

Symbiont switching and alternative resource acquisition strategies drive mutualism breakdown

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Cooperative interactions among species, termed mutualisms, have played a crucial role in the evolution of life on Earth. However, despite key potential benefits to partners, there are many cases in which two species cease to cooperate and mutualisms break down. What factors drive the evolutionary breakdown of mutualism? We examined the pathways toward breakdowns of the mutualism between plants and arbuscular mycorrhizal fungi. By using a comparative approach, we identify ~25 independent cases of complete mutualism breakdown across global seed plants. We found that breakdown of cooperation was only stable when host plants (i) partner with other root symbionts or (ii) evolve alternative resource acquisition strategies. Our results suggest that key mutualistic services are only permanently lost if hosts evolve alternative symbioses or adaptations.

cooperation | mutualism | symbiosis | macroevolution | mycorrhizae

Mutualisms, cooperative partnerships among different species, have shaped much of Earth's biodiversity, allowing organisms to outsource crucial functions like nutrition, cleaning, transport, and defense (1, 2). Theoretical and empirical work has provided us with a good understanding of the mechanisms, such as cotransmission and sanctions, that stabilize mutualism and maintain cooperation among species (3–5). Because of these mechanisms, beneficial interactions can be maintained over millions of years, and in some cases give rise to extreme mutualistic dependence (6–8).

Despite reciprocal benefits, mutualisms do not always persist, and conflict among partners can remain. Theoretical and experimental work suggests that, even when mutual benefits occur, fitness interests of both partners are generally not perfectly aligned, potentially selecting for cheaters and exploiters of mutualism (5, 9–12). This finding is further reinforced by the observation that, in many mutualisms, there are mechanisms to evaluate partner quality and reward cooperation or sanction noncooperative cheats (13–15). Furthermore, over ecological time, short-term breakdowns of cooperation in response to shifting environmental conditions have been observed in many mutualisms, including plant rhizobial and mycorrhizal mutualisms, coral symbioses, and protection and pollination mutualisms (16–20). Together, these observations raise the question of in which conditions we should expect cooperation among species to fail and partners in previously successful mutualisms to cease cooperating.

Even mutualisms that have become highly dependent over millions of years of coevolution have broken down in some occasions. This is the case, for example, when free-living fungi evolved from a previously lichenized lifestyle, or when parasitic moths evolved from pollinating ancestors (21–23). However, although we have a good understanding of why mutualistic cooperation is favored, we lack a general understanding of the drivers of these evolutionary breakdowns of mutualisms. A number of nonexclusive reasons for the breakdown of mutualisms have been proposed (24, 25). Benefits provided by a mutualistic partner can become redundant through the evolution of alternative adaptations. In these cases, one of the partners switches from relying

on another species to acquiring a function autonomously. For example, the evolution of large amounts of small-diameter pollen enabled the reversion back to an autonomous, wind-pollinated lifestyle in some angiosperms (26). A second trajectory occurs when one side of the interaction is replaced with a new mutualist species. Although partner switching, by definition, leads to the evolution of a new partnership, the ancestral interaction is lost and therefore a previously functional mutualism breaks down. This is illustrated in cases in which plant species stop cooperating with birds and switch to insect pollination (27).

Our aim was to study the ancient and ubiquitous mutualism between plants and arbuscular mycorrhizal (AM) fungi to understand pathways toward mutualism breakdown. We focus on the plant–AM mutualism for three reasons. First, AM fungi (Glomeromycota) are among the most important terrestrial mutualists. AM fungi form extensive hyphal networks in the soil (as large as 100 m·cm⁻³ soil), providing plants with a key solution to the problem of extracting immobile nutrients, especially phosphorus (28). The partnership is crucial for plant growth, providing hosts with primarily phosphorus, but also nitrogen, water, and trace elements (29). Second, even though the large majority of plants can be successfully colonized by AM fungi, 10–20% of plant species across a number of divergent clades do not interact with any AM fungi (28, 30). These repeated losses of the interaction, separated by millions of years of evolution, enable us to test general patterns and explanatory factors driving cooperation loss in a comparative framework. Third, the tools and databases

Significance

Cooperative interactions among species—mutualisms—are major sources of evolutionary innovation. However, despite their importance, two species that formerly cooperated sometimes cease their partnership. Why do mutualisms break down? We asked this question in the partnership between arbuscular mycorrhizal (AM) fungi and their plant hosts, one of the most ancient mutualisms. We analyze two potential trajectories toward evolutionary breakdown of their cooperation, symbiont switching and mutualism abandonment. We find evidence that plants stop interacting with AM fungi when they switch to other microbial mutualists or when they evolve alternative strategies to extract nutrients from the environment. Our results show vital cooperative interactions can be lost, but only if successful alternatives evolve.

