

1 **Symbiont switching and alternative resource acquisition strategies drive**  
2 **mutualism breakdown**

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20

21 **Abstract**

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

32

33

## 34 **Significance Statement**

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

45

## 46 **Introduction**

47 Mutualisms, cooperative partnerships among different species, have shaped much of Earth's  
48 biodiversity, allowing organisms to outsource crucial functions like nutrition, cleaning,  
49 transport and defence (1, 2). Both theoretical and empirical work has provided us with a good  
50 understanding of the mechanisms, such as co-transmission and sanctions, that stabilise  
51 mutualism and maintain cooperation among species (3–5). Because of these mechanisms,  
52 beneficial interactions can be maintained over millions of years, and in some cases give rise  
53 to extreme mutualistic dependence (6, 7).

54

55 Despite reciprocal benefits, mutualisms do not always persist, and conflict among partners  
56 can remain. Theoretical and experimental work suggests that even when mutual benefits  
57 occur, fitness interests of both partners are generally not perfectly aligned, potentially  
58 selecting for cheaters and exploiters of mutualism (5, 8–11). This finding is further reinforced  
59 by the observation that in many mutualisms, there are mechanisms to evaluate partner quality  
60 and reward cooperation or sanction non-cooperative cheats (12–14). Furthermore, over  
61 ecological time, short-term breakdowns of cooperation in response to shifting environmental  
62 conditions, have been observed in many mutualisms, including plant rhizobial and  
63 mycorrhizal mutualisms, coral symbioses, protection and pollination mutualisms (15–19).  
64 Together, these observations raise the question in which conditions we should expect  
65 cooperation among species to fail, and partners in previously successful mutualisms to cease  
66 cooperating.

67

68 Even mutualisms that have become highly dependent over millions of years of co-evolution,  
69 have broken down in some occasions. This is the case, for example, when free-living fungi  
70 evolved from a previously lichenised lifestyle, or when parasitic moths evolved from  
71 pollinating ancestors (20–22). Yet, while we have a good understanding of why mutualistic  
72 cooperation is favoured, we lack a general understanding of the drivers of these evolutionary  
73 breakdowns of mutualisms. A number of non-exclusive reasons for the breakdown of  
74 mutualisms have been proposed (23, 24). Benefits provided by a mutualistic partner can  
75 become redundant through the evolution of alternative adaptations. In these cases, one of the  
76 partners switches from relying on another species to acquiring a function autonomously. For  
77 example, the evolution of large amounts of small-diameter pollen enabled the reversion back  
78 to an autonomous, wind-pollinated lifestyle in some angiosperms (25). A second trajectory  
79 occurs when one side of the interaction is replaced with a new mutualist species. While  
80 partner switching by definition leads to the evolution of a new partnership, the ancestral  
81 interaction is lost and thus a previously functional mutualism breaks down. This is illustrated  
82 in cases where plant species stop cooperating with birds and switch to insect pollination (26).

83

84 Our aim was to study the ancient and ubiquitous mutualism between plants and arbuscular  
85 mycorrhizal (AM) fungi to understand pathways towards mutualism breakdown. We focus on  
86 the plant-AM mutualism for three reasons. First, AM fungi (Glomeromycota) are among the  
87 most important terrestrial mutualists. AM fungi form extensive hyphal networks in the soil  
88 (up to 100 m cm<sup>-3</sup> soil), providing plants with a key solution to the problem of extracting  
89 immobile nutrients, especially phosphorus (27). The partnership is crucial for plant growth,  
90 providing hosts with primarily phosphorus, but also nitrogen, water and trace elements (28).  
91 Second, even though the large majority of plants can be successfully colonised by AM fungi,  
92 10-20% of plant species across a number of divergent clades do not interact with any AM  
93 fungi (27, 29). These repeated losses of the interaction, separated by millions of years of  
94 evolution, enable us to test general patterns and explanatory factors driving cooperation loss  
95 in a comparative framework. Third, the tools and databases allowing for broad comparative  
96 analyses are becoming available for plants, including a comprehensive phylogeny of seed  
97 plants (30), and large scale databases of plant traits including their association with AM fungi  
98 and other root symbionts (31–33).

