The land flora: a phototroph–fungus partnership?

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Numerous mutualistic associations between phototrophs and fungi exist in the extant land biota. Some are widespread, such as lichens and mycorrhizae, but some are less well known or restricted to special ecological conditions, such as endophytes in plants and algae. Recent molecular data and fossils suggest that associations arose repeatedly and that some of them are ancient, and even ancestral in the case of land plants. Mutualism, that provides various adaptations to terrestrial constraints, may have played a crucial role during terrestrialization and evolution of land phototrophs.

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Living on emerged land raises several problems: water may be rare or absent, implying (1) that mineral nutrition is more or less impaired, (2) radiation (mainly toxic UV-rays) is not filtered out and (3) strong fluctuations of temperature are not prevented. In spite of those adversities, Precambrian land was colonized by phototrophic microorganisms, probably prokaryotes[1], and multicellular terrestrial phototrophs arose during the Silurian, about 450 million years ago[2]. How did phototrophs overcome land stresses? Nowadays, the pioneer colonizers are mainly lichens – not simple phototrophs, but a mutualistic association of a phototroph with a fungus. At closer inspection, almost all present-day phototrophs adapted to terrestrial ecosystems form mutualistic associations with fungi (Box 1), which are often lost in secondarily aquatic phototrophs, as documented for mycorrhizal symbiosis[3]. The fungi involved in these associations, the 'mycobionts', are non-septate Zygomycotina and septate Ascomycotina and Basidiomycotina. Fossil record[4] and molecular clock data[5] (Box 2) suggest that terrestrial non-septate fungi originated during the Cambrian, and septate fungi during the Devonian. They thus had opportunities to interact with the terrestrial flora during much of its evolution. Here, we review new evidence that (1) mutualism with fungi is widespread among extant phototrophs and ancient in land ecosystems and (2) that it allowed various critical adaptations during the evolution of land phototrophs.

Terrestrial algae repeatedly interacted with fungi

Terrestrial microscopic phototrophs, unicellular or filamentous, belong to cyanobacteria and green algae (e.g. *Trentepohlia*, *Pleurococcus*) and are either free-living or more frequently associated with mutualistic fungi, to form lichens[6]. As shown by molecular approaches[7], lichen-forming fungi arose many times during the evolution of both Ascomycotina and Basidiomycotina. Lichens, in which the alga is protected by the fungal stroma, tolerate a wide range of conditions[8] under which neither vascular plants nor free-living partners could survive: they tolerate drought, cold and heat, intense light, barren rocky substrates.

Such conditions may well have prevailed on land before colonization, so that one could wonder whether lichens were the first the land colonizers. Fossils and molecular data indicate that Basidiomycotina and Ascomycotina, the extant mycobionts of lichens, did not arise before the Devonian[2,5], or at most the Silurian[9] (Box 2). Poorly understood Paleozoic fossils, such as the Devonian *Spongiophyton*[11] or the Siluro–Devonian nematophytes[12], have been interpreted as lichen structures, but this awaits further evidence. Less-disputed fossils from the Eocene are available[13], and a Permo–Triassic origin has been suggested for ascomycete lichens[8]. Basidiomycete lichens are likely to have evolved more recently, such as the genus *Omphalina*, in which intermediates of the transition to lichenization still exist[14]. Present-day lichens thus probably originated after land colonization by plants, and early Paleozoic lichens should involve a non-septate or an ancestral septate fungus. An extant non-septate fungus, *Geosiphon pyriforme*, related to the Glomales[15] (the VA fungi – see below), forms bladders containing endosymbiotic *Nostoc* (Fig. 1). This association, capable of photosynthetic carbon assimilation and N-fixation[16], and living on poor loamy soil, could be similar to primitive lichens. A 400 million year-old fossil lichen from the Rhynie Chert[17], consisting of coccoid cyanobacteria and a zygomycete fungus, supports the existence of Paleozoic non-septate lichens.

Lichens involving cyanobacteria and non-septate fungi can be expected to have existed until 1000 million years ago, as fungi and other main eukaryotic lineages radiated. However, Precambrian fossil lichens, such as *Thuchomyces lichenoides* from South Africa, are often considered dubious[2]. The enigmatic Ediacarian fossils were recently proposed to be lichens, because of their resistance to burial compaction[10], but few anatomical data have been recovered from those compressed structures. We still know very little about possible Precambrian lichens, and phototrophs found in Precambrian paleosoils were able to live on land independently of symbiosis[1], as do present-day free-living algae and cyanobacteria. Lichenization would thus seem secondary in algal and cyanobacterial evolution. Nowadays, lichenization provides these phototrophs a broader ecological niche. Earlier in their evolution, it probably allowed them to stay and diversify on land after the rise of multicellular plants: the latter probably outcompeted simple free-living algae.

