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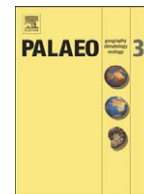
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## Carbon isotopes support the presence of extensive land floras pre-dating the origin of vascular plants

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## ABSTRACT

Multiple lines of evidence indicate that Earth's land masses became green some 2.7 Ga ago, about 1 billion years after the advent of life. About 2.2 billion years later, land plants abruptly appear in the fossil record and diversify marking the onset of ecologically complex terrestrial communities that persist to the present day. Given this long history of land colonization, surprisingly few studies report direct fossil evidence of emergent vegetation prior to the continuous record of life on land that starts in the mid-Silurian (ca. 420–425 Ma ago). Here we compare stable carbon isotope signatures of fossils from seven Ordovician–Silurian (450–420 Ma old) Appalachian biotas with signatures of coeval marine organic matter and with stable carbon isotope values predicted for Ordovician and Silurian liverworts (BRYOCARB model). The comparisons support a terrestrial origin for fossils in six of the biotas analyzed, and indicate that some of the fossils represent bryophyte-grade plants. Our results demonstrate that extensive land floras pre-dated the advent of vascular plants by at least 25 Ma. The Appalachian fossils represent the oldest direct evidence of widespread colonization of continents. These findings provide a new search image for macrofossil assemblages that contain the earliest stages of land plant evolution. We anticipate they will fuel renewed efforts to search for direct fossil evidence to track the origin of land plants and eukaryotic life on continents further back in geologic time.

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## 1. Introduction

## 1.1. The fossil record of life on land

Sedimentological, geochemical, and isotopic lines of evidence indicate that Earth's land masses, or at least their fringes adjacent to the oceans, became green some 2.7 Ga ago, ca. 1 billion years after the advent of life (Buick, 1992; Rye and Holland, 2000; Watanabe et al., 2000; Beukes et al., 2002). About 2.3 billion years later, at the beginning of the Devonian, embryophytic land plants, present in the dispersed palynomorph record as early as the mid-Ordovician (Strother et al., 1996), abruptly diversify in the fossil record marking the onset of ecologically complex terrestrial communities that persist to the present day (Gensel and Edwards, 2001). Given this long history of land colonization, it is surprising that few studies (e.g. Horodyski and Knauth, 1994; Westall et al., 2006; see also review by Retallack, 2000) report direct fossil evidence of emergent vegetation or microbial mats in terrestrial settings prior to the continuous record of life

on land from the mid-Silurian (ca. 420–425 Ma ago) to the present (Gensel and Edwards 2001).

The mid-Silurian marks the sudden appearance in the fossil record of polysporangiphytic and tracheophytic plants (e.g. *Cooksonia*, *Baragwanathia*) which were forming extensive communities soon thereafter. It is a widely held view in plant phylogeny that polysporangiphytes (represented in extant floras by lycophytes, psilotophytes, horsetails, ferns, and seed plants) and tracheophytes (vascular plants) represent derived embryophyte lineages and, as such, they are phylogenetic newcomers among the land plants (Mishler and Churchill, 1985; Garbary and Renzaglia, 1998; Shaw and Goffinet, 2000; Lewis and McCourt, 2004). Apparently, the time interval before the mid-Silurian witnessed not only the earliest phases of polysporangiphyte and tracheophyte evolution, but also the earlier evolution of more basal land plant groups such as the three bryophyte lineages (liverworts, hornworts, and mosses). Unraveling these major evolutionary events is crucial for an in-depth understanding of plant phylogeny and evolution, yet the fossil record of pre-mid-Silurian life on land is frustratingly sparse and cryptic. Direct evidence for these crucial evolutionary events comes exclusively from dispersed microfossils in the form of spores and phytodebris of which only few are assignable to recognizable groups of land plants (the earliest are known from the Middle-Ordovician; Strother et al., 1996; Gensel and Edwards, 2001;

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Wellman et al., 2003). Reliably dated body macrofossils that are unequivocally assigned to land plants or other non-animal organisms of terrestrial origin are not reported from strata older than the mid-Silurian (see review by Retallack, 2000) with the exception of an early Silurian (Llandovery) biota at Passage Creek, Virginia (USA) first reported by Pratt et al. (1978) and further investigated by Tomescu and Rothwell (2006).

### 1.2. Appalachian biotas: promise and challenges

Here we report on seven fossil assemblages from the Appalachian basin whose study represents a step toward a better understanding not only of the early evolution of land plants, but also of the advent of other macroscopic, multicellular eukaryotes in terrestrial ecosystems. These fossil assemblages, referred to as the Appalachian biotas, are composed primarily of macroscopic carbonaceous compressions. Their stratigraphic distribution spans the late Ordovician through late Silurian. Five of the seven assemblages are previously unreported, so much work remains to be done for further elucidation of their paleoecological and evolutionary significance. The two studied assemblages are those of the Passage Creek biota and the Bloomsburg Formation assemblages at Port Clinton (Pratt et al., 1978; Niklas and Smocovitis, 1983; Strother, 1988, 1993; Tomescu and Rothwell, 2006).

The Appalachian biotas are important because their chronostratigraphic placement pre-dates or overlaps the mid-Silurian apparition of derived embryophytes (polysporangiophytes, tracheophytes), providing a transition to the older terrestrial fossil record of microbial mats. It is intriguing that the Appalachian biotas consist exclusively or primarily of thalloid morphologies that are distinctly different from the typical axial morphology of sporophytes that dominate the macrofossil record of land plants starting in the mid-Silurian. The thalloid morphology of Appalachian fossils is reminiscent of embryophyte (liverwort, hornwort) gametophytes, as well as other terrestrial organisms such as algae, fungi, lichens, and some cyanobacterial colonies. Most of these groups of organisms have been postulated as early colonizers on land (Retallack, 2000). Studies of the early Silurian Passage Creek biota have revealed a broad diversity of internal structures in the thalloid fossils, consistent with broad taxonomic diversity of their producers. The internal organization of some of the fossils comprises distinct layers with different structures, consistent with tissue differentiation and indicating that eukaryotes with complex multicellular body plans were present on land 440 Ma ago. These observations suggest that the fossil assemblages of the Appalachian biotas could represent homologues of extant biological soil crust communities (Belnap and Lange, 2001), with which they share taxonomic diversity and thalloid forms (Tomescu and Rothwell, 2006).

These alluring features of the Appalachian biotas come with a set of challenges. First, in the absence of diagnostic internal anatomical characters, the thalloid morphology of fossils is uninformative taxonomically since several groups of organisms have thalloid forms. Second, preservation of the Appalachian fossils as carbonaceous compressions (see Schopf, 1975) has obliterated most of the internal organization of the original organisms, making it impossible to draw any direct anatomical comparisons between the fossils and living organisms. However, differences in internal structure between individual fossils are conspicuous and even sub-micrometer-size structures are preserved (Tomescu and Rothwell, 2006). These differences could provide valuable diagnostic characters when deciphered using experiments that mimic the effects of diagenesis on the anatomy and ultrastructure of living organisms from different systematic groups.

Third, depositional environments at some of the fossil localities are not resolved in detail. This is an important issue because some of the rock units that host the Appalachian biotas cover several depositional environments (e.g. marine, continental, and transitional). Given the potential significance of the Appalachian fossils, correct identification of depositional environments, with an eye toward the reconstruction of their terrestrial provenance, is crucial. However, a confounding issue in

transitional marine-continental settings is the absence, prior to the Devonian, of the most reliable indicator of continentality, plant roots or their characteristic bioturbation structures. As a result, such transitional sequences can be interpreted as shallow marine by the practicing geologists. That is why, when evaluating these sequences, it is important to keep in mind that even in recent depositional settings there is little difference between a “continental” deposit and a “nearshore” setting in terms of plant debris and plant-derived palynological content (Scheihing and Pfefferkorn, 1984; Pfefferkorn et al., 1988; Gastaldo and Huc, 1992; Gastaldo et al., 1996). In the case of the Appalachian biotas, since at present the fossils themselves cannot be identified as terrestrial organisms based on morpho-anatomical characters alone, information from independent sources could provide decisive evidence on their origin (terrestrial vs. marine). One such independent line of evidence, which can help refine the interpretation of sedimentary environments of the Appalachian biotas, and possibly contribute to resolving the systematic affinities of the fossils, is provided by stable carbon isotopic signatures of the organic matter.

### 1.3. Scope of the study

In this paper 1) we present the results of stable carbon isotope measurements of organic matter in fossils from seven Appalachian biotas; 2) we compare these results with values from a worldwide stable carbon isotopic signature database of Ordovician–Silurian marine organic matter, compiled by us from the literature; and 3) we compare our results with the stable carbon isotope signatures predicted for Ordovician and Silurian liverworts based on the BRYOCARB model proposed by Fletcher et al. (2006). Comparisons with marine organic matter provide strong support for terrestrial origin of fossils in six of the seven Appalachian biotas analyzed (hosted in five geologic units: the Reedsville Formation–Oswego Sandstone transition, Massanutten Sandstone, Tuscarora Formation, Rose Hill Formation, and Bloomsburg Formation), and for marine origin of fossils in the Martinsburg Formation. These results concur with the conclusions of most broad-scale sedimentological studies of these rock units. Comparisons with predictions based on the BRYOCARB model (Fletcher et al., 2006) indicate that at least some of the thalloid fossils in the six biotas represent bryophyte-grade plants, as suggested by coeval occurrences of dispersed spores attributable to bryophytes.

## 2. Material and methods

### 2.1. Fossil localities and depositional environments

We analyzed fossils from seven localities in six geologic units in the Appalachian basin (Fig. 1, Table 1). The localities are: Frystown (Pennsylvania; 40° 28' 18" N, 76° 20' 50" W) in the Martinsburg Formation (early Katian, ca. 453 Ma); Conococheague Mountain (Pennsylvania; 40° 14' 41" N, 77° 40' 22" W) in the Reedsville Formation–Oswego Sandstone transition (mid-late Katian, ca. 449 Ma); Passage Creek (Virginia; 38° 56' 43" N, 78° 18' 18" W) in the lower Massanutten Sandstone, and Hardscrabble (Virginia; 38° 33' 18" N, 79° 33' 21" W) and Moatstown (West Virginia; 38° 33' N, 79° 24' W) in the Tuscarora Formation (the lower Massanutten and Tuscarora are stratigraphically equivalent units of Rhuddanian age, ca. 441 Ma); Monterey (Virginia; 38° 25' 18" N, 79° 35' 21" W) in the Rose Hill Formation (Telychian, ca. 432 Ma); and Port Clinton (Pennsylvania; 40° 35' 14" N, 76° 01' 34" W) in the Bloomsburg Formation (Homerian–Gorstian, ca. 423 Ma).

