





ORIGINAL ARTICLE

No support for the emergence of lichens prior to the evolution of vascular plants

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Abstract

The early-successional status of lichens in modern terrestrial ecosystems, together with the role lichen-mediated weathering plays in the carbon cycle, have contributed to the long and widely held assumption that lichens occupied early terrestrial ecosystems prior to the evolution of vascular plants and drove global change during this time. Their poor preservation potential and the classification of ambiguous fossils as lichens or other fungal–algal associations have further reinforced this view. As unambiguous fossil data are lacking to demonstrate the presence of lichens prior to vascular plants, we utilize an alternate approach to assess their historic presence in early terrestrial ecosystems. Here, we analyze new time-calibrated phylogenies of ascomycete fungi and chlorophytan algae, that intensively sample lineages with lichen symbionts. Age estimates for several interacting clades show broad congruence and demonstrate that fungal origins of lichenization postdate the earliest tracheophytes. Coupled with the absence of unambiguous fossil data, our work finds no support for lichens having mediated global change during the Neoproterozoic–early Paleozoic prior to vascular plants. We conclude by discussing our findings in the context of Neoproterozoic–Paleozoic terrestrial ecosystem evolution and the paleoecological context in which vascular plants evolved.

KEYWORDS

carbon cycle, fungi, terrestrial ecosystems, weathering

1 | INTRODUCTION

Terrestrial vegetation contributes to carbon cycling through photosynthetic carbon fixation and rock weathering and has been invoked as a cause or significant feedback in nearly all major shifts in atmospheric composition and climate since at least the Neoproterozoic, including the rise of oxygen, deposition of coal, and major glaciations (Kennedy, Droser, Mayer, Pevear, & Mrofka, 2006; Knauth & Kennedy, 2009; Lenton, Crouch, Johnson, Pires, & Dolan, 2012; Lenton et al., 2016; Nelsen, DiMichele, Peters, & Boyce, 2016; Pagani, Caldeira, Berner, & Beerling, 2009; Robinson, 1990). Biotic influence on the chemical weathering of silicate rocks—a principal

sink of atmospheric CO₂ over geologic timescales—is largely via vascular plants (tracheophytes) and their mycorrhizal fungi in extant ecosystems (Berner, 1998, 2004; Taylor et al., 2009); however, vascular plants do not appear in the fossil record until the later Silurian (Gensel, 2008; Rickards, 2000), and did not achieve deep-rooting via the tree habit until the Middle Devonian (Algeo & Scheckler, 1998). What contributed to these processes before vascular plants?

Prior to the evolution of tracheophytes, any biological mediation of weathering would have been restricted to soil and rock surfaces with little biotic infiltration into the substrate. Since the Neoproterozoic–early Paleozoic terrestrial fossil record is scant and taxonomic identities are ambiguous, community composition during

the original emergence and evolution of early terrestrial ecosystems is frequently inferred through molecular-clock age estimates or assumed to follow the sequence of ecological succession seen on rock surfaces, desert soils, and other analogous modern ecosystems (Gadd, 2007; Gray & Shear, 1992; Grube & Hawksworth, 2007; Heckman et al., 2001; Schwartzman, 1999; Tomescu, Rothwell, & Honegger, 2009). Non-vascular land plants are often implicated as drivers of global change during this time, but do not appear in the fossil record until the Middle Ordovician (Rubinstein, Gerrienne, de la Puente, Astini, & Steemans, 2010), with molecular dates suggesting a mid-Cambrian to early Ordovician appearance (Morris et al., 2018). Cyanobacterial and eukaryotic algal crusts also weather silicates (Olsson-Francis, Simpson, Wolff-Boenisch, & Cockell, 2012), and indirect lines of evidence suggest the presence of terrestrial radiations of cyanobacteria and eukaryotic algae during the Proterozoic (Lenton & Daines, 2017; Wellman & Strother, 2015). Finally, as common pioneers of barren substrates in extant systems, lichens have long (since at least the 1850's) and frequently been suggested as a precursor vegetation—perhaps as early as the Neoproterozoic—which could have accommodated carbon cycle feedbacks and formed soil prior to the evolution of tracheophytes (Berner, 1992; Church, 1921; Eriksson, 2005; Gadd, 2007; Golubic & Schneider, 2003; Gray & Boucot, 1977; Grube & Hawksworth, 2007; Heckman et al., 2001; Kennedy et al., 2006; Knauth & Kennedy, 2009; Lenton et al., 2016; Lindsay, 1856; Porada et al., 2016; Schwartzman, 1999; Schwartzman & Volk, 1989; Tomescu et al., 2009; Weber, Büdel, & Belnap, 2016). Consequently, ascertaining the age of lichens is also important for understanding their contribution to biogeochemical cycles and climate change over time.