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allowing for broad comparative analyses are becoming available for plants, including a comprehensive phylogeny of seed plants (31) and large-scale databases of plant traits including their association with AM fungi and other root symbionts (32–34).

In our analysis of the plant–AM mutualism, we take a plant-centric perspective. We are interested in cases in which plants completely cease to interact with all AM fungi and this lack of interaction persists over evolutionary time, i.e., the loss of the interaction is not followed by host plant extinction. Thus, we do not study when plant–AM cooperation dissolves in the short term as a result of ecological conditions, such as under high-nutrient conditions. Rather, our aim is to first quantify stable losses of cooperation and then test the importance of two types of evolutionary breakdown: partner switching and mutualism abandonment. By partner switching, we mean a situation in which a host plant that ancestrally interacted with AM fungi switched to interacting with a novel root symbiont with similar function and ceased interacting with AM fungi. We analyze switches to other mycorrhizal fungi, as well as to N₂-fixing symbioses with rhizobial and *Frankia* bacteria (29, 35). We refer to mutualism abandonment, when plants have evolved an alternative strategy to acquire resource in a nonsymbiotic way, for instance, carnivory or cluster roots (36, 37).

Results

Evolutionary Reconstruction of the Plant–AM Fungal Mutualism. Our first aim was to quantify the evolutionary stability of the plant–AM mutualism by determining the number of losses of plant–AM interactions across the plant phylogeny. We compiled a global database of plant mycorrhizal fungal status across the seed plants (angiosperms and gymnosperms). We scored the reported interactions of plants with AM fungi in 3,736 plant species present in the most recent and comprehensive phylogeny of gymnosperms and angiosperms (31). We then established patterns of AM loss and gain by using a hidden rate model (HRM) approach to ancestral state reconstructions (38). This technique permits variation in the speed of binary character evolution so we can detect changes in rates of evolution, such as shifts in the evolutionary stability of plant–AM associations.

Our reconstructions revealed that the evolution of AM interactions across seed plants was best characterized by heterogeneity in speed of evolution: the best evolutionary model contains three different rate classes of evolution (Table 1). Specifically, we find strong evidence for the existence of an evolutionary class in which AM interactions are strongly favored (which we termed stable AM), a class in which an absence of AM interactions is strongly favored (stable non-AM), and a class in which AM interactions are evolutionarily labile (*SI Appendix, Fig. S1*).

Mapping these different evolutionary states back onto the phylogeny (*SI Appendix, Fig. S2*), we found that (i) association with AM fungi was likely the ancestral state of seed plants (99.6% likelihood), (ii) stable AM fungal associations have been widely retained throughout the seed plants for more than 350 My and represent the large majority of all historical and contemporary plant species and families (Table 2); and (iii) some plant lineages evolve to an evolutionarily labile state or a state in which AM fungi are disfavored (Table 2 and *SI Appendix, Fig. S1*).

Table 1. AICc values and weights for all HRM models

No. of rate classes	No. of parameters	AICc	ΔAICc	AICc weight, %
1	2	3,231.25	770.0	0.0
2	8	2,597.78	136.5	0.0
3	14	2,461.25	0	74.8
4	20	2,463.44	2.2	25.1
5	26	2,473.79	12.5	0.1

Table of the five different HRMs explored to analyze our AM fungal association data (*Methods*). We used AICc weights to determine the model with the best fit (bold).

