Single-species dinoflagellate cyst carbon isotope ecology across the Paleocene-Eocene Thermal Maximum

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ABSTRACT
We present the first ever species-specific fossil dinoflagellate cyst stable carbon isotope (δ13C) records, from the Bass River Paleocene-Eocene Thermal Maximum (PETM) section in New Jersey (USA), established using a novel coupled laser ablation–isotope ratio mass spectrometer setup. Correspondence with carbonate δ13C records across the characteristic negative carbon isotope excursion indicates that the δ13C of dissolved inorganic carbon exerts a major control on dinocyst δ13C. Pronounced and consistent differences between species, however, reflect different habitats or life cycle processes and different response to pCO2. Decreased interspecimen variability during the PETM in a species that also drops in abundance suggests a more limited niche, either in time (seasonal) or space. This opens a new approach for ecological and evolutionary reconstructions based on organic microfossils.

INTRODUCTION
Fossilizable organic cyst–producing dinoflagellates are ecologically diverse unicellular eukaryotes, and include freshwater and marine, heterotrophic, mixotrophic, autotrophic, and polar and tropical species (Matthiessen et al., 2005). Dinoflagellate cyst (dinocyst) assemblages are widely used for paleoceanographic reconstructions of Triassic to modern seas and oceans, particularly along continental margins (Slujs et al., 2005).

We explore the stable carbon isotopic composition (δ13C) of fossil dinocysts as a paleoceanographic indicator. Dinocyst δ13C likely reflects the δ13C of dissolved inorganic carbon (δ13CDIC) (Slujs et al., 2007). Culturing experiments have shown that dinoflagellate 13C fractionation increases with seawater CO2 concentrations, indicating potential for a CO2 proxy (Hoins et al., 2016b, 2015; Van de Waal et al., 2013). Because cysts directly derive from dinoflagellates, δ13CDIC and seawater CO2 concentrations are expected to affect dinocyst δ13C. A recently developed analytical setup allows for the analysis of ten nanograms of particulate organic matter for δ13C (Van Roij et al., 2017). This setup, laser ablation–nano combustion–gas chromatography–isotope ratio mass spectrometry (LA-nC-GC-IRMS) has been shown to achieve precise and accurate δ13C values for higher plant pollen (Van Roij et al., 2017).

Over the first millennia of the Paleocene-Eocene Thermal Maximum (PETM, ca. 56 Ma), global surface temperatures warmed by ~5 °C (Dunkley Jones et al., 2013; Frieling et al., 2017). This warming roughly coincided with deep ocean carbonate dissolution and a negative stable carbon isotope excursion (CIE) of several per mill recorded globally in sedimentary components, implying massive 13C-depleted carbon injection into the ocean-atmosphere system (Dickens et al., 1997; Zachos et al., 2005).

The magnitude and structure of the CIE differ between bulk carbonate, species- and/or genus-specific foraminifera, bulk organic matter, and bulk dinocyst records at the Ocean Drilling Program Leg 174AX core at Bass River, New Jersey, USA (Cramer et al., 1999; John et al., 2008; Slujs et al., 2007) (Fig. DR1 in the GSA Data Repository¹). Moreover, pronounced regional biogeochemical and paleoenvironmental change has been recorded during the PETM (e.g., Gibbs et al., 2012; Kopp et al., 2009). The Bass River PETM section therefore presents a suitable case to (1) test the dependence of dinocyst δ13C to large changes in both δ13CDIC and seawater carbonate chemistry, (2) evaluate whether this dependence is species specific, and (3) assess if dinocyst δ13C values reflect changes in population ecology and/or biosynthesis. To this end, we analyzed the δ13C of 4 dinocyst species with different ecological preferences across the CIE.

MATERIALS AND METHODS
In the siliciclastic sequence with biogenic carbonate and organic matter at Bass River, a change from glauconite-rich sandy silts to clay and the onset of the CIE at ~357.3 m below surface (mbs) marks the transition from the Paleocene into the PETM (Cramer et al., 1999). Well-preserved (i.e., showing no signs of degradation) dinocysts are abundant throughout the record (Slujs and Brinkhuis, 2009).