The depositional environments of the six geologic units as inferred from sedimentological studies of broad geographic scope are summarized in Table 2. The lower Massanutten Sandstone is a fluvial unit (Dennison and Wheeler, 1975; Pratt et al., 1978; Tomescu and Rothwell, 2006) deposited by extensive braided river systems flowing from the Taconic Highlands west into an epeiric sea. The contemporaneous

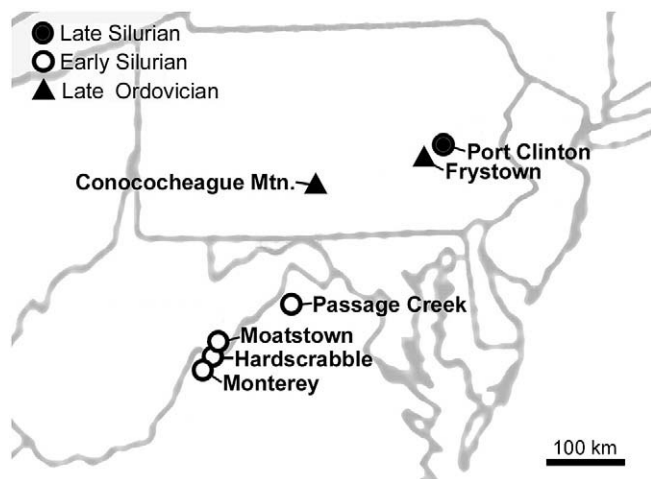


Fig. 1. Geographic location of the Appalachian biotas analyzed.

Tuscarora Formation is part of the same blanket of clastic sediments of which the Massanutten is the most proximal member. The Tuscarora Formation comprises a number of depositional environments ranging from nearshore marine distally (northwestward) to progressively more continental (paralic, to estuarine, to coastal plain, to fluvial) proximally (southwestward) (Yeakel, 1962; Smith, 1970; Dennison and Wheeler, 1975; Whisonant, 1977; Cotter, 1978, 1982, 1983; Diecchio and Dennison, 1996; Castle, 1998, 2001a,b).

The Rose Hill Formation and the Bloomsburg Formation also contain a wide range of depositional environments. The Rose Hill has been interpreted as containing shallow marine, intertidal, and coastal deposits (Diecchio and Dennison, 1996; Cotter, 1998; Dorsch, 1999). The palynomorph content of macrofossil containing layers of the Rose Hill at Monterey, analyzed by one of us (PKS), is consistent with a tidal flat environment (also supported by trace fossil assemblages; Diecchio and Dennison, 1996). The Bloomsburg has been variously interpreted as containing shallow marine to fluvial, including coastal paralic and delta plain sediments at different localities and by different authors (Hoskins, 1961; Dennison and Wheeler, 1975; Driese et al., 1992; Cotter, 1998). Strother (1988) argued for a non-marine origin of the fossiliferous Bloomsburg deposits at the Port Clinton locality. This predominantly red bed unit contains numerous fossil soil horizons (Driese et al., 1992) in addition to *Nematothallus* (Strother, 1988) and spores (Strother and Traverse, 1979).

In contrast to the above stratigraphic units, the Martinsburg Formation is widely regarded as a *bona fide* marine unit. However, in its upper reaches the Martinsburg reflects progressive shallowing of depositional environments which correspond to shallow shelf settings (Dennison and Wheeler, 1975; Diecchio, 1985; Woodcock et al., 2000;

McKinstry et al., 2001) that often grade into continental deposits of overlying fluvial units. One such case is the transition between the marginal marine deposits of the Reedsville Formation (stratigraphically equivalent with most of the Martinsburg Formation except for its bottom section; Diecchio, 1985) and the deltaic and fluvial/floodplain deposits of the Oswego Sandstone (Horowitz, 1966; Dennison and Wheeler, 1975; Lehmann et al., 2000, 2001; Castle, 2001b; however, Diecchio, 1985 interprets the Oswego Sandstone as shallow marine in Virginia and West Virginia). This transition is recorded in the upward-shallowing sequence sampled by us at Conococheague Mountain where thalloid fossils occur throughout the whole spectrum of depositional environments.

## 2.2. Fossil material and sampling

All of the Appalachian biotas analyzed are preserved as carbonaceous compressions (the main morphological types in our samples are summarized in Table 2), and some are associated with trace fossils. The most intensely studied of these biotas, the Rhuddanian Passage Creek biota of Virginia (Pratt et al., 1978; Tomescu and Rothwell, 2006), contains extensive assemblages of principally thalloid and crustose (mat-like) organisms and fragments (Fig. 2), with the largest fossils exceeding 10 cm in overall size. A few of the Passage Creek fossils have been attributed to cyanobacteria (Tomescu et al., 2006, 2008), but the overwhelming majority of the thalloid fossils have unresolved taxonomic affinities. Preliminary experimental comparative studies suggest green algal, bryophyte and lichen affinities (Mack et al., 2007). The Port Clinton biota of Pennsylvania, dated at the Homerian–Gorstian boundary, has been studied by Strother (1988, 1993) who has described there three new species of the enigmatic fossil genus *Nematothallus*. Most compressions are <2 cm in overall size at Port Clinton (Fig. 3 A–C), but in some samples fossils are so abundant that they form continuous carbonaceous layers. The mid-late Katian Conococheague Mountain biota of Pennsylvania has seen only a preliminary study assessing the morphology of fossils (Fig. 3 H, I) that sometimes exceed 4 cm in overall size. Nevertheless, this study has revealed unexpected morphological diversity in the apparently monotonous assemblages of thalloid and fragmentary fossils (McDonald and Tomescu, 2006). The other four biotas (Frystown, Hardscrabble, Moatstown, and Monterey; Fig. 3 D–G, J, K) have not been studied to date.

Stable carbon isotope signatures ( $\delta^{13}\text{C}$ ) were measured on four types of samples. The number and types of samples analyzed at each locality are summarized in Table 1.

- (1) organic matter in bulk sediment (whole-rock samples) from fossiliferous layers (5 samples);
- (2) carbonaceous material scraped directly from fossil compressions (33 samples). Individual fossils that were large enough to provide sufficient material were analyzed separately. Fossils that were too small to provide individual samples were pooled together in composite samples from the same small area of a rock slab;

**Table 1**  
The fossil localities analyzed: age, geologic unit, types and numbers of samples.

Period	Stage	Age (Ma)	Locality	Geologic unit	Samples				Total
					Fossil compressions	Whole rock	Residual kerogen		
							Fossil compressions	Whole rock	
Silurian	Homerian–Gorstian	423	Port Clinton	PA Bloomsburg Fm.	5	–	–	2	7
	Telychian	432	Monterey	VA Rose Hill Fm.	3	–	–	–	3
	Rhuddanian	441	Moatstown	WV Tuscarora Fm.	2	–	–	–	2
			Hardscrabble	VA Tuscarora Fm.	2	–	–	–	2
			Passage Creek	VA Lower Massanutten Ss.	14 <sup>a</sup>	–	3	3	20
Ordovician	Katian (mid-late)	449	Conococheague Mtn.	PA Reedsville Fm.–Oswego Ss. transition	4	–	2	2	8
	Katian (early)	453	Frystown	PA Martinsburg Fm.	3	5	–	–	8
Total					33	5	5	7	50

<sup>a</sup> Includes 2 measurements by Niklas and Smocovitis (1983).



**Table 2**

Types of fossils and depositional environments of the six geologic units studied, as inferred from sedimentological studies of broad geographic scope.

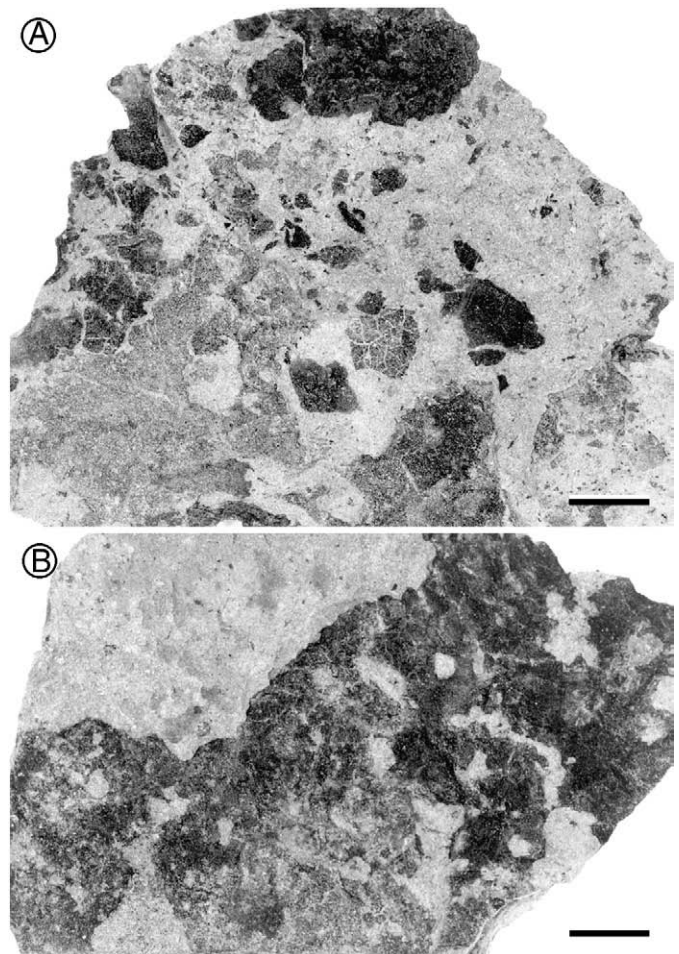
Locality	Fossils	Rock unit	Depositional environments	References
Port Clinton	PA Thalloid and strap-shaped compressions, <i>Nematothallus</i> , <i>Prototaxites</i>	Bloomsburg Formation	Shallow marine to fluvial, coastal paralic and delta plain	Dennison and Wheeler (1975), Strother (1988), Driese et al. (1992), Cotter (1998)
Monterey	VA Thalloid and strap-shaped compressions, arthropod trackways	Rose Hill Formation	Shallow marine, intertidal, and coastal	Diecchio and Dennison (1996), Cotter (1998), Dorsch (1999)
Moatstown	WV Thalloid compressions, eurypterid fragments	Tuscarora Formation	Near-shore marine distally (NW) to paralic, estuarine, coastal plain, and fluvial proximally (SE)	Yeakel (1962), Smith (1970), Dennison and Wheeler (1975), Whisonant (1977), Pratt et al. (1978), Cotter (1978, 1982, 1983), Diecchio (1985), Diecchio and Dennison (1996), Castle (1998, 2001a,b), Tomescu and Rothwell (2006)
Hardscrabble Passage Creek	VA Thalloid compressions VA Thalloid and strap-shaped compressions	Lower Massanutten Sandstone	Fluvial	
Conococheague Mtn.	PA Thalloid compressions	Reedsville Formation–Oswego sandstone transition	Upward-shallowing succession; marginal marine grading upward to deltaic, fluvial and floodplain	Horowitz (1966), Dennison and Wheeler (1975), Diecchio (1985), Lehmann et al. (2000, 2001), Castle (2001b)
Frystown	PA Thalloid compressions	Martinsburg Formation	Upward-shallowing marine sequence; shallow shelf grading into overlying fluvial units	Dennison and Wheeler (1975), Diecchio (1985), Woodcock et al. (2000), McKinstry et al. (2001)

- (3) residual kerogen from whole-rock samples (7 samples). These and type 4 samples were obtained as byproducts of solvent extractions for bitumen characterization;
- (4) residual kerogen in carbonaceous fossil material obtained as for type 2 samples (5 samples). The distribution of samples by stratigraphic units is: Massanutten Sandstone and Tuscarora Formation – 24 samples (these include two Massanutten Sandstone samples measured by Niklas and Smocovitis, 1983); upper Martinsburg Formation and the Reedsville–Oswego transitional sequence – 8 samples each; Bloomsburg Formation – 7 samples; Rose Hill Formation – 3 samples. Multiple measurements were taken for some of the samples (as detailed in Table 3).

