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1 **Symbiotic options for the conquest of land**

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12

13 **The domination of Earth's landmasses by plants starting in the Ordovician Period**
14 **drastically altered the development of the biosphere and the composition of the**
15 **atmosphere, with far-reaching consequences for all life ever since. It is widely thought**
16 **that symbiotic soil fungi facilitated the colonisation of the terrestrial environment by**
17 **plants. However, recent discoveries in molecular ecology, physiology, cytology and**
18 **palaeontology have brought into question the hitherto-assumed identity and biology of**
19 **the fungi engaged in symbiosis with the earliest diverging lineages of extant land plants.**
20 **We reconsider the existing paradigm and show that the symbiotic options available to**
21 **the first plants emerging onto the land were more varied than previously thought.**

22

23 **The current paradigm**

24 The colonisation of the terrestrial environment by plants was a major turning point in Earth's
25 evolutionary history. In conquering the land, plants have sculpted the biosphere and
26 geosphere through their influence on global carbon, nutrient and water cycles [1], leading to
27 the development of climates and habitats essential for supporting the diverse array of life that
28 now exists in terrestrial environments [2,3]. It is estimated that more than 80% of living plant
29 species form symbioses with horizontally-transmitted filamentous fungi, representing 92% of
30 plant families worldwide [4] (Figure 1). These intimate associations are known as
31 'mycorrhizas', or 'mycorrhiza-like' when referring to plants lacking true roots such as non-
32 vascular plants. Through mycorrhizas and mycorrhiza-like associations, plants assimilate
33 fungus-acquired mineral nutrients far beyond their roots' soil depletion zones and from soil
34 pores too narrow for their root hairs. In return, plants supply their fungal symbionts with
35 carbohydrates fixed from atmospheric carbon dioxide through photosynthesis [5].

36 Plant life diversified on land some 70 MY after the diversification of most major
37 animal lineages in the seas during the Cambrian explosion (Fig. 1) [6]. Classic palaeoclimate

38 modelling shows that land plants diversified against a backdrop of falling atmospheric CO₂
39 [7] (Figure 2), likely driven by growing demand and evolving capacity for carbon
40 assimilation of the burgeoning Earth flora [8,9]. Long before land plants emerged, however,
41 the terrestrial environment had been colonised by fungi [10,11]. Among the early-branching
42 fungal lineages were those that today form mutualistic associations with most plants (Figure
43 1). The microfossil record for non-vascular plants is even more fragmentary than that for
44 vascular plants, though the study of microfossils is now providing key new data [12,13], and
45 molecular studies that date land plant origins and the divergence of the non-vascular groups
46 (i.e. liverworts, mosses and hornworts [14]), remain imprecise compared to those on
47 flowering plant evolution [15]. This has resulted in some doubt over the precise evolutionary
48 relationships both among non-vascular plants themselves and between non-vascular and
49 vascular plants [16,17]. Despite these lacunae, it has been proposed by plant and fungal
50 biologists [31,32] that initial plant colonisation of the terrestrial environment was facilitated
51 through interactions with symbiotic fungi. Such hypotheses are supported by data spanning
52 five decades of research and three scientific disciplines. Firstly, the incredibly well preserved
53 early-Devonian Rhynie chert microfossils show individual vascular plant cells harbouring
54 fungal structures that are strikingly similar to those formed by arbuscular mycorrhizal (AM)
55 fungi of the phylum Glomeromycota within living cells of modern vascular plants [18,19]
56 (see Box 1). Secondly, molecular evidence indicates that genes and biochemical signalling
57 pathways initiating and regulating the arbuscular mycorrhizal symbiosis are ubiquitous across
58 all living land plant lineages [20-23]. Thirdly, physiological data demonstrate nutritional
59 mutualisms between Glomeromycota fungi and living members of the earliest branching
60 lineages of land plants [24,25].

61 The resulting paradigm is that the earliest, rootless, terrestrial plants co-evolved with
62 Glomeromycota fungi [4,26-30] that in exchange for plant photosynthates, enhanced access

63 to soil mineral nutrients (Box 2). This hypothesis was elaborated in classic papers by T. H.
64 Nicolson, K. A. Pirozynski and D. W. Malloch [31,32]. All other mycorrhizal symbioses (i.e.
65 ectomycorrhizal, ericoid, orchid) are thought to have evolved through later switches to other
66 fungal lineages [33]. Even symbioses between flowering plants and nitrogen-fixing bacteria,
67 such as *Rhizobium*, must have evolved from the ancient AM symbiosis [21]. These diverse
68 plant-microbe partnerships continue to drive the establishment and development of terrestrial
69 ecosystems [33,34]. Recent discoveries showing that the earliest diverging groups of land
70 plants form mutualistic associations with fungi other than Glomeromycota mean it is now
71 timely to challenge the existing paradigm for plant and fungal colonisation of land. In this
72 review we bring together the most recent advances and emerging perspectives in the field,
73 leading to the development of a new paradigm for the conquest of the terrestrial environment.

