.1146/annurev.phyto-080508-081831

Punja, M., Coggill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., … Clements, J., et al. (2012). The Pfam protein families database. Nucleic Acids Research, 40, D290–D301. https://doi.org/10.1093/nar/gkr1065

Ranf, S., Gisch, N., Schaffer, M., Illig, T., Westphal, L., Knirel, Y. A., … Scheel, D. (2015). A lectin S-domain receptor kinase mediates lipo-poly saccharide sensing in Arabidopsis thaliana. Nature Immunology, 16(4), 426–433. https://doi.org/10.1038/ni.3124

Rate, D. N., Cuenca, J. V., Bowman, G. R., Gutmann, D. S., & Greenberg, J. T. (1999). The gain-of-function Arabidopsis acd6 mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. The Plant Cell, 11(9), 1695–1708. https://doi.org/10.1105/tpc.11.9.1695

Recchia, G. H., Konzen, E. R., Cassieri, F., Caldas, D. G. G., & Tsai, S. M. (2018). Arbuscular mycorrhizal symbiosis leads to differential regulation of drought-responsive genes in tissue-specific root cells of common bean. Frontiers in Microbiology, 9, 1339. https://doi.org/10.3389/fmicb.2018.01339

Reinhold-Hurek, B., Bunger, W., Burbano, C. S., Sabale, M., & Hurek, T. (2015). Roots shaping their microbiome: Global hotspots for microbial activity. Annual review of Phytopathology, 53, 403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Schussler, A., Schwarzott, D., & Walker, C. (2001). A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycological Research, 105, 1413–1421. https://doi.org/10.1017/S0953755201005196

Sekhwali, M. K., Li, P., Lam, I., Wang, X., Cloutier, S., & You, F. M. (2015). Disease resistance gene analogs (RGAs) in plants. International Journal of Molecular Sciences, 16(8), 19248–19290. https://doi.org/10.3390/ijms160819248

Smith, S. E., & Read, D. (2008). Mycorrhizal symbiosis. Amsterdam, the Netherlands: Academic Press.
Sugawara, M., Epstein, B., Badgley, B. D., Unno, T., Xu, L., Reese, J., ... Sadowsky, M. J. (2013). Comparative genomics of the core and accessory genomes of 48 Sinorhizobium strains comprising five genospecies. *Genome Biology, 14*(2), R17. https://doi.org/10.1186/gb-2013-14-2-r17

Sugimura, Y., & Saito, K. (2017). Comparative transcriptome analysis between *Solanum lycopersicum* L. and *Lotus japonicus* L. during arbuscular mycorrhizal development. *Soil Science and Plant Nutrition, 63*, 127–136. https://doi.org/10.1080/00380768.2017.1280378

Takasaki, T., Hatakeyama, K., Suzuki, G., Watanabe, M., Isogai, A., & Hinata, K. (2000). The S receptor kinase determines self-incompatibility in *Brassica* stigma. *Nature, 403*(6772), 913–916. https://doi.org/10.1038/35002628

Team, R. C. (2018). *R*: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Tedersoo, L., & Brundrett, M. C. (2017). Evolution of ectomycorrhizal symbiosis in plants. *Biogeography of Mycorrhizal Symbiosis*. L. Tedersoo. Switzerland, Springer.

Tobias, P. A., & Guest, D. I. (2014). Tree immunity: Growing old with out antibodies. *Trends in Plant Science, 19*(6), 367–370. https://doi.org/10.1016/j.tplants.2014.01.011

Trappe, J. M. (1977). Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annual Review of Phytopathology, 15*, 203–222. https://doi.org/10.1146/annurev.py.15.090177.001223

Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., ... Rohkhar, D. (2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science, 313*(5793), 1596–1604. https://doi.org/10.1126/science.1128691

van der Linde, S., Suz, L. M., Orme, C. D. L., Cox, F., Andreae, H., Asi, E., ... Bidartondo, M. I. (2018). Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature, 558*(7709), 243–248. https://doi.org/10.1038/s41586-018-0189-9

Van Geel, M., Yu, K., Ceulemans, T., Peeters, G., van Acker, K., Geerts, W., ... Honnay, O. (2018). Variation in ectomycorrhizal fungal communities associated with Silver linden (*Tilia tomentosa*) within and across urban areas. *FEMS Microbiology Ecology, 94*(12).

Vangelisti, A., Natali, L., Bernardi, R., Sbrana, C., Turrini, A., Hassan-Pak, K., ... Giordani, T. (2018). Transcriptome changes induced by arbuscular mycorrhizal fungi in sunflower (*Helianthus annuus* L.) roots. *Scientific Reports, 8*, 4. https://doi.org/10.1038/s41598-017-18445-0

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., ... Viola, R. (2010). The genome of the domesticated apple (*Malus x domestica* Borkh.). *Nature Genetics, 42*(10), 833–839. https://doi.org/10.1038/ng.654

Wan, J., Zhang, X.-C., Neece, D., Ramonell, K. M., Clough, S., Kim, S.-Y., ... Stacey, G. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in arabidopsis. *The Plant Cell, 20*, 471–481. https://doi.org/10.1105/tpc.107.056754

Wang, G., Ellendorff, U., Kemp, B., Mansfield, J. W., Forsyth, A., Mitchell, K., ... Thomma, B. P. (2008). A genome-wide functional investigation into the roles of receptor-like proteins in Arabidopsis. *Plant Physiology, 147*(2), 503–517. https://doi.org/10.1104/pp.108.119487

Wang, B., & Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza, 16*(5), 299–363. https://doi.org/10.1007/s00572-005-0033-6

Wang, Z. X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., ... Sasaki, T. (1999). The Pib gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *The Plant Journal, 19*(1), 55–64. https://doi.org/10.1046/j.1365-313X.1999.00498.x

Weiberg, A., Wang, M., Lin, F. M., Zhao, H., Zhang, Z., Kaloshian, I., ... Jin, H. (2013). Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science, 342*(6154), 118–123. https://doi.org/10.1126/science.1239705

Yang, S., Tang, F., Gao, M., Krishnan, H. B., & Zhu, H. (2010). R gene-controlled host specificity in the legume-rhizobia symbiosis. *Proceedings of the National Academy of Sciences of the United States of America, 107*(43), 18735–18740. https://doi.org/10.1073/pnas.1011957107

Yang, S., Zhang, X., Yue, J. X., Tian, D., & Chen, J. Q. (2008). Recent duplications dominate NBS-encoding gene expansion in two woody species. *Molecular Genetics and Genomics, 280*(3), 187–198. https://doi.org/10.1007/s00438-008-0355-0

Yue, J. X., Meyers, B. C., Chen, J. Q., Tian, D., & Yang, S. (2012). Tracing the origin and evolutionary history of plant nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes. *New Phytologist, 193*(4), 1049–1063. https://doi.org/10.1111/j.1469-8137.2011.04006.x

**SUPPORTING INFORMATION**

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

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