99

100 In our analysis of the plant – AM mutualism, we take a plant-centric perspective. We are  
101 interested in cases where plants completely cease to interact with *all* AM fungi and where

102 this lack of interaction persists over evolutionary time: *i.e.* where the loss of the interaction is  
103 not followed by host plant extinction. Thus, we do not study when plant-AM cooperation  
104 dissolves in the short-term due to ecological conditions, such as under high nutrient  
105 conditions. Rather, our aim is to first quantify stable losses of cooperation, and then test the  
106 importance of two types of evolutionary breakdown: partner switching and mutualism  
107 abandonment. By partner switching, we mean a situation where a host plant that ancestrally  
108 interacted with AM fungi, switched to interacting with a novel root symbiont with similar  
109 function and ceased interacting with AM fungi. We analyse switches to other mycorrhizal  
110 fungi, as well as to N<sub>2</sub>-fixing symbioses with rhizobial and *Frankia* bacteria (28, 34). We  
111 refer to mutualism abandonment, when plants have evolved an alternative strategy to acquire  
112 resource in a non-symbiotic way, for instance carnivory or cluster roots (35, 36).

113

114

## 115 **Results**

### 116 *Evolutionary reconstruction of the plant-AM fungal mutualism*

117 Our first aim was to quantify the evolutionary stability of the plant-AM mutualism,  
118 determining the number of losses of plant-AM interactions across the plant phylogeny. We  
119 compiled a global database of plant mycorrhizal fungal status across the seed plants  
120 (angiosperms and gymnosperms). We scored the reported interactions of plants with AM  
121 fungi in 3,736 plant species present in the most recent and comprehensive phylogeny of  
122 gymnosperms and angiosperms (30). We then established patterns of AM loss and gain using  
123 a Hidden Rate Model (HRM) approach to ancestral state reconstructions (37). This technique  
124 permits variation in the speed of binary character evolution so we can detect changes in rates  
125 of evolution, such as shifts in the evolutionary stability of plant-AM associations.

126

**Table 1: AICc-values and weights for all HRM models**

Number of rate classes	Number of parameters	AICc	$\Delta$ -AICc	AICc-weight
1	2	3231.25	770.0	0.0%
2	8	2597.78	136.5	0.0%
<b>3</b>	<b>14</b>	<b>2461.25</b>	<b>0</b>	<b>74.8%</b>
4	20	2463.44	2.2	25.1%
5	26	2473.79	12.5	0.1%

*Table of the five different HRMs explored to analyse our AM fungal association data (see Methods). We used AICc-weights (corrected Akaike information criterion) to determine the model with the best fit (bold).*

127

128 Our reconstructions revealed that the evolution of AM interactions across seed plants was  
129 best characterised by heterogeneity in speed of evolution: the best evolutionary model  
130 contains three different rate classes of evolution (Table 1). Specifically, we find strong  
131 evidence for the existence of an evolutionary class where AM interactions are strongly  
132 favoured (which we termed Stable AM), a class where an absence of AM interactions is  
133 strongly favoured (Stable Non-AM), and a class where AM interactions are evolutionarily  
134 labile (SI Figure 1).

135

136 Mapping these different evolutionary states back onto the phylogeny (SI Figure 2), we found  
137 that: (i) Association with AM fungi was likely the ancestral state of seed plants (99.6%  
138 likelihood); (ii) Stable AM fungal associations have been widely retained throughout the seed  
139 plants for over 350 million years, and represent the large majority of all historical and  
140 contemporary plant species and families (Table 2); (iii) some plant lineages evolve to either  
141 an evolutionarily labile state or a state where AM fungi are disfavoured (SI Figure 1, Table 2).  
142 Specifically, (iv) there have been an estimated ~25 evolutionary losses of the AM mutualism  
143 throughout the history of seed plants, found across 69 families (median over 100 bootstrap  
144 phylogenies 25.4, SD: 7.73). Which evolutionary trajectories are most important in  
145 explaining these breakdowns of cooperation among plants and AM fungi?

146

**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	2613 (SD 178)	172	171 (SD 12)
Labile	829	833 (SD 180)	77	79 (SD 13)
Stable Non-AM	291	288 (SD 21)	8	7 (SD 3.7)

*Numbers of contemporary species and families per AM stability class.*

*Median value and SD across 100 bootstrap phylogenies are indicated.*

147

148 *Symbiont switching and mutualism abandonment drive breakdown*

149 We tested the hypotheses that AM loss is driven by shifts to other symbionts (partner  
150 switching) or by alternative adaptations for resource acquisition (abandonment). We  
151 generated a database of other major root symbionts with functional roles (providing  
152 phosphorus and nitrogen) similar to AM fungi. Specifically, based on a previously published  
153 database, we included presence or absence of a potential to interact with symbiotic N<sub>2</sub>-fixing  
154 bacteria (both rhizobial and *Frankia* bacteria) for all our host plant species (34, 38). We also  
155 included reported interactions with other mycorrhizal fungi (*i.e.* non-AM fungi that live in