### 2.3. Sample processing and $\delta^{13}\text{C}_{\text{org}}$ analyses

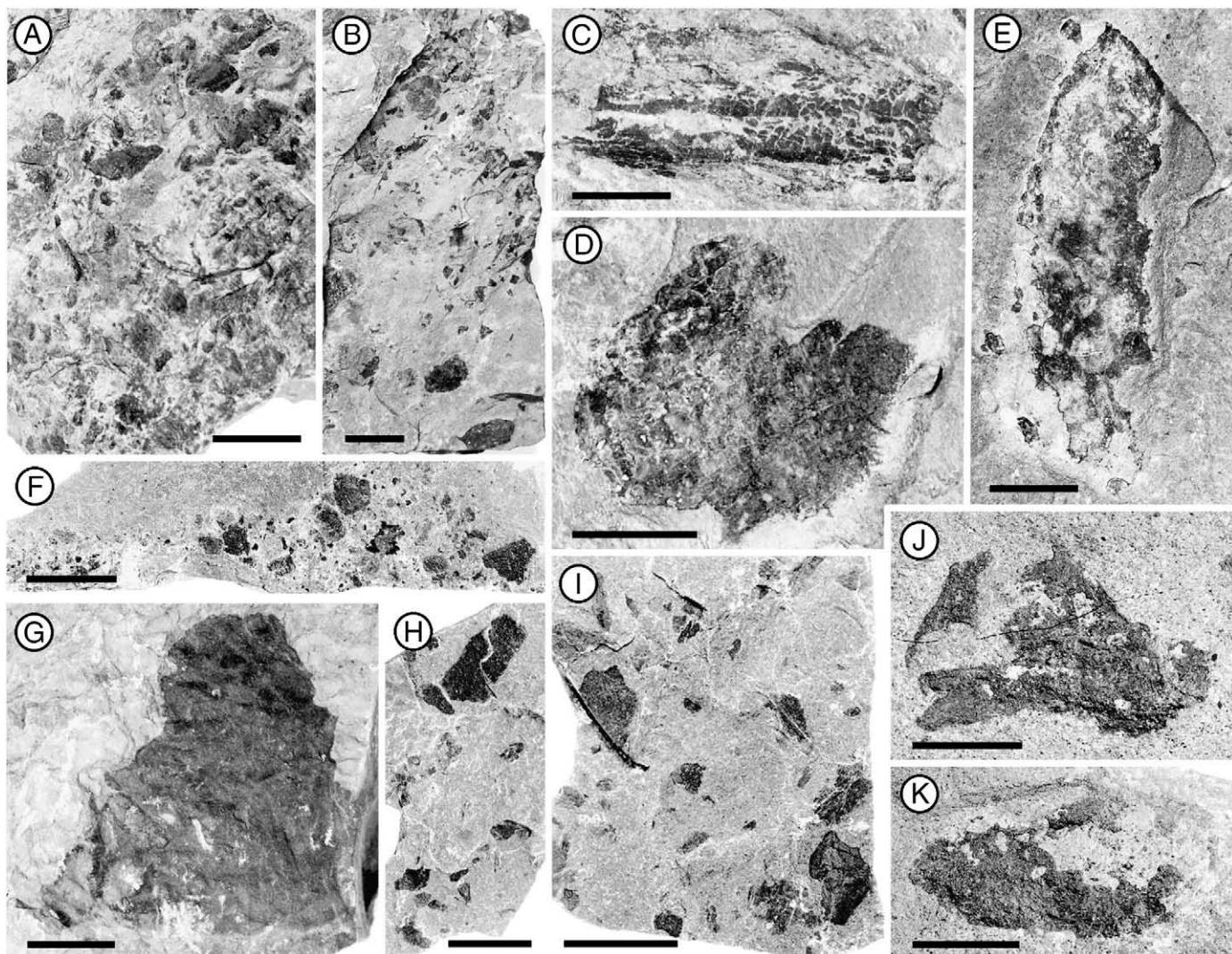
Stable carbon isotope analyses were performed in the Biogeochemical Laboratories, Department of Geological Sciences, Indiana University – Bloomington. Whole-rock samples were ground to powder in a ball mixing mill. To avoid contamination from one sample to the next, the mill was cleaned between successive samples by grinding clean quartz sand (washed with 1 N HCl and baked at 500 °C for 10 h) and rinsing with acetone. Scrapings of fossil compressions were ground by hand with a mortar and pestle. Between samples, the mortar and pestle were carefully cleaned with acetone. The ground samples destined exclusively to stable carbon isotope analysis (both whole-rock and fossils scrapings) were reacted with 1 N HCl at 60 °C for 14 h, then rinsed in de-ionized water, filtered on glass filters (Whatman GF/C), dried on the filters at 50 °C, scraped off the filters, and reground manually with a mortar and pestle (thoroughly cleaned with acetone between successive samples). Samples used for bitumen characterization were solvent extracted with a 4:1 v:v  $\text{CH}_2\text{Cl}_2$ : $\text{CH}_3\text{OH}$  mixture in a Dionex ASE200 accelerated solvent extractor. Extraction residues including kerogen were reacted with 1 N HCl at 50 °C for 12 h, rinsed in de-ionized water, filtered on glass filters, dried on the filters at 50 °C, scraped off the filters, and reground manually with a mortar and pestle (thoroughly cleaned with acetone between successive samples). All samples were combusted in a Costech Instruments ECS4010 elemental combustion system and carbon isotope concentrations were measured on the evolved  $\text{CO}_2$  gas with an in-line Thermo Finnigan Delta Plus XP mass spectrometer.

Precision of measurements was calculated from five measurements of the same acetanilide ( $\text{C}_8\text{H}_9\text{NO}$ ) and corn starch standards at



**Fig. 2.** Characteristic morphology of fossils in the Passage Creek biota (lower Massanutten Sandstone, Rhuddanian). A. Thalloid, crustose, and fragmentary fossils. B. Extensive crustose specimen. Scale bars 10 mm. Published with permission from the Geological Society of America.





**Fig. 3.** Fossils from six Ordovician–Silurian Appalachian localities. A–C. Port Clinton (Bloomsburg Formation, Homerian–Gorstian). D, E. Monterey (Rose Hill Formation, Telychian). F. Hardscrabble. G. Moatstown (F and G Tuscarora Formation, Rhuddanian). H, I. Conococheague Mountain (Reedsville Formation–Oswego Sandstone transition, mid-late Katian). J, K. Frystown (Martinsburg Formation, early Katian). Scale bars 10 mm except C, D, G 5 mm.

the beginning and end of each run of 30 samples, as well as from measurements of three acetanilide and three corn starch standards midway through the runs:  $\delta^{13}\text{C} \pm 0.016\text{‰}$  (acetanilide standard) and  $\delta^{13}\text{C} \pm 0.019\text{‰}$  (corn starch standard).

#### 2.4. Ordovician–Silurian marine $\delta^{13}\text{C}_{\text{org}}$ database

A compilation of published marine  $\delta^{13}\text{C}_{\text{org}}$  data for the Ordovician and Silurian worldwide was prepared for comparison to our data set (Appendix 1, 2, Fig. 4; the digital version of the dataset is available from the authors upon request). All the data were assigned to standard chronostratigraphic stages (Gradstein et al., 2004; complemented by Bergstrom et al., 2006, for Ordovician stage names) based on the litho- and biostratigraphic information and, where available, absolute ages. To improve the resolution of the wealth of data points published for the Katian, we arbitrarily divided that stage into early Katian (455.8 to 452.7 Ma – upper limit of the Chatfieldian; Gradstein et al., 2004), middle Katian (452.7 to 450.2 Ma – including the Edenian and Maysvillian, to the lower limit of the Ashgill–Bolindian; Gradstein et al., 2004), and late Katian (450.2 to 445.6 Ma). The database includes 1160 measurements (701 in the Ordovician and 459 in the Silurian) from the USA, the Canadian Arctic, China, Great Britain, Czech Republic, Latvia, Estonia, Belgium, Sweden,

Australia, Syria, and Saudi Arabia. The density of data by stratigraphic stage is shown in Fig. 5. To date, the highest data density is available for the Katian (410 measurements) and Hirnantian (266 measurements) stages in the Ordovician, and for the Rhuddanian (142 measurements) and Homerian (87 measurements) in the Silurian. Data coverage is very scarce for the Pridoli and Ludfordian, and no  $\delta^{13}\text{C}_{\text{org}}$  data were available for three Ordovician stages – Tremadocian, third stage (unnamed), and Darriwilian.

