Lichen metabolism identified in Early Devonian terrestrial organisms

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ABSTRACT

We used $\delta^{13}C$ values to identify lichen metabolism in the globally distributed Early Devonian (409–386 Ma) macrofossil *Spongiophyton minutissimum*, which had been alternatively interpreted as a green plant of bryophyte grade or as a lichen, based on its morphology. Extant mosses and hornworts exhibited a range of $\delta^{13}C_{tissue}$ values that was discrete from that of extant lichens. The $\delta^{13}C_{tissue}$ values of 96 *S. minutissimum* specimens coincided with $\delta^{13}C_{tissue}$ values of extant lichens. In contrast, *S. minutissimum* $\delta^{13}C_{tissue}$ values showed no similarity to bryophyte carbon isotope values. The identification of large global populations of lichens during the Early Devonian may indicate that lichen-accelerated soil formation fostered the development of Paleozoic terrestrial ecosystems.

Keywords: Devonian, lichen, bryophyte, carbon isotope ratios, terrestrial ecosystems, *Spongiophyton minutissimum*.

INTRODUCTION

Bryophytes (mosses, hornworts, and liverworts) are considered prime candidates to have made the evolutionary transition of photosynthesis onto land because of their basal placement within the embryophyta. Most extant bryophytes live in continuously moist or periodically wet terrestrial habitats that could be considered similar to the first transitional terrestrial environments. Examinations of microfossil tetrads have revealed that embryophytes of bryophyte grade have existed at least since the Ordovician (Strother, 2000). However, the macrostructure of such bryophytes is not known. Here we examined the $\delta^{13}C$ value of one enigmatic and particularly widespread macrofossil, *Spongiophyton minutissimum* (Fig. 1B), an abundant and globally distributed Early to Middle Devonian (409–377 Ma) fossil found in the sediments of Brazil, Bolivia, Ghana, Canada, Poland, and elsewhere (Gensel et al., 1991). In many of these deposits, the fossil is densely abundant, suggesting that large populations of *S. minutissimum* were a common element of early terrestrial ecosystems. Based on its morphology, *S. minutissimum* was first interpreted (Chaloner et al., 1974) as a nonvascular plant (i.e., a bryophyte; e.g., Fig. 1A). Gensel et al. (1991) examined the inert covering of the fossil and suggested that the pattern of cell outlines was reminiscent of the inner surface of embryophyte cuticle. Stein et al. (1993) investigated the morphology of internal structures and demonstrated a reticulate network of hyphae, which led them to interpret the fossil as a lichen (i.e., a symbiotic association of fungus with algae; e.g., Fig. 1C). Because bryophytes fix carbon directly, while within the lichen carbon is transferred to the fungal associate only after being fixed by algae, we hypothesized that bryophyte and lichen metabolism might result in different $\delta^{13}C_{tissue}$ values, and sought to apply this distinction to the kingdom-level identification of *S. minutissimum*.

SAMPLES AND METHODS

Shale containing abundant branching thalli (Fig. 1B) was collected from the Halquist Quarry in Waukesha County, Wisconsin. Morphological evaluation of the dichotomizing, perforate thalli led to its identification as *Spongiophyton minutissimum* Kraüsel (Kuglitsch et al., 1998), known from the Lower Devonian of New Brunswick and Quebec (Gensel et al., 1991). The shale filled a large fissure in the Waukesha Formation (Silurian) and was notably devoid of acritarchs and other marine palynomorphs (Kuglitsch et al., 1998). Identification of palynomorphs isolated from the shale placed the deposit within the Early Devonian according to spore zones defined by Richardson and McGregor (1986). Examination by light microscopy of the organic thalli revealed thick-walled cutinite fragments and vitrinite-like material, both with remnant cell structure. Overall, the thalli were excellently preserved with average reflectivity of 0.23% R_O, corresponding to a thermal alteration index = 1. In preparation for carbon isotope analysis, thalli were chemically separated from whole rock via digestion in 60% HF for 24 h, then handpicked under 40× magnification. We analyzed 96 individual fossil thalli, as well as extant bryophytes

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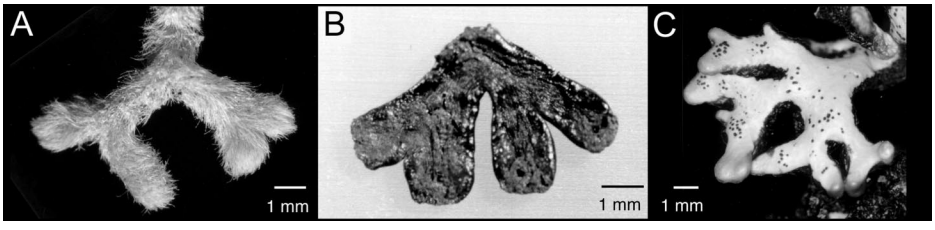

Figure 1. Morphology of *S. minutissimum* (B) to *Trichocolea tomentosa*, an extant bryophyte (A), and *Hypogymnia enteromorpha*, an extant lichen (C). Both extant organisms were included in species analyzed for $\delta^{13}C$ value in this study.