Macroscopic and pseudoparenchymatous algae – that is, green, red and brown algae – are not common in land ecosystems. However, such algae living in the tidal zone lead a semi-terrestrial existence during low tide. Interestingly, they form a less well-known association (the so-called 'mycophycobiosis', Box 1) with ascomycetes living intercellularly, inside the algal thallus[18]. The best known is the brown alga *Ascophyllum nodosum* (Fig. 2) which is always infected by *Mycosphaerella ascophylli*. The association is facultative,

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Box 1. Glossary of extant phototroph–fungi mutualisms

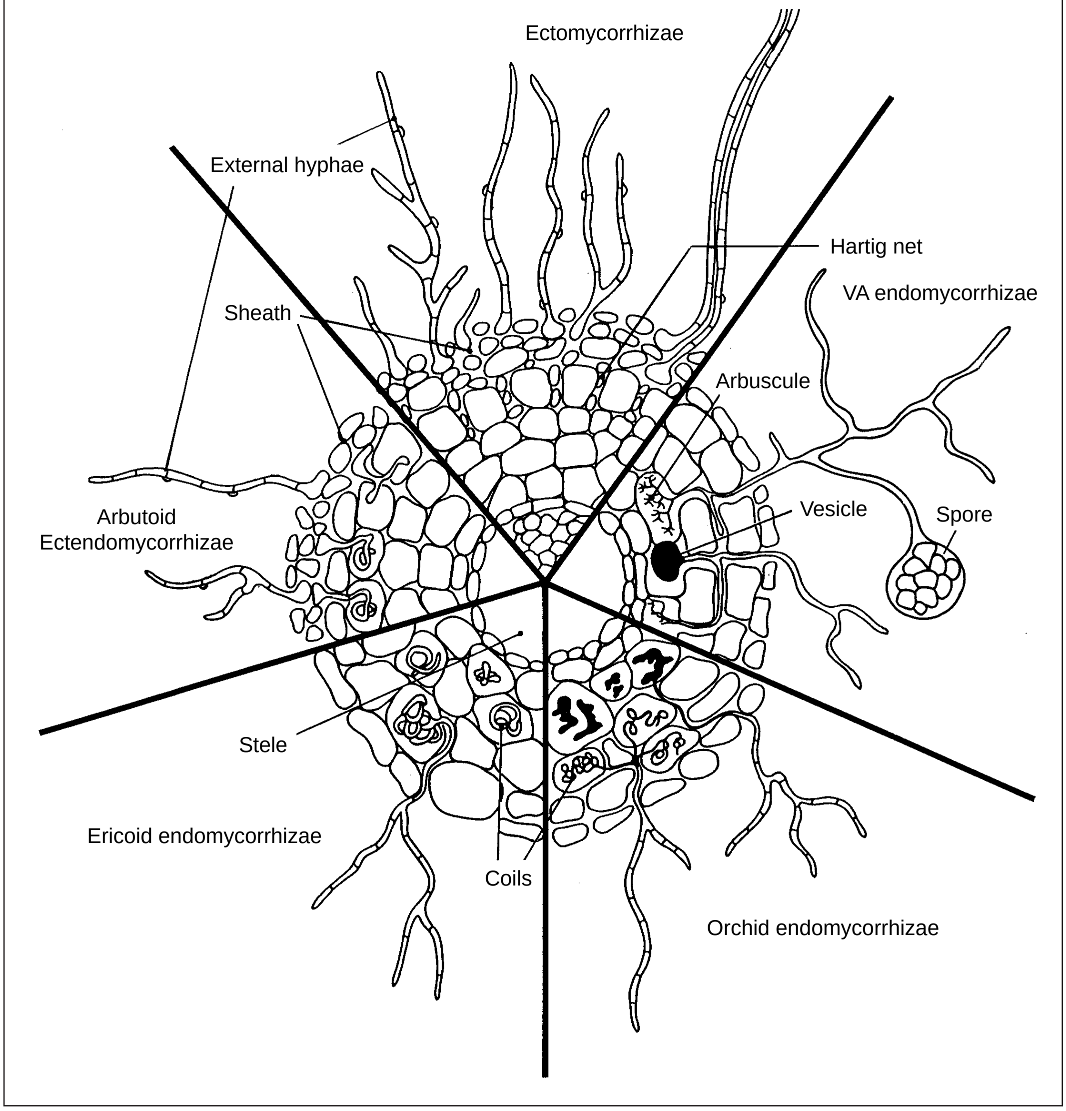
Lichens are composed of a cyanobacterium or a green alga embedded in a vegetative stroma of an ascomycete or, less frequently, a basidiomycete[6]. *Geosiphon pyriforme* (Fig. 1) is a non-septate fungus that harbors intracellular cyanobacteria[16].

Mycophycobioses are composed of an ascomycete embedded in the thallus of a multicellular alga[18] (Fig. 2).

Mycorrhiza are composed of septate or non-septate fungi within a root, but also exploring the surrounding soil[20] (see figure). In ectomycorrhiza, a septate fungus forms a sheath around the root ('sheath-forming mycorrhiza') and grows between cortical cells forming the so-called Hartig net. Endomycorrhiza involves non-septate Glomales (VA mycorrhiza) or septate fungi (ericoid and orchid mycorrhiza). In both cases, the fungus penetrates the cortical cells where it forms respectively arbuscules or coils. Ectendomycorrhiza formed by basidiomycetes in some Ericales (e.g. *Arbutus*) shares features of both ecto- and endo-mycorrhizae, i.e. a sheath and intracellular coils.

Mycorrhizome and **mycothallus** are composed of septate or non-septate fungi within a rhizome and a thallus (Fig. 4) of a land plant respectively[21].