#### 2.5. Calculation of predicted liverwort $\delta^{13}\text{C}$

Predicted liverwort stable carbon isotope signatures ( $\delta^{13}\text{C}_{\text{lw}}$ ) were calculated for different time intervals as follows:

$$\delta^{13}\text{C}_{\text{lw}} = 100(\delta^{13}\text{C}_a - \Delta^{13}\text{C}) / (\Delta^{13}\text{C} + 100) \quad (1)$$

Eq. (1) was obtained from the Farquhar et al. (1982) quantification of the isotopic discrimination against  $^{13}\text{C}$  by plants:

$$\Delta^{13}\text{C} = (\delta^{13}\text{C}_a - \delta^{13}\text{C}_p) / (1 + \delta^{13}\text{C}_p / 100) \quad (2)$$

**Table 3**

$\delta^{13}\text{C}_{\text{org}}$  and organic carbon content of fossils and associated sediments from Appalachian biotas. Values used for comparisons with marine  $\delta^{13}\text{C}_{\text{org}}$  data are in boldface. (1) and (2) represent duplicate measurements performed on the same sample. \* designates the mean of four samples (in parentheses) that represent the part and counterpart, respectively, of the same fossils. Sample type: F – individual carbonaceous fossil, they are thalloid unless otherwise indicated; FC – composite sample of several carbonaceous fossils; B – bulk sediment (whole rock); RK – sample that was solvent extracted for bitumens and contains residual kerogen; crust – carbonaceous compressions with crustose morphology; cyano – cyanobacterial colony (Tomescu et al., 2006); eury – a eurypterid prosoma. Samples designated <sup>a</sup> and <sup>b</sup> were used for the between-layer comparison at Passage Creek (section B of Table 4). <sup>c</sup> designates samples measured by Niklas and Smocovitis (1983) for which carbon content was not published (not included in discussions of precision of measurements). <sup>d</sup> designates mean carbon content for samples with two measurements. Samples designated <sup>e</sup> and <sup>f</sup> were used for comparisons between fossils and bulk sedimentary organic matter (section D of Table 4).

Period	Stage	Locality	Geologic unit	$\delta^{13}\text{C}_{\text{org}}$ (‰PDB)			C (%)	Sample type
				(1)	(2)	Mean		
Silurian	Homerian–Gorstian	Port Clinton	Bloomsburg Fm.	–28.78			24.13	F
				–26.89	–26.92	–26.90	3.93 <sup>d</sup>	FC
				–27.19	–27.30	–27.24	4.39 <sup>d</sup>	FC
				–27.90			1.48	FC
				–26.80			1.68	FC
				–27.17			0.71	B–RK
				–26.59			0.31	B–RK
		Telychian	Rose Hill Fm.	–26.82	–26.85	–26.84	1.70 <sup>d</sup>	F
				–26.52			33.49	F
				–26.16			0.35	FC
	Rhuddanian	Moatstown	Tuscarora Fm.	–26.13			0.44	F
				–25.79			0.39	F (eury)
				–27.96			0.58	FC
				–28.23			0.73	FC
		Passage Creek	Lower Massanutten Ss.	–26.80	–26.68	–26.74	2.15 <sup>d</sup>	F (crust)
				–26.65	–26.74	–26.70 <sup>a</sup>	7.35 <sup>d</sup>	F (crust)
				–26.46	–26.50	–26.48 <sup>a</sup>	15.53 <sup>d</sup>	F (cyano)
				(–26.46)	(–26.32)	–26.39	2.47 <sup>d</sup>	FC part
				(–26.40)	(–26.30)	–26.35	2.34 <sup>d</sup>	FC c-part
						–26.37 <sup>a, *</sup>		FC
Silurian	Rhuddanian	Passage Creek	Lower Massanutten Ss.	–26.30 <sup>a</sup>			1.42	FC
				–24.94 <sup>a</sup>			1.08	FC
				–26.23 <sup>a, e</sup>			5.99	F–RK (crust)
				–26.15 <sup>a, e</sup>			3.90	FC–RK
				–26.12 <sup>a, f</sup>			0.12	B–RK
				–25.96 <sup>a, f</sup>			0.03	B–RK
				–26.51 <sup>b</sup>			1.49	F (crust)
				–26.63	–26.54	–26.58 <sup>b</sup>	1.98 <sup>d</sup>	F
				–26.36	–26.32	–26.34 <sup>b</sup>	2.03 <sup>d</sup>	FC
				–26.36	–26.31	–26.33 <sup>b</sup>	2.49 <sup>d</sup>	FC
				–26.45 <sup>b</sup>			1.19	FC
				–26.55 <sup>b</sup>			0.94	FC
				–26.21 <sup>b, e</sup>			3.84	FC–RK
				–26.19 <sup>b, f</sup>			0.16	B–RK
				–25.60 <sup>c</sup>	–25.60 <sup>c</sup>	–25.60 <sup>c</sup>	–	FC
				–26.40 <sup>c</sup>	–26.10 <sup>c</sup>	–26.20 <sup>c</sup>	–	FC
	Katian (mid-late)	Conococheague Mt.	Reedsville Fm.–Oswego Ss. transition	–27.81			0.43	F
				–27.66	–27.58	–27.62	0.56 <sup>d</sup>	F
				–27.18	–27.10	–27.14	0.71 <sup>d</sup>	FC
				–27.41	–27.39	–27.40	0.64 <sup>d</sup>	FC
				–27.50 <sup>e</sup>			0.30	F–RK
				–27.35 <sup>e</sup>			0.63	FC–RK
				–28.35 <sup>f</sup>			0.13	B–RK
				–28.51 <sup>f</sup>			0.09	B–RK
	Katian (mid)	Frystown	Martinsburg Fm.	–27.78	–27.76	–27.77 <sup>e</sup>	1.23 <sup>d</sup>	F
				–27.76	–27.76	–27.76 <sup>e</sup>	2.13 <sup>d</sup>	F
				–27.52 <sup>e</sup>			0.43	FC
				–28.36 <sup>f</sup>			0.94	B
				–28.77 <sup>f</sup>			0.55	B
				–28.63 <sup>f</sup>			0.32	B
				–28.81 <sup>f</sup>			0.30	B
				–28.77 <sup>f</sup>			0.19	B

where  $\delta^{13}\text{C}_p$  is the stable carbon isotopic signature of plants (in our case liverworts),  $\delta^{13}\text{C}_a$  is the stable carbon isotopic signature of atmospheric  $\text{CO}_2$ , and  $\text{D}^{13}\text{C}$  is the isotopic discrimination against  $^{13}\text{C}$ .

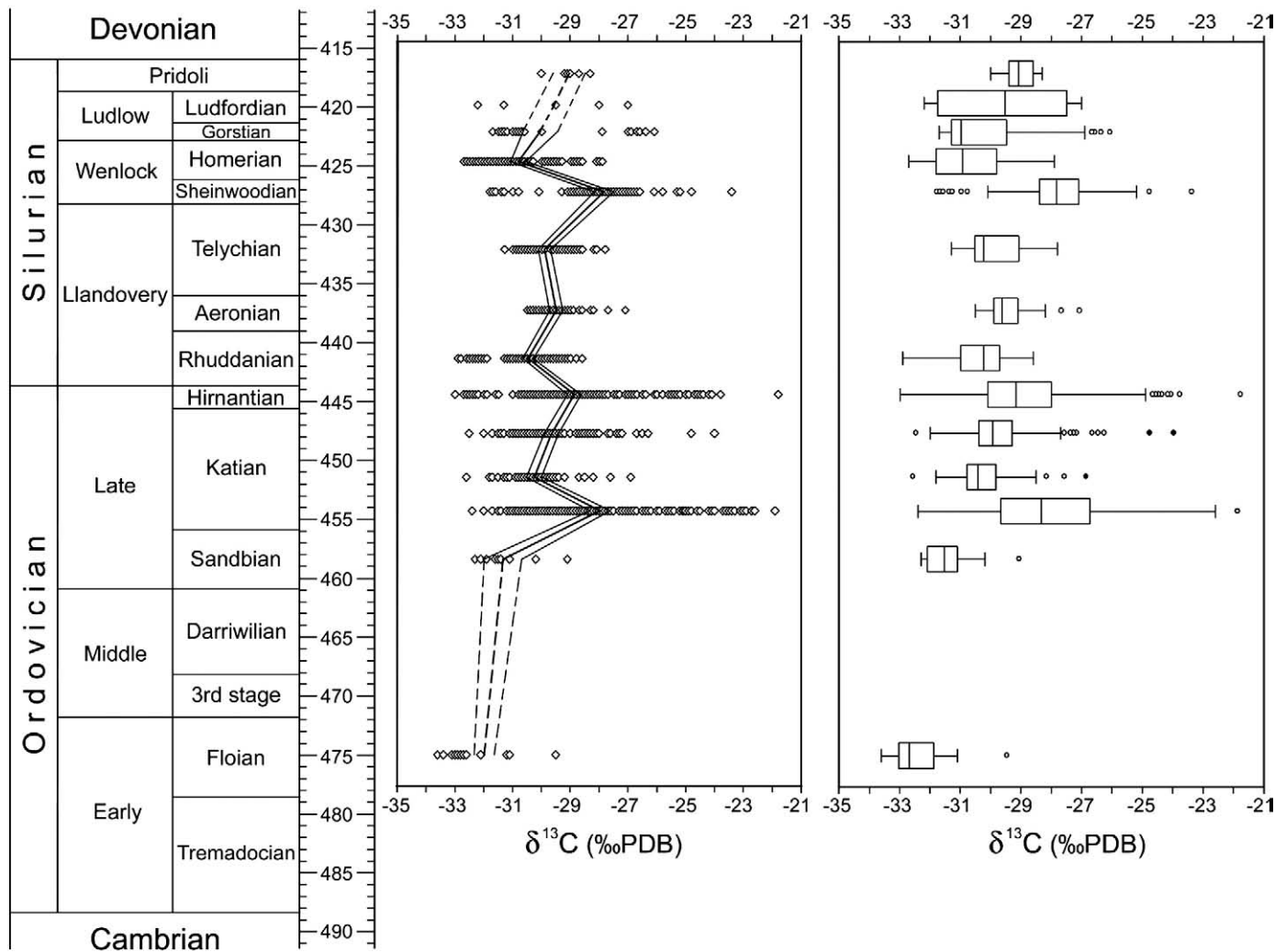
For  $\text{D}^{13}\text{C}$  we used values predicted by the BRYOCARB model (Fletcher et al., 2006) based on experiments with the thalloid liverworts *Marchantia* and *Lunularia*. For our interval of interest the model predicts a plateau at  $\text{D}^{13}\text{C} = 26.45$ .