Lichens—an archetype of symbiosis (de Bary, 1879; Frank, 1877)—are stable, extracellular associations between fungi (mycobiont) and eukaryotic algae and/or cyanobacteria (photobiont) in which the exhabitant mycobiont forms a thallus around the photobiont, which feeds the fungus photosynthetically derived carbohydrates (Ahmadjian, 1993; Jahns, 1988). Lichens dominate about ~7% of the earth's terrestrial surface (Larson, 1987) and modify numerous aspects of extant biocrust-dominated ecosystems. Carbon and nitrogen are drawn into the ecosystem via photosynthesis and nitrogen-fixation by lichen symbionts, enabling the development and growth of lichen thalli and nutrient transference upon decomposition or consumption. They enhance ecosystem complexity by supporting diverse intra-thalline bacterial and eukaryotic communities and serve as a habitat and food source to a range of soil invertebrates (Weber et al., 2016). Lichen thalli stabilize the soil in these ecosystems by extending rhizines into the substrate, and increase the trapping of aeolian dust via their surface roughness (Weber et al., 2016). The presence of lichens may raise surface temperatures by lowering albedo (Belnap, 1995; Rutherford et al., 2017) and influences a number of soil properties, such as soil porosity and pore formation. While effects vary, lichens overall tend to increase water infiltration and retention, while stabilizing soil and reducing erosion (Belnap, 2006; Weber et al., 2016). Finally, lichens play important roles in mineral weathering and pedogenesis, thereby impacting

the carbon cycle and, potentially, atmospheric CO₂ levels (Ascaso & Galvan, 1976; Banfield, Barker, Welch, & Taunton, 1999; Chen, Blume, & Beyer, 2000; Gadd, 2007; Porada, Weber, Elbert, Pöschl, & Kleidon, 2013; Quirk et al., 2015). Given their wide range of ecosystem service contributions in extant biocrusts, lichens could have substantially modified soil biogeochemistry, stability, and hydrology, as well as the complexity of early terrestrial ecosystems—if they were present. While substantial progress has been made in clarifying the evolution of lichens, a synthetic perspective linking the macro-evolutionary histories of lichen-forming fungi (LFF) and algae (LFA) is lacking. A refined understanding of the evolutionary sequence, stability, and timing of one of the oldest and best-known symbioses is critical to understand the evolutionary origins of lichens, and their contributions to geochemical cycles and climatic change over geologic timescales.

In contrast to the identification of a single taxon from the fossil record, the unambiguous identification of a fossil lichen association requires the presence of *both* symbionts *and* evidence of a physiological interaction, or the unambiguous assignment to an extant lichenized lineage based on external morphology (Lücking & Nelsen, 2018; Taylor, Hass, & Kerp, 1997). Support for an early origin of lichens includes lichen-like fossils from as early as the Neoproterozoic, but these all lack features diagnostic of lichens and have been rejected or regarded as ambiguous (Antcliffe & Hancy, 2013; Karatygin, Snigirevskaya, & Vikulin, 2009; Lücking & Nelsen, 2018; Matsunaga, Stockey, & Tomescu, 2013; Poinar, Peterson, & Platt, 2000; Taylor et al., 1997; Tomescu, Klymiuk, Matsunaga, Bippus, & Shelton, 2016). Similarly, a variety of thalloid fossils are known from the Silurian and Devonian, but preservation is generally not adequate to distinguish between microbial mats, land plant gametophytes, extinct algal experiments, or lichens. A small number of charcoalified fragments from the Silurian-Devonian containing stratified thalli are certainly comparable to lichens, but positive taxonomic and ecological identification is contingent on some caveats of preservation (see Supporting information), and are no older than vascular plants related to modern lycopsids (Edwards, Axe, & Honegger, 2013; Edwards, Honegger, Axe, & Morris, 2018; Honegger, Edwards, & Axe, 2013). Thus, there is a paucity of convincing fossil evidence to support the existence of pre-tracheophyte lichens. Furthermore, experimental replication of taphonomic processes demonstrate the destruction of key characteristics of modern lichen thalli, suggesting breakthrough fossils may be unlikely to be forthcoming (Graham, Trest, Cook, & Dunn, 2017; Honegger et al., 2013; Lücking & Nelsen, 2018; Tomescu, Tate, Mack, & Calder, 2010). Given the challenges of obtaining incontrovertible evidence of lichens in the fossil record, molecular phylogenetic studies may provide a complementary approach to estimate the age of lichen associations.

Reconstructing the temporal origins of an ecological mode, rather than a systematic entity, requires both the inference of a time-scaled phylogeny, and the identification of which nodes in the phylogeny represent evolutionary acquisitions of the ecological mode. Previous ancestral state reconstructions are most consistent with multiple transitions to the lichen-forming habit (Gargas, DePriest,

Grube, & Tehler, 1995; Gueidan et al., 2008; Lutzoni, Pagel, & Reeb, 2001; Schoch et al., 2009), and separate molecular-dating studies suggest a Paleozoic (541–252.2 Ma) to Mesozoic age for clades of LFF (Amo de Paz, Cubas, Divakar, Lumbsch, & Crespo, 2011; Beimforde et al., 2014; Ertz & Diederich, 2015; Gueidan, Ruibal, de Hoog, & Schneider, 2011; Lücking & Nelsen, 2018; Lumbsch & Rikkinen, 2017; Pérez-Ortega, Garrido-Benavent, Grube, Olmo, & de los Ríos, 2016). However, recent work has instead relied on a non-quantitative approach to disentangle where lichenization evolved in fungi, and asserted a single ascomycete origin of lichenization as early as the Ordovician—concurrently with or prior to tracheophytes (Lutzoni et al., 2018). Thus lichens could still have been responsible, in theory, for Ordovician environmental impacts that pre-dated tracheophytes. However, several clades of LFF (Ertz & Diederich, 2015; Lücking et al., 2008; Lumbsch, Zimmermann, & Schmitt, 2009; Nelsen et al., 2009; Nelsen, Lücking, et al., 2011; Printzen, Cezanne, Eichler, & Lumbsch, 2012; Reeb, Lutzoni, & Roux, 2004) not included in these studies (some of which are lacking from the alignment utilized (James et al., 2006) in recent work (Lutzoni et al., 2018)) have recently been shown to occupy isolated phylogenetic positions within Pezizomycotina (the largest ascomycete subphylum), or were recovered from larger clades of non-lichen-forming fungi (NLFF), thereby confounding the interpretation of previous studies lacking these important lineages. Consequently, the phylogenetic position and age of lichenization in fungi remains unclear.