Table 2. Number of contemporary species and families in three AM classes

Stability class	Species numbers		Family numbers	
	Best tree	Median	Best tree	Median
Stable AM	2,616	2,613 ± 178	172	171 ± 12
Labile	829	833 ± 180	77	79 ± 13
Stable non-AM	291	288 ± 21	8	7 ± 3.7

Numbers of contemporary species and families per AM stability class. Median value and SD across 100 bootstrap phylogenies are indicated.

Specifically, (iv) there have been an estimated ~25 evolutionary losses of the AM mutualism throughout the history of seed plants [median over 100 bootstrap phylogenies, 25.4 ± 7.73 (SD)], found across 69 families. Which evolutionary trajectories are most important in explaining these breakdowns of cooperation among plants and AM fungi?

Symbiont Switching and Mutualism Abandonment Drive Breakdown.

We tested the hypotheses that AM loss is driven by shifts to other symbionts (i.e., partner switching) or by alternative adaptations for resource acquisition (i.e., abandonment). We generated a database of other major root symbionts with functional roles (providing phosphorus and nitrogen) similar to AM fungi. Specifically, based on a previously published database, we included presence or absence of a potential to interact with symbiotic N₂-fixing bacteria (rhizobial and *Frankia* bacteria) for all our host plant species (35, 39). We also included reported interactions with other mycorrhizal fungi (i.e., non-AM fungi that live in symbiotic association with plant roots). This included ectomycorrhizal (EM), ericoid mycorrhizal (ER), orchid mycorrhizal (ORM), and arbutoid mycorrhizal (ARB) fungi. All AM fungi belong to the division Glomeromycota, whereas other mycorrhizal fungi are only distantly related, belonging to a wide range of divisions, mainly Basidiomycota (ECM, ARB, ORM, and some ER fungi) and Ascomycota (some ECM and ORM and most ER fungi). Some plant species interact with multiple types of mycorrhizal fungi (29, 40).

We scored our species for the reported presence of alternative resource acquisition strategies. These included parasitism as a plant strategy (plants parasitizing other plants and full mycoheterotrophs, i.e., plants parasitizing mycorrhizal fungi) (41, 42), carnivory (36), and cluster roots (37) (Fig. 1). These strategies have in common that they represent alternative solutions to the problem of acquiring scarce mineral resources: they acquire resources by seizing them from other organisms (i.e., plant parasitism), through direct predation (i.e., carnivorous plants), or through investing in a unique root architecture characterized by a high density of finely branched roots and root hairs, known as cluster roots (Fig. 1). To study congruence between losses of AM interactions and alternative strategies, we again performed ancestral state reconstructions to study the origins of (i) other symbionts (i.e., non-AM mycorrhizal fungal symbionts or symbiotic N₂ fixation), which were present in 820 of our 3,736 plant species; and (ii) alternative resource acquisition strategies, present in 109 plant species.

We found a high degree of congruence between the different origins of AM losses and various AM alternatives (*SI Appendix, Figs. S3–S9 and Table S1*). To study this quantitatively, we compared models of dependent vs. independent evolution (43), analyzing the relationship between AM loss and presence of alternative partners or alternative resource acquisition strategies. We studied a binary variable coding for presence of any AM alternative and found that a dependent model of evolution vastly outperformed an independent model [Δ -AICc (corrected Akaike information criterion), 428.90; AICc weight, 99.9%]. This means that, over evolutionary time, AM loss (i.e., shift from the bottom left plane to the top right in the transitions matrix; Fig. 2) is strongly associated with the presence of another mycorrhizal fungal partner or alternative resource strategy. Thus, partner switching and mutualism abandonment are important