$\delta^{13}\text{C}_a$  can be deduced from the carbon isotopic signature of marine carbonates ( $\delta^{13}\text{C}_c$ )

$$\delta^{13}\text{C}_a = \delta^{13}\text{C}_c - f \quad (3)$$

where  $f$  is the stable carbon isotopic fractionation factor between ocean surface carbonates and atmospheric  $\text{CO}_2$ . According to Strauss and Peters-Kottig (2003),  $f = 7\%$ ; but they cite Ekart et al. (1999) who





**Fig. 4.** Published Ordovician–Silurian marine  $\delta^{13}\text{C}_{\text{org}}$  data (based on Appendix 1). A. Data point distribution by stratigraphic stage; lines represent the mean and 95% confidence interval of the mean for each stage; a 95% confidence interval has not been calculated for the Ludfordian due to the scarcity of and extensive range of the data. B. Box plots of the data by stage; white dots – mild outliers ( $< 3$  IQR); solid dots – severe outliers ( $> 3$  IQR).

used a fractionation factor of 8‰. In our calculations we used  $f=6\%$  and  $f=8\%$  in order to define a range of predicted liverwort  $\delta^{13}\text{C}$  values for each time interval. The values of  $\delta^{13}\text{C}_c$  for our intervals of interest were taken from the Phanerozoic curve of Veizer et al. (1999).

### 3. Results

#### 3.1. Precision of measurements, part-to-counterpart and between-fossil variation

We performed duplicate measurements on 16 samples from five of the fossil localities (values in columns 1 and 2 in Table 3). The modulus of the difference between duplicate measurements has a mean of  $0.06 \pm 0.02\%$ .

Comparison of  $\delta^{13}\text{C}$  values obtained at Passage Creek for two composite samples consisting one of the parts, and the other of the counterparts of the same fossils from a 14 cm<sup>2</sup> area of a bedding plane, shows no significant differences [–26.46 and –26.32‰ (mean = –26.39‰) for the part; –26.40 and –26.30‰ (mean = –26.35‰) for the counterpart]. On the other hand, a comparison of  $\delta^{13}\text{C}$  values measured on a carbonaceous crust [–26.65 and –26.74‰ (mean = –26.70‰)] with the value obtained for a composite sample of small

thalloid fossils (–26.30‰) located within 8 cm of distance from the crust, on the same hand sample from Passage Creek, shows a difference of 0.4‰.

#### 3.2. Within-locality variability and between-locality comparison

The variability in  $\delta^{13}\text{C}$  values spans a range just a little over 4‰ across all Appalachian localities (Table 4), from –28.81‰ (Martinsburg Fm.) to –24.94‰ (lower Massanutten Ss.). Ranges of variation of measured  $\delta^{13}\text{C}_{\text{org}}$  in individual rock units are narrower: 0.68‰ for the three Rose Hill Fm. samples, to 2.44‰ for the four Tuscarora Fm. samples (representing two localities). Interestingly, the 20 measurements from the lower Massanutten Ss. (best sampled unit) cluster within a relatively tight range of only 1.80‰. The Martinsburg Fm. presents the lightest mean (–28.30‰,  $n=8$ ) and the lower Massanutten Sandstone the heaviest mean (–26.25‰,  $n=20$ ) of the six rock units. In between, with increasingly heavier means, are the Reedsville–Oswego transitional sequence, Bloomsburg Fm., Tuscarora Fm., and Rose Hill Fm.

A comparison between two fossiliferous layers sampled in the lower Massanutten Ss. (Table 4) shows a four-fold range of variation in one layer (layer 10) compared to the other. However, the means obtained for the two layers, –26.14‰ ( $n=9$ ; layer 10) and –26.40‰ ( $n=8$ ,



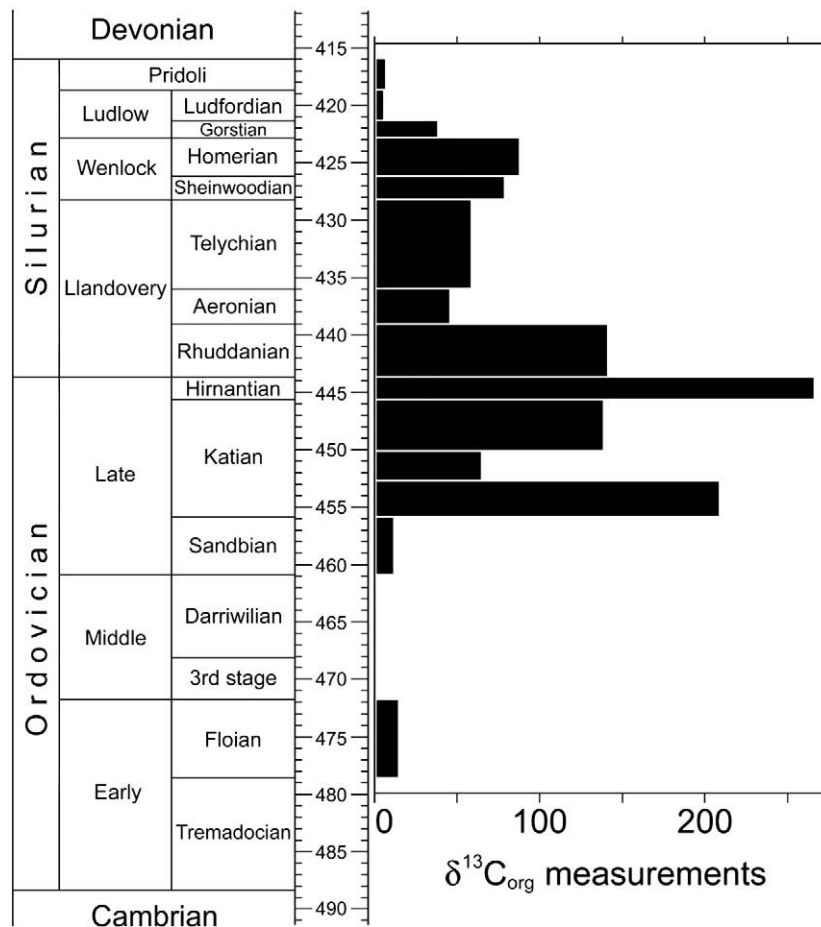


Fig. 5. Data density by stratigraphic stage for published Ordovician–Silurian marine  $\delta^{13}\text{C}_{\text{org}}$  measurements.

layer 12) are similar. The difference in  $\delta^{13}\text{C}_{\text{org}}$  signatures between the two layers is minimal.

### 3.3. Effect of bitumen extraction on fossil $\delta^{13}\text{C}_{\text{org}}$

We can test for the effect of bitumen extraction on  $\delta^{13}\text{C}_{\text{org}}$  signatures based on data from the Reedsville–Oswego transitional sequence and the lower Massanutten Ss. Comparison of measurements from untreated fossils (insoluble kerogen + soluble bitumens) vs. fossil material consisting of residual kerogen only (as a result of bitumen extraction) show no significant effect in either of the units (Table 4). Mean values obtained in the Reedsville–Oswego transitional sequence are  $-27.49\%$  ( $n=4$ ) for untreated fossils, and  $-27.43\%$  ( $n=2$ ) for extracted fossils. In the lower Massanutten Ss. the means are also similar ( $-26.36\%$ ,  $n=12$ , for untreated fossils, and  $-26.15\%$ ,  $n=3$ , for extracted fossils), despite very different ranges of variation for the two treatments.

### 3.4. Effect of sample type

Because of the makeup of our dataset, for comparisons illustrating the effect of sample type (fossil compressions versus bulk sedimentary organic matter) we used samples processed for bitumen extraction from the lower Massanutten Ss. and the Reedsville–Oswego transitional sequence, and samples untreated by extraction from the Martinsburg Fm. The results of these comparisons are somewhat conflicting (Table 4), although the small numbers of samples compared in each instance lower the inferential value of the results. In the lower Massanutten Ss. the  $\delta^{13}\text{C}$  signatures of residual kerogen from compression fossils and corresponding bulk sedimentary organic matter from the same hand

Table 4

Summary statistics of  $\delta^{13}\text{C}_{\text{org}}$  signatures in Appalachian biotas;  $n$  – number of samples. A Within-locality variability and between-locality comparison. B Between-layer comparison (Passage Creek). C Effect of bitumen extraction. D Effect of sample type.

	$n$	Min	Max	Mean	Range
A Bloomsburg all samples	7	−28.78	−26.59	−27.34	2.19
Rose Hill all samples	3	−26.84	−26.16	−26.51	0.68
Tuscarora all samples	4	−28.23	−25.79	−27.03	2.44
Lower Massanutten all samples	20	−26.74	−24.94	−26.25	1.80
Reedsville–Oswego all samples	8	−28.51	−27.14	−27.71	1.37
Martinsburg all samples	8	28.81	27.52	−28.30	1.29
B Lower Massanutten – layer 10 all samples	9	−26.70	−24.94	−26.14	1.76
Lower Massanutten – layer 12 all samples	8	−26.58	−26.19	−26.40	0.39
C Lower Massanutten all fossils	12	−26.74	−24.94	−26.36	1.80
Lower Massanutten all fossils residual kerogen	3	−26.23	−26.20	−26.15	0.08
Reedsville–Oswego all fossils	4	−27.81	−27.14	−27.49	0.67
Reedsville–Oswego all fossils residual kerogen	2	−27.50	−27.35	−27.43	0.15
D Lower Massanutten fossils residual kerogen	3	−26.23	−26.15	−26.20	0.08
Lower Massanutten whole OM residual kerogen	3	−26.19	−25.96	−26.09	0.23
Reedsville–Oswego fossils residual kerogen	2	−27.50	−27.35	−27.43	0.15
Reedsville–Oswego whole OM residual kerogen	2	−28.51	−28.35	−28.43	0.16
Martinsburg fossils	3	−27.77	−27.52	−27.68	0.25
Martinsburg whole OM	5	−28.81	−28.36	−28.67	0.45

samples are not significantly different (the fossils are on average lighter by 0.11‰ than the whole sedimentary organic matter). However, both the residual kerogen samples from the Reedsville–Oswego transitional sequence, and the un-extracted samples from the Martinsburg Fm. reveal fossils heavier than the bulk sedimentary organic matter by 1‰.

### 3.5. Stable carbon isotope signatures of Ordovician and Silurian marine organic matter

Ordovician and Silurian marine  $\delta^{13}\text{C}_{\text{org}}$  signatures in our compiled dataset of Fig. 4 cover broad ranges. In the Ordovician they are more negative than  $-33.5\text{‰}$ , and all the way to  $-22\text{‰}$ , whereas the Silurian is characterized by values ranging between  $<-33\text{‰}$  and  $-26\text{‰}$ . The broadest ranges are recorded in the Hirnantian and Katian. Although in-depth analysis of this marine dataset is beyond the scope of this paper, it is worth noticing that marine  $\delta^{13}\text{C}_{\text{org}}$  signatures have a bimodal distribution in the Gorstian.