Palynological residues of Slujs and Brinkhuis (2009) were washed using sieving (15 µm) and ultrasonic cleaning in Milli-Q water to exclude contamination with amorphous particulate organic matter. Individual dinocysts of the species Apectodinium homomorphum, Areoligoiera volata, Spinniferites ramosus (and closely resembling specimens), and Eo cladopoxyxis peniculata were pressed on a nickel sample tray, which was then placed in a miniaturized ablation chamber. In our LA-nC-GC-IRMS setup (Van Roij et al., 2017), molecular dinocyst fragments resulting from deep ultraviolet LA are transported into capillaries on a helium carrier gas, and subsequently oxidized in a combustion oven. The CO2 formed is transported to a GC combustion interface, and subsequently into a ThermoFisher DeltaV Advantage IRMS for δ13C analysis.

Analyses of the International Atomic Energy Agency CH-7 polystyrene standard (PE; certified δ13C value −32.151‰ ± 0.050‰; σ<sup>13C</sup> 0.41‰ precision and 0.36‰ accuracy for analyses yielding peak areas of at least 4 voltseconds (Vs), equivalent to >42 ng C (Van Roij et al., 2017), Precision decreases at lower yields, so that multiple specimens need to be analyzed to achieve low standard errors. We therefore typically perform 20–50 analyses of individual specimens of the relatively thick-walled A. homomorphum and large A. volata. Because of their thin cyst wall, 3–5 specimens of S. ramosus and E. peniculata were analyzed simultaneously for a single measurement. Calibration to the Vienna PeeDee belemnite (VPDB) scale was achieved through bracketing series of analyses by the PE standard (σ>4 Vs).

¹GSA Data Repository item 2018018, Figures DR1–DR3 (site location, all isotope results in histograms, and dinocyst assemblages), and Table DR1 (raw isotope data), is available online at http://www.geosociety.org/datarepository/2018/ or on request from editing@geosociety.org.
RESULTS AND DISCUSSION

Data Quality and Variability

Individual ablations yielded between 0.1 and 3.4 Vs and produced a broad range of δ13C values (Fig. DR2). At a similar signal range, PE analyses show a 1σ uncertainty of ~0.5‰~1.7‰ (Van Roij et al., 2017), which for most samples and species is similar to the general variability observed between the dinocysts studied here. It is unclear, however, if the PE standard is homogeneous on the micrometer scale (Van Roij et al., 2017). This result implies that the variability between specimens within most samples does not significantly exceed the analytical uncertainty. Shapiro-Wilk tests show that the distributions of the populations are (close to) normal (Fig. DR2). Moreover, small peak areas correspond to relatively high scatter, and high peak areas correspond to δ13C values that are close to the mean of the populations. We recorded no sample-size dependency (Fig. DR2).

For a few samples and species, particularly Paleocene A. volata, the variance in dinocyst δ13C significantly (p < 0.05) exceeds that of the standard (Fig. DR2). Part of the variance therefore relates to variability within populations. It is interesting that the variance in A. volata is significantly smaller than that of the standard in PETM sample at 356.84 mbs (n = 43), suggesting that this population is isotopically more homogeneous than the PE standard.

CIE in Dinocyst δ13C Records

Normality of the data sets implies that the absolute values of the populations can be assessed by means and standard errors of the means (Fig. 1). Mean δ13C values of S. ramosus are ~24.9‰ just below the CIE. At the onset of the CIE, at 357.3 mbs, δ13C values shift to a mean of ~27.1‰, implying a CIE of 2.2‰ (±0.46, 1 standard error, SE). Only one data point could be generated below the CIE for A. homomorphum and above the CIE for A. volata due to scarcity of these species in these intervals. The difference between the means of uppermost Paleocene and lowest PETM samples suggest a CIE of 1.8‰ (±0.36, 1 SE) for A. volata and 4.0‰ (±0.54, 1 SE) for A. homomorphum. Regardless of these species-specific differences, the results imply that dinocyst δ13C is primarily controlled by the δ13C of DIC at the time of formation.