As LFF derive their carbohydrates from LFA for nutrition, this also raises questions about which algal lineages could have associated with the earliest LFF. Most LFF associate with eukaryotic green algae, and the phylogenetic distribution and abundance of LFF associated with chlorophytan algae (90%) (Högnabba, Stenroos, Thell, & Myllys, 2009; Honegger, 2009; Miadlikowska et al., 2014) is consistent with these algae facilitating the early evolution and diversification of most LFF lineages. Despite their importance to LFF, these LFA represent only a small proportion (<2%) of all chlorophytan species (Guiry & Guiry, 2019; Honegger, 2009; Voytsekhovich, Mikhailuk, & Darienko, 2011) and occupy isolated positions within larger clades of non-LFA (Friedl & Bhattacharya, 2002; Malavasi et al., 2016; Sanders, Pérez-Ortega, Nelsen, Lücking, & Ríos, 2016). Consequently, these isolated lineages are thought to represent independent origins of LFA (Friedl & Bhattacharya, 2002). The vast majority of LFF associate with LFA derived from just two of these putative origins (Trebouxiaceae: ≥50%; Trentepohliaceae: ≥25%), highlighting the importance of these clades for the evolution of LFF (Ahmadjian, 1993; Nelsen, Rivas Plata, Andrew, Lücking, & Lumbsch, 2011). While previous efforts have been made to date chlorophytan algae (De Wever et al., 2009; Lutzoni et al., 2018), they have not concentrated on LFA or have yielded highly uncertain age estimates; consequently, robust age estimates of most clades of LFA are lacking.

Here, we inferred new time-calibrated molecular phylogenies of fungi and used ancestral state estimates to infer origins of LFF. We also inferred a new dated algal phylogeny to obtain ages of clades representing putative origins of LFA (Friedl & Bhattacharya, 2002). We did not explicitly reconstruct ancestral states on the algal tree, as

LFA regularly occur free-living (Friedl & Büdel, 2008), and inferences of LFA ancestry does not positively indicate the ancestral presence of lichen associations. We then compared ages of LFF, fossil tracheophytes, and clades including LFA, to determine whether lichens pre-date tracheophytes.

2 | METHODS

All analyses were performed on laboratory clusters, personal computers, the Research Computing Cluster at the University of Chicago, and the Cipres Portal (Miller, Pfeiffer, & Schwartz, 2010). A stepwise approach was taken to obtain age estimates of Ascomycota taxa. This approach was taken due to computational constraints and analytical challenges encountered in analyses in BEAST analyses. Alternate Bayesian dating software, such as mcmctree, were not used because of its restriction to a single data type. A restricted dataset was first assembled and a relaxed-clock analysis performed. A larger dataset was then assembled and age estimates of shared nodes between the restricted and broad phylogenies were used as used as age constraints when time-scaling the broad phylogeny.

2.1 | Fungal analyses

Stepwise molecular-clock analyses were performed to estimate ages of Ascomycota taxa—a clade which encompasses approximately 98% of all LFF (Honegger, 2009). A restricted dataset was constructed to provide age estimates that could be used as secondary calibrations for time-scaling a more densely sampled phylogeny. This dataset was also used to estimate the crown nodes of several lichenized fungal lineages, which were then compared with ages of interacting algal clades (see Supporting information). Sequences (18S, 28S, mtSSU, RPB1, RPB2, TEF1α) were retrieved from NCBI and supplemented with newly generated RPB2 and mtSSU sequences (Table S1). Sequences were aligned, and a partitioned maximum likelihood (ML) analysis was conducted in RAxML 8.2.9 (Stamatakis, 2014) with support estimated through 1,000 rapid bootstrap pseudoreplicates (Stamatakis, Hoover, & Rougemont, 2008) (Figure S1). Minimum ages for fossil calibrations were identified (Table S2), and the consistency between fossil age estimates was evaluated with previously established methods (Dornburg, Beaulieu, Oliver, & Near, 2011; Marshall, 2008). Minimum ages were largely determined based on the upper limit of the stratigraphic interval in which fossils were found. Following the methods of Dornburg et al., (2011) and Marshall (2008), fossil age estimates were evaluated for consistency (Table S2). This facilitated the retention of consistent calibrations while removing those that were potentially misplaced or distant from the actual time of divergence. Three BEAST 2 (Bouckaert et al., 2014) chains were run without any calibration priors for just under 50 million generations each, sampling every 20,000 generations. A burn-in period was discarded, and chains combined. Following Ref (Dornburg et al., 2011; Marshall, 2008), trees were subsequently rescaled to a height of 1 and the empirical scaling factor (s_j) (Marshall, 2008)