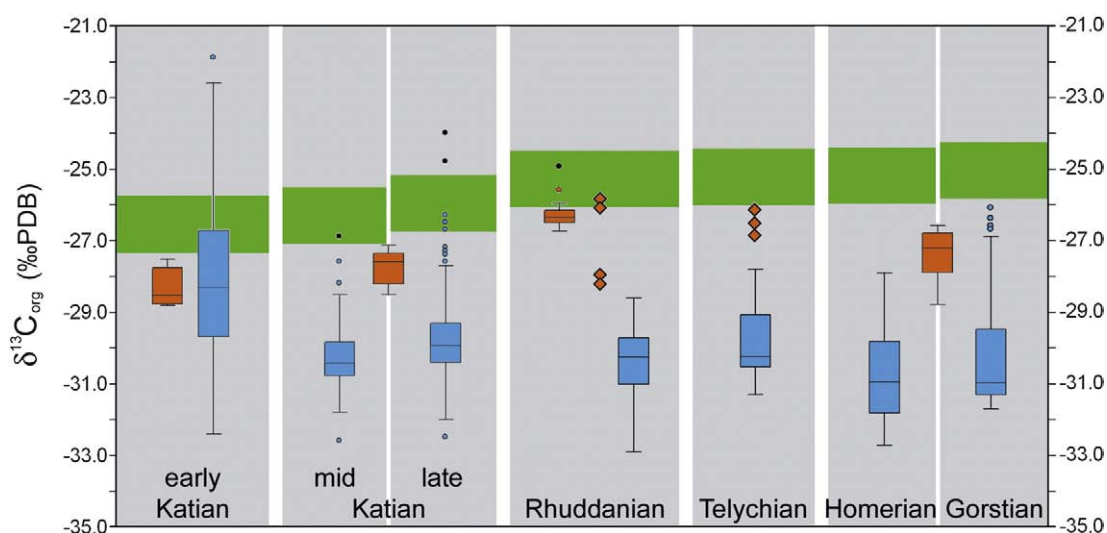
Stable carbon isotope signatures of organic matter in marine sediments are influenced by many factors among which the most important are surface water temperature;  $\text{CO}_2$  partial pressure in seawater, which is a function of temperature and atmospheric  $\text{CO}_2$  partial pressure; biological productivity; and influx of terrestrial organic matter (Wang et al., 1993). Marine  $\delta^{13}\text{C}_{\text{org}}$  values can therefore vary widely for the same interval depending on local conditions specific to each basin (e.g., Panchuk et al., 2006). Hence, combining data from such widely different marine settings in one dataset is in many cases not advisable. Our worldwide dataset shows such a wide variability expected for compilations of values representing widely different marine settings. Moreover, this dataset combines  $\delta^{13}\text{C}_{\text{org}}$  values of total sedimentary organic carbon with those obtained from discrete marine fossils. However, given the broad scope of our comparison (i.e. establishment of marine vs. non-marine nature of organic matter), combining all marine values available for each stratigraphic stage is not only acceptable, but recommended, because it reflects the broadest possible range of marine values for a given time interval, and thus provides the most conservative comparison.

### 3.6. Comparisons between Appalachian fossils and coeval marine organic matter

The Bloomsburg Formation is dated somewhere around the Homerian/Gorstian boundary (Strother, 1988), so this unit was compared with the marine isotopic records of both stages. Similarly, the Reedsville–Oswego transitional sequence was compared both the middle and late Katian. All of these comparisons, as well as comparisons for the lower Massanutten Sandstone, Tuscarora Formation, and Rose Hill Formation, show  $\delta^{13}\text{C}$  signatures of fossils and organic matter in these geologic units significantly different and much heavier ( $^{13}\text{C}$  enriched) as compared to coeval marine organic matter (Fig. 6, Table 6). The mean of the Reedsville–Oswego values falls within the mild outliers of both the mid- and the late Katian datasets; all data points of the Rhuddanian lower Massanutten Sandstone and Tuscarora Formation, and of the Telychian Rose Hill Formation fall outside of the two IQR intervals around the marine means for the respective stages; the mean of the Bloomsburg Formation data also falls outside of the two IQR intervals around the marine means for the Homerian marine dataset, and inside that interval but very close to the two IQR fences when compared to the Gorstian marine dataset. The only unit that yielded  $\delta^{13}\text{C}_{\text{org}}$  signatures similar to those of coeval marine sedimentary organic matter is the Martinsburg Formation (early Katian).

### 3.7. Comparisons between Appalachian fossils and predicted Ordovician–Silurian liverwort $\delta^{13}\text{C}$ signatures

The predicted values of liverwort  $\delta^{13}\text{C}$  signatures vary between a minimum of  $-27.35\text{‰}$  for the early Katian and a maximum  $-24.25\text{‰}$  for the Gorstian (Table 5). Not surprisingly, they show a steady trend towards heavier values in time. This is due to the steady trend toward heavier  $\delta^{13}\text{C}$  values of marine carbonates, and the constant value of the isotopic discrimination against  $^{13}\text{C}$ , for the studied interval (Veizer et al., 1999; Fletcher et al., 2006). In general, the  $\delta^{13}\text{C}$  signatures of Appalachian fossils and organic matter are lighter than the values predicted for liverworts for the corresponding intervals. However, they are not very different from the predicted values and they present a few significant results.



**Fig. 6.** Comparisons of  $\delta^{13}\text{C}_{\text{org}}$  signatures of Appalachian biotas with those of coeval marine organic matter and with ranges of predicted liverwort  $\delta^{13}\text{C}$  signatures. Appalachian biotas – brown box plots and diamonds (diamonds represent individual measurements in localities for which low numbers of measurements preclude computing of box plots); marine organic matter – blue box plots; predicted liverwort values – green bands; open dots – mild outliers ( $<3$  IQR); black dots – severe outliers ( $>3$  IQR). Data for Appalachian biotas from Table 3. Box plots for marine data from Fig. 4. Predicted liverwort values based on the BRYOCARB model (Fletcher et al., 2006) and marine carbonate  $\delta^{13}\text{C}$  values (Veizer et al., 1999); upper and lower limits of predicted liverwort values from Table 5. Appalachian biotas whose age falls close to a boundary between two stratigraphic stages are compared with both stages. Early Katian: Martinsburg Formation. Mid/late Katian: Reedsville–Oswego transitional sequence. Rhuddanian: lower Massanutten Sandstone (box plot) and Tuscarora Formation (diamonds). Telychian: Rose Hill Formation. Homerian/Gorstian: Bloomsburg Formation.

**Table 5**

Calculation of predicted liverwort stable carbon isotope signatures ( $\delta^{13}\text{C}_p$ );  $\Delta^{13}\text{C}$  — isotopic discrimination against  $^{13}\text{C}$  by liverworts as predicted by the BRYOCARB model (Fletcher et al., 2006);  $\delta^{13}\text{C}_c$  — stable carbon isotopic signature of ocean surface carbonates (from Veizer et al., 1999);  $\delta^{13}\text{C}_a$  — stable carbon isotopic signature of atmospheric  $\text{CO}_2$ .

Stage	$\Delta^{13}\text{C}$	$\delta^{13}\text{C}_c$ (‰ PDB)	$\delta^{13}\text{C}_a$ (‰ PDB)	$\delta^{13}\text{C}_p$ (‰ PDB)
Gorstian	26.45	1.78	−4.22 −6.22	−24.25 −25.84
Homerian	26.45	1.59	−4.41 −6.41	−24.40 −25.99
Telychian	26.45	1.55	−4.45 −6.45	−24.44 −26.02
Rhuddanian	26.45	1.47	−4.53 −6.53	−24.50 −26.08
Katian (late)	26.45	0.62	−5.38 −7.38	−25.17 −26.75
Katian (middle)	26.45	0.18	−5.82 −7.82	−25.52 −27.10
Katian (early)	26.45	−0.13	−6.13 −8.13	−25.77 −27.35

Most interestingly, stable carbon isotope signatures of fossils and organic matter in the Tuscarora Formation, and especially the lower Massanutten Sandstone, overlap at the heavier ends of their ranges with  $\delta^{13}\text{C}$  values predicted for Rhuddanian liverworts (Fig. 6). Fossils and organic matter in the Martinsburg Formation, the Reedsville–Oswego transitional sequence, and the Rose Hill Formation, fall short of overlapping, at the heavier end of their  $\delta^{13}\text{C}$  values, with the lighter end of the range predicted for liverworts. Similar to the comparisons of the Appalachian biotas with coeval marine values, here the Reedsville–Oswego transitional sequence is compared with middle and late Katian predicted values, and the Bloomsburg Formation with Homerian and Gorstian values. The heaviest value in the Reedsville–Oswego sequence is only 0.04‰ lighter than the lighter end of the range predicted for middle Katian liverworts. On the other hand, fossils and organic matter in the Bloomsburg Formation are at least 0.6‰ lighter than predicted Homerian or Gorstian liverwort values.

## 4. Discussion

### 4.1. General considerations

The  $\delta^{13}\text{C}_{\text{org}}$  signatures of the Appalachian biotas are characterized by strong internal consistency of the carbon isotope datasets within each locality. The close similarity between our measurements for the Passage Creek biota and those published by Niklas and Smocovitis (1983) (Table 3) demonstrate the high degree of reproducibility of the  $\delta^{13}\text{C}_{\text{org}}$  signatures.

The isotopic composition of fossil organic matter is influenced by original biology as well as diagenetic history. Differences between

localities are difficult to interpret because they could be due to different depositional environments with different sources of organic matter, as well as different diagenetic histories. In contrast, within-locality comparisons control for the diagenetic factor (Boyce et al., 2007), therefore the variability of  $\delta^{13}\text{C}_{\text{org}}$  signatures in the Appalachian biotas (some with ranges of variation around 2‰) must reflect some of the original taxonomic diversity. At the scale of a hand sample, the 0.4‰ difference between fossils in a sample from the lower Massanutten Sandstone, paralleled by different morphologies (crustose vs. thalloid), is significant. At the scale of the locality, the close similarity in overall  $\delta^{13}\text{C}_{\text{org}}$  signatures in the two layers sampled in the lower Massanutten implies stasis in carbon fixation mechanisms and sources at the community level, possibly associated with conserved taxonomic structure of communities, especially since lithology and diagenesis are homogeneous at the scale of the locality, and the fossil assemblages are very similar morphologically.

According to Underwood et al. (1997), the difference in  $\delta^{13}\text{C}_{\text{org}}$  signatures between total organic carbon (insoluble kerogen + soluble bitumens) and kerogen represents the isotopic effect of mobile hydrocarbons migrated into the sediment during diagenesis. We found no significant effect of bitumen extraction on  $\delta^{13}\text{C}_{\text{org}}$  signatures of fossils and sedimentary organic matter in the Reedsville–Oswego transitional sequence and the lower Massanutten Sandstone. Consequently, these two units are not contaminated by mobile hydrocarbons and the stable carbon isotope values obtained represent exclusively organic matter present in the system at the onset of diagenesis. This observation justifies combining measurements from both bitumen-extracted samples (kerogen) and non-extracted samples in comparisons with coeval marine organic matter.