74

75 **Early interactions between plants and fungi**

76 In early land plants associated with symbiotic fungi, movement of carbohydrates from
77 photosynthetic tissues would likely extend into the associated fungal hyphae [35]. In turn,
78 symbiotic fungi might have used those plant carbohydrates to power resource extraction and
79 plant provisioning from surrounding soil, greatly increasing the surface area for nutrient
80 capture and uptake in the earliest rootless plants - one of the most important but least
81 discussed adaptations to life on land. Compared to unicellular non-vascular plant rhizoids
82 and vascular plant root hairs, the multicellular filaments of fungi are much thinner, far longer
83 and able to branch and fuse, creating complex underground networks. The resulting
84 enhancement of biological mineral weathering by mycorrhizal and mycorrhiza-like
85 associations has been linked to the dramatic 90% decline in atmospheric CO₂ concentration
86 [36] and to pedogenesis in the Palaeozoic [2,37,38] (Figure 2). The acceleration of mineral
87 weathering by the first rooted vascular plants during the Devonian could have been preceded

88 by an earlier peak driven by rootless non-vascular plants during the Ordovician as suggested
89 by an experiment with living non-vascular plants [39]. Non-vascular plants are still major
90 contributors to global nitrogen and carbon cycling [40].

91 Some of the first plants to emerge onto land were morphologically similar to modern-
92 day liverworts [3,41], while others were unlike any extant species (Figure 1). As non-
93 vascular plants are the closest living relatives of the first land plants [42,43], they are the
94 most appropriate organisms for inferring experimentally the ecology and evolution of
95 partnerships between early land plants and fungi. Until recently this opportunity remained
96 untapped; the existing models (the moss *Physcomitrella patens* and the liverwort *Marchantia*
97 *polymorpha*) both lack symbiotic fungi (see Box 3) and no efforts had been made to
98 experimentally test the functioning of mycorrhiza-like associations in non-vascular plants.
99 Thus, evidence for homology in terms of both fungal identity and function relied solely on
100 morphological congruence [27] and the ecological nature of the intimate interactions between
101 non-vascular plants and fungi remained pure speculation [5].

102

103 **Fungi in extant early-diverging land plants**

104 Some commonalities in fungal symbiont morphologies between non-vascular and vascular
105 plants supported the long-held assumption that fungi observed in non-vascular plants belong
106 to the Glomeromycota [19] (see Box 1 and Figure 3c,d). Molecular identification of the
107 symbionts of some complex and simple thalloid liverworts supported this view [24,25].
108 Symbioses - of still untested function - of the more recently evolved fungal phyla
109 Basidiomycota (e.g., *Sebacina* and *Tulasnella*) and Ascomycota (e.g., *Pezoloma*) with
110 derived liverwort clades [19,44] demonstrate that fungal partners in modern liverworts are
111 not always conserved among groups. Nonetheless, given the early branching of liverworts
112 during land plant evolution (Figure 1), it was generally assumed that their fungal symbioses

113 predated those of vascular plants [4]. The unexpected identification of the fungal symbionts
114 of some liverworts as only being members of derived Glomeromycota (order Glomerales)
115 that are widespread in vascular plants, raised the alternative possibility that the earliest
116 lineages of symbiotic fungi were replaced by more derived ones through secondary shifts
117 from vascular to non-vascular plants [45,46].

118 However, this Glomeromycota-centred understanding of the nature of fungal
119 symbioses in land plants is now being revised. Through the application of inclusive
120 molecular detection methods, in 2011 Bidartondo et al. [47] discovered that the earliest
121 branching lineage of liverworts – the class Haplomitriopsida – do not harbour
122 Glomeromycota fungal partners, but instead associate with another group of early-diverging
123 fungi, the Mucoromycotina, considered either basal or sister to the Glomeromycota [48,49]
124 (Figure 1 and Figure 3b). Further investigation has revealed that symbiotic associations in
125 many other early divergent liverworts, most hornworts, and some early branching vascular
126 plants include unexpectedly diverse members of the Glomeromycota and/or diverse
127 Mucoromycotina fungi [47,50-52]. These findings raise the novel hypothesis that plant-
128 Mucoromycotina associations represent an ancestral land plant-fungal symbiosis. It is
129 possible that facultatively biotrophic and sexual Mucoromycotina fungi were gradually
130 replaced by the now strictly biotrophic and asexual Glomeromycota fungi as land plants grew
131 in stature, soil organic matter accumulated and atmospheric CO₂ levels dropped. This could
132 have resulted in shifts in the dynamics and trade-offs of early plant-fungal joint ventures [53].