Symptomless endophytes (often ascomycetes, such as *Clavicipitaceae* in grasses) live intercellularly in various plant organs[40]. Some may be transmitted by seeds. The name **mycophylla** has been proposed for associations between leaves and endophytes[42].



since axenic cultures are possible, but the fungus seems to protect the alga against desiccation[19]. The same fungus is also present in *Pelvetia canaliculata*, the most exposed brown alga in the tidal zone of temperate regions. Other mycophycobioses have been reported in green (*Prasiola, Blidingia*) and red algae (*Apophlaea*). They are thus polyphyletic and probably recent, since they involve secondarily aquatic ascomycetes. Nevertheless, they suggest that a phototroph having the organizational complexity of a pseudoparenchymatous alga can take advantage of a mycobiont to survive terrestrial conditions. This could be an analogue of the first land plants.

Plants are ancestrally mutualistic with Glomales

Land colonization by multicellular phototrophs involved the radiation of the 'Plantae' (a particular subgroup of green algae phylogenetically independent of terrestrial green microalgae) during the Silurian, with possible precursors during the Ordovician[2] (Box 2). These phototrophs, probably derived from Charophyta, share an egg-protecting archegonium and are divided into two main lineages: Atrachaetae (mosses, hepatics and hornworts) and Tracheophytae (vascular plants). Vascular plants, but also hepatics and hornworts, have various mycobionts inhabiting different vegetative organs (Box 1).

Non-septate fungi form mutualistic associations with about 90% of these phototrophs. The fungus grows intercellularly and forms vesicles and intracellular arbuscules (Box 1). The vesicular–arbuscular (VA) associations occur in roots of vascular plants (the so-called VA mycorrhiza)[20], but also in rhizomes and gametophytes of ferns and their allies[21] and in hepatics such as Metzgeriales and Marchantiales[22] (mycorrhizomes and mycothalli). VA fungi facilitate mineral nutrition of the phototroph, mainly the uptake of phosphorus and microelements whose mobility in soil is often low. They also protect the root system against soil-borne pathogens[23].

VA fungi belong to a small group of about 200 species of zygomycetes, the Glomales. They are strictly biotrophic, asexual, and only distantly related to other zygomycetes – a feature suggesting a long evolutionary stasis in a stable and protected biological niche. VA symbiosis is so widespread among the extant flora that it was suspected to be ancestral in Plantae[24]. Fossil Glomales are reported at least since the Devonian[25] and molecular clock data suggest that Glomales arose between 350 and 460 million years ago[26], that is, at the time of land colonization. Indeed, hyphae and vesicles had long been reported in fossils of the oldest well-preserved vascular flora, the Rhynie Chert. A recent re-examination of the rhizome of *Aglaophyton* (= *Rhynia*) *major*, a fossil often considered as ancestral of vascular plants, showed nicely

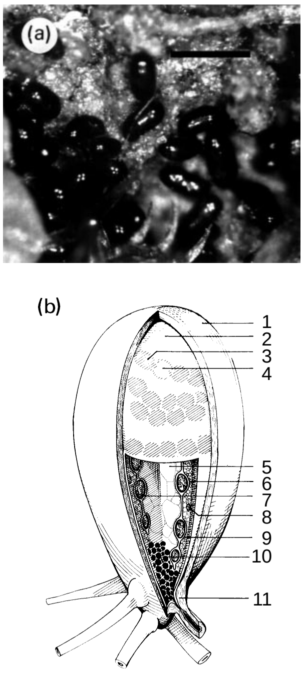


Fig. 1. *Geosiphon pyriforme*, a non-septate fungus related to the Glomales, forms endosymbiosis with photosynthetic and N_2-fixating *Nostoc*. (a) Bladders growing in the natural habitat on mineral soil (Spessart mountains, Germany. Bar: 1 mm) – *Courtesy of D. Mollenhauer*. (b) Structure of a coenocytic bladder containing *Nostoc* filaments: 1, cell wall; 2, fungal plasmalemma; 3, internalized *Nostoc* filaments; 4, heterocyst; 5, fungal vacuoles; 6, peripheral cytoplasm; 7, section of a *Nostoc* filament; 8, mitochondriae; 9, sequestration membrane; 10, lipid droplets; 11, non-septate hypha linking the bladders. *Courtesy of M. Kluge*.

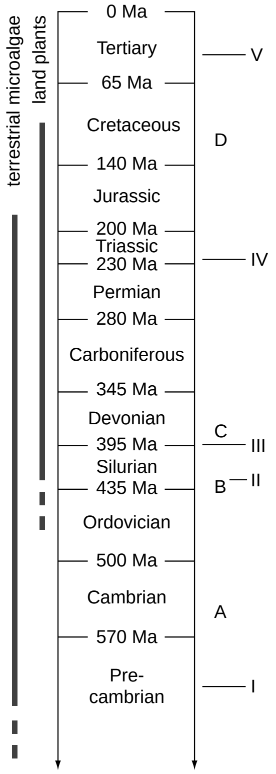
Box 2. Geological timescale and milestones in evolution of terrestrial phototrophs and fungi

Molecular clock data

- **A** Divergence between chytrids and terrestrial fungi[5].
- **B** Divergence between Glomales and other Zygomycotina[26].
- **C** Divergence between Ascomycetes and Basidiomycetes[5].
- **D** Emergence of Holobasidiomycotina, among which extant ectomycorrhizal basidiomycetes[5].