Comparing the  $\delta^{13}\text{C}_{\text{org}}$  signatures of Appalachian fossils and total organic carbon in the sedimentary matrix at the same localities produced two results. First, there is no significant difference between the two types of samples in the lower Massanutten Sandstone. This situation could be due to either (1) diagenetic homogenization of carbon isotopic signatures between fossils and organic matter of a different nature in the host sediment, or (2) to the fact that fossils represent the only source of organic matter in the host sediment. Since comparisons of kerogen and total organic matter preclude mobile hydrocarbons, the second explanation is more plausible. It also supports interpretations of taphonomy at Passage Creek (Tomescu and Rothwell, 2006), where the fossil assemblages are thought to represent communities of overbank areas preserved in many cases in situ. These communities were very likely the only source of organic matter within the braided river system, where the widely migrating and possibly ephemeral channels were unfavorable to development of a rich freshwater biota.

Second, the Martinsburg Formation and the Reedsville–Oswego transitional sequence show differences of 1‰ between fossil  $\delta^{13}\text{C}_{\text{org}}$  values and those of the sedimentary matrix. In theory, such differences would indicate that the depositional environments received organic carbon from other sources than the fossils themselves. In the case of the two Appalachian localities the 1‰ difference between fossils and the surrounding mineral matrix is much smaller than the within-species and even within-individual variability documented for several groups of living organisms (Fletcher et al., 2004, 2005). Also, the differences documented in the two Appalachian localities are based on small numbers of samples, which lowers their interpretive value. Thus, at present, we interpret these differences as being too small to be biologically and geologically meaningful. Since our carbon isotopic evidence points to two of the three Appalachian biotas above being continental (see next section), the data are concurrent with the results of Strauss and Peters-Kottig (2003) and Peters-Kottig et al. (2006), who found no systematic difference in average  $\delta^{13}\text{C}_{\text{org}}$  between fossils and total organic matter in sediment in their survey of Paleozoic and Mesozoic terrestrial  $\delta^{13}\text{C}_{\text{org}}$  signatures. The lack of major differences between fossil  $\delta^{13}\text{C}_{\text{org}}$  signatures and those of the host rocks justifies combining both types of data points in comparisons with coeval marine organic matter.

**Table 6**

Summary statistics of comparisons between  $\delta^{13}\text{C}_{\text{org}}$  in Appalachian biotas and coeval marine  $\delta^{13}\text{C}_{\text{org}}$  signatures. Included are means and numbers of measurements, as well as probability levels for two-tailed Mann–Whitney  $U$  test (non-parametric samples).

Comparison	Marine	Appalachian biota	$P$
Gorstian — Bloomsburg Fm.	−30.05 ( $n=38$ )	−27.34 ( $n=7$ )	0.00880
Homerian — Bloomsburg Fm.	−30.80 ( $n=87$ )	−27.34 ( $n=7$ )	0.00002
Rhuddanian — Massanutten Ss.	−30.46 ( $n=142$ )	−26.25 ( $n=20$ )	0.00000
Late Katian — Reedsville–Oswego transition	−29.66 ( $n=138$ )	−27.71 ( $n=8$ )	0.00007
Mid. Katian — Reedsville–Oswego transition	−30.22 ( $n=64$ )	−27.71 ( $n=8$ )	0.00003
Early Katian — Martinsburg Fm.	−27.99 ( $n=208$ )	−28.23 ( $n=8$ )	0.90134



#### 4.2. Terrestrial vs. marine nature of the Appalachian biotas

The most important finding of the present study is that  $\delta^{13}\text{C}_{\text{org}}$  signatures of fossils and sedimentary organic matter at six of the seven fossil localities studied are significantly heavier than  $\delta^{13}\text{C}_{\text{org}}$  values of coeval marine organic matter documented by the global compilation (Fig. 6). Discussing the value of stable carbon isotope data in the geologic record, Hayes et al. (1999, p. 114) noted that “the issue is not exactly what can be learned about ancient organisms or environmental conditions from these crude data but instead what if anything can be learned. How might these observations be understood and in what ways do they fit existing ideas? Where do they not fit?” The answer to the first question is that comparisons with coeval  $\delta^{13}\text{C}_{\text{org}}$  signatures of known provenance can provide strong evidence for the origin of the organic matter, at least in broad terms. The broad and partially overlapping ranges of  $\delta^{13}\text{C}_{\text{org}}$  signatures from different types of modern environments and the examples of such high variability in the past from the marine sediments of the Ordovician–Silurian stratigraphic stages in our marine database (Fig. 4), or the upper Paleozoic  $\delta^{13}\text{C}$  signatures of terrestrial organic matter (Peters-Kottig et al., 2006), point to an answer to Hayes et al.'s second question, in that the direct use of  $\delta^{13}\text{C}_{\text{org}}$  signatures as indicators of the origin of organic matter has very wide margins of error.

Thus, the significant difference from coeval marine  $\delta^{13}\text{C}_{\text{org}}$  signatures indicates a non-marine origin for the organic matter that produced the  $\delta^{13}\text{C}$  signatures measured at Conococheague Mountain (in the Reedsville–Oswego transitional sequence), Passage Creek (Massanutten Sandstone), Hardscrabble and Moatstown (Tuscarora Formation), Monterey (Rose Hill Formation), and Port Clinton (Bloomsburg Formation). Consequently, the fossils analyzed at these localities represent terrestrial organisms, and the sedimentary organic matter is of terrestrial origin or, at least, includes a significant terrestrial component. The autochthony of fossils at most of these localities cannot be demonstrated (except for some fossils at Passage Creek; Tomescu and Rothwell, 2006), and the exact origin of particulate sedimentary organic matter cannot be inferred from stable carbon isotope values only, as mixing of organic matter of different origin (e.g. terrestrial and aquatic) is likely to occur in sediments. As a result, our data do not necessarily resolve the details of depositional environments of the geologic units at the six localities, although they generally concur with most published interpretations.

In contrast to those six localities, the  $\delta^{13}\text{C}_{\text{org}}$  values obtained for fossils and sedimentary organic matter in the Martinsburg Formation at Frystown fall within the marine  $\delta^{13}\text{C}_{\text{org}}$  range documented for the early Katian (Fig. 6), and are in accord with the marine origin of that unit. However, if the Martinsburg Formation includes the *Climacograptus bicornis* biozone at Frystown (R. Ganis, pers. comm., 2007), then its absolute age straddles the Sandbian–Katian boundary. Compared to Sandbian marine  $\delta^{13}\text{C}_{\text{org}}$  values (Fig. 4), Martinsburg Formation fossils are significantly heavier, which would suggest non-marine origin. However, the Sandbian marine  $\delta^{13}\text{C}_{\text{org}}$  signature is based on only 11 values, so the predictive value of the comparison in terms of the nature (terrestrial vs. marine) of the fossils is relatively low. Thus, the nature of carbonaceous compression fossils of the Martinsburg Formation remains equivocal.

Fossils from the other six Appalachian localities represent terrestrial organisms. This raises two questions. Are the  $\delta^{13}\text{C}_{\text{org}}$  signatures at these localities in accord with those of coeval terrestrial organic matter? What can these  $\delta^{13}\text{C}_{\text{org}}$  signatures tell us about the taxonomic affinities of the organism at the origin of the fossils? The  $\delta^{13}\text{C}$  measurements of terrestrial organic matter are extremely sparse for most of the interval covered by our study: the only published values are those reported for the final part of the Silurian by Peters-Kottig et al. (2006) in their synthesis of the Late Paleozoic plant  $\delta^{13}\text{C}$  record. Port Clinton (in the Homerian–Gorstian Bloomsburg Formation) is the only one of our localities that falls within the time interval covered by the Peters-Kottig et al. study. Comparison of the two datasets shows that Port Clinton

fossils and sedimentary organic matter have  $\delta^{13}\text{C}_{\text{org}}$  signatures similar to those of coeval terrestrial organic matter. Although direct comparisons are not warranted, carbon isotope values of organic matter and fossils at the five older Appalachian localities fall within the same range as Late Paleozoic terrestrial organic matter. Moreover, they fit in general the trend of progressive  $^{13}\text{C}$  enrichment seen at the older end of the curve of Peters-Kottig et al. (2006). As such, our data extend the terrestrial  $\delta^{13}\text{C}_{\text{org}}$  record back to the late Ordovician.

#### 4.3. $\delta^{13}\text{C}_{\text{org}}$ signatures and fossil taxonomy

The taxonomic affinities of most of the fossils in the Appalachian localities are unresolved to date. For instance, we know from morpho-anatomical and palynological studies (Pratt et al., 1978; Tomescu and Rothwell, 2006) that Rhuddanian communities represented by the thalloid fossils at Passage Creek included a broad diversity of organisms. For a small number of these fossils we were able to infer better-or-less constrained taxonomic identity (Pratt et al., 1978; Niklas and Smocovitis, 1983; Tomescu et al., 2006, 2008). However, attempts at defining the taxonomy of most of the fossils at Passage Creek by direct morpho-anatomical and ultrastructural comparisons with living organisms have failed. Fossils at the other Appalachian localities have not undergone any detailed morpho-anatomical studies to date.

Chemistry can offer some answers to questions addressing the nature of sedimentary organic matter and fossils, and examples of such studies abound in the literature. As part of the chemical makeup, stable carbon isotopic signatures of organic matter have been used to address questions of fossil taxonomy. One type of approach at that is exemplified by the study of Jahren et al. (2003) in which comparisons with  $\delta^{13}\text{C}$  signatures of modern liverworts, mosses, and lichens pointed to the latter as the group to which the enigmatic Devonian *Spongiophyton* belongs. Taking a similar approach, it is tempting to compare the  $\delta^{13}\text{C}_{\text{org}}$  signatures of fossils from the Appalachian localities directly with the  $\delta^{13}\text{C}$  signatures of modern organisms, and to use these comparisons for resolving fossil taxonomy. However, as shown by Fig. 7, such a comparison is uninformative since the range of  $\delta^{13}\text{C}_{\text{org}}$  values of Appalachian fossils overlaps with those of numerous groups of organisms, both terrestrial and aquatic.