133

134 **Plant-Glomeromycota symbiosis**

135 The vast majority of modern mycorrhizas are formed between plants and the arbuscule-
136 forming fungi of the Glomeromycota (Figure 1) (see Box 1). Glomeromycota fungi are
137 strictly biotrophic, relying entirely on their plant partners for organic carbon and to complete

138 their strictly asexual life cycle. The routes by which plants and fungi exchange carbon for
139 nutrients have been studied extensively in a wide range of flowering plants, by far the largest
140 group of extant plants. Flowering plants rapidly diversified and dominated the land during
141 the last 100 million years, outcompeting most earlier plant lineages in the majority of
142 terrestrial ecosystems - Darwin's "abominable mystery". The classic 2006 compilation by
143 Wang & Qiu [4] of mycorrhizal and mycorrhiza-like associations in 3,617 plant species from
144 659 publications confirmed the wide host range of AM fungi, indicating that the evolution of
145 Glomeromycota likely coincided with that of the earliest diverging land plants. Therefore, it
146 was firmly concluded that AM fungi formed the ancestral mycorrhizas, a view consistent
147 with the hitherto most widely-held hypothesis on the origin and evolution of land plant-
148 fungus symbiosis [54,55]. However, the functional significance of mycorrhiza-like
149 associations in non-vascular plants - although key to unravelling the role of plant-fungal
150 interactions in the evolution and diversification of land plants through the Phanerozoic - was
151 conjectural [27], having been bypassed by physiological research. Further, at that time it was
152 unknown that a wide range of non-vascular and early divergent vascular plant lineages
153 regularly form symbiotic associations with fungi of the Mucoromycotina [47,52,53,56].

154 We now know that liverwort-Glomeromycota symbioses can be mutualistic, involving
155 reciprocal exchange of organic carbon and nutrients between partners [25] with measureable
156 fitness benefits to both plant and fungus [24]. Similarly, the first studies on mycorrhizal
157 functioning in early branching lineages of vascular plants show their root fungal symbionts
158 are also mutualistic [25,53]. However, whilst vascular plants harbouring Glomeromycota
159 benefit from lower, near modern day atmospheric CO₂ in terms of reduced carbon costs per
160 nutrients gained from their fungal partners, the symbiotic efficiency of liverwort-
161 Glomeromycota associations is severely compromised by simulated Palaeozoic drops in CO₂
162 [25]. Given recent evidence indicating that the transporter proteins characteristic of vascular

163 plant-Glomeromycota symbioses are not operational in non-vascular plants [22], it is likely
164 that alternative nutrient exchange and translocation pathways are at work in these more
165 ancient partnerships thus resulting in their different responses to atmospheric CO₂
166 concentrations.

167

168 **Ancestral plant-Mucoromycotina symbiosis?**

169 The still-unfolding awareness that partnerships with Mucoromycotina fungi in non-vascular
170 and early branching lineages of vascular plants are widespread has major ramifications
171 throughout modern mycorrhizal research. The Mucoromycotina encompasses species that,
172 until the rise of molecular phylogenetics in the 1990s, were classified together with
173 Glomeromycota [30]. They include saprotrophic and sexual lineages such as *Endogone*,
174 *Sphaerocreas* and at least ten unnamed lineages [47,50-52,57] largely neglected by biologists
175 since the 1980s as the study of Glomeromycota arbuscular mycorrhizas blossomed. Both
176 Glomeromycota and Mucoromycotina have coenocytic hyphae occupied by specific
177 Mollicutes-related endobacteria [51]. Some Mucoromycotina have recently been cultured
178 axenically from host plants (Figure 3a) [53], so they should prove considerably more
179 orthodox and tractable in experiments than the obligate biotrophic Glomeromycota fungi.

180 Within hornworts, either fungal group might have been ancestral, the fungi occupy
181 varied locations within plants in close association with cyanobacteria, and symbiotic function
182 remains to be tested [50]. Within liverworts, so far only the Haplomitriopsida genera
183 *Haplomitrium* and *Treubia* are known to associate exclusively with Mucoromycotina. In
184 *Treubia* the fungus has distinct inter- and intracellular colonization zones in the thallus
185 midrib whilst in *Haplomitrium* it is confined to the epidermal layers in mucilage-secreting
186 subterranean axes. In both genera, the fungus produces abundant thin-walled intracellular
187 hyphal coils and unique short-lived swellings, which might serve as sites of carbon-for-

188 nutrient exchange and storage, potentially functioning like mycorrhizal arbuscules. Studies
189 thus far show unequivocally that Haplomitriopsida-Mucoromycotina associations are
190 mutualistic in terms of carbon-for-nutrient exchange, on a par with liverwort-Glomeromycota
191 partnerships and vascular plant arbuscular mycorrhizas [53]. These findings lend support to
192 the notion that associations with Mucoromycotina could be an ancestral state, giving way to
193 plant-Glomeromycota fungal associations as the atmosphere and soils developed and plants
194 diversified through the Palaeozoic.