Fossil record

- **I** Precambrian: fossil paleosoils with free-living microphototrophs[1]; fossils of uncertain affinities interpreted as lichens by some authors[2,10].
- **II** Silurian: rise of a multicellular land flora[2], some fossils could be interpreted as lichens[10,11]. Earliest putative fossil ascomycetes[9].
- **III** Early Devonian: Rhynie Chert flora, with a lichen involving cyanobacteria and a zygomycete[17], fossil vascular plants showing VA associations[27] and biotrophic associations within a charophyte[28].
- **IV** Middle Triassic: evidence for diversification of VA fungi from fossil cycad roots of Antarctica[30].
- **V** Eocene: oldest definite lichen involving extant septate fungi[13] and oldest ectomycorrhiza on *Pinus*[32].



preserved arbuscules[27] (Fig. 3) and established beyond doubt the existence of VA associations in the Lower Devonian. The intact host cell wall and the strictly cortical localization of the infection suggest a biotrophic association. It could explain how the rootless *Aglaophyton* coped with its mineral supply[23], in a way reminiscent of the rootless mycorrhizome of *Psilotum*[21], an extant pteridophyte of uncertain affinity, which resembles early land plants.

Older fossil plants are often compressed, making the presence of endophytes difficult to assess. However, some Siluro–Devonian plants of unknown systematic affinities have been claimed to contain fungal structures[10–12] (see above). Remembering the actual case of mycophycobioses, it has often been speculated that land plants arose from a charophycean alga associated with a non-septate fungus; terrestrial fungi were also proposed to have originated from such associations[5]. Although no convincing fossils of the hypothesized symbiosis have been found to date, reports on biotrophic interactions within the charophycean *Palaeonitella*[28] from the Devonian show that the fossil record may be used to test this hypothesis. A small group of present-day land plants, the hornworts (also called Anthocerotophyta), could represent an intermediate state: their simple thallus of single-plast cells is very reminiscent of algae and harbors VA endophytes[29].

Glomales diversified during the late Paleozoic and the Mesozoic[26] (two glomalean families, the *Gigasporaceae* and the *Glomalaceae*, are reported from Triassic cycad roots[30]), but the relation to the evolution of land flora remains poorly understood. In the course of plant and hepatic evolution, VA mycobionts were sometimes lost, leading to an aposymbiotic habit. About 10% of plants, including whole angiosperm families (e.g. *Joncaceae*, *Chenopodiaceae* and *Brassicaceae*), are non-mycorrhizal and compensate by developing special root system architectures[31]. Disturbed habitats, where mycorrhizal inoculum is lacking, and wet or aquatic habitats, where access to mineral resources is easier and oxygen diffusion limits fungal growth, often harbour non-mycorrhizal species[3]. Mosses are the only high-rank taxon of Plantae where associations with fungi are lacking. Their low morphological and anatomical differentiation, together with poïkilohydric strategy, is reminiscent of the free-living terrestrial algae. This nutrient stress-tolerance is a successful strategy in some, but not all, terrestrial conditions.

Plants repeatedly interacted with septate fungi

Although the majority of advanced groups, such as grasses, retained the primitive VA mycorrhiza, other plants associated with septate fungi. Numerous trees and some shrubs (Gymnosperms, Gnetales and Angiosperms) form

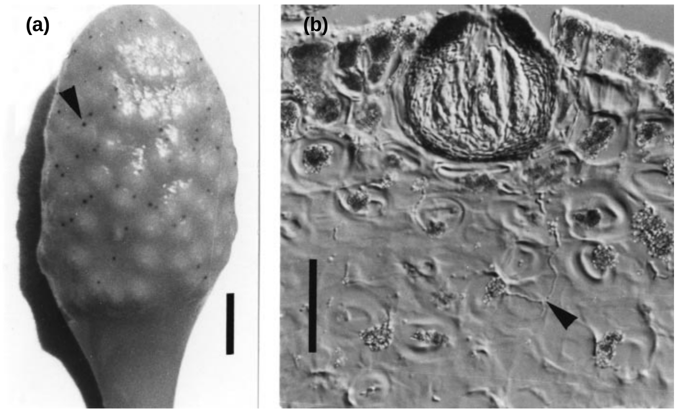


Fig. 2. *Ascophyllum nodosum*, a brown alga of the tidal zone, forms mycophycobiosis with the ascomycete *Mycosphaerella ascophylli*. (a) Fertile receptacle of *A. nodosum*, with several pale gamete-bearing conceptacles and numerous dark-pigmented ascocarps of *M. ascophylli* (arrowhead), achieving synchronous reproduction (bar: 2 mm). (b) Section of a receptacle in Nomarski interference contrast, showing an ascocarp with asci and systemic intercellular hyphae of the endophyte (arrowhead, bar: 50 mm). *Courtesy of J. Kohlmeyer*.