calculated for each calibration over the pool of trees (Table S2). The empirical scaling factor provides an estimate for the root age if only that fossil calibration were used. The 95% HPD for each calibration was calculated and the density of s_i values for each calibration were compared (Dornburg et al., 2011). The calibration with the highest s_i value was selected, and priors with lognormal distributions were assigned to all calibrations whose 95% HPD overlapped with that of the calibration with the highest mean s_i value, while uniform priors were assigned to all others. The upper limit for calibrations with a lognormal shape was determined by using the $FA_{0.95}$ of (Marshall, 2008). Four BEAST chains were run for approximately 45 million generations each under these priors, with the ucl.d.mean estimated, and a gamma-distributed prior with a shape of 0.001 and scale of 1,000 was set for the ucl.d.mean. The first 10% of each run was discarded and the remaining combined. ESS values were over 200 for all parameters. The MCC tree with 95% HPD node age estimates is included in Figure S2.

Age estimates from the above analyses were then used to date a larger and more detailed dataset under penalized likelihood (PL). The detailed dataset was constructed by iteratively adding sequences to the core alignment that was then manually refined and trimmed (Table S3). A ML analysis was conducted utilizing the methods described above for the restricted fungal dataset, and the detailed ML tree and all trees inferred from bootstrap analyses (BS) (Figure S3) were time-scaled (penalized likelihood) with ages for nodes shared with the relaxed-clock maximum clade credibility (MCC) tree obtained from the restricted dataset (ML-M, and BS-M, respectively). For each analysis, a log rate penalty was used, and a cross-validation procedure was conducted to identify the optimal scaling factor (1e-6 to 1,000). To further account for uncertainty in both topology and age estimates, the ML tree was time-scaled with a sample (S) of 100 randomly selected post-burn in trees from the restricted analysis (ML-S), and 100 bootstrap replicates were time-scaled with 50 of these randomly selected post-burn in trees (BS-S). In all analyses, node ages shared with those from the restricted dataset were fixed to reflect node ages derived from these analyses. Together, this resulted in the analysis of over 5,500 trees varying in their topology and/or age constraints.

Individual species were coded as lichen-forming or not. Ancestral state reconstruction (ASR) was then performed on all sets of time-scaled trees using the hidden rates model in corHMM, with the most recent common ancestor (MRCA) of Ascomycota constrained to be non-lichen-forming (Beaulieu & O'Meara, 2017; Beaulieu, O'Meara, & Donoghue, 2013). Models with 1–2 rate categories were fit and the small size-corrected Akaike information criterion (AICc) values compared on all of the over 5,500 trees, and the model with the best value was retained. The number of transitions and ages of state changes were determined by comparing the most likely state at each node. Ages (Figure S4) and numbers of changes (Figure S5) were then summarized across trees in an effort to accommodate topological uncertainty. We then compared age estimates for the origin of LFF with a fossil-based age estimate of ~425 Ma representing the origin of crown-group tracheophytes (Supporting information).

2.2 | Algal analyses

Lichens (by definition) include a photobiont; however, most LFA occur free-living (Friedl & Büdel, 2008). Thus, the occurrence of clades containing LFA prior to tracheophytes does not demonstrate the presence of lichens. Despite this, estimating the age of clades containing LFA provides insight into which clades were available for LFF to associate with when LFF evolved. Here, we compared the ages of putative origins of LFA (Friedl & Bhattacharya, 2002) with that of LFF and fossil tracheophytes. We also compared crown node ages of several algal clades known to interact with specific clades of LFF (Supporting information) to ascertain whether there was broad temporal concordance among interacting lineages. Taxa were selected to meet these two objectives (see Supporting information), and embryophytes and rhodophyten algae were included only to utilize their fossil records for dating clades of LFA. Five of the longest and most conserved chloroplast loci were selected (*atpA*, *atpB*, *psbA*, *psbB*, *rbcl*) and the 18S ribosomal rRNA gene were selected for a core set of taxa, and their sequences retrieved from GenBank and parsed (Table S4). Data were supplemented with newly generated chloroplast (*atpB* and *rbcl*) and 18S ribosomal RNA sequences. Sequences were aligned and manually refined. Additional taxa were then added to this core, and alignments trimmed. A search for the best-scoring ML tree was then run in RAxML 8.2.9 (Stamatakis, 2014) and support estimated by performing 1,000 rapid bootstrap pseudoreplicates (Stamatakis et al., 2008) (Figure S6). Calibrations and their priors (Table S5) for relaxed-clock analyses were selected as described in the Supporting information. Twelve runs of up to 43 million generations were run in BEAST 2, burn-in discarded, and chains resampled and combined (Figures S7–S8).

Together, this study relies on larger and more densely sampled Ascomycota and Chlorophyta datasets with a greater number of Ascomycota fossil calibrations relative to recent work (Lutzoni et al., 2018). Our work further differs through the use of molecular clocks and quantitative approaches (ancestral state reconstruction) to disentangle where lichenization evolved in fungi. Further details on taxon selection and methods employed are included in the Supporting information; associated code and files are available on GitHub (<https://github.com/mpnelsen/pha-li>).