195 The occurrence of Mucoromycotina and/or Glomeromycota symbioses across extant
196 early lineages of non-vascular and vascular plants challenges the hitherto monolithic
197 paradigm of arbuscular mycorrhizas as the ancestral land plant-fungal symbiosis, and it raises
198 the probability that early plant lineages, through their long evolutionary history, variously
199 became independent of fungi and then recruited them again. Similarly, some
200 Mucoromycotina and Glomeromycota fungi appear to have switched symbioses, e.g. some
201 *Endogone* form ectomycorrhizas with trees and *Geosiphon* harbour cyanobacteria,
202 respectively. The current, albeit fragmentary due to limited plant and fungal sampling,
203 understanding of the phylogenetic distribution of fungal symbioses in liverworts points to the
204 potential loss of ancestral Mucoromycotina symbiosis and the subsequent reacquisition of the
205 same plus Glomeromycota. Thus, Mucoromycotina fungi occur in the earliest diverging
206 Haplomitriopsida, are lacking in the early Marchantiopsida orders Sphaerocarpaceae and
207 Blasiales [19] and co-occur with Glomeromycota across complex and simple thalloid
208 lineages. Exclusive associations with Glomero-, Basidio- and Ascomycota are restricted to
209 the more derived groups [19,47].

210 The first functional study of liverwort-Mucoromycotina symbiosis [53] showed that
211 nutritional benefits to the host plant remained the same or even increased under modern day
212 atmospheric CO₂ concentrations, in sharp contrast to the response observed in liverwort-

213 Glomeromycota associations [25]. If liverwort-Mucoromycotina symbioses perform much
214 better than glomeromycotean symbioses under modern atmospheric scenarios, why did the
215 first apparently lose out to the latter? With latest research indicating that a wide range of
216 extant early divergent land plant clades engage in partnerships with diverse members of both
217 the Mucoromycotina and Glomeromycota, sometimes simultaneously [50,52], an emerging
218 evolutionary scenario would envisage that dual fungal partnerships might have been a
219 prevailing strategy among land pioneers. It is possible that Haplomitriopsida liverworts
220 never evolved the capacity to form symbioses with Glomeromycota fungi and remained
221 Mucoromycotina-specific through their 400+ million year evolutionary history. Conversely,
222 liverworts known to harbour exclusively Glomeromycota fungi, including some species in the
223 genera *Marchantia*, *Conocephalum* and *Preissia*, most likely diverged during the Cretaceous,
224 a period of rapid flowering plant and fern radiation [58]. We speculate that during this
225 period, major changes in both abiotic and biotic, below- and above-ground dynamics led to
226 the predominance of strictly biotrophic Glomeromycota fungi in plant roots. It is possible
227 that Glomeromycota-specific liverworts evolved in Glomeromycota-dominated environments
228 and subsequently never engaged in associations with Mucoromycotina fungi. Thus, it is
229 possible that these later diverging, Glomeromycota-specific liverworts either lack or have lost
230 the ability to engage with Mucoromycotina fungi.

231

232 **Varied options to conquer land**

233 Did early land colonists rely on more symbiotic strategies than hitherto assumed? The
234 phylogenetic distribution of partnerships involving one, both or neither of the early diverging
235 fungal lineages Mucoromycotina and Glomeromycota, in early diverging lineages of extant
236 plants suggests that this was indeed the case. Additional support for the possible antiquity of
237 dual fungal symbioses comes from the fossil record. Whilst it seems likely that all Rhynie

238 chert plants interacted with Glomeromycota fungi [11,18], recent palaeontological research
239 points to more diverse early symbiotic plant-fungus encounters. A re-examination of the
240 early Devonian fossil plant *Horneophyton lignieri* reveals that its fungal endophytes have
241 characteristic features of Glomeromycota and Mucoromycotina [59], including intercellular
242 fungal proliferation, a distinct character of Mucoromycotina colonization in extant
243 Haplomitriopsida liverworts, hornworts and lycopods. This observation places
244 Mucoromycotina-only and dual Mucoromycotina and Glomeromycota-plant associations
245 within the timeframe of early plant terrestrialization and diversification, pointing to more
246 versatile and shifting evolutionary scenarios in plant-fungal symbioses than previously
247 assumed.

248 Whilst the potential nutritional benefits of dual partnerships with both
249 Glomeromycota and Mucoromycotina fungi in living plants remain to be tested, it is tempting
250 to speculate that for small plants occupying highly heterogeneous and dynamic environments,
251 the ability to associate with more than one fungal partner might be a winning strategy. For
252 the earliest land colonizing plants, and similarly for many modern plants occupying
253 equivalent wide ranges of stable and disturbed habitats, a bet-hedging strategy whereby a
254 plant can ‘pick and choose’ a facultative symbiont (i.e. Mucoromycotina), an obligate one
255 (i.e. Glomeromycota), both, or neither, can yield significant fitness advantages and additional
256 plasticity. Parallel functional studies to those on Glomeromycota- [25] and Mucoromycotina-
257 specific [53] land plant symbioses are needed to determine the potential benefits of
258 contrasting strategies across the carbon dioxide gradient that accompanied the evolution and
259 diversification of land plants. Axenic isolation and symbiotic resynthesis experiments
260 coupled with cytological investigation can reveal subtle diagnostic differences in the plant-
261 fungus intracellular interface between Glomeromycota and Mucoromycotina colonizations in
262 plants. Given that structures close in morphology to arbuscules, the diagnostic feature of

263 glomeromycotean colonization (see Box 1), can be produced by Mucoromycotina, we can no
264 longer be certain that the presence of these structures in plant cells is diagnostic of exclusive
265 partnerships with Glomeromycota fungi unless inclusive identification methods are applied.