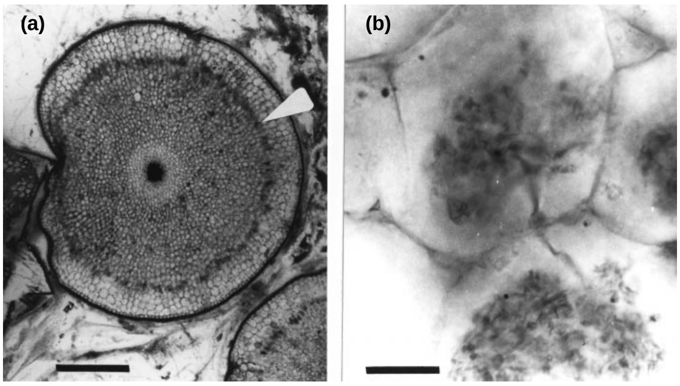


Fig. 3. *Aglaophyton* (= *Rhynia*) *major*, a 400 million year-old fossil plant from the Rhynie Chert, is associated with Glomales[27]. (a) Section of a *A. major* rhizome showing the central stele and the cortical parenchyma with a dark-colored zone (arrow) where hyphae, vesicles and arbuscules have been described (bar: 1 mm). (b) Two parenchymal cells exhibiting arbuscules with nicely preserved trunks (bar: 10 μm). *Courtesy of T.N. Taylor*.

the so-called ectomycorrhizae (EcM) with septate fungi, Ascomycotina or Basidiomycotina, that grow intercellularly in the roots (Box 1)[20]. The oldest known fossil EcM were found on *Pinus* roots from the Eocene[32], demonstrating that EcM were already established at least 50 million years ago. Molecular clock data suggests that the Holobasidiomycotina, which include the EcM basidiomycetes, radiated during the Cretaceous (130 million years ago)[5], but earlier EcM ascomycetes cannot be rejected. It may be speculated that EcM originated during or after the radiation of major extant EcM plant taxa – that is, a Mesozoic origin – but this still requires paleontological investigation. Assuming the ancestral nature of VA mycorrhiza, EcM fungi probably replaced the VA partners at the time of their emergence. However, even today, coexistence of VA and EcM fungi occurs in root systems of some angiosperms (such as Salicaceae, Myrtaceae) and gymnosperms[33]. EcM are also polyphyletic for the mycobionts[34], thought to have evolved from saprotrophic septate fungi, which existed in terrestrial paleoecosystems since the Paleozoic[4]. Extant EcM fungi still have saprotrophic abilities, that give access to organic nitrogen as well as phosphorus. EcM association thus allows colonization of soils where organic matter accumulates, for instance, in temperate and boreal zones.

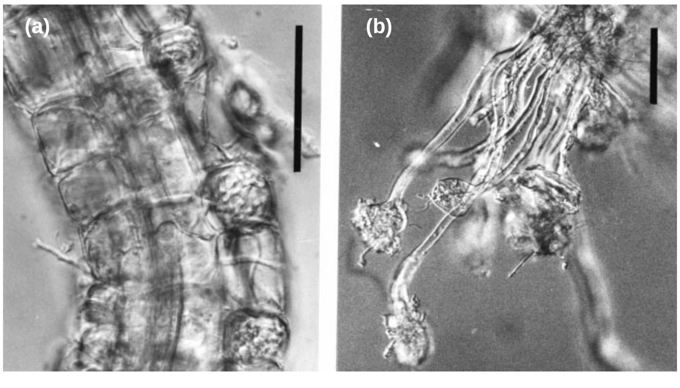


Fig. 4. Some ascomycetes colonize the cells of both ericalean species and hepatics on organic-rich soils (bar: 50 μm). (a) Fine root of *Calluna vulgaris* with epidermal swollen cells: some are invaded by fungal coils. (b) Rhizoids of the hepatic *Telaranea nematodes* with swollen tips invaded by fungal hyphae. *Courtesy of D.G. Duckett*.

Other root associations with septate fungi involve more-restricted plant taxa. Ericales associate intracellularly with fungi, mainly ascomycetes[20], forming endo- or ectendomycorrhizae (Box 1 and Fig. 4). These associations allow utilization of dead organic matter and the successful colonization of heathland soils, where nitrogen and phosphorus are slowly or not mineralized. Intracellular coil-forming associations with basidiomycetes occur in the Orchidaceae (Box 1); orchids are completely dependent on fungal carbohydrates at the protocorm level, and throughout life in achlorophyllous species[20]. Both orchid and ericalean associations probably arose early in the evolution of the host taxa, in which they are highly frequent, but this remains difficult to date precisely. Mycothalli with features similar to orchid and ericalean mutualisms are also known in hepatics: basidiomycetes intracellularly colonize some Jungermanniales and Metzgeriales in peaty soils[35]; some Jungermanniales found on organic-rich soils host ascomycetes identical to ericoid fungi, infecting rhizoids and flagelliform subterranean axes[36] (Fig. 4). A lateral transfer of symbiont, rather than a common ancestral association, is likely to explain such similarities[36]. Other specialized root associations may exist; for instance, some plants of high arctic and alpine environments are often associated with the so-called 'dark septate fungi'[37].