156 symbiotic association with plant roots). This included ectomycorrhizal (EM), ericoid  
157 mycorrhizal (ER), orchid mycorrhizal (ORM) and arbutoid mycorrhizal (ARB) fungi. All  
158 AM fungi belong to the division *Glomeromycota*, while other mycorrhizal fungi are only  
159 distantly related, belonging to a wide range of divisions, mainly *Basidiomycota* (ECM, ARB  
160 and ORM, some ER) and *Ascomycota* (some ECM and ORM and most ER). Some plant  
161 species interact with multiple types of mycorrhizal fungi (28, 39).

162

163 We scored our species for the reported presence of alternative resource acquisition strategies.  
164 These included parasitism as a plant strategy (both plants parasitising other plants and full  
165 mycoheterotrophs, *i.e.* plants parasitising mycorrhizal fungi) (40, 41), carnivory (35) and  
166 cluster roots (36) (Figure 1). These strategies have in common that they represent alternative  
167 solutions to the problem of acquiring scarce mineral resources: they acquire resources by  
168 seizing them from other organisms (plant parasitism), through direct predation (carnivorous  
169 plants), or through investing in a unique root architecture characterised by a high density of  
170 finely-branched roots and root hairs, known as cluster roots (Figure 1). To study congruence  
171 between losses of AM interactions and alternative strategies, we again performed ancestral  
172 state reconstructions, to study the origins of: (i) other symbionts (*i.e.* non-AM mycorrhizal  
173 fungal symbionts or symbiotic N<sub>2</sub>-fixation), which were present in 820 of our 3,736 plant  
174 species; and (ii) alternative resource acquisition strategies, present in 109 plant species.

175

176 We found a high degree of congruence between the different origins of AM losses and of  
177 various AM-alternatives (SI Table 1, SI Figures 3-9). To study this quantitatively, we  
178 compared models of dependent *vs.* independent evolution (42), analysing the relationship  
179 between AM loss and presence of alternative partners or alternative resources acquisition  
180 strategies. We studied a binary variable coding for presence of any AM alternative and found  
181 that a dependent model of evolution vastly outperformed an independent model ( $\Delta$ -AICc  
182 428.90, AICc-weight 99.9%). This means that over evolutionary time, AM loss (shift from  
183 the bottom left plane to the top right in the transitions matrix, Figure 2) is strongly associated  
184 with the presence of another mycorrhizal fungal partner, or alternative resource strategy.  
185 Thus, partner switching and mutualism abandonment are important in enabling evolutionary  
186 breakdown of the ancestral plant-AM fungal mutualism throughout the seed plants.

187

188 More specifically, from the inferred transition matrix and associated ancestral state  
189 reconstruction (Figure 2), we conclude that: (i) The AM mutualism is generally highly stable  
190 - transition rates towards the AM state (green) are about ten times as high as losses (from  
191 green to yellow) (ii) AM fungal loss is only stable when an alternative is present (orange to  
192 red) and not without (green to yellow). (iii) While evolutionary stability is high when plants  
193 associate with either AM fungal symbionts (green state) or an alternative symbiont or  
194 acquisition strategy (red), having neither (yellow) is evolutionarily unstable. For instance, all  
195 the origins of this type (*e.g.* in the Brassicales) have occurred relatively recently in  
196 evolutionary terms (within the last 30 million years). (iv) Similarly, it is evolutionarily less  
197 stable to have both AM and an alternative simultaneously (orange).

198

199 Our reconstructions show that both the evolutionary scenario of initial AM loss followed by  
200 alternative strategy evolution and the reverse order are possible. Initial acquisition of an AM-  
201 alternative (move from green to orange state), in some cases may have resulted in relaxed  
202 selection to maintain the AM interaction, allowing for its subsequent evolutionary breakdown  
203 (orange to red). In other cases, the AM interaction was lost first (yellow state), for instance  
204 through symbiosis gene loss (43), and survival of host plants was subsequently favoured  
205 when rapidly evolving an AM alternative state. Thus, our analysis indicates there is no single  
206 dominant trajectory in the transition from an AM plant to a stable non-AM plant, but that  
207 both routes can occur.