TABLE 1. $\delta^{13}C$ OF EXTANT BRYOPHYTES

Family	Species	$\delta^{13}C$ (‰)	σ (‰)
Liverwort: class Jungermanniopsida			
Aneuraceae	*Riccardia fucoides*	−30.72	0.06
Bryopteridaceae	*Bryopteris fruticulosa*	−26.59	0.29
Lepidoziaceae	*Bazzania trilobata*	−29.31	0.31
Pelliaceae	*Pellia epiphylla*	−30.23	0.49
Scapaniaceae	*Scapania nemorosa*	−40.25	0.91
Trichocoleaceae	*Trichocolea tomentosa*	−26.17	0.79
Liverwort: class Marchantiopsida			
Marchantiaceae	*Marchantia polymorpha*	−27.87	0.68
Moss:			
Amblystegiaceae	*Drepanocladus ucinatas*	−26.74	0.67
Brachytheciaceae	*Brachythecium laetum*	−28.84	0.56
Brachytheciaceae	*Brachythecium* spp.	−30.97	0.73
Climaciaceae	*Climacium dendroides*	−28.08	0.62
Dicranaceae	*Dicranum scoparium*	−27.27	2.03
Ephemeraceae	*Micromitrium cirrosum*	−26.86	0.39
Fontinalaceae	*Fontinalis antipyretica*	−37.24	0.86
Grimmiaceae	*Grimmia pilifera*	−26.19	1.98
Hylocomiaceae	*Pleurozium schreberi*	−30.97	0.31
Hypnaceae	*Hypnum imponena*	−29.41	0.89
Polytrichaceae	*Polytrichum commune*	−26.13	0.04
Polytrichaceae	*Dawsonia pullei*	−30.70	0.57
Prionodontaceae	*Prionodon densus*	−26.91	0.15
Sphagnaceae	*Sphagnum subsicundum*	−32.72	0.50
Sphagnaceae	*Sphagnum squarrosum*	−26.06	0.26
Tetraphidaceae	*Tetraphis pellucida*	−28.55	0.49

Note: Standard deviation (σ) of the carbon isotope value ($\delta^{13}C$) measured in three individuals.

TABLE 2. $\delta^{13}C$ OF EXTANT LICHENS

Species	$\delta^{13}C$ (‰)	σ (‰)
Basidiomycete		
Dictyonema montanum†	−17.50	0.03
Clade II*		
Umbilicaria caroliniana	−19.86	0.30
Umbilicaria papulosa	−22.61	0.25
Lecanorales*		
Alectoria nidulifera	−22.15	0.34
Anaptychia palmatula	−23.56	0.27
Caloplaca cerina	−23.30	0.34
Cetraria islandica	−23.96	0.06
Cetrelia chicitae	−23.67	0.21
Cladonia capitata	−24.11	0.15
Evernia mesomorpha	−23.52	0.07
Heterodermia hypoleuca	−23.38	0.57
Hypogymnia enteromorpha	−22.94	0.21
Leptogium azureum†	−20.14	0.25
Parmelia aurulenta	−23.88	0.18
Parmelia subrudecta	−22.47	0.25
Peltigera canina†	−22.89	0.32
Peltigera praetextata†	−22.55	0.15
Platismatia tuckermanii	−24.02	0.21
Pycnothelia papillaria	−25.35	0.07
Stereocaulon spp.	−22.06	0.22
Usnea spp.	−24.95	0.11

Note: Standard deviation (σ) of the carbon isotope value ($\delta^{13}C$) measured in three individuals.

*Clade assignment according to the phylogenic hypothesis for the lichen-forming ascomycetes (Stenroos and DePriest, 1998).

†Lichen contains a cyanobacteria symbiont (cf., chlorophyta).

and lichens (discussed later), for $\delta^{13}C$ values using a Eurovector automated combustion system in conjunction with an Isoprime stable isotope mass spectrometer at Johns Hopkins University. All carbon stable isotope values are reported in standard delta notation [δ,‰] relative to the Peedee belemnite (PDB) standard; analytical uncertainty associated with each measurement was ±0.05‰.