It is interesting that the CIEs in these single-species records are somewhat smaller in magnitude than that recorded in a previously published bulk dinocyst δ13C record, based on an optimally sieved palynological residue that dominantly comprised dinocysts (Sluijs et al., 2007) (Fig. 1). While absolute δ13C values of this record correspond well with the species-specific records in the Paleocene, bulk values are 1‰–2‰ more 13C-depleted in the early stages of the CIE. This is unexpected, as the bulk record should reflect a weighted average of Spiniferites and Apectodinium, the dominant dinocysts within these samples. We therefore also analyzed the δ13C of amorphous organic matter, using LA-nC-GC-IRMS, which was originally considered to be a negligible factor based on light microscopic observations (Sluijs et al., 2007). The amorphous organic matter, which is almost absent in the Paleocene but present in the PETM, is, however, very depleted in 13C (Fig. 1), implying that even a mass contribution of 10%–20% would skew bulk dinocyst records toward recorded low values and result in a large CIE. Considering δ13C constraints on Paleocene–Eocene terrestrial and marine organic matter (Hayes et al., 1999; Sluijs and Dickens, 2012), the extremely low value suggests that the amorphous matter is of marine origin.

The bulk dinocyst record was generated to demonstrate that the observed onset of the Apectodinium acme (Crouch et al., 2001) prior to the onset of the CIE, as measured on carbonate, was not an artifact of selective bioturbation. Here our species-specific record confirms that the abundant Apectodinium at 357.58 mbs (Fig. DR3) yield Paleocene δ13C values and thus that the Apectodinium acme precedes the CIE. The magnitude of the CIE in A. homomorphum (4‰) is slightly larger than that recorded in the mixed-layer foraminifer Acarinina spp. at Bass River (3.4‰; John et al., 2008), possibly indicating an increase in 13C fractionation due to the PETM rise in CO2 (Hoins et al., 2015).

Dinocyst δ13C Ecology

The records also show differences in δ13C values between species, related to differences in ecology and/or biosynthesis (Fig. 1). Populations of A. volata are enriched in 13C relative to S. ramosus for all 4 samples where both could be analyzed, typically by ~1‰. The PETM δ13C value of E. peniculata is strikingly high, broadly comparable to Paleocene values of the other taxa. Just as remarkable is the changing offset between S. ramosus and A. homomorphum across the PETM: A. homomorphum is relatively 13C enriched in the latest Paleocene and toward the top of the PETM, while the species are statistically indistinguishable in the lowermost part of the CIE. This implies that at least one of these species underwent a significant change in carbon acquisition or ecological niche across the onset of the PETM.

Differences in dinocyst species-specific δ13C values could originate from several ecological factors. Dinoflagellates may be autotrophic, mixotrophic, or heterotrophic, which may affect cyst δ13C. Such strategies are known for many extant species and evolutionary lineages (Spiniferites and Eocladopyxis) but not well constrained for extinct groups (Areoligera and Apectodinium). Culturing experiments on autotrophic taxa have
shown species-specific fractionation resulting from differences in carbon acquisition (Hoins et al., 2015, 2016a, 2016b; Rost et al., 2006). Many dinoflagellates take up both dissolved CO₂ and HCO₃⁻ for fixation, but their relative contributions differ between species (Hoins et al., 2016b; Rost et al., 2006). Because seawater CO₂ is depleted in ¹³C relative to HCO₃⁻ by 8%–12% depending on temperature (Mook et al., 1974), this may contribute appreciably to differences between species. Dinoflagellate ¹³C fractionalation might also increase because of changing influx and leakage of inorganic carbon through the cell per unit of time. Increased fluxes of CO₂ result in more efficient replenishment of the intracellular stock of ¹²C, so that the resulting fractionalation of the cell is closer to the maximum of RubisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase; Hoins et al., 2016b; Sharkey and Berry, 1985). In addition, changes in seawater δ¹³C related to the seasonal cycle or depth habitat may result in species-specific δ¹³C differences. Some photosynthetic dinoflagellate species may deplete seawater from ¹³C as they occur in massive blooms of as many as 10⁷ cells/L of seawater (e.g., Heiskanen, 1993).

