An organic record of terrestrial ecosystem collapse and recovery at the

Triassic–Jurassic boundary in East Greenland

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Abstract

Terrestrial ecosystem collapse at the end of the Triassic Period coincided with a major mass extinction in the marine realm and has been linked to increasing atmospheric carbon dioxide, global warming, and fire activity. Extractable hydrocarbons in samples from the fluvial Triassic–Jurassic boundary section at Astartekløft, East Greenland were analyzed to investigate the molecular and isotopic organic record of biotic and environmental change during this event. Carbon isotopic compositions of individual plant wax lipids show a >4‰ negative excursion coinciding with peak extinction and a further decrease of 2‰ coinciding with peak $p$CO$_2$ as estimated from the stomatal indices of fossil Gingkoales. An increase of ~30‰ in the hydrogen isotopic compositions of the same plant wax lipids coincides with ecosystem collapse, suggesting that the biotic crisis was accompanied by strong hydrologic change. Concentrations of polycyclic aromatic hydrocarbons related to combustion also increase together with abrupt plant diversity loss and peak with fossil charcoal abundance and maximum plant turnover, supporting the role of fire in terrestrial extinctions. Anomalously high concentrations of a monoaromatic diterpenoid related to gymnosperm resin derivatives (and similar to dehydroabietane) occur uniquely in samples from the boundary bed, indicating that environmental stress factors leading to peak plant extinction stimulated increased resin production, and that plant resin derivatives may be effective biomarkers of terrestrial ecosystem stress.
1. INTRODUCTION

Global warming due to increased atmospheric concentrations of greenhouse gases, initiated by the breakup of the supercontinent Pangaea and eruption of the Central Atlantic Magmatic Province (CAMP; Marzoli et al., 1999; Schaller et al., 2011), led to mass extinctions in the marine and terrestrial realms ~200 million years ago at the end of the Triassic period (Raup and Sepkoski, 1982; McElwain et al., 1999; Schoene et al., 2010; Blackburn et al., 2013). Stomatal indices from fossil Gingkoales leaves recovered from terrestrial Triassic–Jurassic boundary (TJB) sections in East Greenland and Scania, Sweden point to a fourfold increase in $pCO_2$ (Fig. 1a) coinciding with an 80% extinction of plant species and negative excursions in the stable carbon isotopic composition ($\delta^{13}C$) of fossil leaves (McElwain et al., 1999; Bacon et al., 2011) and wood (Hesselbo et al., 2002; Fig. 1b). Subsequent $pCO_2$ reconstructions based on stomatal indices (Steinthorsdottir et al., 2011; Fig. 1a) and pedogenic carbonates (Schaller et al., 2011) have strengthened this interpretation. Decreasing leaf width and increasing leaf dissection across the TJB section at Astartekløft, East Greenland suggest selection for an ability to mitigate heat stress (McElwain et al., 1999). The resulting, relatively narrow-leaved floras would have been more susceptible to fire, which increased significantly at the boundary as evidenced by fossil charcoal concentrations (Belcher et al., 2010).

Global carbon cycle perturbation across the TJB was initially recognized from $\delta^{13}C$ records of bulk organic matter and carbonate (Palfy et al., 2001; Ward et al., 2001; Hesselbo et al., 2002; Guex et al., 2004; Galli et al., 2005; Kuerschner et al., 2007; Ward et al., 2007; Williford et al., 2007; van de Schootbrugge et al., 2008; Ruhl et al., 2009;
Bachan et al., 2012). Stratigraphic records of bulk organic δ\(^{13}\)C are affected by changes in the relative contribution of source components with different isotopic compositions, but some of this uncertainty is removed with compound specific isotope analysis (CSIA; Hayes et al., 1990). For instance, long chain \(n\)-alkanes with odd carbon numbers, largely derived from terrestrial plant leaf waxes (Eglinton and Hamilton, 1967) in communication with the atmosphere, offer a more specific proxy for the δ\(^{13}\)C of atmospheric CO\(_2\) than bulk organic matter. CSIA of long chain \(n\)-alkanes has recently been employed to resolve TJB carbon cycle perturbation in marine (Ruhl et al., 2011) and lacustrine (Whiteside et al., 2010) settings.

Plant lipids record the hydrogen isotopic composition (δ\(^2\)H) of source water available during growth, thus enabling the use of these compounds as tracers of paleohydrologic change (Sachse et al., 2012). Fidelity of compound specific δ\(^2\)H records declines with thermal maturity, as carbon-bound hydrogen exchanges with formation water over geologic timescales (Sessions et al., 2004). However, the preservation of hydrogen isotopic offsets consistent with biosynthetic processes on 10\(^8\) yr timescales suggests that such exchange is negligible in some ancient sedimentary organic matter of low maturity (Dawson et al., 2004; Dawson et al., 2005; Dawson et al., 2007).

Beyond \(n\)-alkanes, the distribution and abundance of other lipid biomarkers records paleoecological change in the environment of deposition. For example, increases in isorenieratane and aryl-isoprenoids (biomarkers indicative of photic zone euxinia) in marine rocks suggest that hydrogen sulfide toxicity may have played a role in both the end-Permian (Grice et al., 2005) and end-Triassic (Richoz et al., 2012; Jaraula et al., 2013) mass extinctions.
Here we report the distribution and isotopic composition of individual organic molecules (biomarkers, molecular fossils) in an investigation of the response of a terrestrial ecosystem to increasing atmospheric CO₂ and global warming across the TJB. The reader should carefully consider the context of the data presented here: nine discreet “time slices” from an evolving, but generally fluvial environment over perhaps more than a million years. The four samples analyzed within the “boundary interval” (46.48–46.98 m) are likely to be separated in time to some extent, but it is difficult to constrain the degree to which they were reworked during or after deposition. Nevertheless, the organic record from Astartekløft offers a valuable complement to similar records from marine and lacustrine environments.