RESULTS

We hypothesized that it would be possible to distinguish between lichen and bryophyte metabolism using $\delta^{13}C$ values because the two organisms acquire carbon through very different processes. Bryophytes have cuticles (waxy surficial coatings that inhibit water loss) with fixed pores (perforations or stomata) through which CO_2 diffuses into the organism. In contrast, the external surfaces of lichens are composed of a continuous cortical fungal layer (however, in some lichens the lower cortex is perforated by cyphellae). Because both water loss and CO_2 diffusion occur readily across the entire surface of the lichen, Nash (1996) suggested that models of land plant photosynthesis could not be used to understand lichen photosynthesis. In both types of organisms, CO_2 is fixed by the Rubisco enzyme (ribulose 1,5-biphosphate carboxylase-oxygenase). In bryophytes, the site of carboxylation is within the numerous chlorophyll-bearing cells that line the gas-filled chambers of the pores. In lichens, carboxylation occurs within the photobiont, and carbon is then transferred to the mycobiont as glucose, ribitol, sorbitol, or erythritol (Nash, 1996). Assimilation of these nutrients by the mycobiont involves facilitated diffusion and/or carrier proteins that actively transport nutrients across the fungal plasma membrane to the site of heterotrophic metabolism (Robson, 1999). We hypothesized that these different processes of carbon uptake and assimilation in bryophytes versus lichens might lead to differences in $\delta^{13}C_{tissue}$ values.

Because published $\delta^{13}C$ values of extant lichens and bryophytes are limited and restricted to a few species in sometimes extreme conditions, we measured the $\delta^{13}C_{tissue}$ values of 23 species of extant bryophytes and 21 species of extant lichens. The choice of species sampled was designed to span the full range of taxonomic diversity found in each group. Air-dried bryophyte gametophyte and lichen tissues from three separate individuals of each species listed in Tables 1 and 2 were subsampled from the collections of the National Museum of Natural History at the Smithsonian Institution. Bryophytes sampled included both liverworts and mosses (Table 1); liverworts are divided into Class Jungermanniopsida and Class Marchantiopsida, and both were represented in the species sampled. Although no agreement exists regarding a higher-level classification of mosses, three of the four proposed major groups were represented in the species sampled: Sphagnopsida, Polytrichopsia, and Bryopsida. The fourth group of mosses, Andreaeopsida, is not diverse and was probably not abundant throughout the Phanerozoic. Lichens have historically been classified with fungi, the majority of which are within the Division Ascomycota, recently subdivided into four clades (Clades I, II, III and IV) based on small subunit rDNA analyses (Stenroos and DePriest, 1998). The suggestion that the organization of *S. minutissimum* most resembles the Lecanoralean lichens (Retallack, 1994; Stein et al., 1993) led us to focus on Clade III (Lecanorales) lichens (Table 2): 18 species were included. In addition, the sample set included two species of Clade II lichens, and one lichen from the Division Basidiomycota (Table 2). The $\delta^{13}C_{bryophyte}$ value of extant species sampled ranged from −40.25‰ to −26.06‰ with a mean value of −29.34‰ (Fig. 2). The $\delta^{13}C_{lichen}$ value of extant species sampled ranged from −25.35‰ to −17.50‰ with a mean value of −22.80‰ (Fig. 2). Lichens with cyanobacterial photobionts ($n = 4$; Table 2) tended to have the highest $\delta^{13}C_{lichen}$ values of those observed.

Carbon isotope measurements of *S. minutissimum* fossils showed a $\delta^{13}C$ range of −25.77‰ to −21.09‰, with a mean value of −24.19‰ ($n = 96$; Fig. 2). The congruence of measured $\delta^{13}C$ values for the fossils with measured $\delta^{13}C_{lichen}$ values led us to conclude that *S. minutissimum* had a carbon metabolism that closely resembled that

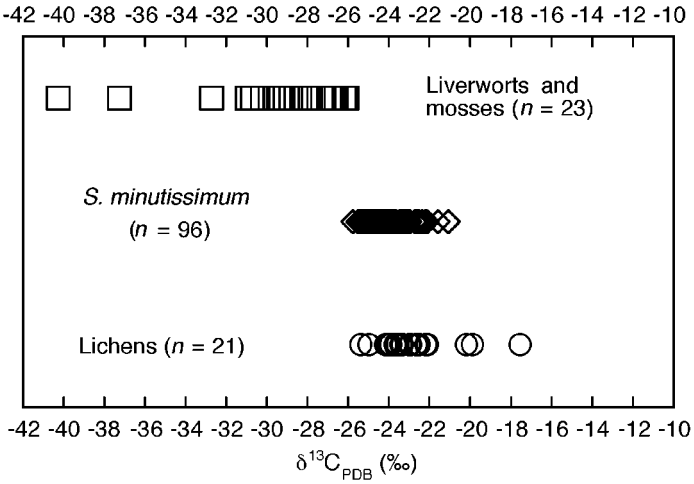

Figure 2. Carbon isotope composition (PDB is Peedee belemnite) of fossil *S. minutissimum* (open diamonds), extant bryophytes (open squares), and extant lichens (open circles).

of extant Lecanoralean lichens (Fig. 2). We also stress that $\delta^{13}C$ values of *S. minutissimum* showed no overlap with measured $\delta^{13}C_{bryophyte}$ values (Fig. 2).