3 | RESULTS

Ancestral state reconstruction (ASR) on all four sets of trees demonstrated that all nodes reconstructed as lichen-forming postdated tracheophytes, except in exceedingly rare instances. The earliest gains of LFF in the ML-M tree postdated tracheophytes (248.62 Ma) (Figure 1). While gains ranged from 431 Ma to 230.79 Ma in the BS-M trees (Figure S7, Table 1), they preceded the first fossil appearance of crown-group vascular plants in only 1% of the trees. In these cases, the MRCAs of Saccharomycotina—which contains no extant lichen-forming taxa—and Pezizomycotina were inferred to be lichen-forming, with subsequent losses of lichenization in each subclass,

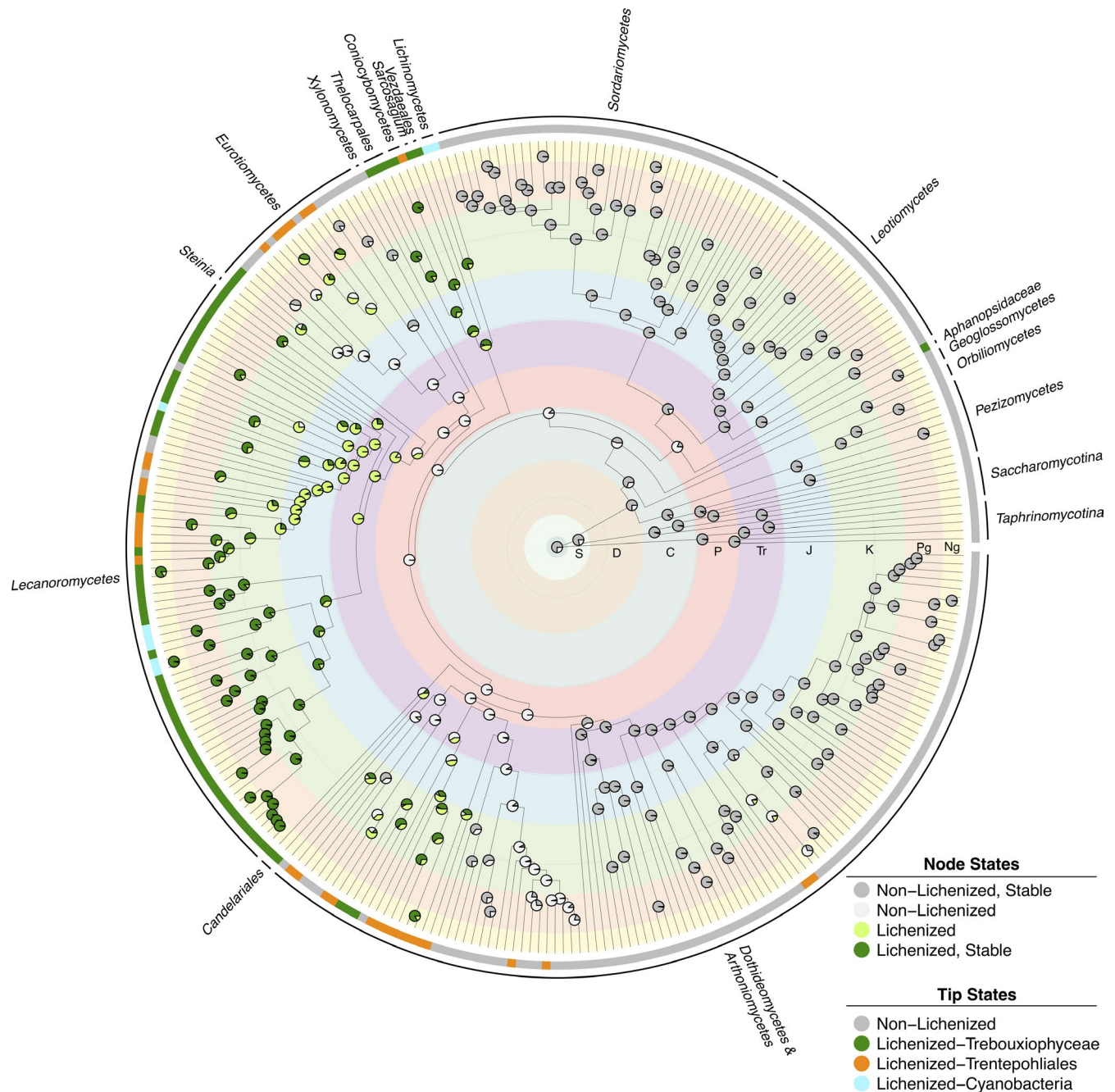


FIGURE 1 Time-scaled, ML tree derived from detailed fungal (Ascomycota) dataset. Pezizomycotina includes clades Pezizomycetes-Dothideomycetes/Arthoniomycetes. Tip states are colored according to photobiont type (if any), and nodes shaded proportionally to the marginal ASR. Node states are inferred from the analysis to be in a character state (lichenized vs. non-lichenized), and a rate category (unstable vs. stable). Rate category transitions only occur within a character state, and character state transitions only occur in the unstable rate category. Shaded rings underlying the phylogeny indicate geological time periods, and dashed lines occur in 100 Myr intervals. Geological periods are shaded and abbreviated as: Neogene (Ng), Paleogene (Pg), Cretaceous (K), Jurassic (J), Triassic (T), Permian (P), Carboniferous (C), Devonian (D), Silurian (S)