2. SAMPLES AND METHODS

Detailed lithology and paleontology for the Astartekløft locality, including identification and numbering of individual plant beds, are discussed in a previous publication (McElwain et al., 2007). Briefly, the locality exposes river channel sandstones interbedded with shaley, organic rich, immature, and fossiliferous overbank deposits, or “plant beds,” of the Rhaetian–Hettangian Primulaev Formation, one of three formations that comprise the Kap Stewart Group (Surlyk, 2003). One mudstone sample from each of plant Beds 1, 2, 3, 4, 6, 7, 8 and 9, and three samples from the TJB Bed 5 were analyzed in this study. Sample heights and organic geochemical data are given in Table 1.
2.1. Sample processing

Fresh, unweathered subsamples were prepared from larger hand samples collected by J. McElwain in 2002. Exterior surfaces of all subsamples were washed by sonicating for 10 min each in deionized water, dichloromethane (DCM) and hexane. Washed samples were ground to a fine powder using a vibratory ring mill, cleaned between each sample by grinding combusted quartz sand three times for 60 seconds and rinsing with methanol, DCM and hexane.

Approximately 20 g of each sample were extracted using a Dionex Accelerated Solvent Extractor and a 9:1 mixture of DCM:methanol at 1500 psi and 100°C. Elemental sulfur was removed by passing the extracts over solvent-rinsed, HCl-activated Cu powder. Extracts were brought to incipient dryness by evaporating solvent under a stream of high purity nitrogen. Recovered extracts ranged in mass from 6 to 104 mg. Total lipid extracts were separated into aliphatic, aromatic and polar fractions by adding to the top of a column (20 cm x 0.9 cm internal diameter) of activated silica gel (120°C, 8 hrs) and eluting with hexane, 3:7 (v/v) DCM in hexane and 1:1 (v/v) DCM:methanol, respectively.

2.2. Gas chromatography-mass spectrometry (GC-MS)

Aliphatic and aromatic fractions were analyzed by GC-MS using a Hewlett Packard (HP) 5973 mass selective detector interfaced to a 6890 GC. Fractions were dissolved in n-hexane and injected by the HP6890 autosampler into a split-splitless
injector using pulsed-splitless mode. A DB-5MS (J&W Scientific, 5% phenylmethylsiloxane stationary phase) GC column was used with He as the carrier gas. The GC oven was programmed from 40°C to 310°C at a rate of 3°C/minute and held isothermally for 30 minutes. Data were acquired in full-scan ($m/z$ 50–500) mode with an ionization energy of 70 eV.

Individual compound abundances were quantified using relative GC-MS peak areas on the total ion chromatogram. After fraction collection and transfer to 2 ml autosampler vials, appropriate masses of squalane and deuterated terphenyl standards were injected into saturate and aromatic fractions, respectively, such that the concentration of the standard compound in the fraction would approximate that of the most abundant sample compound (n.b. aromatic fractions containing the deuterated terphenyl standard were not processed on the instrument used for δ²H measurements). A single response factor was calculated for each sample analysis by dividing the peak area of the standard compound in the total ion chromatogram by its known concentration in the analysis. Semi-quantitative individual compound abundances were calculated as μg per g total organic carbon (TOC; calculated using starting sample mass and weight percent organic carbon as previously measured by elemental analyzer).

2.3. Compound specific carbon and hydrogen isotope analysis

Compound specific δ¹³C and δ²H were measured on aliphatic fractions using gas chromatography-isotope ratio mass spectrometry (GCIRMS) following a protocol described previously by Dawson et al. (2004). Isotope ratio analyses were performed
using a HP 6890 GC equipped with a HP 6890 autosampler and coupled to a Micromass isotope ratio monitoring mass spectrometer. The GC oven was held at 50ºC for 1 min, increased to 310ºC at 3ºC/min, and held isothermally for 20 min, using a J&W Scientific DB-5MS GC column (60 m x 0.25 mm i.d. x 0.25 mm film thickness) and He as the carrier gas with a flow rate of 1ml/min.

For δ¹³C, CO₂ was produced during the oxidation of each chromatographically separated component after being passed through a quartz furnace containing copper oxide pellets (heated to 850ºC). Values of δ¹³C were determined by integrating mass 44, 45 and 46 ion currents of the CO₂ peaks. For hydrogen isotope measurements, H₂ was produced during pyrolysis of each chromatographically separated component, after passing through a quartz furnace containing chromium powder and heated to 1050ºC. H₃⁺ correction was performed by measuring mass 3 at different H₂ reference gas pressures. Values of δD were determined by integrating the mass 2 and 3 ion currents of the H₂ peaks. Samples were analysed 1–3 times (depending upon the quantity of aliphatic fraction recovered), and the average δ¹³C and δ²H values and standard deviations are reported relative to CO₂ reference gas calibrated to Vienna Peedee Belemnite (VPDB) and H₂ reference gas calibrated to Vienna Standard Mean Ocean Water (VSMOW).