208

### 209 *Sensitivity Analyses*

210 To verify the robustness of our results, we considered the sensitivity of our main conclusions  
211 to two forms of uncertainty, (1) phylogenetic uncertainty and (2) uncertainty in the  
212 underlying AM data. We analysed phylogenetic uncertainty by replicating our initial AM  
213 fungal reconstruction analysis over 100 bootstrap phylogenies, and found highly similar  
214 relative loss rates of the plant AM-interaction throughout 100% of our bootstrap replicates  
215 (SI Figure 10) and highly similar ancestral state reconstructions (SI Figure 11). We also  
216 found that across the 100 bootstrap phylogenies a dependent model of evolution always  
217 outperformed an independent model (mean  $\Delta$ -AICc 390.52). This further confirms the deep  
218 evolutionary link between AM loss and the evolution of other symbionts and resource  
219 acquisition strategies regardless of the details of the phylogenies used (SI Figure 12).

220

221 A second main source of uncertainty is in the AM status of plants. This is because AM fungi  
222 are notoriously difficult to score: it is easy to misidentify other fungi as AM fungi (false  
223 positive) or to miss AM hyphae (false negative). To address this, we implemented a re-  
224 simulation approach which takes into account the number of independent reports of AM  
225 status, and allows us to test separate false positive and false negative rates for these  
226 underlying reports. We found that even if one in four of the AM reports in our database is  
227 incorrect (e.g. a saprotrophic fungus) while simultaneously 25% of our AM absence reports  
228 in fact were mycorrhizal, we still draw highly similar conclusions (SI Figure 13, SI Table 2).  
229 Therefore, even if we assume the underlying mycorrhizal data are of very poor quality, we  
230 recover qualitatively highly similar patterns. Thus, overall, we conclude that all our main  
231 conclusions are robust to substantial phylogenetic and data uncertainty.

232  
233 As a final analysis, we compared our results with a recent comparative analysis of plant-  
234 mycorrhizal symbioses (29). This analysis used an alternative scoring approach that divided  
235 plant species in four categories: AM plants, non-mycorrhizal plants (NM-plants), ECM plants,  
236 and plants that are commonly found in either AM or NM states (AMNM-plants), and found  
237 that transitions from AM towards NM states primarily go through the AMNM state. We re-  
238 confirmed this result, in that we find in our best HRM-model of plant-AM interactions that  
239 plants transition through the labile state to the stable non-AM state, where the loss of plant-  
240 AM mutualism becomes evolutionarily entrenched (SI Figure 1). We also find that the  
241 species-level percentage of observations with AM-presence has a median value of 100% (SE:  
242 0.89%; mean 83.4%) for species inferred to be in the stable AM class and 0% in the stable  
243 non-AM class (SE: 0.73%; mean 1.58%), while in the evolutionarily labile class this is 16.7%  
244 (SE: 1.57%; mean 22.0%; SI Figure 14). This indicates that the labile state inferred under our  
245 deep evolutionary model effectively recovers the notion of an AMNM presupposed by  
246 Maherali *et al.* While their analysis allows for direct inclusion of AMNM and ECM states,  
247 with our approach of binary coding the presence or absence of AM and other mycorrhizal  
248 interactions we can answer different questions: (i) It allows us to infer the *variation* in loss  
249 rate of the AM mutualism across seed plant evolutionary history (which is only possible in  
250 the HRM-framework for binary traits (37)) (ii) Rather than *a priori* defining an intermediate  
251 state, it allows us to verify if an evolutionarily labile state is actually inferred in our best  
252 model. (iii) It allows us to study the dependent evolution of AM and other mycorrhizal  
253 interactions as separate traits. This is especially important because, while rare, dual  
254 colonisation of plants by two types simultaneously is possible and could represent an



255 important evolutionary intermediary state, as confirmed by our analysis (Figure 2). (4) It  
256 allows us to include in our analysis not just ECM fungi, but also other root symbionts such as  
257 symbiotic N<sub>2</sub>-fixation, ericoid (ERM) and orchid (ORM) mycorrhizal fungi, which turned out  
258 to be drivers of major evolutionary losses of the plant-AM mutualism (Figure 2).

259

## 260 **Discussion**

261 Our analyses revealed that the ancient and ubiquitous plant-AM fungal mutualism has  
262 broken down in ~25 cases across the seed plants. We found that stable and persistent  
263 mutualism breakdown is driven both by acquisitions of other root symbionts (partner  
264 switching) and by the evolution of alternative non-symbiotic resource acquisition strategies  
265 (mutualism abandonment).