266

267 **Widening views of plant-fungal symbiosis**

268 The biochemical and genetic mechanisms regulating and promoting the interactions between
269 plants and Glomeromycota fungi appear largely conserved throughout the plant kingdom
270 [21]. The mechanisms by which Mucoromycotina fungi engage with plants remain to be
271 investigated. Recently identified diffusible plant and fungal signalling molecules engage
272 plant roots and AM fungi in dialogue. The plant molecular SYM pathway, or "symbiotic
273 toolkit", guides entry and accommodation of fungi (and in legumes, of nitrogen-fixing
274 bacteria) within plant root tissues [23]. The release of biochemical signals into soil by
275 vascular plants engages AM fungi in the processes of contacting and entering roots. The
276 production of plant signals, including strigolactones, has been detected in some green algae,
277 non-vascular and flowering plants. However, in algae and non-vascular plants they do not
278 engage fungi; instead they behave as hormones stimulating plant rhizoid elongation [60-62].
279 Despite this, there is conservation of the 'toolkit' of proteins, microRNAs and other small
280 molecules involved in initiation and regulation of symbiosis across the land plant phylogeny,
281 even in non-symbiotic lineages such as mosses (see Box 3). Together with the ubiquitous
282 presence of SYM genes across all plant lineages, intriguingly including the Mucoromycotina-
283 specific Haplomitriopsida [20], these findings suggest that the ability to form mycorrhizas or
284 mycorrhiza-like associations has been conserved from symbiotic gametophytes of non-
285 vascular plants through to symbiotic sporophytes of vascular plants. It remains to be
286 determined, however, which plant genes originally permitted the accommodation of which

287 fungi within plant tissues. An alternative but untested hypothesis is that their evolution was
288 linked to harbouring an overlooked or extinct microbial symbiont of green algae [22].

289 The assumption that function of mycorrhizal and mycorrhiza-like fungal associations
290 is also conserved across land plant evolution has, until recently, been based on striking
291 homology between fossilised arbuscule-like structures in early Devonian plants and the
292 Glomeromycota arbuscular structures of most living plants [63] (see Box 1). Based on
293 shared arbuscule morphology it is assumed that any nutritional gains in well-studied vascular
294 plants would be echoed in the earliest branching lineages of non-vascular plants and, by
295 extension, in the earliest land plants to colonise the inhospitable Ordovician landmasses.
296 However, while we know for instance that functioning in modern vascular plant-
297 Glomeromycota symbiosis changes according to atmospheric CO₂ concentrations [64], the
298 only evidence for similar responses in early-branching lineages of vascular plants is based on
299 a single fern [25] and two liverworts [24,25]. Recent research demonstrates that the
300 exclusively Mucoromycotina symbioses of the Haplomitriopsida are also responsive to
301 changes in CO₂ [53], but the functional significance of dual fungal colonisations across early
302 branching lineages of land plants remains to be elucidated on a comprehensive range of
303 liverworts, hornworts and early vascular plants.

304

305 **Concluding remarks**

306 Typically asymptomatic microbes are universal symbionts in land plants, as in the
307 microbiomes of other multicellular eukaryotes, but there is accumulating evidence in the few
308 non-vascular plants that have been examined so far that important nutritional mutualisms
309 occur with diverse fungi. Associations with newly discovered Mucoromycotina and/or well-
310 known Glomeromycota fungi occur in several non-vascular and vascular plants. Research is
311 required to assess the extent to which Mucoromycotina colonize land plants, particularly

312 within liverworts and early-branching lineages of both non-flowering and flowering vascular
313 plants; how often Mucoromycotina and Glomeromycota co-colonize plants; and whether the
314 resulting associations are consistently mutualistic. Combined molecular, cytological,
315 physiological and ecological studies are essential to understand the biology of
316 Mucoromycotina, their intimate interactions with plants, the significance of dual fungal
317 colonizations (viz. how carbon gains and nutrient costs are shared by different co-occurring
318 symbionts) and their potential to partition ecosystems. It is possible that, given their shared
319 symbionts, pioneer non-vascular plants facilitated vascular plant colonization and
320 diversification in the past, and might still be doing the same today. Further, it is critical that
321 research prioritizes understanding the physiological and molecular mechanisms by which
322 plant specificity towards Mucoromycotina versus Glomeromycota fungi, and viceversa,
323 arises and is regulated. An unprecedented approach merging palaeontology, eco-physiology,
324 biogeochemistry, developmental biology, and -omics disciplines including neglected non-
325 vascular plants, early-branching vascular plants, and fungi promises fascinating discoveries
326 into the intertwined past, present and future of plants, microbes, soils and the biosphere.
327

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335

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508 **Boxes**

509

510 **Glossary**

511 **Arbuscular mycorrhizal fungi:** members of the phylum Glomeromycota that form cell-to-
512 cell nutritional associations with plant roots and extend filaments into soil to forage for
513 minerals, colonize other roots and form spores. They often produce characteristic highly
514 branched tree-like structures known as arbuscules within colonised plant cells.