To summarize, as formerly hypothesized with regard to lichen evolution, septate mycobionts arose secondarily in plant evolution. This diversification of mutualism allowed colonization of new terrestrial ecosystems mainly by exploiting the organic matter of the environment. Finally, several species evolved in a heterotrophic habit with the help of mycorrhizal fungi[38]. Plant 'mycotrophy' arose in monocots and dicots, but also in subterranean perennial gametophytes of some pteridophytes[21] and in some hepatics[35], entailing a loss of phototrophy. The septate, or more rarely non-septate, mycobiont exploits either soil resources or other phototrophs through mycorrhizal association[39].

However, mutualism between plants and septate fungi extends far beyond trophic relationships within root tissues. Symptomless endophytes, mainly ascomycetes, living intercellularly in plants, are increasingly being discovered[40]. They have been extensively studied in grasses and sedges, where clavipitaceous endophytes proved to be mutualistic. This symbiosis is interpreted as a defence mutualism, thanks to fungal alkaloids[40]. The association was also shown to improve nitrogen utilization and drought resistance through enhanced osmotic adjustment and lower stomatal conductance[41]. Other endophytic associations with fungi, particularly in the woody species, may await discovery: although latent pathogens and saprotrophs could be represented, some endophytes that do not colonize dead tissues may represent true biotrophs, that could protect their host against pathogens[42]. Conversion of a pathogenic ascomycete into a symptomless and protective endophyte was shown to require a single mutation[43], and may thus be a rather common event.

Why 1 + 1 > 2 in terrestrialization

Adaptation to terrestrial life has often arisen through symbiosis, which brought together genetic material having different and complementary characteristics. The two partners are partly 'preadapted' to live on land. The mycelial habit is well adapted to three-dimensional exploration of the substrate. Some mycobionts have a huge weathering potential, that allows access to non-soluble mineral elements[44], or even saprotrophic ability, that compensates for the low availability of mineral resources in most soils. On the other hand, phototrophs are well preadapted to gas exchange and photon collection. The symbiotic association is thus well-suited for exploiting the interface between soil and atmosphere.

The emergence of new (= symbiogenetic) properties in the symbiotic association also contributes to the success of mutualism in land ecosystems. Symbiosis entails reciprocal modifications of both partners: hormone-like substances induce morphological changes in both parasitic (tumors and galls) and mutualistic symbioses, as described in mycorrhiza[45]. Strikingly, lichen fungi exhibit highly differentiated vegetative stroma, in contrast to non-lichenized fungi[8]. The presence of a fungus may have facilitated the emergence of thick parenchymatous tissues in plants, whose charophycean ancestors had simple morphology. Hyperplasia of organs may enhance protection and increase opportunities for new adaptations[46]. Symbiosis-related modifications are also evident at the physiological and biochemical levels, for instance improvement of drought-resistance[19,41] by systemic endophytes of grasses and fungi in mycophycobioses. Reciprocal induction of genes and metabolic pathways may lead to their constitutive expression, as exemplified by lichen products (aromatic compounds that are synthesized by the fungus only in the presence of the algal partner[6,47]) or plant phenylpropanoids (such as flavonoids that are elicited by mycobionts such as VA fungi[48] or EcM[49]). Both kinds of compound improve resistance to various stresses, mainly UV radiation and parasitic or grazing organisms[47,50]. Systemically induced defensive compounds could have been of significant advantage for primitive land associations[17], which were presumably slow-growing and perhaps less tolerant of pathogens and grazing. Stimulation of phenylpropanoid metabolism may have given opportunities for the synthesis of lignin[46], a novelty of major consequence for vascular plants.

Symbiosis, which allows evolutionary jumps by creating new entities with new abilities, was repeatedly used during land colonization by algae and plants, and is still helpful (if not necessary) to most terrestrial phototrophs. In some lineages, mutualism with fungi is an ancestral feature that probably allowed terrestrialization (e.g. VA symbiosis in plant emergence); in other lineages, it has favored the colonization of extreme environments (e.g. mycophycobioses, lichens and ericalean mycorrhizae). New fossil data, mainly from early land phototrophs, would help to clarify the origin and antiquity of these various associations. Awareness of the symbiotic status of terrestrial phototrophs is rising among physiologists and ecologists[23]; however, the role of extant mycobionts, mainly systemic endophytes, still requires much investigation.

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References

1 Horodyski, R.J. and Knauth, L.P. (1994) **Life on land in the Precambrian,** *Science* 263, 494–498

2 Taylor, T.N. and Taylor, E.L. (1993) *The Biology and Evolution of Fossil Plants,* Prentice Hall

3 Peat, H.J. and Fitter, A.H. (1993) **The distribution of arbuscular mycorrhizas in the British flora,** *New Phytol.* 125, 845–854

4 Taylor, T.N. (1990) **Fungal associations in the terrestrial palaeoecosystem,** *Trends Ecol. Evol.* 5, 21–25

5 Berbee, M.L. and Taylor, J.W. (1993) **Dating the radiations of the true fungi,** *Can. J. Bot.* 71, 1114–1127

6 Ahmadjian, V. (1993) *The Lichen Symbiosis,* Wiley

7 Gargas, A. *et al.* (1995) **Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny,** *Science* 268, 1492–1495

8 Hawksworth, D.L. (1988) **Coevolution of fungi with algae and cyanobacteria in lichen symbioses,** in *Coevolution of Fungi with Plants and Animals* (Pirozynski, K.A. and Hawksworth, K.A., eds), pp. 125–148, Academic Press