and occasional regains in Dothideomycetes (Pezizomycotina). Similarly, the earliest nodes reconstructed as LFF in the ML-S (334.8–193.4 Ma) topologies, and most of the BS-M trees (98.8%), postdated tracheophytes (Figure 2; Figure S7, Table 1). Inclusion of the stem was also consistent with a post-tracheophyte origin of LFF in the ML-M (296.63 Ma) and ML-S trees (375.7–249.8 Ma) and most of the BS-M (97.8%) and BS-S (95.2%) trees. Crown node age of

Pezizomycotina were also younger than vascular plants in all ML-M and BS-M trees, and most time-scaled ML-S (98%) and BS-S (95.7%) trees (Figure 1; Table 1). In all sets of analyses, multiple gains and losses of lichenization were typically inferred (Figure S8 and Table S6).

When considering the ages of clades representing putative origins of lichenization among Chlorophyta, our data suggest

TABLE 1 Age estimates of first LFF lineage Pezizomycotina derived from time-scaling trees from the densely sampled dataset

Tree	Cals	Node	Position	Min	2.5%	Mean	97.5%	Max
ML	Median	LFF	Crown	248.6	248.6	248.6	248.6	248.6
Bootstraps	Median	LFF	Crown	230.8	248.6	288.6	350.2	431.0
ML	Sample	LFF	Crown	193.4	210.5	257.8	332.7	334.8
Bootstraps	Sample	LFF	Crown	193.6	232.6	296.9	396.9	527.0
ML	Median	LFF	Stem	296.6	296.6	296.6	296.6	296.6
Bootstraps	Median	LFF	Stem	261.9	287.3	323.3	376.3	456.2
ML	Sample	LFF	Stem	249.8	252.6	300.6	354.3	375.7
Bootstraps	Sample	LFF	Stem	237.7	269.5	333.5	450.5	539.9
ML	Median	Pezizomycotina	Crown	377.6	377.6	377.6	377.6	377.6
Bootstraps	Median	Pezizomycotina	Crown	360.0	360.0	369.5	394.1	401.8
ML	Sample	Pezizomycotina	Crown	312.5	319.7	363.2	420.3	469.2
Bootstraps	Sample	Pezizomycotina	Crown	312.5	324.0	373.9	459.7	498.6
ML	Median	Pezizomycotina	Stem	431	431	431	431	431
Bootstraps	Median	Pezizomycotina	Stem	431	431	431	431	431
ML	Sample	Pezizomycotina	Stem	406.5	409.2	436.3	504.9	530.3
Bootstraps	Sample	Pezizomycotina	Stem	406.5	408.4	438.1	527.0	530.3

Note: The trees (*Tree*: densely sampled ML tree vs. densely sampled bootstrap replicates) and calibration method (*Cals*: median node age estimates from MCC of relaxed-clock analysis of sparsely sampled dataset vs. a sample of randomly sampled trees from posterior of relaxed-clock analysis of sparsely sampled dataset), the lineage investigated (*Node*: first LFF vs. Pezizomycotina), position (*Position*: crown vs. stem) and minimum (*Min*), mean, maximum (*Max*), and 2.5% and 97.5% quantile age estimates obtained. Age estimates for the densely sampled ML tree were calibrated with median node age estimates from the sparsely sampled MCC tree (*Tree*: ML; *Cals*: Median), while the topologies derived from 500 bootstrap analyses of the densely sampled dataset were calibrated the with median node age estimates from the MCC tree of the sparsely sampled dataset (*Tree*: Bootstrap; *Cals*: Median). Alternatively, the densely sampled ML tree was calibrated with a sample of 100 randomly sampled trees from the posterior of the relaxed-clock analysis of sparsely sampled dataset (*Tree*: ML; *Cals*: Sample), or 100 of the densely sampled bootstrap topologies were each time-scaled with 50 randomly sampled trees from the posterior of the relaxed-clock analysis of the sparsely sampled dataset (*Tree*: Bootstrap; *Cals*: Sample).