515 **Biotroph:** an organism nutritionally dependent on another living organism.

516 **Coenocytic:** refers to a multinucleate mass of cytoplasm, characteristic of non-septate
517 filamentous fungi.

518 **Depletion zone:** the volume of soil surrounding a root that becomes depleted of nutrients.

519 **Embryophyte:** plants in which the embryo is retained within maternal tissues, includes all
520 clades of living land plants.

521 **Endophyte:** typically cryptic and asymptomatic microbe living within a plant, or any
522 microbe within or between intact fossilized plant cells.

523 **Gametophyte:** haploid stage in the alternation of generations during the life cycle of plants,
524 it is dominant in non-vascular plants.

525 **Glomeromycota:** a fungal phylum composed of biotrophs including all known arbuscular
526 mycorrhizal fungi and a mutualistic fungus of photosynthetic bacteria (i.e. Geosiphon). It
527 includes the orders Archaeosporales, Paraglomerales, Diversisporales and Glomerales.

528 **Hypha:** a fungal filament made up of tubular cells.

529 **Mucoromycotina:** a fungal subphylum of early diverging fungal lineages composed of
530 saprotrophs, parasites and mutualists. It includes the orders Endogonales (pea truffles) and
531 Mucorales (pin moulds).

532 **Mutualism:** a symbiosis that increases the fitness of all partners.

533 **Mycorrhiza:** "fungus-root", a typically mutualistic symbiosis between plant roots and fungi.
534 "Mycorrhiza-like" is used to refer to mutualistic fungal symbiosis in plants without true roots.
535 **Non-vascular plants:** land plants without true (i.e. lignin-containing) vascular tissue, also
536 known as bryophytes. They include three phyla: Marchantiophyta (liverworts),
537 Anthocerotophyta (hornworts) and Bryophyta (mosses).
538 **Rhizoid:** unicellular tubular extension of an epidermal plant cell into the substrate
539 functioning in attachment and resource uptake.
540 **Saprotroph:** an organism that fulfils its nutritional needs from dead organic matter, a
541 decomposer.
542 **Sporophyte:** diploid stage in the alternation of generations during the life cycle of plants. It
543 is dominant in vascular plants.
544 **Symbiosis:** the living together of unlike organisms, sometimes restricted to persistent
545 mutualisms.
546 **Thallus:** body of some plants and fungi that is not differentiated into organs.
547 **Vascular plants:** land plants with lignified vascular tissue (i.e. xylem), also known as
548 tracheophytes. They include the phyla Lycopodiophyta (lycopods or clubmosses),
549 Pteridophyta (ferns and allies), Pinophyta (conifers), Cycadophyta (cycads), Ginkgophyta
550 (Ginkgo), Gnetophyta (Gnetum, Welwitschia and Ephedra), and Magnoliophyta (flowering
551 plants or angiosperms).

552

553 **Box 1. Structure of arbuscular mycorrhizal associations**

554 Mycorrhizal fungi of the Glomeromycota in flowering plants preferentially colonize
555 root cortical cells. The principal sites for nutrient exchange through phosphorus, nitrogen
556 and carbon transporter proteins between the symbiotic partners are intracellular tree-like
557 fungal structures known as arbuscules. Depending on the plant they colonise, the fungi take

558 two main forms inside plant root tissues. In ‘Arum’ colonisation, fungi grow through plant
559 intercellular spaces before penetrating root cells and branching to form characteristic
560 arbuscules and/or vesicles. ‘Paris’-type colonisation is common in many non-vascular plants
561 including liverworts and hornworts. The latter is typical of many plants with minimal
562 intercellular spaces where colonisation is characterised by extensive intracellular growth
563 producing fungal coils, sometimes without producing arbuscules or vesicles [5].

564

565 **Box 2. Evolutionary stability of plant-fungal symbioses**

566 It has been postulated that for mutualistic interactions between plants and fungi to persist
567 across evolutionary timescales they require stabilisation in the form of reciprocal rewards, i.e.
568 ‘generous’ plant partners are rewarded with more nutrient returns from fungal partners in
569 exchange for enhanced plant carbon investment on fungi. Kiers et al. [65] carried out one of
570 the few investigations to take into account the fact that most plants have multiple
571 simultaneous Glomeromycota fungal partners and that likewise, most symbiotic fungi
572 simultaneously associate with multiple plants. Accordingly, it is hypothesised that the plant-
573 mycorrhizal mutualism is stabilised because both plants and fungi are able to detect variation
574 in phosphorus and carbon resources exchanged, allowing them to adjust resource allocation -
575 individuals are able to withhold nutrients until maximum “reward” for their “investment” is
576 achieved. The reciprocity of the exchange, with partners able to regulate allocation of their
577 resources to each other, should generate evolutionary stability. It has been shown that this
578 could also apply to nitrogen and carbon resources, with carbon supply by host plants resulting
579 in increased nitrogen allocation by fungal partners [66] and that fungi might be able to
580 discriminate between plant partners based on their carbon wealth [67]. This in turn
581 corroborates a previous study suggesting the symbiosis operates on a “tit-for-tat” basis [68].
582 However, stability does not mean invincibility. Mutualisms are open to the evolution of