### 2.3.1. Precision and accuracy

In order to monitor the precision and accuracy of compound specific isotope analyses, a mixture of six reference compounds (n-C₁₁, n-C₁₃, n-C₁₄, n-C₁₇, n-C₁₈, n-C₁₉, n-C₂₅) whose isotopic compositions had been previously measured by elemental analyzer.
isotope ratio mass spectrometry (EA-irMS) was analyzed using the same $\delta^{13}$C or $\delta^2$H methods as sample analysis before, after, and sometimes during analytical sessions. Data from these analyses are available in the supplemental material (Figs. S1–S3). For all analyses of the standard mixture, the difference between returned and known values for each compound (accuracy) and the standard deviation (precision) for each compound were calculated. For 17 $\delta^{13}$C analyses of the reference mixture, the differences between returned and known values ranged between 0 and 0.6‰, and the average difference was –0.03‰. Standard deviations for the $\delta^{13}$C analyses of individual reference compounds ranged from 0.25‰ to 0.45‰ with an average of 0.35‰. For 38 $\delta^2$H analyses of the reference mixture, the difference between returned and known values ranged from 0 to 11‰, with an average of 0‰. Standard deviations for repeat analyses of individual compounds ranged from 2‰ to 8‰ with an average of 5‰.

Average peak height for all $\delta^2$H measurements of $n$-C$_{25}$–$n$-C$_{29}$ alkanes in this study was 1.8 nA (major area $\approx 1 \times 10^{-8}$), and the maximum peak height was 4.3 nA (major area $\approx 2.5 \times 10^{-8}$). In samples 3363, 3771, and 4137, no $n$-alkane $\delta^2$H measurement had a peak height above 0.2 nA (major area $\approx 1 \times 10^{-9}$), outside the linearity range of the instrument. Because we estimate that the reduction in accuracy of measurements with peak heights lower than 0.2 nA is sufficient to affect our interpretations, $\delta^2$H data from these samples are excluded from figures and discussion. Similarly, the relatively low concentration of hopanoids and of the resin derivative (in samples other than those in and slightly above the boundary interval) relative to $n$-alkanes led to $\delta^{13}$C analyses of these compounds with peak heights as low as 0.4 nA. In contrast to analyses of $\delta^2$H, however, the estimated reduction of accuracy in the $\delta^{13}$C
measurements is insufficient (<2‰, or ~3x the typical 2S uncertainty of 0.7‰) to affect our interpretations of the data.

2.4. Isolation of unknown resin (molecular weight 270)

A total of 280 g of ground rock from bed 5 was extracted in a large Soxhlet apparatus using a 9:1 mixture of DCM: methanol for ca. 48 hours. Activated copper was used in the round bottom flask of the Soxhlet apparatus in order to remove elemental sulfur. The extract was brought to incipient dryness by evaporating solvent under a stream of high purity nitrogen. The total lipid extract was separated into saturate, aromatic and polar fractions by adding to the top of a column (20 cm x 0.9 cm i.d.) of activated silica gel (120°C, 8 hrs) and eluting with hexane, 3:7 (v/v) DCM:hexane and 1:1 (v/v) DCM:methanol, respectively. The saturate fraction (18 mg) was subjected to 5A molecular sieving to separate straight chain and branched/cyclic fractions (Grice et al., 2008). Preparative GC was performed on the branched/cyclic fraction using a HP 5890 GC fitted with a flame ionization detector (FID). An aliquot of the branched/cyclic fraction was analyzed by GC-FID in order to establish the retention time of the resin derivative of interest. One third of the fraction was separated by preparative GC 125 times over a three-month period. The sample (ca. 2 µL of an original aliquot of 5 mg of the branched/cyclic fraction dissolved in 1.5 ml of cyclohexane) was subjected to preparative GC column (60 m x 0.32 mm i.d.) using a DB-5MS GC column with helium as the carrier gas. The FID was removed from the GC just prior to the retention time at which the resin eluted. The GC oven was programmed from 40°C to 300°C at a rate of
3°C/min and the sample was collected manually by using a Pasteur pipette inserted into the FID port. The sample was vaporized into the Pasteur pipette, and the compound was collected with a hexane rinse.

2.5. $^1$HNMR and heteronuclear single quantum correlation spectroscopy (HSQC)

Approximately 0.5 mg of the resin derivative was obtained for $^1$HNMR analyses. The minimum volume (~0.4 ml) of CDCl$_3$ was first placed in an NMR tube and the $^1$H spectrum was recorded on a Bruker Avance 600 MHz NMR Spectrometer. The CDCl$_3$ was transferred into the sample container to dissolve the sample and then pipetted back into the same NMR tube using the same Pasteur pipette. The $^1$H signals due to the isopropyl protons (16-Me, 17-Me and H15) match those reported in the literature (Hanari et al., 2002) similar to the isopropyl identified in dehydroabietane (Fig. 6) showing a doublet at 1.23 ppm relative to CDCl$_3$ at 7.62 ppm and a septet at 2.84 ppm relative to CDCl$_3$ at 7.62 ppm. The sample was too small to obtain a normal $^{13}$C spectrum. However, it is sometimes possible to determine $^{13}$C chemical shifts of carbons with attached protons using an HSQC experiment, a proton-detected 2D method that correlates $^1$H signals with those of their directly attached carbon. The HSQC experiment, recorded over a 72 h period, showed extremely weak signals, and only the signals due to the isopropyl methyl groups were identified. These $^{13}$C signals due to the isopropyl methyls (C16, C17) are consistent with values reported in the literature (Nishida et al., 1977; Delcorral et al., 1994).
2.6. Geochemical indices and statistics

Two maturity indices were used in this study. The methylphenanthrene index (MPI-1) estimates the degree to which less stable methylphenanthrene isomers (1-MP and 9-MP) and phenanthrene transformed into methylphenanthrene isomers of higher stability (2-MP and 3-MP) as a result of thermal alteration, and the index is calculated as follows:

\[
MPI-1 = 1.5 \times \frac{(2\text{-MP} + 3\text{-MP})}{(P + 1\text{-MP} + 9\text{-MP})}
\]  

with lower values indicating lower maturity (Radke et al., 1982; Radke and Welte, 1983).