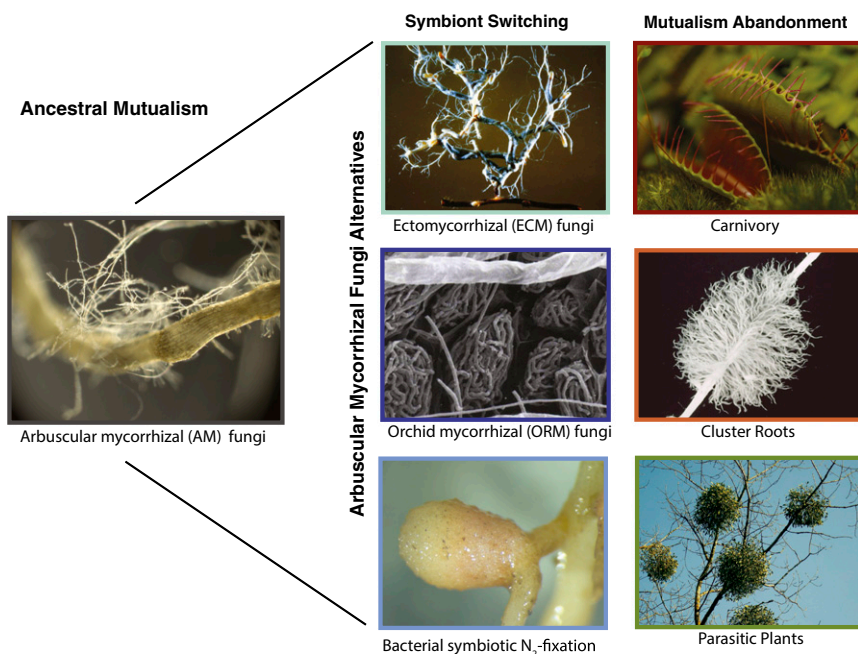


Fig. 1. We explored the evolution of various alternatives to interacting with AM fungi, the ancestral state of seed plants. Examples of six important alternatives we considered are depicted, with columns indicating two potential pathways toward evolutionary breakdown of the plant–AM mutualism. In both pathways, the ancestral mutualism with AM fungi breaks down. Colored borders match colored bars in Fig. 2, indicating distribution of these traits across global seed plants. Image of AM fungi courtesy of Yoshihiro Kobae (Rakuno Gakuen University, Ebetsu, Japan). Other images courtesy of (Clockwise from Top Right) Pixabay/PublicDomainPictures; adapted with permission from ref. 69; Pixabay/Egle_pe; Euan James (The James Hutton Institute, Dundee, United Kingdom); adapted with permission from ref. 70; and Wikimedia Commons/B. Zak.

in enabling evolutionary breakdown of the ancestral plant–AM fungal mutualism throughout the seed plants.

More specifically, from the inferred transition matrix and associated ancestral state reconstruction (Fig. 2), we conclude that (i) the AM mutualism is generally highly stable, i.e., transition rates toward the AM state (Fig. 2, green) are approximately 10 times as high as losses (Fig. 2, from green to yellow); and (ii) AM fungal loss is only stable when an alternative is present (Fig. 2, orange to red) and not without (Fig. 2, green to yellow). In addition, (iii) although evolutionary stability is high when plants associate with AM fungal symbionts (Fig. 2, green state) or an alternative symbiont or acquisition strategy (Fig. 2, red), having neither (Fig. 2, yellow) is evolutionarily unstable. For instance, all of the origins of this type (e.g., in the Brassicales) have occurred relatively recently in evolutionary terms (within the past 30 My). (iv) Similarly, it is evolutionarily less stable to have both AM and an alternative simultaneously (Fig. 2, orange).

Our reconstructions show that the evolutionary scenario of initial AM loss followed by alternative strategy evolution and the reverse order are possible. Initial acquisition of an AM alternative (i.e., a move from green to orange state in Fig. 2), in some cases, may have resulted in relaxed selection to maintain the AM interaction, allowing for its subsequent evolutionary breakdown (Fig. 2, orange to red). In other cases, the AM interaction was lost first (Fig. 2, yellow state), such as through symbiosis gene loss (44), and survival of host plants was subsequently favored when rapidly evolving an AM-alternative state. Thus, our analysis indicates there is no single dominant trajectory in the transition from an AM plant to a stable non-AM plant, but that both routes can occur.

Sensitivity Analyses. To verify the robustness of our results, we considered the sensitivity of our main conclusions to two forms of uncertainty: (i) phylogenetic uncertainty and (ii) uncertainty in the underlying AM data. We analyzed phylogenetic uncertainty by replicating our initial AM fungal reconstruction analysis over 100 bootstrap phylogenies, and found highly similar relative loss rates of the plant–AM interaction throughout 100% of our bootstrap replicates (SI Appendix, Fig. S10) and highly similar ancestral state reconstructions (SI Appendix, Fig. S11). We also found that, across the 100 bootstrap phylogenies, a dependent model of evolution always outperformed an independent model (mean Δ -AICc, 390.52). This further confirms the deep evolutionary link between AM loss and the evolution of other

symbionts and resource acquisition strategies regardless of the details of the phylogenies used (SI Appendix, Fig. S12).