266

267 These results in turn raise the question of what underlying ecological factors favour  
268 transitions to these alternative solutions, and the mechanisms that enable them.  
269 Mechanistically, an important step is likely the loss of key genes in the ‘symbiotic toolkit’  
270 encoding crucial root mutualism effectors (43, 44). This must either be followed or preceded  
271 by molecular evolution in the genes encoding alternative symbioses or resource acquisition  
272 traits. Ecologically, these alternatives can potentially be favoured by a range of ultimate  
273 factors, such as environmental change, habitat shifts (for instance to high-nutrient soils),  
274 migration, invasion or partner abundance (22, 24, 45–49), although discriminating these over  
275 deep evolutionary time is challenging. One hypothesis is that switching from the AM nutrient  
276 uptake strategy to rarer alternative strategies has enabled plants to compete in a range of  
277 (micro)habitats. Evolution of carnivory in temperate swamps (35), cluster-roots in extremely  
278 phosphorus-impoverished soils (36), cold-resistant ectomycorrhizal interactions in lower  
279 temperature habitats (50) and ericoid mycorrhizal fungi in resource-poor heath lands (51) has  
280 helped host plants to thrive in environments where the more common AM interaction is a less  
281 successful solution to obtain nutrients.

282

283 We emphasise that our estimate of ~25 breakdowns represents a conservative lower bound,  
284 since we study plants that stopped interacting with *all* AM fungal species and subsequently  
285 persisted over evolutionary times. The number of breakdowns of plant mutualism with  
286 specific AM fungal species or lineages while cooperation with other AM continued, is likely  
287 to be considerably higher. More generally, studies that analyse breakdowns of symbiotic  
288 interactions with entire taxa of organisms - such as among corals and any photosynthetic

289 dinoflagellates (52, 53) – will underestimate the number of breakdowns with specific  
290 symbiont species.

291

292 We find that dual symbioses – simultaneously being able to interact with two types of root  
293 symbionts - is unlikely to be evolutionarily stable (Figure 2). This is a different pattern  
294 compared to what is documented in insect endosymbioses, which often acquire secondary  
295 partners while retaining the ancestral mutualism (54, 55). In insects, maintenance of two  
296 endosymbionts could be favoured by different microbial partners subsequently specialising  
297 on different mutualistic functions (56, 57). In root symbioses, nutritional benefits provided by  
298 AM fungi and by alternative root symbionts may often be too similar to outweigh the costs of  
299 maintaining them both. For instance, while AM fungi are thought to provide primarily  
300 phosphorus, they also contribute nitrogen to their hosts (58). This could help explain why  
301 plants only rarely associate with both ECM fungi and AM fungi simultaneously (131 species  
302 in our dataset). However, our reconstructions suggest that such dual symbioses can be a  
303 transitory state on the path towards a complete switch and breakdown of the original  
304 mutualism (Figure 2), as was previously hypothesised (59). Other root symbionts may  
305 provide more complementary benefits to their plant hosts, which could select for maintenance  
306 of dual symbioses. For instance, AM fungi and N<sub>2</sub>-fixing rhizobial bacteria are often thought  
307 to provide complementary benefits to their legume hosts (60), although a meta-analysis did  
308 not generally find synergistic effects on host growth (61).

309

310 If breakdown of the AM fungal mutualism is driven by acquisition of other root symbionts or  
311 alternative resources strategies, how can we explain plants that have neither AM fungi nor an  
312 alternative (yellow in Figure 2)? Recently, a member of the Brassicaceae, a family generally  
313 lacking mycorrhizal symbionts, was found to engage in a specific and beneficial interaction  
314 with fungi from the order Helotiales (Ascomycota), which provides soil nutrients (phosphate)  
315 to their hosts (62). While we do not know how widespread this phenomenon yet is, it raises  
316 the intriguing possibility that some of our species without AM fungi have in fact evolved  
317 interactions with yet unknown beneficial root symbionts functionally similar to mycorrhizal  
318 fungi. This would further strengthen the relationship we observed among AM loss and  
319 switches to alternative symbionts. Another, non-mutually exclusive possibility is that plants  
320 abandoning the AM fungal mutualism - without evolving alternatives - are likely to go  
321 extinct after an evolutionarily short period of time, or rapidly re-establish the mutualism. In  
322 line with this, all cases of AM breakdown not coupled to an alternative (yellow state; Figure

323 2), have evolved fairly recently (<30 MYA), compared to many much older losses associated  
324 with symbiont switching or alternative strategies (e.g. the switch to ECM fungi in Pines,  
325 more than 200 MYA).