The odd-to-even predominance (OEP-2) estimates thermal maturity based on the relative abundance of odd versus even carbon-numbered \( n \)-alkanes, and is calculated as follows:

\[
OEP-2 = \frac{(C_{25} + 6C_{27} + C_{29})}{(4C_{26} + 4C_{28})}
\]

with values significantly above 1 indicating low maturity (Peters et al., 2005).

Average Chain Length (ACL) and Carbon Preference Index (CPI) were also calculated, using the following formulae:

\[
ACL = \frac{(25C_{25} + 27C_{27} + 29C_{29} + 31C_{31} + 33C_{33})}{(C_{25} + C_{27} + C_{29} + C_{31} + C_{33})}
\]  

and
CPI = 0.5*[(C_{25} + C_{27} + C_{29} + C_{31} + C_{33})/(C_{24} + C_{26} + C_{28} + C_{30} + C_{32})] + 0.5*[(C_{25} + C_{27} + C_{29} + C_{31} + C_{33})/(C_{26} + C_{28} + C_{30} + C_{32} + C_{34})]

(Peters et al., 2005), using peak areas in GC-MS total ion chromatograms for individual \( n \)-alkane abundances.

Least squares linear regressions were used to assess relations between variables, with the coefficient of determination \((r^2)\) calculated using the difference between the total sum of squares (sum of squared differences between individual values and the average value of the dependent variable) and the residual sum of squares (sum of squared differences between the actual and predicted values of the dependent variable) divided by the total sum of squares. Estimated linear regression slopes were tested for significance at the 95% confidence level \((\alpha = 0.05)\) by calculating the probability \((p)\) of exceeding a critical F value by chance using the FDIST function in the StatPlus software package (AnalystSoft Inc.).

3. RESULTS

Total organic carbon (TOC) decreases from 1.9% at the bottom of the section to a minimum of 0.7% at 41.37 m, increases to 1.6% within the boundary interval, and increases again to maximum values between 2 and 4% near the top of the sampled interval. Fig. 2 shows GC-MS chromatograms of aliphatic and aromatic fractions.
separated from 13.58, 46.88, and 94.03 m, adjusted for concentration and varying TOC in order to show relative abundance of individual compounds. Aliphatic fractions of the twelve samples analyzed include a series of \( n \)-alkanes with odd-over-even predominance from 1.2 to 1.6, hopanes and steranes.

A monoaromatic diterpenoid compound (similar to dehydroabietane) elutes in both the aliphatic and aromatic fractions of most samples. Our quantitation method suggests that the compound elutes predominantly in the aliphatic fraction: only three samples had more than 1 µg/g TOC in their aromatic fractions, and the maximum concentration observed in an aromatic fraction was 5 µg/g TOC (as compared to between 100 and 200 µg/g TOC in the aliphatic fractions of the boundary crossing samples).

Unidentified compounds with similar mass spectra have been reported with abundant triterpenoids of higher plant origin in Miocene sediments from Baffin Bay off the west coast of Greenland (ten Haven et al., 1992) and in a sub-bituminous coal from the East Elbian coal basin (Hazai et al., 1986). Relative ion peak values for the most abundant monoaromatic diterpenoid observed in this study are 270 (100), 255 (70), 185 (20), 160 (10), 146 (45), 131 (35), whereas the values reported by Hazai et al. (1986) are 270 (100), 255 (70), 185 (30), 160 (45), 146 (70), 131 (40). By contrast, relative peak values for dehydroabietane are as follows: 270 (40), 255 (100), 185 (60), 173 (80), 159 (80), 143 (40), 129 (50): particularly notable are the abundance of the \( m/z \) 255 ion relative to the base peak and presence of large \( m/z \) 173 and 159 peaks in dehydroabietane (Righi et al., 2013). The mass spectrum of the unidentified monoaromatic diterpenoid is shown in Fig. 6, and \(^1\)H-NMR data support an isopropyl moiety as in dehydroabietane (Fig. 7).
Concentration of long chain \(n\)-alkanes varies considerably throughout the section, with the lowest values (<50 µg/g TOC) occurring in beds 2–4, intermediate values (100–200 µg/g TOC) in the boundary interval (with the exception of a low value in bed 5B), and highest values (>200 µg/g TOC) above bed 6. The resin derivatives are present in all samples, but in the boundary crossing interval (46.48 to 46.98 m) the concentration of these compounds increases by a factor of ~40. Pristane to phytane ratios (Pr/Ph) are >1 (avg. = 3) in all samples except 60.86 m, which has a ratio of 0.8. ACL decreases from ~28 at the bottom of the section to a minimum of ~27 at 41.37 m, increases to ~28 again within the TJB interval, and remains relatively constant throughout the rest of the sampled interval.

Aromatic fractions also show significant changes across the TJB at Astartekløft. Polycyclic aromatic hydrocarbons (PAHs) including retene, benzo\((g,h,i)\)perylene, and coronene are present only at low (1–5 µg/g TOC) concentrations in beds 1–3. Coronene and benzo\((g,h,i)\)perylene increase to 8 and 17 µg/g TOC, respectively, in bed 4, and retene increases to 33 µg/g TOC in bed 5. Retene decreases somewhat in the uppermost sample from bed 5, but remains relatively elevated (4–17 µg/g TOC) through the rest of the section. Benzo\((g,h,i)\)perylene and coronene return to lower concentrations above bed 5.