A second main source of uncertainty is in the AM status of plants. This is because AM fungi are notoriously difficult to score: it is easy to misidentify other fungi as AM fungi (i.e., false positive) or to miss AM hyphae (i.e., false negative). To address this, we implemented a resimulation approach that takes into account the number of independent reports of AM status and allows us to test separate false-positive and false-negative rates for these underlying reports. We found that, even if one in four of the AM reports in our database is incorrect (e.g., a saprotrophic fungus), while simultaneously 25% of our AM absence reports in fact were mycorrhizal, we still draw highly similar conclusions (SI Appendix, Fig. S13 and Table S2). Therefore, even if we assume the underlying mycorrhizal data are of very poor quality, we recover qualitatively highly similar patterns. Thus, overall, we conclude that all our main conclusions are robust to substantial phylogenetic and data uncertainty.

As a final analysis, we compared our results with a recent comparative analysis of plant–mycorrhizal symbioses (30). This analysis used an alternative scoring approach that divided plant species into four categories—AM plants, nonmycorrhizal (NM) plants, ECM plants, and plants that are commonly found in either AM or NM states (AMNM plants)—and found that transitions from AM toward NM states primarily go through the AMNM state. We reconfirmed this result in that we find in our best HRM model of plant–AM interactions that plants transition through the labile state to the stable non-AM state, in which the loss of plant–AM mutualism becomes evolutionarily entrenched (SI Appendix, Fig. S1). We also find that the species-level percentage of observations with AM presence has a median value of 100% (SE, 0.89%; mean, 83.4%) for species inferred to be in the stable AM class and a median value of 0% in the stable non-AM class (SE, 0.73%; mean, 1.58%), whereas, in the evolutionarily labile class, this is 16.7% (SE, 1.57%; mean, 22.0%; SI Appendix, Fig. S14). This indicates that the labile state inferred under our deep evolutionary model effectively recovers the notion of an AMNM presupposed by Maherali et al. (30). Although their analysis allows for direct inclusion of AMNM and ECM states, with our approach of binary-coding the presence or absence of AM and other mycorrhizal interactions, we can answer different questions as follows. (i) It allows us to infer the variation in loss rate of the AM mutualism across seed plant evolutionary history [which is possible only in the HRM framework for binary traits (38)] (ii) Rather than a priori defining an intermediate state, it allows us to