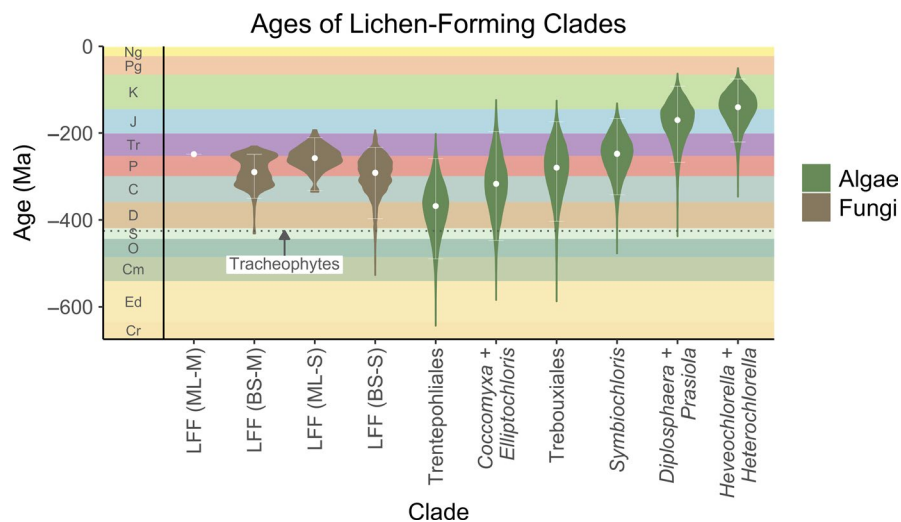


FIGURE 2 Crown age estimates for LFF and putative origins of LFA (distribution, median [white point] and 95% HPD [white bars]). The ML-M estimate consists of a single tree, and LFF age estimates for the ML and BS trees were time-scaled with the MCC tree (M) or a sample from the posterior (S). The horizontal dashed line indicates the minimum, fossil-based age estimate for the origin of tracheophytes. Horizontal, shaded bands indicate geological periods and are abbreviated as in Figure 1 with the addition of the Ordovician (O), Cambrian (Cm), Ediacaran (Ed), and Cryogenian (Cr)

that most LFA-containing clades have median crown ages in the Mesozoic-Cenozoic (252.2 Ma-present)—substantially postdating the evolution of tracheophytes (Figure 2). The 95% HPD interval

of *Diplosphaera* + *Prasiola*, *Symbiochloris* and *Heveochlorella* + *Heterochlorella* are all inferred to have mid-late Mesozoic crown ages, while *Trebouxiales* is inferred to be mid-Paleozoic to mid-Mesozoic

in age—all postdating tracheophytes. The *Coccomyxa* + *Elliptochloris* clade is inferred to have Mesozoic-Paleozoic origins, with only a small proportion (5.4%) of age estimates pre-dating tracheophytes. Similarly, the order Trentepohliales is also inferred to be Mesozoic-Paleozoic in age, with only 17.6% of all crown age estimates pre-dating tracheophytes. Finally, age estimates of the earliest inferred LFF overlap with the ages of many of these algal clades (Figure 2). Stem ages often reinforce patterns relative to tracheophytes and between symbionts, but ages are treated with caution, as sister-group relationships are typically unclear, and sampling not designed to capture them (Table 1; Table S7). Additional comparisons of several independently dated clades of interacting LFF and LFA illustrate broad congruence at coarse temporal and phylogenetic scales, providing further support for our age estimates, while also illustrating the close evolutionary coupling between LFF and LFA lineages (Figure S9).

4 | DISCUSSION

4.1 | Lichens postdate vascular plants

Our analyses indicate with a high probability that lichens evolved after vascular plants and therefore could not have mediated global change during the Neoproterozoic-early Paleozoic. Ascomycete LFF are inferred to have evolved after tracheophytes with high confidence. Even if one assumes that the fungal clade Pezizomycotina was ancestrally lichen-forming (Eriksson, 2005), our age estimates consistently recover the crown node of Pezizomycotina as younger than tracheophytes. A small number of LFF not studied here are also known from Basidiomycota, but these are restricted to five isolated lineages within Agaricomycetes, a group that postdates tracheophytes, and whose MRCA was not lichen-forming (Floudas et al., 2012; Kohler et al., 2015). Thus, the ages of extant lineages of LFF are consistent with lichens postdating the origin of tracheophytes. Accepting the early presence of LFF requires one to invoke the evolution of lichenization in clades lacking any extant, lichenized descendants outside Pezizomycotina and Agaricomycetes.

Age estimates of algal clades including LFA—which are phylogenetically isolated and considered only putative origins of LFA (Friedl & Bhattacharya, 2002)—generally postdate tracheophytes. The 95% HPD of the *Coccomyxa*-*Elliptochloris* clade slightly pre-dates tracheophytes as does that of Trentepohliales. However, the ages of these clades likely overestimate the true age of LFA within them, as they contain non-LFA (and aquatic algae in the case of the *Coccomyxa*-*Elliptochloris* clade), suggesting lichenization may have evolved more recently than the MRCA of each clade (López-Bautista, Waters, & Chapman, 2002; Malavasi et al., 2016; Rindi & López-Bautista, 2008). Additionally, LFA are not obligately lichen-forming and may have persisted in the free-living state (Friedl & Büdel, 2008; Mukhtar, Garty, & Galun, 1994). Thus, the evolution of lineages containing lichen-forming photobionts indicates the *potential* to form lichens but does not require it. Consequently, earlier age estimates of clades containing LFA would not necessitate the occurrence of

lichens. Stem ages also largely agree with a post-tracheophyte origin, but are treated with caution as sister-group relationships are often unknown, and sampling was not designed to capture sister groups; consequently, these may overestimate true stem ages. While age estimates of lichen-associated cyanobacteria are few, those examined—including the *Nostoc* symbiotic clade and *Rivularia*—also postdate the evolution of tracheophytes (Ortiz-Álvarez, de los Ríos, Fernández-Mendoza, Torralba-Burrial, & Pérez-Ortega, 2015; Shih, Hemp, Ward, Matzke, & Fischer, 2017). Together, this suggests a relative paucity of suitable photobiont lineages with which any hypothetical LFF could have associated with in pre-tracheophyte ecosystems. Thus, the acceptance of lichens in a pre-tracheophyte world requires the acceptance of associations with a restricted set of algae, a deeper acquisition of the lichen-forming ability among algae, or the presence of now-extinct lineages of LFA.