9 Sherwood-Pike, M.A. and Gray, J. (1985) **Silurian fungal remains: probable records of the class Ascomycetes,** *Lethaia* 18, 271–285

10 Retallack, G.J. (1994) **Were the Ediacarian fossils lichens?** *Paleobiology* 20, 523–544

11 Stein, W.E., Harmon, G.D. and Hueber, F.M. (1993) ***Spongiophyton* from the Lower Devonian of North America reinterpreted as a lichen,** *Am. J. Bot.* 80, 93

12 Burgess, N.D. and Edwards, F.L.S. (1988) **A new palaeozoic plant closely allied to *Prototaxites* Dawson,** *Bot. J. Linn. Soc.* 97, 189–203

13 Sherwood-Pike, M.A. (1985) ***Pelicothallos* Dilcher, an overlooked fossil lichen,** *Lichenologist* 17, 114–115

14 Lutzoni, F. and Vilgalys, R. (1995) ***Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichens,** *Crypt. Bot.* 5, 71–81

15 Gehrig, H.H., Schussler, A. and Kluge, M. (1996) ***Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (Cyanobacteria), is an ancestral member of the Glomales – evidence by SSU rRNA analysis,** *Mol. Evol.* 43, 71–81

16 Mollenhauer, D. and Kluge, M. (1994) ***Geosiphon pyriforme*,** *Endocytobiosis Cell Res.* 10, 29–34

17 Taylor, T.N., Hass, H. and Kerp, H. (1997) **A cyanolichen from the Lower Devonian Rhynie Chert,** *Am. J. Bot.* 84, 992–1004

18 Kohlmeyer, J. and Kohlmeyer, E. (1979) *Marine Mycology: the Higher Fungi,* Academic Press

19 Garbary, D.J. and London, F.J. (1995) **The *Ascophyllum*, *Polysiphonia*, *Mycosphaerella* symbiosis – V. Fungal infection protects *A. nodosum* from desiccation,** *Bot. Mar.* 38, 529–533

20 Smith, S.E. and Read, D.J. (1997) *Mycorrhizal Symbiosis* (2nd edn), Academic Press

21 Boullard, B. (1979) **Considérations sur la symbiose fongique chez les Ptéridophytes,** *Syllogeus* 19, 1–58

22 Ligrone, R. and Lopes, C. (1989) **Cytology and development of a mycorrhiza-like infection in the gametophyte of *Conocephalum conicum* (L.) Dum. (Marchantiales, Hepatophyta),** *New Phytol.* 111, 423–433

23 Newsham, K.K., Fitter, A.H. and Watkinson, A.R. (1995) **Multi-functionality and biodiversity in arbuscular mycorrhizas,** *Trends Ecol. Evol.* 10, 407–411

24 Pirozynski, K.A. and Malloch, D.W. (1975) **The origin of land plants: a matter of mycotrophism,** *BioSystems* 6, 153–164

25 Pirozynski, K.A. and Dalpé, Y. (1989) **Geological history of the *Glomaceae* with particular reference to mycorrhizal symbiosis,** *Symbiosis* 7, 1–36

26 Simon, L. *et al.* (1993) **Origin and diversification of endomycorrhizal fungi and coincidence with vascular plants,** *Nature* 363, 67–69

27 Taylor, T.N. *et al.* (1995) **Fossil arbuscular mycorrhizae from the early Devonian,** *Mycologia* 87, 560–573

28 Taylor, T.N., Hass, H. and Remy, W. (1992) **Devonian fungi: interactions with the green alga *Paleonitella*,** *Mycologia* 84, 901–910

29 Ligrone, R. (1988) **Ultrastructure of a fungal endophyte in *Phaeoceros laevis* (L.) Prosk. (Anthocerotophyta),** *Bot. Gaz.* 149, 92–100

30 Phipps, C.J. and Taylor, T.N. (1997) **Mixed arbuscular mycorrhizae from the Triassic of Antarctica,** *Mycologia* 88, 707–714

31 Hetrick, B.A.D. (1991) **Mycorrhizas and root architecture,** *Experientia* 47, 355–362

32 LePage, B.A. *et al.* (1997) **Fossil ectomycorrhizae from the middle Eocene,** *Am. J. Bot.* 84, 410–412

33 Cazares, E. and Smith, J.E. (1996) **Occurrence of vesicular–arbuscular mycorrhizae in *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in Oregon Coast Range soils,** *Mycorrhiza* 6, 65–67

34 Lobuglio, K.F., Berbee, M.L. and Taylor, J.W. (1996) **Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among ascomycetes,** *Mol. Phyl. Evol.* 6, 287–294

35 Ligrone, R. *et al.* (1993) **A comparative ultrastructural study of endophytic basidiomycetes in the parasitic achlorophyllous hepatic *Cryptothallus mirabilis* and the closely allied photosynthetic**

species *Aneura pinguis* (Metzgeriales), *Can J. Bot.* 71, 666–679

36 Duckett, J.G. and Read, D.J. (1995) **Ericoid mycorrhizas and rhizoid–ascomycete associations in liverworts share the same mycobiont: isolation of the partners and resynthesis of the associations *in vitro*,** *New Phytol.* 129, 439–447