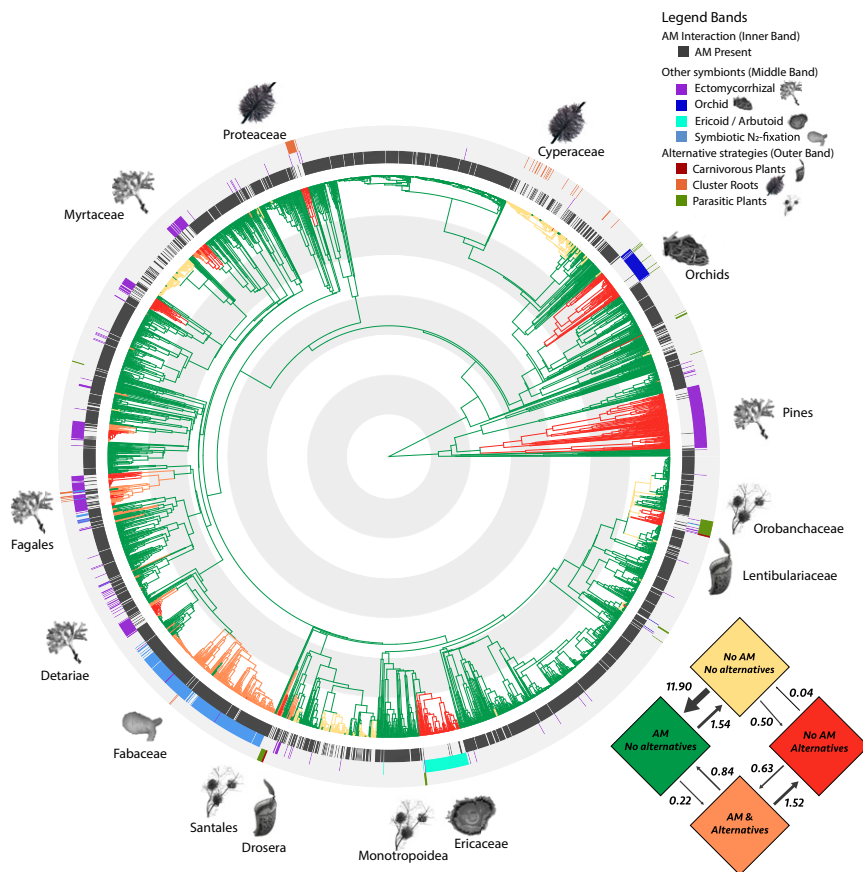


Fig. 2. Transition rates and ancestral state reconstruction of the dependent evolutionary model for plant AM status and AM alternatives. The four potential evolutionary states are represented by colors in the transition matrix and on the seed plant phylogeny. Transition rates are expressed as number of transitions per 100 My per lineage. The ancestral state is AM presence with no AM alternatives (green), and red indicates the switch to one of the AM alternatives (i.e., another mycorrhizal fungus, symbiotic N₂ fixation, parasitism, carnivory, or cluster roots). From inside to outside, colored bands around the phylogeny indicate the presence or absence of (i) AM interactions, (ii) other root symbionts, and (iii) alternative resource acquisition strategies. Key clades that have lost AM fungal interactions are indicated with schematic images of their evolved alternatives. Gray and white concentric circles indicate 50 My. An expanded version with fully legible species labels for all 3,736 species is available online (*SI Appendix*). Images courtesy of (Ectomycorrhizal) Wikimedia Commons/B. Zak; (Orchid) adapted with permission from ref. 70; (Ericoid/Arbutoid) adapted with permission from David Midgley (photographer); (Symbiotic N₂-fixation) Euan James (The James Hutton Institute, Dundee, Scotland); (Carnivorous Plants) Pixabay/PublicDomainPictures; (Cluster Roots) adapted with permission from ref. 69; and (Parasitic Plants) Pixabay/Egle.pe.

verify if an evolutionarily labile state is actually inferred in our best model. (iii) It allows us to study the dependent evolution of AM and other mycorrhizal interactions as separate traits. This is especially important because, although rare, dual colonization of plants by two types simultaneously is possible and could represent an important evolutionary intermediary state, as confirmed by our analysis (Fig. 2) (4). It allows us to include in our analysis not just ECM fungi, but also other root symbionts such as symbiotic N₂-fixation, ERM, and ORM mycorrhizal fungi, which turned out to be drivers of major evolutionary losses of the plant–AM mutualism (Fig. 2).

Discussion

Our analyses revealed that the ancient and still ubiquitous plant–AM fungal mutualism has broken down in ~25 cases across the seed plants. We found that stable and persistent mutualism breakdown is driven by acquisitions of other root symbionts (i.e., partner switching) and by the evolution of alternative nonsymbiotic resource acquisition strategies (i.e., mutualism abandonment).

These results in turn raise the question of what underlying ecological factors favor transitions to these alternative solutions, and what mechanisms enable them. Mechanistically, an important step is likely the loss of key genes in the “symbiotic toolkit” encoding crucial root mutualism effectors (44, 45). This must be followed or preceded by molecular evolution in the genes encoding alternative symbioses or resource-acquisition traits. Ecologically, these alternatives

can potentially be favored by a range of ultimate factors, such as environmental change, habitat shifts (e.g., to high-nutrient soils), migration, invasion, or partner abundance (23, 25, 46–50), although discriminating these over deep evolutionary time is challenging. One hypothesis is that switching from the AM nutrient uptake strategy to rarer alternative strategies has enabled plants to compete in a range of specific but relatively rare (micro)habitats. Evolution of carnivory in temperate swamps (36), cluster roots in extremely phosphorus-impooverished soils (37), cold-resistant EM interactions in lower-temperature habitats (51), and ER fungi in resource-poor heath lands (52) has helped host plants to thrive in environments in which the more common AM interaction is a less successful solution to obtain nutrients.