We consider the latter factor a likely explanation for the high δ¹³C values of E. peniculata. This species is evolutionary related to modern Pyrodinium bahamense, which causes harmful blooms (McLean, 1976). The high δ¹³C values are thus in line with a short, intense growing season, similar to P. bahamense in present oceans, which locally depleted seawater DIC from ¹²C. This interpretation is also consistent with very high δ¹³C values (to −18.9‰) of Eocladopyxis-dominated (73%; 10⁵ cysts/g) palynological residues at the onset of the PETM in a Nigerian section (Frieling et al., 2017).

Recent controlled growth experiments have indicated that ¹³C fractionation in the dinoflagellate Gonyaulax spinifera, which produces S. ramosus dinocysts, increases with higher CO₂ concentrations due to increased leakage (Hoins et al., 2015, 2016b). S. ramosus, however, does not show a larger CIE relative to biogenic carbonate that would be expected from the increase in pCO₂ during the PETM (Fig. 1). Concentrations of CO₂ across the PETM were likely higher than those in the culturing experiments, so possibly maximum ¹³C fractionalization was already reached prior to the CIE. Alternatively, a shift of the dominant season of production or depth habitat might have reduced the difference between dinocyst and carbonate δ¹³C. Similar to other proxies, a CO₂ proxy based on dinocysts may thus possibly be biased during phases of massive ecological change in such marginal settings.

**Added Value of Single-Specimen Isotopic Analyses**

A. volata exhibits relatively high δ¹³C values. Areoligeria was likely autotrophic and is typically associated with relatively shallow, high-energy settings (Brinkhuis, 1994; Sluijs and Brinkhuis, 2009). With the available information, it is not possible to determine whether it occurred in seasonal blooms, used relatively high amounts of HCO₃⁻ during carbon acquisition, leaked little CO₂, or fixed carbon close to the sea surface. Regardless of the absolute δ¹³C values, A. volata exhibits considerable variability prior to the CIE (1σ = 2.2%–2.5%), while variability is significantly smaller in the sample within the CIE (1σ = 1.26%; Fig. 2). This suggests that the niche of this species, geographically or in (annual) duration of presence, became limited during the CIE. This species is present in very low numbers only during the PETM at the study site (Fig. DR3), hypothesized to reflect sea-level rise (Sluijs and Brinkhuis, 2009). Comparison to the variance in carbon isotopic values in A. homomorphum shows that the fitness of this species is not affected across the PETM, which is in line with its acme, as A. volata collapsed. Crucially, this indicates that the variance in single specimen δ¹³C populations can yield information on the ecological fit and/or fitness of species.

**CONCLUSIONS**

We present the first ever species-specific dinoflagellate cyst δ¹³C records. Apectodinium homomorphum, Areoligeria volata, and Spiniferites

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**Figure 2.** Frequency distribution of δ¹³C analyses of individual Areoligeria volata and Apectodinium homomorphum specimens (mbs—m below surface). Error bars reflect 1 standard error of the mean of dinocyst δ¹³C analyses and the International Atomic Energy Agency CH-7 polyethylene (PE) standard. The variance of A. volata in the sample marked by # is significantly smaller than that of the PE standard, while stars indicate samples in which dinocyst δ¹³C variance significantly exceeds that of the standard (p < 0.05).
ramous all show the characteristic negative carbon isotope excursion across the PETM at Bass River, New Jersey. Differences in absolute values and the magnitude of the CIE are attributed to changes in carbon acquisition, food source, and dominant season and depth of production. The records confirm that the first anomalous *Apectodinium* abundance during the PETM, underlining the potential of this fitness of all show the characteristic negative carbon isotope excursion.

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