It is becoming increasingly apparent that direct comparisons of stable carbon isotope ratios cannot provide easy answers to questions of fossil taxonomy. A careful anatomical analysis of *Spongiophyton* (Taylor et al., 2004) has confirmed the conclusions of the Jahren et al. (2003) study, supporting the idea of lichen affinities for this fossil genus. However, in another study of *Spongiophyton*, Fletcher et al. (2004) assembled a considerably expanded database of stable carbon isotopic signatures of modern organisms. When compared with values in the expanded database,  $\delta^{13}\text{C}_{\text{org}}$  signatures of *Spogiophyton* indicated affinities not only with lichens, but also with many other groups of organisms, including liverworts and mosses. These results point to the same conclusion reached by LoDuca and Pratt (2002) in a very different type of environment, that only relative isotopic comparisons between taxa from the same geologic unit and locality are valid for taxonomic purposes. From that point of view, as discussed above, the variability of stable carbon isotope signatures in the Appalachian biotas supports ideas of taxonomic diversity, and corroborates, at Passage Creek, the findings of morpho-anatomical studies.

Fletcher et al. (2005, 2006) experimented with factors controlling bryophyte stable carbon isotope signatures and produced a model describing bryophyte isotopic discrimination against  $^{13}\text{C}$  throughout the Phanerozoic. The model, called BRYOCARB (Fletcher et al., 2006), is based on the liverworts *Marchantia* and *Lunularia*, but in theory it would apply to any plant with the same simple type of photosynthetic apparatus (i.e., devoid of stomata and carbon concentration mechanisms, and based on passive gas diffusion) where carbon isotope fractionation during photosynthesis is primarily controlled by atmospheric  $^{13}\text{C}$  concentrations. Such models, which unfortunately

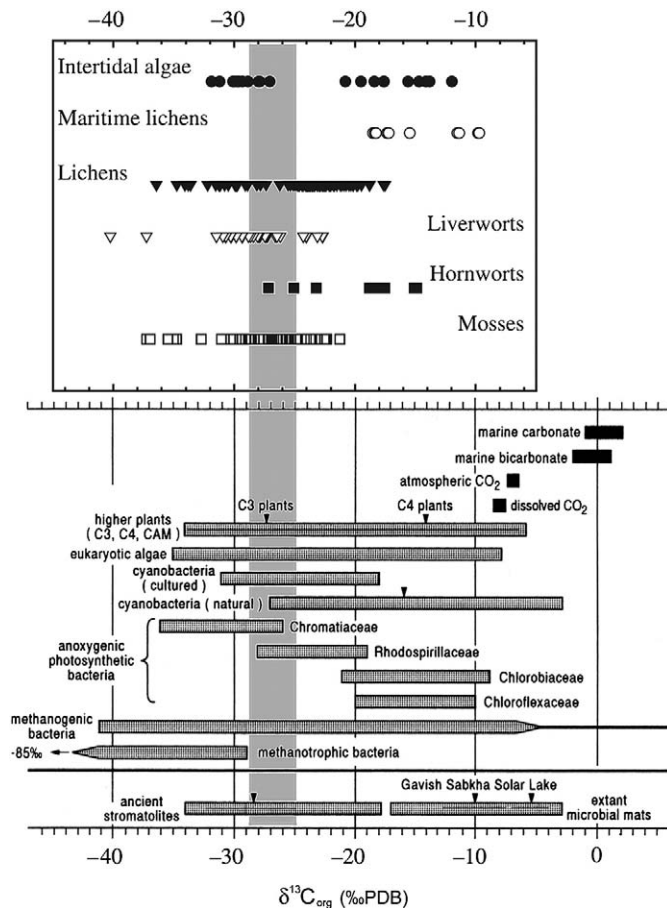


Fig. 7. Comparisons of  $\delta^{13}\text{C}_{\text{org}}$  signatures of Appalachian biotas (vertical grey band) and ranges of  $\delta^{13}\text{C}$  values measured in different groups of living organisms (upper panel from Fletcher et al., 2004, published with permission from Blackwell Publishing; lower panel from Schidlowski, 2000, published with permission from Springer Verlag).

are not yet available for other groups of organisms or photosynthetic mechanisms, provide a much more refined approach to isotopic comparisons between fossils and living organisms, with highly improved interpretive value.

We used BRYOCARB, along with reconstructions of the variation of marine carbonate stable carbon isotope ratios (Veizer et al., 1999), to calculate liverwort  $\delta^{13}\text{C}$  signatures for the time intervals corresponding to the ages of the Appalachian biotas. The comparisons (Fig. 6) show overlap between some of the thalloid fossils in the Rhuddanian Passage Creek biota and predicted liverwort  $\delta^{13}\text{C}$  signatures, which supports bryophyte affinities for those fossils. The presence of bryophyte-grade plants in the Passage Creek biota had been proposed earlier by Pratt et al. (1978) based on the trilete spores found at the locality. Niklas and Smocovitis (1983) have described from Passage Creek a strand of tubular cells interpreted as potential conducting tissue of a nonvascular land plant. Earlier, in an analysis of bitumens extracted from Passage Creek fossils, Niklas and Pratt (1980) had identified compounds that they interpreted as potential products of lignin degradation. Together with the stable carbon isotope values of Passage Creek fossils, these data indicate that some of those fossils are indeed bryophyte-grade plants that represent a snapshot in the evolution of the lignin biosynthetic pathway and of vascular tissues.

It is also worth noting that all other stable carbon isotope ratios measured in the Appalachian biotas are only slightly lighter than predicted liverwort values (<1‰ lighter than the lighter limit of the predicted liverwort intervals) (Fig. 6). Given that fossil diagenesis might alter the original stable carbon isotope ratios of organic matter, one might ask: are the differences between Appalachian fossils and predicted

liverwort values large enough to exclude the possibility of any of the fossils being liverworts? Some studies (e.g. Chung and Sackett, 1979) have suggested that diagenesis does indeed lead to relative  $^{12}\text{C}$  enrichment, and thus lighter stable carbon isotope ratios of fossils. However, other authors have inferred diagenetic  $^{13}\text{C}$  enrichment, or have proposed that patterns of diagenetic  $\delta^{13}\text{C}$  change are more complex than simple unidirectional variations (Schopf, 1994; Underwood et al., 1997; Kump et al., 1999; Czimczik et al., 2002; Fernandez et al., 2003; Poole et al., 2004). Peters-Kottig et al. (2006) provided a useful summary and discussion of results of research on diagenetic effects on stable carbon isotopic signatures of plant material. They outlined the contradictory nature of many results and the complex patterns in the amount and direction of isotopic shifts documented by different studies. One obvious conclusion of their discussion is that there is no general rule governing the direction of diagenetic  $\delta^{13}\text{C}$  changes in organic matter. However, another conclusion that emerged from the work of Peters-Kottig et al. is that diagenesis causes carbon isotopic changes of less than 2‰, and commonly less than 1‰ in sedimentary organic matter. The isotopic differences between the Appalachian fossils and predicted liverwort values fall within these narrow limits of diagenetically-induced change. Combined with the fact that bryophytes originated at least as early as the mid-Ordovician (Strother et al., 1996), this makes it very likely that some of the fossils at Conococheague Mountain, Moatstown, Hardscrabble, and Port Clinton represent liverworts too. The reports of embryophyte spores in the microfossil content at Port Clinton (Strother and Traverse, 1979) corroborates this conclusion.

Finally, the Martinsburg Formation is intriguing in that some of the stable carbon isotope signatures of organic matter fall within the range of diagenetic variability of predicted liverwort  $\delta^{13}\text{C}$  signatures, and yet all of the Martinsburg  $\delta^{13}\text{C}_{\text{org}}$  values overlap with early Katian (although not with Sandbian) marine  $\delta^{13}\text{C}_{\text{org}}$  signatures. This apparent paradox must be due to the vagaries of isotopic fractionation between different carbon sinks and temperature, at a global scale, which led to a significant overlap between carbon isotope ratios of early Katian marine organic matter and coeval predicted liverwort values.

## 5. Conclusions

We documented stable carbon isotopic signatures of carbonaceous fossils and sedimentary organic matter from six Ordovician and Silurian geologic units, at seven localities in the Appalachian Basin (referred to as the Appalachian localities/fossils). Fossil assemblages produced by these localities demonstrably or potentially represent terrestrial communities including complex multicellular eukaryotes (the oldest such organisms known to date on continents) and are thus of utmost importance to studies of the colonization of land. Due to equivocal thalloid morphology and to preservation by compression, the taxonomic origin of most of these Appalachian fossils is unresolved to date. Additional uncertainty in interpretation of these fossils comes from ambiguities in the identification of the exact depositional environments responsible for accumulation of the fossil assemblages. Thus, our stable carbon isotope investigation was aimed at confirming or establishing a terrestrial origin of the Appalachian fossils, and at elucidating their taxonomic affinities.

Comparison with an extensive dataset of published worldwide Ordovician and Silurian stable carbon isotopic signatures of marine organic matter shows that Appalachian fossils and sedimentary organic matter are significantly heavier isotopically than coeval marine organic matter at all but one of the studied localities. The heavy carbon indicates that fossils analyzed in the Reedsville–Oswego transitional sequence (mid-late Katian), Massanutten Sandstone and Tuscarora Formation (Rhuddanian), Rose Hill Formation (Telychian), and Bloomsburg Formation (Homerian–Gorstian) represent terrestrial organisms. The significance of  $\delta^{13}\text{C}_{\text{org}}$  signatures of fossils and organic matter in the early Katian Martinsburg Formation is more equivocal, although they most likely point to marine origin. These results are generally in accord with sedimentological interpretations of depositional environments at the

different localities. Comparisons with stable carbon isotope values predicted for liverworts of different ages based on the BRYOCARB model of Fletcher et al. (2006) indicate that some of the Massanutten Sandstone fossils, and possibly some of the terrestrial fossils at the other localities, could represent hepatic bryophytes.

The importance of this study resides in the strong support that it provides to the terrestrial origin of intriguing fossils that are placed stratigraphically in positions that are key to illuminating some of the most important aspects of the history of life on land: the origin of complex terrestrial eukaryotes and the advent of land plants. Strong support for a terrestrial origin of these fossils underscores their importance and provides impetus for detailed morpho-anatomical, ultrastructural, and chemical studies to resolve their taxonomic affinities. Support for bryophytic affinities of some of the Appalachian thalloid fossils brings us a step closer to resolving the systematic affinities of these important fossils. Moreover, it designates them as potentially the oldest land plant fossils (those in the Reedswille–Oswego transitional sequence, Massanutten Sandstone, Tuscarora Formation, and Rose Hill Formation). Along with coeval microscopic evidence for bryophytic and fungal life on land (Pratt et al., 1978; Strother et al., 1996; Gensel and Edwards, 2001; Wellman et al., 2003), the isotopic evidence provided by the Appalachian biotas helps paint a picture of Ordovician and Silurian terrestrial landscapes colonized by diverse communities including bryophytic-grade land plants and analogous to modern biological soil crusts, prior to the evolution of vascular plants.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.palaeo.2009.09.002.

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