We emphasize that our estimate of ~25 breakdowns represents a conservative lower bound, as we studied plants that stopped interacting with all AM fungal species and subsequently persisted over evolutionary times. The number of breakdowns of plant mutualism with specific AM fungal species or lineages while cooperation with other AM continued is likely to be considerably higher. More generally, studies that analyze breakdowns of mutualistic interactions with entire taxa of organisms—such as among corals and any photosynthetic dinoflagellates (53, 54)—will similarly underestimate the number of breakdowns with specific pairs of species.

We find that dual symbioses—simultaneously being able to interact with two types of root symbionts—is unlikely to be evolutionarily stable (Fig. 2). This is a different pattern compared with

what is documented in many insect endosymbioses, which often acquire secondary partners while retaining the ancestral mutualism (55, 56). In insects, maintenance of two endosymbionts could be favored by different microbial partners subsequently specializing in different mutualistic functions (56, 57). In root symbioses, nutritional benefits provided by AM fungi and by alternative root symbionts may often be too similar to outweigh the costs of maintaining them both. For instance, although AM fungi are thought to provide primarily phosphorus, they also contribute nitrogen to their hosts (58). This could help explain why plants only rarely associate with ECM fungi and AM fungi simultaneously (131 species in our dataset). However, our reconstructions suggest that such dual symbioses can be a transitory state on the path toward a complete switch and breakdown of the original mutualism (Fig. 2), as was previously hypothesized (59). Other root symbionts may provide more complementary benefits to their plant hosts, which could select for maintenance of dual symbioses. For instance, AM fungi and N_2 -fixing rhizobial bacteria are often thought to provide complementary benefits to their legume hosts (60), although a meta-analysis did not generally find synergistic effects on host growth (61).

If breakdown of the AM fungal mutualism is driven by acquisition of other root symbionts or alternative resource strategies, how can we explain plants that have neither AM fungi nor an alternative (Fig. 2, yellow)? Recently, a member of the Brassicaceae, a family generally lacking mycorrhizal symbionts, was found to engage in a specific and beneficial interaction with fungi from the order Helotiales (Ascomycota), which provides soil nutrients (i.e., phosphate) to their hosts (62). Although we do not yet know how widespread this phenomenon is, it raises the intriguing possibility that some of our species without AM fungi have in fact evolved interactions with yet unknown beneficial root symbionts functionally similar to mycorrhizal fungi. This would further strengthen the relationship we observed among AM loss and switches to alternative symbionts. Another, non-mutually exclusive possibility is that plants abandoning the AM fungal mutualism without evolving alternatives are likely to go extinct after an evolutionarily short period of time or rapidly reestablish the mutualism. In line with this, all cases of AM breakdown not coupled to an alternative (Fig. 2, yellow state) have evolved fairly recently (<30 Mya) compared with many much older losses associated with symbiont switching or alternative strategies (e.g., the switch to ECM fungi in pines more than 200 Mya).

An alternative potential reason for mutualism breakdown is when cheaters, low-quality partners, or parasites arise in one of the partner lineages (24, 25). This can drive the interaction from mutual benefit to parasitism and cause the other partner to abandon the interaction (10). Theory and empirical work suggests that hosts are particularly vulnerable to cheating when partners are acquired directly from the environment, like AM fungi (4, 5, 63–65). However, in bacteria, phylogenetic work has shown that, although transitions toward cooperative states are common, loss of mutualist status is rare for bacterial symbionts (66, 67). When these losses occur, bacteria are more likely to revert to a free-living state than to become parasites (66, 67). In our case, such a reversion to a free-living state would correspond to a plant evolving an abiotic adaptation to replace AM fungi, such as cluster roots. Although most of our ~25 losses can be explained in terms of symbiont switches or alternative resource strategies (Fig. 2), some of the switches to other root symbionts or resource strategies we observed could initially have been driven by the fitness cost of parasitic AM fungi.