Carbon isotopic compositions of \(n\)-alkanes from \(n\)-C\(_{25}\) to \(n\)-C\(_{29}\) track the composition of fossil wood and leaves (that have slightly higher \(\delta^{13}C\)) in the lower part of the section (Fig. 1b). A 1.5‰ increase in \(\delta^{13}C\) between 37.71 and 41.37 m is followed by a ~4.5‰ negative excursion, with a TJB minimum value of –29.8‰ at 46.88 m. A slight increase in \(\delta^{13}C\) is followed by a further decrease to a low for the section of –30.5‰ at
60.86 m, nearly 4‰ lower than previously reported values for fossil wood and leaves from this bed. Values for \( n \)-alkanes increase to \(-27\%\) at 72.69 m, approaching values for fossil wood and leaves, and then decrease again at the top of the sampled interval, \(-3\%\) lower than fossil wood and leaves. The most abundant hopanoid (\( C_{30} \) 17\( \alpha \),21\( \beta \)) in the TJB sample at 46.48 m has an anomalously low \( \delta^{13}C \) of \(-55 \pm 1\%\), in contrast to other samples in the section within which \( \delta^{13}C \) of this compound varies between \(-30\) and \(-45\%\) (Fig. 1c).

An increase of \(-30\%\) in the weighted average \( \delta^{2}H \) of odd numbered \( n-C_{25-29} \) alkanes (\( \delta^{2}H_{n-C_{25-29\text{avg}}} \)) from the base of the section to the boundary interval coincides with the increase in CO\(_2\) and abrupt ecosystem collapse, and is followed by a decrease in \( \delta^{2}H \) of \( \sim30\%\) from the TJB to the top of the sampled interval (Fig. 1d). In samples for which it was possible to measure the quantity, the offset in \( \delta^{2}H \) between the \( n-C_{29} \) alkane and phytane varies between 75 and 130\%, and this offset is not correlated with \( \delta^{2}H_{n-C_{29}} \) (\( r^2 = 0.03 \)). It is therefore unlikely that post-depositional hydrogen exchange was a significant driver of stratigraphic trends in \( \delta^{2}H \) (see Discussion).

4. DISCUSSION

4.1. Trends in \( \delta^{13}C \) of \( n \)-alkanes

Plant lipids are typically \( ^{13}C \)-depleted by up to 10\% on average relative to bulk wood and leaves due to the kinetic effect of the decarboxylation of pyruvic acid (DeNiro and Epstein, 1977). This relative depletion is lower at Astartekløft, more similar in
magnitude to that observed in lacustrine sediments from the Newark and Hartford basins, USA (Whiteside et al., 2010) than marine sediments from Kuhjoch and Hochalpgraben, Austria (Ruhl et al., 2011), perhaps reflecting the relatively pristine nature of organic matter in the Austrian sections. Only odd-numbered \( n \)-alkanes (\( n-C_{25}, n-C_{27}, n-C_{29} \)) are plotted in Fig. 1 for clarity, and because these compounds derive from plant waxes, whereas even-numbered \( n \)-alkanes in this range likely represent remnants of partially cleaved molecules with higher original carbon numbers. It is possible that some component of odd-numbered \( n \)-alkanes also derives from cleavage. For this and other reasons, including variable production of different chain lengths by different plant groups and varying biosynthetic fractionation in alkanes of different chain lengths (Diefendorf et al., 2011), we hesitate to draw conclusions about trends in individual \( n \)-alkanes. In the Electronic Annex (EA), we report data from all \( n \)-alkanes present in sufficient concentrations to obtain isotope measurements in all samples analyzed (\( n-C_{25} \) to \( n-C_{29} \)), but we argue that these data are best considered in aggregate (e.g., using the concentration-weighted average \( n-C_{25-29 \text{avg}} \)).

Values of \( \delta^{13}C_{n-C_{25-29}} \) are not correlated with CPI (\( r^2 = 0.0004 \)), and an apparent negative relation with ACL is not significant at the 95% confidence level (\( r^2 = 0.29; p = 0.07; \) Fig. 3; EA). Changes in the distribution of long chain \( n \)-alkanes have been used as a proxy for paleoecological change, based on the reasoning that different plant groups synthesize different characteristic distributions (Cranwell, 1973). However, a recent and comprehensive compilation of published \( n \)-alkane distribution data showed large heterogeneities among individual plant groups and concluded that biosynthetic responses to local environmental change may be as important as ecological change in determining
the distribution of \( n \)-alkanes within a given ecosystem (Bush and McInerney, 2013).

Given the uncertainty about relations between \( n \)-alkane distributions and ecology in studies of modern ecosystems, we are unable to determine the extent to which changing \( n \)-alkane distributions at Astartekløft were driven by ecological or environmental effects.

The high relative abundance of herbaceous taxa (e.g., 78% relative abundance of ferns; McElwain et al., 2007; Mander et al., 2010) may contribute indirectly to the low \( \delta^{13}C \) value in bed 6. Leaves of herbaceous taxa close to the forest floor typically have low \( \delta^{13}C \) values relative to those of woody canopy taxa due to photosynthetic recycling of soil-respired CO\(_2\) (the “canopy effect”; Vandermerwe and Medina, 1991). Notably, this is the only sample that has a Pr/Ph < 1 (0.8), which may suggest relatively reducing conditions during deposition, consistent with the interpretation of bed 6 as a “coal swamp”.