326

327 An alternative potential reason for mutualism breakdown is when cheaters, low quality  
328 partners or parasites, arise in one of the partner lineages (23, 24). This can drive the  
329 interaction from mutual benefit to parasitism, and cause the other partner to abandon the  
330 interaction (9). Theory and empirical work suggests that hosts are particularly vulnerable to  
331 cheating when partners are acquired directly from the environment, like AM fungi (4, 5, 63–  
332 65). However, in bacteria, phylogenetic work has shown that while transitions towards  
333 cooperative states are common, loss of mutualist status is rare for bacterial symbionts (66,  
334 67). When these losses occur, bacteria are more likely to revert to a free-living state than to  
335 become parasites (66, 67). In our case, such a reversion to a free-living state would  
336 correspond to a plant evolving an abiotic adaptation to replace AM fungi, such as cluster  
337 roots. While most of our ~25 losses can be explained in terms of symbiont switches or  
338 alternative resource strategies (Figure 2), some of the switches to other root symbionts or  
339 resource strategies we observed could initially have been driven by the fitness cost of  
340 parasitic AM fungi.

341

342 Our analyses show that cooperation among plants and AM fungi has generally persisted in a  
343 highly stable state for over 350 million years. This illustrates the importance of mutualistic  
344 services provided by AM fungi for most host plant species. Yet, even ancient and versatile  
345 mutualists like AM fungi can be completely and permanently lost in the right circumstances:  
346 we estimate this happened ~25 times. In general, mutualistic partnerships allow organisms to  
347 outsource crucial functions to other species, thereby obtaining these services more efficiently  
348 (5). Our results highlight how a key mutualistic service like nutrient acquisition is only  
349 permanently lost if hosts evolve either symbiotic or abiotic alternatives to obtain these  
350 functions.

351

## 352 **Methods**

353 More detailed Extended Methods can be found in the online Supporting Information.

354

355 *Mycorrhizal status database*

356 We compiled our database of reported plant mycorrhizal status by obtaining data from both primary  
357 literature and publicly accessible databases. Our full data source list, as well as our scoring criteria  
358 can be found in the Extended Methods (Supporting Information). Our analysed database contained  
359 data for a total of 3,736 spermatophyte species (3,530 angiosperms, 206 gymnosperms, 61 orders, 230  
360 families and 1,629 genera) that overlapped with the phylogeny used in our analysis (30), is available  
361 online (Supporting Data 1).

362

### 363 *Reconstruction of the evolution of AM interactions*

364 We used a Hidden Markov Model approach called ‘Hidden Rate Models’ (HRMs) which allows for  
365 heterogeneity in the loss and gain rates of a binary trait across a phylogeny (37). We used the R-  
366 package *corHMM* (37)(version 1.18) in R 3.2.3 to analyse our mycorrhizal data and explored HRMs  
367 with one to five rate classes, using AICc-weights to select the best HRM among this family of  
368 candidate models (Table 1). We used the marginal method to perform ancestral state reconstructions  
369 and employed Yang’s method to compute the root state (68). We *a posteriori* labelled the three rate  
370 classes under the best model ‘Stable AM’, ‘Labile’ and ‘Stable Non-AM’ (SI Figure 1).

371

### 372 *Database Alternative Resource Acquisition Strategies*

373 We generated a second database for all our 3,376 analysed species and scored each species for the  
374 presence or absence of three main resource strategies, which each represent an alternative way of  
375 extracting minerals from the environment: carnivory (35), parasitism (40, 41) and cluster roots (36).  
376 Based on our previously generated database of plant species associating with symbiotic nitrogen-  
377 fixing bacteria (34, 38), we also assigned all analysed species a binary symbiotic nitrogen-fixation  
378 status. We describe our full data sources and scoring procedures in the Extended Methods.

379

### 380 *Correlated evolution of AM interactions and AM-alternatives*

381 We generated HRM-models (37) of both non-AM mycorrhizal fungi and adaptations for resource  
382 acquisition (SI Table 1), plotted them onto our AM ancestral state reconstruction and visually  
383 identified the origins of these AM-alternatives (SI Figure 3-9). We then tested the potential for  
384 correlated evolution among AM fungi, other mycorrhizal fungi and resource acquisition adaptations.  
385 Using AIC-criteria, we compared models of dependent and independent evolution (42, 69) among the  
386 binary variables AM and AM-alternatives. We utilised the Maximum Likelihood implementation of  
387 the Discrete-module in *BayesTraits V2*, and constrained the ancestral node of the phylogeny to have  
388 AM fungi but none of the alternatives, as that is what our previous analyses had revealed (SI Figures  
389 3-9).