Our analyses show that cooperation among plants and AM fungi has generally persisted in a highly stable state for more than 350 My. This illustrates the importance of mutualistic services provided by AM fungi for most host plant species. However, even ancient and versatile mutualists like AM fungi can be completely and permanently lost in the right circumstances: we estimate this happened ~25 times. In general, mutualistic partnerships allow organisms to outsource crucial functions to other species, thereby obtaining

these services more efficiently (5). Our results highlight how a key mutualistic service like nutrient acquisition is only permanently lost if hosts evolve symbiotic or abiotic alternatives to obtain these functions.

Methods

More details of study methods are provided in *SI Appendix, Extended Methods*.

Mycorrhizal Status Database. We compiled our database of reported plant mycorrhizal status by obtaining data from primary literature and publicly accessible databases. Our full data source list, as well as our scoring criteria, are provided in *SI Appendix, Extended Methods*. Our analyzed database contained data for a total of 3,736 spermatophyte species (3,530 angiosperms, 206 gymnosperms, 61 orders, 230 families, and 1,629 genera) that overlapped with the phylogeny used in our analysis (31) and is available online ([Dataset S1](#)).

Reconstruction of the Evolution of AM Interactions. We used a hidden Markov model approach called HRMs, which allows for heterogeneity in the loss and gain rates of a binary trait across a phylogeny (38). We used the R package corHMM (38) (version 1.18) in R 3.2.3 to analyze our mycorrhizal data and explored HRMs with one to five rate classes by using AICc weights to select the best HRM among this family of candidate models (Table 1). We used the marginal method to perform ancestral state reconstructions and employed Yang's method to compute the root state (68). We a posteriori labeled the three rate classes under the best model: "stable AM," "labile," and "stable non-AM" (*SI Appendix, Fig. S1*).

Database Alternative Resource Acquisition Strategies. We generated a second database for all our 3,376 analyzed species and scored each species for the presence or absence of three main resource strategies, which each represent an alternative way of extracting minerals from the environment: carnivory (36), parasitism (41, 42), and cluster roots (37). Based on our previously generated database of plant species associating with symbiotic nitrogen-fixing bacteria (35, 39), we also assigned all analyzed species a binary symbiotic nitrogen-fixation status. Full data sources and scoring procedures are provided in *SI Appendix, Extended Methods*.

Correlated Evolution of AM Interactions and AM Alternatives. We generated HRM models (38) of non-AM mycorrhizal fungi and adaptations for resource acquisition (*SI Appendix, Table S1*), plotted them onto our AM ancestral state reconstruction, and visually identified the origins of these AM alternatives (*SI Appendix, Fig. S3–S9*). We then tested the potential for correlated evolution among AM fungi, other mycorrhizal fungi, and resource acquisition adaptations. By using Akaike information criteria, we compared models of dependent and independent evolution (43) among the binary variables AM and AM alternatives. We used the maximum-likelihood implementation of the discrete module in BayesTraits V2 and constrained the ancestral node of the phylogeny to have AM fungi but none of the alternatives, as that is what our previous analyses had revealed (*SI Appendix, Figs. S3–S9*).

Sensitivity Analysis to Phylogenetic and Data Uncertainty. We studied the robustness of our main conclusions to two main sources of uncertainty: phylogenetic uncertainty and uncertainty in the underlying mycorrhizal data. We reran our key models (three rate class HRM and correlated evolution models in BayesTraits) across 100 bootstrap phylogenies (31) (*SI Appendix, Figs. S11 and S12*). To test for effects of data uncertainty, we used a resimulation approach that for each species takes into account the number of observations of a given mycorrhizal state and simulates different error rates for underlying mycorrhizal observations (*SI Appendix, Fig. S13 and Table S2*). The full approach to the sensitivity analyses is detailed in the *SI Appendix, Extended Methods*.

Data Availability. Our full dataset, including the number of reports of various mycorrhizal states across databases (*SI Appendix, Extended Methods*), our resulting assignment of AM, ECM, ORM, ER, ARB, and symbiotic N_2 -fixation states, and our assignment of alternative resources acquisition strategies (carnivory, parasitism, mycoheterotrophy) is available online ([Dataset S1](#)).

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