Although significant divergence in \( \delta^{13}C \) is observed between \( n \)-alkanes and fossil wood and leaves from the TJB to the top of the sampled interval, a negative relation between the weighted average \( \delta^{13}C_{n-C25-29} \) and estimated atmospheric CO\(_2\) concentration with \(-0.0009 \leq \text{slope} \leq -0.005\) (95% CI; \( r^2 = 0.74, p = 0.01 \)) is observed (Fig. 1a,b). Peak CO\(_2\) (Steinthorsdottir et al., 2011) corresponds to the \( \delta^{13}C \) minimum in bed 6, a coincidence that could record stable photosynthetic fractionation under a relatively \( ^{13}C \)-depleted atmosphere after peak extinction or increased photosynthetic fractionation under high \( p\text{CO}_2 \). Notably, the prominent gymnosperm resin derivative exhibits a minimum \( \delta^{13}C \) value for the section (–28.1‰) in bed 6, 2.5‰ lower than the average value for this compound in other samples in this study.
Most marine Triassic–Jurassic boundary sections around the world record a relatively short decrease in $\delta^{13}C$ coinciding with extinction, and some record an increase of longer duration sometime during the Hettangian (Williford et al., 2007; Bartolini et al., 2012). Paired $\delta^{13}C_{\text{carb}}$ and $\delta^{13}C_{\text{org}}$ records show an increase in $\Delta^{13}C (\delta^{13}C_{\text{carb}} - \delta^{13}C_{\text{org}})$ in the recovery interval (Bachan et al., 2012). This is consistent with the view that the $^{13}C$-depleted values of individual compounds above the extinction interval at Astartekløft resulted from preferential preservation (in lipids relative to fossil wood or leaves) of increased photosynthetic fractionation under increased atmospheric $pCO_2$.

4.2. Trends in $\delta^{2}H$ of $n$-alkanes

During the interval with rising CO$_2$ levels prior to the TJB, $\delta^{2}H_{nC_{25-29}}$avg shifts positively from $-133\%$ to $-104\%$ (Fig. 1d). After peak plant extinctions, $\delta^{2}H$ of $n$-alkanes (1) decreases, but remains relatively elevated through bed 6, which records the highest CO$_2$ and lowest $\delta^{13}C$ values, (2) decreases during the CO$_2$ decrease and $\delta^{13}C$ increase between bed 6 and 7, and (3) decreases slightly to the lowest recorded values between beds 7 and 9 (Fig 1c). The various environmental and biological factors that control the $\delta^{2}H$ of lipids in phototrophic organisms were recently reviewed by Sachse et al. (2012). In the discussion below, we consider the relative contribution of the following factors towards the observed positive shift in $\delta^{2}H$ of $n$-alkanes that co-occurs with end-Triassic ecosystem collapse at Astartekløft: 1) equilibrium hydrogen exchange during thermal maturation of organic matter, 2) vegetation change, and 3) temperature. As is the case for all such sedimentary successions, isotopic trends observed at Astartekløft are
driven by a combination of local and global effects. This is, to our knowledge, the first TJB record of \(n\)-alkane \(\delta^2\)H – a small dataset from a single locality; similar evaluation of well-preserved organic matter from other TJB localities can constrain the degree to which the changes observed at Astartekløft are representative of global biogeochemical change.

4.2.1. Hydrogen exchange

Equilibrium isotopic exchange of carbon bound hydrogen with formation water over geologic timescales can obscure original isotopic compositions (Sessions et al., 2004), especially at high maturity levels (Dawson et al., 2005). Two parameters were used to evaluate the relative thermal maturity of the organic matter in the Astartekløft sediments: the methylphenanthrene index (MPI-1; Radke et al., 1982; Radke and Welte, 1983) and odd-to-even predominance (OEP-2; Peters et al., 2005). Previous studies comparing MPI-1 to \(\delta^D\) of \(n\)-alkanes and isoprenoids show evidence for some exchange in isoprenoids, but little to no apparent exchange in alkanes extracted from rocks having MPI-1 < 0.6 (Dawson et al., 2005; Radke et al., 2005). The samples in this study are of uniformly low maturity (MPI-1=0.38–0.60; OEP-2=1.24–1.64), with a range of calculated vitrinite reflectance values (\(R_c\); Radke et al., 1982) between 0.60 and 0.73. We conclude that that Astartekløft organic matter was not subject to significant exchange due to maturity (Fig. 4a), an interpretation further supported by the preservation of offsets between the \(n\)-C\(_{20}\) alkane and phytane (Wang et al., 2009) ranging between 76 and 129‰. This offset is not correlated with \(\delta^2\)H, and it is therefore unlikely that post-
depositional hydrogen exchange was a significant driver of stratigraphic trends discussed here.

4.2.2. Vegetation change

The TJB at Astartekløft is defined by the large vegetation changes that occur across the boundary interval (Harris, 1926, 1937; McElwain et al., 1999; McElwain et al., 2007; Mander et al., 2010, 2013). Studies of modern ecosystems have demonstrated that δ²H of n-alkanes can vary considerably within and among different plant groups and emphasized the importance of accounting for vegetation change when using δ²H as a paleoproxy (Chikaraishi and Naraoka, 2003; Sachse et al., 2004, 2006; Hou et al., 2007; Sachse et al., 2012). To what degree did these vegetation changes affect the δ²H of sedimentary n-alkanes at Astartekløft?