390

### 391 *Sensitivity analysis to phylogenetic and data uncertainty*

392 We studied the robustness of our main conclusions to two main sources of uncertainty: phylogenetic  
393 uncertainty and uncertainty in the underlying mycorrhizal data. We reran our key models (three rate  
394 class HRM and correlated evolution models in *BayesTraits*) across hundred bootstrap phylogenies  
395 (30) (SI Figure 11 and 12). To test for effects of data uncertainty, we used a resimulation approach  
396 that for each species takes into the number of observations of a given mycorrhizal state and simulates  
397 different error rates for underlying mycorrhizal observations (SI Figure 13, SI Table 2). We detail our  
398 full approach to the sensitivity analyses in the Extended Methods.

399

#### 400 **Data Availability**

401 Our full dataset, including number of reports of various mycorrhizal states across databases  
402 (see Extended Methods), our resulting assignment of AM, ECM, ORM, ER, ARB and symbiotic N<sub>2</sub>-  
403 fixation states, and our assignment of alternative resources acquisition strategies (carnivory,  
404 parasitism, mycoheterotrophy) is available online (Supporting Data 1).

405

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538

### 539 **Acknowledgements**

540 We thank SURFsara ([www.surf-sara.nl](http://www.surf-sara.nl)) for support in using the Lisa Computing Cluster. The  
541 AM and AM-alternative illustrations in Figures 1 and 2 are based on figures in the public domain  
542 (CC0) with the exception of the illustrations for ericoid (courtesy Dr. David Midgley, CC-BY-SA)  
543 and arbuscular mycorrhizae (courtesy dr. Yoshihiro Kobae), root nodules (courtesy Dr. Euan James)  
544 and the illustrations for cluster roots (70) and orchid mycorrhizae (71) which were reprinted with  
545 permission from the respective publishers. The study has been supported by the TRY initiative on  
546 plant traits (<http://www.trydb.org>). GDAW was funded by a Royal Society Newton International  
547 Fellowship and a Junior Research Fellowship at Balliol College Oxford. ETK was funded by  
548 Netherlands Organisation for Scientific Research Grants 836.10.001 and 864.10.005 and European  
549 Research Council ERC Grant Agreement 335542.

550

### 551 **Figure Legends**

552 Figure 1: We explored the evolution of various alternatives to interacting with AM fungi, the ancestral  
553 state of seed plants. Examples of six important alternatives we considered are depicted, with columns  
554 indicating two potential pathways towards evolutionary breakdown of the plant-AM mutualism. In both  
555 pathways, the ancestral mutualism with AM fungi breaks down. Coloured borders match coloured bars in  
556 Figure 2, indicating distribution of these traits across global seed plants.

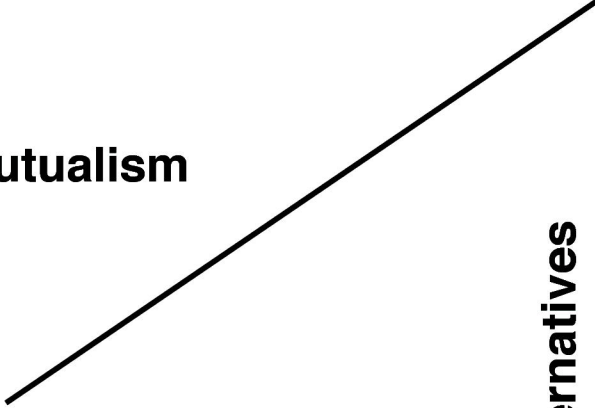
557

558 Figure 2: Transition rates and ancestral state reconstruction of the dependent evolutionary model for plant  
559 AM status and AM-alternatives. The four potential evolutionary states are represented by colours in the  
560 transition matrix and on the seed plant phylogeny. Transition rates are expressed as number of transitions  
561 per 100 million years per lineage. The ancestral state is AM presence with no AM-alternatives (green), red  
562 indicates the switch to one of the AM alternatives (i.e. another mycorrhizal fungus, symbiotic N<sub>2</sub>-fixation,



563 parasitism, carnivory or cluster roots). From inside to outside, coloured bands around the phylogeny  
564 indicate the presence or absence of (i) AM interactions, (ii) other root symbionts and (iii) alternative  
565 resource acquisition strategies. Key clades that have lost AM fungal interactions are indicated with  
566 schematic images of their evolved alternatives. Grey and white concentric circles indicate 50 million years.  
567 An expanded version with fully legible species labels for all 3,736 species is available online.