583 “cheating” whereby a partner fails to “repay” a carbon or nutrient “debt”, becoming parasitic.
584 For example, narrowly specialized non-photosynthetic angiosperms that depend on an
585 arbuscular mycorrhizal fungus and the non-photosynthetic liverwort *Aneura mirabilis* that
586 parasitizes a basidiomycete fungus [69]. Plants that have evolved mycoheterotrophic-to-
587 autotrophic lifecycles are also not taken into account in the model of evolutionary stability.
588 For example, the arbuscular mycorrhizal symbiosis operates on a ‘take now, pay later’ basis
589 in an early-diverging fern lineage [56]. Here, a subterranean gametophyte is supported by
590 fungi until commencement of autotrophy, at which point the fern ‘repays’ the
591 Glomeromycota fungus with photosynthates [56]; specificity and intergenerational fidelity
592 could be important in stabilizing benefits of mutualisms in some plants.

593

594 **Box 3. Mosses are asymbiotic**

595 Given the widespread occurrence of fungal symbioses in liverworts and hornworts, their
596 absence in mosses stands out as anomalous; there are some 10,000 moss species in the same
597 range of habitats as liverworts and hornworts, and mosses possess the genetic toolkit to form
598 fungal symbioses [22]. Though fungal fruiting bodies, including species-specific ones, are
599 often found growing on mosses [70], the fungi are either saprophytes or parasites. There is
600 no physiological evidence for nutritional interdependence or any image showing fungi in
601 symbiosis with healthy cells. Indeed, the extensive incidence of pegs of host cell wall
602 encasing invading hyphae is indicative of an anti-fungus immune response apparatus [19].

603 A likely answer to the absence of symbiotic fungi in mosses relates both to their early
604 evolutionary history and to the acquisition of highly differentiated multicellular rhizoids
605 subsequent to the divergence of the early Sphagnales, Andreaeales and Takakiales. The peat
606 moss *Sphagnum* has limited contact with mineral substrates and obtains nutrients principally
607 from atmospheric sources whilst the same is likely in *Andreaea* which mainly grows on hard

608 siliceous rocks. The ill-differentiated thallus-like structures at the stem bases function solely
609 as organs of attachment [71]. The thickened epidermal cell walls in the rhizomes of *Takakia*
610 are an unlikely fungal interface.

611 The mosses' multicellular rhizoids, found in all later moss lineages with their tip-
612 growing filaments, subapical side branching and differentiated cells differ from the
613 unicellular rhizoids and root hairs of all other land plants. Whereas the latter typically have
614 diameters ca. 20 μ m, branching of moss rhizoids sees a gradual reduction in size to 3-5 μ m
615 (Figure Ia,b), i.e. the same as those of soil-growing fungi. Moreover, unlike unicellular
616 rhizoids and root hairs which are highly vacuolated, the cytology of moss rhizoids mirrors
617 that in the photosynthate-conducting leptoids of large moss stems. Their differentiation
618 involves mixing of the vacuolar and cytoplasmic contents (a striking parallel with phloem
619 sieve elements), and the alignment of mitochondria and plastids along longitudinal arrays of
620 endoplasmic microtubules [72]. The comparative efficiency of nutrient uptake between moss
621 rhizoids and symbiotic rhizoids remains untested.

622 **Figure I.** The two rhizoid types in non-vascular plants. **(a)** Multicellular rhizoids of the
623 moss *Funaria hygrometrica*, arrow points to main axis. **(b,c)** Unicellular rhizoids of the
624 liverwort *Dumortiera hirsuta*, arrows point to fungi. Main moss axes have diameters like
625 liverwort rhizoids, but ultimate moss ramifications are as narrow as fungi. Scale bars: 50 μ m.

626

627 **Outstanding questions**

628 The recent molecular, cytological and physiological studies on plant-fungal symbioses have
629 given unique insights on their biology and evolution. However, several areas remain open to
630 study and should form the focus of future lines of investigation. Many of these questions
631 could be addressed through genomic, metabolomic and eco-physiological investigation of
632 early-branching plant lineages and Mucoromycotina fungi using monoxenic cultures.

633 1) The evolutionary relationships between fungi of the Glomeromycota and Mucoromycotina
634 remain under investigation; studies are now required to establish whether these fungal
635 lineages are sisters or one is older and to reveal the full molecular diversity of symbiotic
636 Mucoromycotina. In resolving the phylogenomic relationships between these key fungal
637 groups, we could be able to infer the ancestral symbiotic type and how it evolved to the
638 present day.