Taken together, there is no support for the occurrence of lichens during the Neoproterozoic-early Paleozoic. Fossil data are lacking, and ASR of LFF indicates a post-tracheophyte origin. Moreover, support for the existence of algal lineages that could potentially form LFA during the Neoproterozoic-early Paleozoic is limited, and evidence for their existence does not necessitate the presence of lichens. As is always the case, the possibility of some unknown and now-extinct early lichen-forming lineage could never be positively ruled out. However, acceptance of a lichen contribution to Neoproterozoic-early Paleozoic environmental change through the enhancement of biotic weathering capacity would require both the evolution *and* extinction of lineages for which there is no evidence. We therefore argue that the burden of proof would lie with any advocate for the existence of pre-tracheophyte lichens. Furthermore, if any unknown early lichen associations were to be speculated, our results demonstrate that such a hypothetical would have to sit outside of the Pezizomycotina and Agaricomycetes that harbor all known lichens and, thus, that the biology and physiology of modern lichens should not be considered a relevant constraint on the potential geobiological implications of such a hypothetical; lichens as we know and understand them come later. Comparative genomic work will ultimately provide a clearer path forward as the underlying genomic machinery required for the evolution of lichenization, but in the absence of any positive evidence or requirement for their existence, we find no support for lichens having pre-dated the appearance of tracheophytes in the fossil record.

4.2 | Terrestrial ecosystems before vascular plants

Our results suggest a need to re-evaluate Neoproterozoic-early Paleozoic terrestrial ecosystems, and the paleoecological context in which tracheophytes evolved. Indirect fossil evidence suggests microbial communities occupied terrestrial ecosystems as early as the Archean (4.0–2.5 Ga) (Lenton & Daines, 2017; Wellman & Strother, 2015), but these systems would have remained as simple mats and soils throughout the Precambrian into the early Paleozoic. The absence of lichens and non-vascular plants in these early systems is expected to have resulted in drier, shallower, and

more unstable soil ecosystems with lower productivity and nutrient input, and simplified food webs. Land plants, rather than lichens, were likely the first to provide the increased surficial texture necessary for enhanced dust capture and stabilization. The delayed evolution of lichens suggests early land plants invaded relatively simple, microbially based, terrestrial ecosystems. Consequently, lichens provided no competitive interactions within or previous modification of these systems. This is distinct from the successional sequence in extant dryland ecosystems, where lichens are early colonizers that provide soil stability for the later colonizing bryophytes (Weber et al., 2016), and further illustrates that the successional trajectory in extant ecosystems need not mirror their evolution (Wellman & Strother, 2015). The ecological and biogeochemical influence of lichens would therefore have evolved in a world already structured by land plants.

While land plants first appeared during the Ordovician, it remains unclear when they first achieved high abundance and a global distribution. Bryophytes are a significant component of some extant biological soil crusts (BSCs); however, the dominant bryophytes in these communities are mosses derived from lineages originating in the Mesozoic-Cenozoic (Newton, Wikström, Bell, Lowe Forrester, & Ignatov, 2007; Weber et al., 2016), with liverworts and hornworts representing small or negligible components, respectively (Weber et al., 2016). Furthermore, rock-inhabiting bryophytes in extant desert environments are largely confined to crevices that collect periodic moisture and provide shade (Scott, 1982); consequently, their distribution was likely restricted to at least periodically moist habitats until they subsequently evolved the capacity to persist as BSCs in arid regions—potentially much later in Earth history, perhaps as late as the Mesozoic. Similarly, suggestions based on the fossil record contend that early embryophytes were initially restricted to wet areas, subsequently displaced by competitive interactions with tracheophytes, and relegated to occupy dry habitats (Wellman, Steemans, & Vecoli, 2013). Thus, initial landscape occupation by bryophyte-grade land plants and the early expression of their weathering potential (Quirk et al., 2015) was likely to have been environmentally restricted, rather than forming contiguous covers across entire continents (Porada et al., 2016). Nonetheless, the earliest fossil occurrence of land plant spores represents a real beginning. There is no evidence of a poorly defined prehistory of lichens to blunt their impact; the Ordovician appearance of land plants represents the advent of terrestrial environments structured by macroscopic eukaryotes.

5 | CONCLUSION

For over 160 years, lichens have been discussed as being among the earliest colonizers of terrestrial ecosystems. Lichens are frequently thought to have formed soil and contributed to global cooling via their feedbacks with silicate weathering and the global carbon cycle—all prior to the evolution of vascular plants. Here, we

demonstrate that lichens are unlikely to have existed prior to vascular plants, and thus were not available to serve as potential drivers of earlier environmental change. Our analyses also revealed that several independently dated clades of interacting LFF and LFA are broadly congruent at coarse temporal and phylogenetic scales, providing support for our age estimates, and illustrating the close evolutionary coupling between LFF and LFA lineages.

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SUPPORTING INFORMATION

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