A negative relation is observed between δ²Hn-C25–29 and ACL with a slope between –40 and –14 (95% CI; r² = 0.71; p = 0.001). As previously mentioned, relations between n-alkane distributions and plant species distributions are unclear, as ACL varies widely within individual plant groups (Bush and McInerney, 2013). An increase in ACL from <29 to >30 coincides with the PETM negative carbon isotope excursion in the Bighorn basin and is interpreted to record an increase in long chain n-alkane production in order to mitigate water loss in plants subjected to rising temperatures (Smith et al., 2007). Whereas we do observe an overall increase in ACL from the base to the top of the Astartekløft section, a decrease in ACL coincides with the onset of ecosystem collapse and isotopic change (Fig. 3). This likely reflects a combination of vegetation change as
well as changes in $n$-alkane production by plants in response to changing environmental conditions.

To test the dependence of $\delta^2$H on vegetation assemblages, regression analysis was performed using the most recent, high-resolution sporomorph record from Astartekløft (Mander et al., 2010). Four distinct vegetation zones were recognized on the basis of sporomorph distribution in samples from plant Beds 1–7: Zone A1 (Beds 1, 1.5, and 2), in which the liverwort *Riccisporites tuberculatus* constitutes 40–50% of the assemblages, Zone A2 (Beds 3 and 4), the base of which is marked by the local acme (up to 51% relative abundance) of the lycopod *Limbosporites lundbladii*, Zone A3 (Bed 5), in which the lycopod *Uvaesporites reisseringerii* and the fern *Baculatisporites comaumensis* co-dominate, and the conifer *Perinopollenites elatoides* reaches a local acme, and Zone A4 (Beds 6 and 7), the base of which is marked by local acmes of the ferns *Deltoidospora* *toralis* (55%) and *Punctatisporites globosus* (25%) (Mander et al., 2013). Fig. 5 shows relations (including least squares lines and $r^2$ values) between $\delta^2$H$_{nC25-29avg}$ and relative abundance of eight sporomorph groups in Beds 1–7. None of the sporomorph groups shows a correlation with $\delta^2$H that that is significant at the 95% level. Mosses show a moderate, negative relation with $\delta^2$H$_{nC25-29avg}$ ($r^2 = 0.62; p = 0.06$). However, the few data that exist comparing mosses to other plant groups in the same environments suggest that mosses have higher $\delta^2$H than trees, attributed in part to increased evaporative leaf water enrichment due to the lack of stomata in mosses (Sachse et al., 2006). We find no compelling evidence that vegetation change exerted a significant control on the $\delta^2$H of sedimentary $n$-alkanes at Astartekløft.
4.2.3. Temperature

The traditional interpretation of changes in δ²H values of sedimentary long chain alkanes with odd carbon numbers is that they reflect changes in δ²H of meteoric water, dominantly driven at low latitudes by the amount of precipitation and at high latitudes by temperature (Dansgaard, 1964; Sachse et al., 2012). Assuming a net fractionation for n-C₂⁹ (ΔnC₂⁹ = δ²H_{H₂O} – δ²H_{nC₂⁹}) of 100 to 130‰ (Sachse et al., 2004), δ²H of Astartekløft n-alkanes indicates a meteoric δ²H shift from values measured in modern temperate environments (~50 to ~20‰) to values measured in low latitude regions with high rates of evaporation such as the Red Sea (0 to 20‰; Bowen and Revenaugh, 2003). However, paleobotanical (McElwain et al., 2007) and paleolimnological (Clemmensen et al., 1998) records from East Greenland suggest increasing humidity during the Triassic–Jurassic transition, indicating that a mechanism other than local aridity was responsible for any change in δ²H of meteoric water recorded by n-alkanes.

During the Late Triassic, Astartekløft was likely at temperate latitudes, drifting north (Kent and Tauxe, 2005). All else being equal, greenhouse warming at the TJB would have led to an increase in δ²H of meteoric source water at Astartekløft in two ways: 1) warmer air has a higher capacity to hold moisture, so less ²H-enriched condensate would have been lost during transport from evaporative source regions, and 2) equilibrium fractionation between vapor and condensate decreases with increasing temperature (Dansgaard, 1964; Sachse et al., 2012). A similar scenario, evidenced by a large negative excursion in δ¹³C and a large positive excursion in δ²H, is recorded in Arctic sediments crossing the PETM (Pagani et al., 2006), although records of δ¹⁸O from
soil carbonates suggest that $^2$H-enrichments would have been slightly lower at mid-latitudes (Fricke et al., 1998).

4.3. Fire activity

High concentrations of combustion-ascribed polycyclic aromatic hydrocarbons (PAHs) in the Early Jurassic of Poland have been interpreted as evidence for increased wildfire activity (Marynowski and Simoneit, 2009), and a fivefold increase in charcoal across the TJB at Astartekløft (Fig. 4c), indicates that fire activity increased with atmospheric CO$_2$ (Belcher et al., 2010) at this locality. We find increased concentrations of combustion-ascribed PAHs coronene and retene (Ramdahl, 1983; Freeman and Cattell, 1990) in the same stratigraphic interval (Fig 3d). Retene is also known to be a degradation product of dehydroabietic acid in sediments (Tavendale et al., 1997), and the similarity observed in the relative abundance of retene and resin byproducts related to dehydroabietane (Fig. 4d–e and discussion below) could be related to early diagenetic transformations. High PAH concentrations in TJB sediments from the Danish-German basin have been attributed directly to CAMP volcanism on the basis of high coronene to benzo$(a)$pyrene ratios (up to 3.46 with an average of 1.11; (van de Schootbrugge et al., 2009). By contrast, the average coronene to benzo$(a)$pyrene ratio for this study is 0.33.