37 Gardes, M. and Dahlberg, A. (1996) **Mycorrhizal diversity in arctic and alpine tundra: an open question**, *New Phytol.* 133, 147–157

38 Leake, J.R. (1994) **The biology of myco-heterotrophic (‘saprophytic’) plants,** *New Phytol.* 127, 171–216

39 Cullings, K., Szaro, T.M. and Bruns, T.D. (1996) **Evolution of extreme specialization within a lineage of ectomycorrhizal parasites,** *Nature* 379, 63–66

40 Redlin, S.C. and Carris, L.M., eds (1996) *Endophytic Fungi in Grasses and Woody Plants*, APS Press

41 Elmi, A.A. and West, C.P. (1995) **Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue,** *New Phytol.* 131, 61–67

42 Caroll, G. (1988) **Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont,** *Ecology* 69, 2–9

43 Freemann, S. and Rodriguez, R.J. (1993) **Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist,** *Science* 260, 75–78

44 Elmi, A.A. and West, C.P. (1995) **Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue,** *New Phytol.* 131, 61–67

44 Lapeyrie, F. *et al.* (1991) **Phosphate solubilizing activities of ectomycorrhizal fungi *in vitro*,** *Can. J. Bot.* 69, 342–346

45 Gogala, N. (1991) **Regulation of mycorrhizal infection by hormonal factors produced by host and fungi,** *Experientia* 47, 331–340

46 Lewis, D.H. (1991) **Mutualistic symbioses in the origin and evolution of land plants,** in *Symbiosis as an Evolutionary Innovation in Speciation and Morphogenesis* (Margulis, L. and Fester, R., eds), pp. 288–300, MIT Press

47 Fahselt, D. (1994) **Secondary biochemistry of lichens,** *Symbiosis* 16, 117–165

48 Harrison, M.J. and Dixon, R.A. (1994) **Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme*,** *Plant J.* 6, 9–20

49 Weiss, M. *et al.* (1997) **Tissue-specific and development-dependent accumulation of phenylpropanoids in larch mycorrhizas,** *Plant Physiol.* 114, 15–27

50 Ensminger, P.A. (1993) **Control of development in plants and fungi**

Can elevated CO_2 affect secondary metabolism and ecosystem function?

Josep Peñuelas and Marc Estiarte

It has generally been assumed that increasing atmospheric CO_2 concentrations will increase plant carbon-based secondary or structural compounds concentrations. These changes may have far-reaching consequences for herbivory and plant litter decomposition. Recent experimental results provide evidence of increases in concentrations of soluble phenolics and condensed tannins but not in lignin, structural polysaccharides or terpenes. They also show significant effects of these plant chemical changes on herbivores and little or any effects on decomposition. However, there is no consistent evidence of any of these effects at the complex long-term ecosystem level.

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It is widely known that the atmospheric concentration of CO_2 has risen about 30% from the pre-industrial concentration and it is continuously rising because of human activities[1]. This increase is likely to affect biota indirectly, via climate change, and directly, by producing changes not only in plant growth and allocation but also in plant tissue chemical composition[1]. Among such composition changes, decreased leaf N concentration has been widely reported[2,3]. The concentration of carbon-based secondary or structural compounds (CBSSC), like phenolics, terpenes or structural polysaccharides (cellulose, hemicellulose and pectin) is also expected to increase[4,5]. It has been generally assumed that such changes could have far-reaching consequences for herbivory[6–8], and plant litter decomposition[5,9,10]. Recently, several experimental studies have provided data that, although heterogeneous and far from sufficient, is worth summarizing as a current attempt to elucidate (1) whether the expected increases in CBSSC under elevated CO_2 really occur or not, (2) when they do occur, whether they are general or particular for different compound classes and different plant species, and (3) whether they really will affect herbivory and decomposition in the field.

Expected increases in carbon based compounds

The concentration of nutrients and especially CBSSC in plants are very variable, because of genetic differences within and between species, and also because of varying availability of resources. Several studies have shown that the balance between the availabilities of carbon and nutrients is a primary determinant of the phenotypic expression of CBSSC (reviewed in Ref. 11).

The widely reported decreases in plant tissue nitrogen concentrations and the consequent increases in C/N ratios under elevated CO_2 atmospheres[2,3] moved some authors[4,12] to predict a larger accumulation of CBSSC in plant tissues in response to increasing atmospheric CO_2 concentrations. Such predictions had their roots in the carbon–nutrient balance hypothesis, which directly relates plant CBSSC concentration to the balance between C and N in the plant[13], and on the growth–differentiation balance hypothesis[11,14], which inversely relates growth and differentiation processes in the plant. The latter hypothesis, which includes and extends the former, considers that any environmental condition that affects photosynthesis (carbon source) and growth (carbon sink) with different intensity will affect the relative carbon pool available for allocation to carbon based compounds. Thus, these hypotheses are in fact source–sink balance hypotheses, a summary of which is given in Box 1.

Factors such as high CO_2 concentration, which increases carbon supply, or nutrient stress, which decreases carbon demand, promote a relative increase of carbon availability

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