**Ancestral Mutualism**

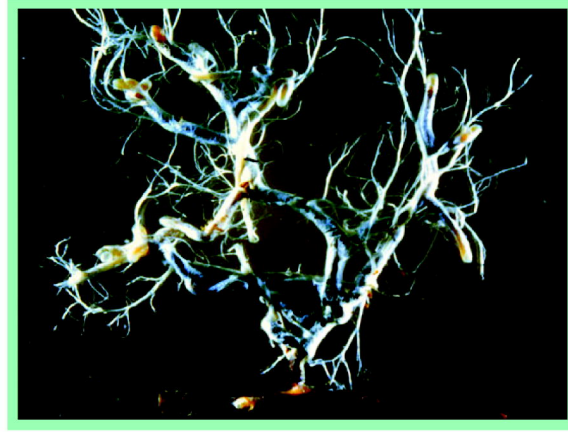


**Arbuscular Mycorrhizal Fungi Alternatives**



Arbuscular mycorrhizal (AM) fungi

**Symbiont Switching**

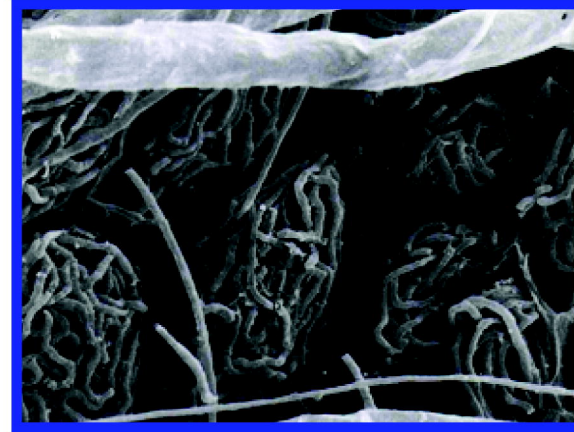


Ectomycorrhizal (ECM) fungi

**Mutualism Abandonment**



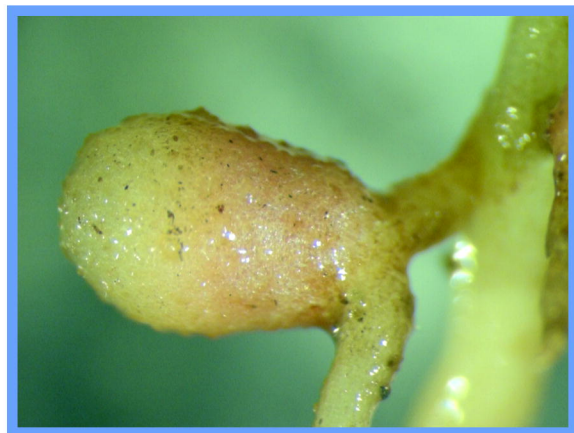
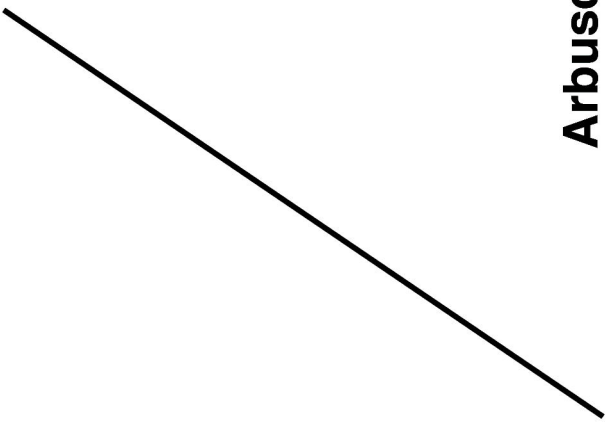
Carnivory



Orchid mycorrhizal (ORM) fungi



Cluster Roots



Bacterial symbiotic N<sub>2</sub>-fixation



Parasitic Plants

### Legend Bands

AM Interaction (Inner Band)

■ AM Present

Other symbionts (Middle Band)

■ Ectomycorrhizal

■ Orchid

■ Ericoid / Arbutoid

■ Symbiotic N<sub>2</sub>-fixation

Alternative strategies (Outer Band)

■ Carnivorous Plants

■ Cluster Roots

■ Parasitic Plants

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