4.4 Resin derivatives as biomarkers of terrestrial ecosystem stress
The rise in CO₂, δ²H, charcoal, and PAHs at the TJB is accompanied by increasing concentration of a monoaromatic diterpenoid likely derived from gymnosperm resins (Fig. 4e, 6). Oleoresins are an important part of the defense system of conifers exposed to herbivory, infection, and wounding stress (Phillips and Croteau, 1999), and significant increases in oleoresin production result from burning (Cannac et al., 2009; Perrakis et al., 2011). We attribute the dramatic increase in gymnosperm-resin derivatives at the TJB to environmental stress including fire, and more generally, we suggest that these compounds are potential biomarkers for terrestrial ecosystem stress. For example, correlations between resin compounds and fungal biomarkers or abundance of herbivorous insects could also record responses to disease and predation stress.

5. CONCLUSIONS

The results presented here are consistent with the view that greenhouse warming due to CAMP eruptions led to severe terrestrial ecosystem stress. Carbon isotopic compositions of individual n-alkanes record a >4‰ negative excursion coinciding with the interval of greatest plant turnover. A further decrease in δ¹³C above the TJB coincides with peak atmospheric CO₂ concentration as calculated from stomatal indices, but may have been amplified by a shift in depositional environment from overbank to coal swamp deposits. A ~30‰ positive shift in δ²H of long chain n-alkanes coinciding with CO₂ rise, global warming, and plant diversity loss is consistent with increased δ²H of local meteoric source water (due to a temperature-driven decrease in rainout of ²H-enriched condensate during vapor transport from evaporative source regions). Increases in the
relative abundance of combustion-derived PAHs and gymnosperm resin derivatives in the TJB interval record a terrestrial ecosystem under extreme stress by heat and fire.

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Table and FigureCaptions

Table 1. Molecular and average isotopic data for Triassic–Jurassic boundary samples from Astartekløft, East Greenland.

Fig. 1. Lithostratigraphy, plant beds, (a) estimated CO₂ concentration (Steinthorsdottir et al., 2011), (b) δ¹³C of C₂₅–C₂₉ n-alkanes, fossil leaf cuticle *(McElwain et al., 1999; Bacon et al., 2011) and fossil wood **(Hesselbo et al., 2002), (c) δ¹³C of C₂₅ hopane, and (d) δ²H of C₂₅–C₂₉ n-alkanes with height at Astartekløft. Gray field in (a) represents full range of stomatal index data in Steinthorsdottir et al. (2011), black line represents average. Gray field in (b) represents the range of leaf cuticle data reported by Bacon et al. (2011). Dotted black line in (b) and (d) represent weighted averages of odd numbered n-C₂₅-2₉ alkanes, and insets show expanded boundary interval (Bed 5). Average analytical uncertainty for individual n-alkane values, (calculated as the average variability at one standard deviation of replicate analyses of individual compounds) is 0.3‰ for δ¹³C and 7‰ for δ²H (see Tables S1 and S2 for individual values). Horizontal, solid gray line indicates the level of 80% plant species turnover, and dotted gray line indicates onset of plant diversity loss (McElwain et al., 1999; McElwain et al., 2007; McElwain et al., 2009).

Fig. 2. GC-MS Total ion chromatograms from aliphatic and aromatic fractions from 13.58, 46.88, and 94.03 m. Chromatogram heights are adjusted for concentration using internal standards (squalane and terphenyl-d14 for aliphatic and aromatic fractions, respectively) and then for variable TOC to show differences in relative abundance of individual compounds (e.g., note anomalously high concentration of resin derivative in the aliphatic fraction from 46.88 m). Compounds discussed in the text are indicated as follows: pristane (Pr), resin derivative (m/z 270), n-alkanes (C₂₅, C₂₇, C₂₉, C₃₁), squalane (Sq), C₃₀ 17α, 21β hopane (C₃₀Hop), naphthalene (N), methyl(naphthalenes (MN), dimethyl(naphthalenes (DMN), phenanthrene (P), methylphenanthrenes (MP), dimethylphenanthrenes (DMP), terphenyl-d14 (t-d14), retene (Ret), benzo(g,h,i)perylene (BePery), coronene (Cor).

Fig. 3. Average chain length (ACL) and carbon preference index (CPI) values with height at Astartekløft.

Fig. 4. Plant beds, (a) the maturity indicators methylphenanthrene index (MPI-1) and odd-over-even preference (OEP-2), (b) sum of long chain (C₂₅–C₃₃) n-alkanes, (c) charcoal content (Belcher et al., 2010), (d) PAH concentration and (e) semi-quantitative concentration (in saturate fraction) of resin derivative with m/z 270 base peak with height at Astartekløft. Horizontal, solid gray line indicates the level of 80% plant species turnover, and dotted gray line indicates onset of plant diversity loss (McElwain et al., 1999; McElwain et al., 2007; McElwain et al., 2009).

Fig. 5. Linear regressions, including least squares fit lines and r² values, between relative abundances of eight sporomorph groups counted in a high resolution record from Beds 1–
7 (Mander et al., 2010) and $\delta^2\text{H}_{n\text{-C}25-29\text{avg}}$ at Astartekløft. None of the apparent correlations is significant at the 95% level.

**Fig. 6.** Mass spectrum of an unidentified monoaromatic tricyclic terpenoid compound (containing iso-propyl group cf. dehydroabietane type structure), likely derived from gymnosperm resins.

**Fig. 7.** Structure of dehydroabietane, with carbon atoms numbered as discussed in text.