639 2) In terms of symbiotic engagement, what were the first signals between fungi and newly
640 evolved land-plants? For example, are comparable fungal and non-vascular plant responses
641 observed upon exposure to plant strigolactones and fungal lipochitooligosaccharides,
642 respectively, as in vascular plants?

643 3) Regarding nutritional exchange between partners, are similar or different phosphorus,
644 nitrogen and carbon transporter proteins operating in arbuscular mycorrhizas and plant-
645 Mucoromycotina symbioses? Are the fungal symbioses of hornworts and lycopods
646 nutritionally mutualistic? How do asymbiotic plants compete with symbiotic plants? What is
647 the significance of plant-fungal-cyanobacterial symbioses known from some hornworts,
648 liverworts and cycads?

649 4) It remains unclear whether and how declining CO₂ through the Palaeozoic favoured the
650 retention of dual fungal associations in many non-vascular plants to the present day.

651 Methods allowing discrimination between fungal partners in vivo will provide clues as to why
652 dual fungal colonisation occurs in some liverworts, hornworts, lycopods and ferns.

653 **Figure legends**

654 **Figure 1.** Land plant phylogeny showing estimated dates of divergence, symbiotic fungi and
655 key features of major land plant clades. A + indicates presence of fungal group within extant
656 members of plant clade. The numbers of living species in each plant group are shown
657 between parentheses and fossilised plant groups are shown at the bottom with approximate
658 dates of fossilisation. Liverworts are the earliest diverging group of extant plants and are
659 shown to associate with Mucoromycotina, Glomeromycota, Basidiomycota and Ascomycota
660 fungi. The only other clade known to associate with all mycorrhizal fungal groups are the
661 conifers. Mosses are asymbiotic, while hornworts, lycopods and ferns are only known to
662 associate with Mucoromycotina and Glomeromycota fungi. The Rhynie chert fossils provide
663 the only detailed snapshot of early vascular plants and their associated fungi, some of which
664 bear resemblance to extant examples of plant-Glomeromycota and/or plant-Mucoromycotina
665 partnerships.

666

667 **Figure 2.** Key abiotic and biotic characteristics of the environment, plants and fungi during
668 colonisation of the land by plants. The first emergent plants were likely liverwort-like,
669 lacking in roots and vasculature. Fossil and molecular evidence indicates that the earliest
670 plants were associated variously with obligately biotrophic Glomeromycota, partially
671 saprotrophic Mucoromycotina or even both fungal groups simultaneously. The fungi likely
672 aided plants in their conquest of the land masses through provision of mineral nutrients in
673 return for photosynthetically-fixed carbohydrates through enhanced biological mineral
674 weathering. With the evolution of increasingly complex plant vasculature and organs and the
675 buildup of organic matter in the soil, atmospheric CO₂ dramatically decreased in line with
676 increasing plant demand. Ultimately, flowering plants rose to supremacy on Earth. The
677 majority of extant land plants now form arbuscular mycorrhizal (or mycorrhiza-like in plants

678 without roots) associations with fungi of the Glomeromycota. However, recent discoveries
679 show Mucoromycotina and dual Mucoromycotina-Glomeromycota fungal associations occur
680 throughout non-vascular plants. Atmospheric carbon dioxide concentrations from
681 GEOCARB III [73]. The first land plants were possibly Ordovician or Silurian cryptophytes
682 [13], though body form and rhizoids are unknown. Drawings are adapted and reproduced
683 with permission from [74-76].

684

685 **Figure 3.** Scanning electron micrographs illustrating key features of the fungi that form
686 intimate symbioses with the most ancient lineages of land plants. **(a)** Mucoromycotina in
687 pure culture; these fungi were recently isolated from a host plant for the first time [53]; **(b)**
688 Mucoromycotina in symbiosis within cells of the Haplomitriopsida liverwort *Treubia*
689 *lacunosa*. **(b)** Within plant cells, Mucoromycotina produce coils (asterisk), and short-lived
690 swellings (arrow). **(c,d)** Glomeromycota in symbiosis with the complex thalloid liverwort
691 *Marchantia pappeana*, showing fungal structures typical of arbuscular mycorrhizas in
692 flowering plants: an intracellular arbuscule and trunk hyphae (c) and a large vesicle (d). **(e)**
693 Arbuscules are not a constant feature of Glomeromycota in early diverging land plants; for
694 example, in the rhizome of the fern *Psilotum nudum*, Glomeromycota fungi only form coils
695 and terminal vesicles. **(f)** Except for Haplomitriopsida liverworts, fungal entry into
696 liverworts is via rhizoids, shown here in the complex thalloid liverwort *Neohodgsonia*
697 *mirabilis*. Note the abundance of fungi within a rhizoid (insert). The arrow points to the
698 fungal colonization zone in the centre of the liverwort. Scale bars: (c, d) 10µm; (b, e) 50µm;
699 (a) 100µm; (f) 1000µm.

700 (d, e) reproduced from Fig. 4f, l in Strullu-Derrien et al., 2014.