DISCUSSION

Carbon isotope discrimination (Δ) during photosynthesis is a measure of $^{13}CO_2$ uptake relative to $^{12}CO_2$ uptake: $\Delta_{organism} = \delta^{13}CO_{2atmosphere} - \delta^{13}C_{tissue}$ (‰). The main factors contributing to $\Delta_{land\ plants}$ are diffusion rates of $^{13}CO_2$ versus $^{12}CO_2$ in air, the isotopically selective activity of Rubisco, and the leaf's internal concentration of CO_2 relative to external conditions as modified by the plant's ecophysiological status (Farquhar et al., 1989). A recent evaluation of 519 $\delta^{13}C_{tissue}$ measurements made on 176 C3 (Rubisco only) vascular land plant species yielded an average $\Delta_{C3\ land\ plant} = 18.7‰$ across a wide range of ecophysiological stresses and atmospheric pCO_2 and $\delta^{13}CO_2$ (Arens et al., 2000). Most bryophytes live in continuously moist or periodically wet terrestrial habitats, while some (e.g., *Sphagnum*) live in partially submerged habitats. These wet environments may contribute to the high values of Δ seen in some extant bryophytes, relative to C3 vascular land plants: Rice (2000) measured $\Delta_{bryophyte}$ values for three species of *Sphagnum* grown under field conditions, and determined $\Delta_{bryophyte} = 22‰–25.5‰$, attributing most of the variability to changing seasonal climate conditions. In contrast, Δ values for cyanobacteria and chlorophytes (the photobionts of lichens) are generally much lower than those observed for C3 land plants, possibly due to the active uptake of HCO_3^- by these algae (Goericke et al., 1994). Chlorophytes exclusively taking up HCO_3^- exhibited $\Delta = 4‰–12‰$ (Sharkey and Berry, 1985). As chlorophyte growth rates increase, Δ decreases: at metabolic rates approaching those necessary for symbiosis (photobionts within lichens transfer not less than 70% of their net carbon gain to the mycobiont [MacFarlane and Kershaw, 1982]), chlorophytes showed $\Delta \leq 18‰$ (Fry and Wainright, 1991). Caution should be used when applying published cyanobacteria and chlorophyte isotopic characterizations to lichens: we note that the photobiont is surrounded by air and fungal tissue (cf. water), and would only have access to HCO_3^- if it were found in mycobiont cell fluid. Additional processes present in lichens, such as the transport of carbon into the mycobiont, or heterotrophic metabolism within the mycobiont, could contribute to the disparate ranges of $\delta^{13}C_{bryophyte}$ vs. $\delta^{13}C_{lichen}$ values we observed.

The extreme environmental tolerances apparent in extant lichens recommend this symbiosis as a likely part of Earth's early subaerial ecosystems; rDNA sequences of extant lichens indicated that lichens arose at least five times in phylogenetically distant groups (Gargas et al., 1995). While lichen communities have been suggested to extend even into the Precambrian (Retallack, 1994), the earliest fossil lichen in which both symbiotic partners have been described comes from the Early Devonian Rhynie Chert (Taylor et al., 1995). Identification of *S. minutissimum* as an abundant lichen of a slightly younger age, with a widespread geographic distribution, affirms the presence of lichens within a period widely thought to have been dominated by plants (LePage and Pfefferkorn, 2000).

During the Devonian, *S. minutissimum* cooccurred with vascular plant flora (Stein et al., 1993). However, if the processes observed in extant lichens and bryophytes were common during the Devonian, basic differences in reproduction may have allowed *S. minutissimum* to arrive in areas that excluded bryophytes. Reproduction in living bryophytes requires a water-fertilization step, while lichens often reproduce through vegetative propagules dispersed by wind in a brittle, dry state (Büdel and Scheidegger, 1996). Extant lichens accelerate mineral dissolution and enhance soil fertility indirectly through microbially mediated processes (Banfield et al., 1999). If these same processes operated throughout the Phanerozoic, *S. minutissimum*-accelerated soil development might have helped foster the establishment, expansion, and diversification of plant ecosystems during the Devonian.

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