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# A comparison of terpenoid and leaf fossil vegetation proxies in Paleocene and Eocene Bighorn Basin sediments



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## ABSTRACT

Plant-derived terpenoids, long recognized as biomarkers, can help reveal the major taxonomic groups of land plants present in ancient environments, even if rocks and sediments do not preserve plant macro- or microfossils. Previous studies have used simple di- to triterpenoid ratios to reconstruct floral changes in the geologic past, but few have compared terpenoid ratios with estimates of floral composition from fossils. Further, reconstructions have not taken into account differences in biomarker production (i.e. concentration relative to leaf biomass) between different types of plants. Here, we have examined terpenoids from early Cenozoic fluvial rocks from the Bighorn Basin (Wyoming, USA), where fossil flora has been studied in detail. We analyzed the distributions of diterpenoids, triterpenoids and *n*-alkanes from leaf wax in a total of 43 samples from 15 stratigraphic horizons of late Paleocene (63 Ma) to early Eocene (53 Ma) in age. In nearly all samples, triterpenoids, derived from angiosperms, were significantly lower in abundance than conifer-specific diterpenoids, a finding that contrasted with plant fossil evidence for the same rocks. This suggests that di- to triterpenoid ratios severely underestimate the abundance of angiosperms in paleovegetation. Angiosperms dominated *n*-alkane production among modern plants, and we propose a new paleovegetation proxy based on the ratio of diterpenoids (conifers) to *n*-alkanes (angiosperms), corrected for lipid production estimated from extant vegetation. Using diterpenoids and alkanes, we infer the composition of paleovegetation to be similar to that inferred from plant fossils. Although the approach works well for the Bighorn Basin, we stress the new paleovegetation proxy will need to be evaluated for other time periods, communities, paleogeography and depositional environments with pollen or megafossil data available.

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## 1. Introduction

Characterizing paleovegetation patterns in response to climate shifts is challenging because of the limited or discontinuous preservation of plant fossils such as leaves and pollen in many ancient environments. Plant terpenoids (defense compounds synthesized from the 5 carbon building block, isoprene) are well known biomarkers (Fig. 1) and high level chemotaxonomic indicators. For example, non-steroid pentacyclic triterpenoids are almost exclusively synthesized by angiosperms, whereas tricyclic diterpenoids are characteristic of gymnosperms, specifically conifers (Erdtman, 1963; ten Haven and Rullkötter, 1988; Sukh Dev, 1989; Otto et al., 1997; Otto and Simoneit, 2001; Otto and Wilde, 2001; Hauteville et al., 2006; Keeling and Bohlmann, 2006; Cox et al.,

2007; Diefendorf et al., 2012). Studies have used simple di- to triterpenoid ratios to reconstruct vegetation distribution, but few compared terpenoid abundance ratios to the relative abundance of angiosperms and conifers seen in pollen or megafossils from the same deposits (Bechtel et al., 2003; Nakamura et al., 2010). These earlier studies also did not account for biomarker production, which differs between different plant functional types (angiosperm vs. conifer; deciduous vs. evergreen), as such data have only recently become available (Diefendorf et al., 2012). As a result, there is significant uncertainty as to the value of terpenoid ratios as paleovegetation proxies in ancient sediments where biomarkers have been significantly altered or lost during early diagenesis (e.g. Wakeham et al., 1980).

To investigate the use of terpenoids as proxies for the relative abundance of angiosperms and gymnosperms in paleovegetation, we examined sediments from the Bighorn Basin (Wyoming, USA), where plant fossils provide an independent means of estimating

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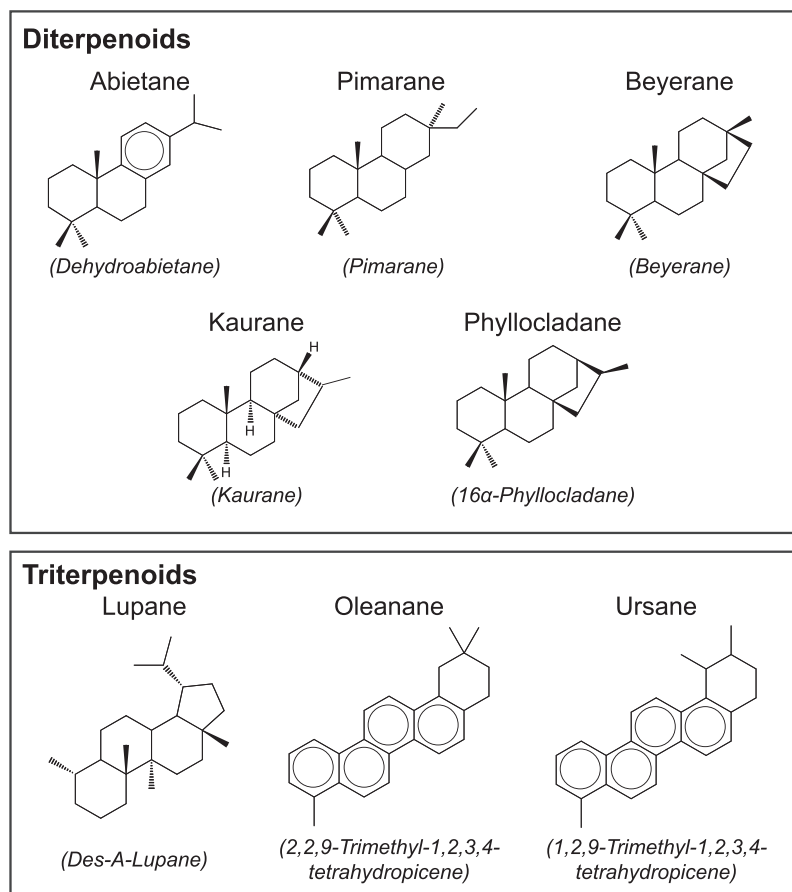


Fig. 1. Di- and triterpenoid classes and commonly found compounds (in italics) from each class found in Bighorn Basin sediments.

the relative abundance of the two groups. Diterpenoids, triterpenoids and leaf wax *n*-alkanes in 43 samples from 15 stratigraphic horizons from the late Paleocene (63 Ma) to early Eocene (53 Ma) were quantified. For the majority of sampling sites, leaf fossil data indicated a mixture of angiosperms and gymnosperms dominated by angiosperms (typically 85–100%).

Simple di- and triterpenoid abundance ratio estimates of paleovegetation and estimates that took into account production differences between plant taxonomic groups and leaf lifespans were not consistent with floral composition predicted from fossils. Angiosperm triterpenoids had lower than expected abundance relative to diterpenoids given the dominance of angiosperms in the leaf flora, and also had low abundance compared with leaf wax *n*-alkanes, which are most common in angiosperms (Diefendorf et al., 2011). The data reveal a preferential loss of triterpenoids and suggest separate controls on di- and triterpenoid preservation. In contrast, after accounting for biomarker production differences, diterpenoid to *n*-alkane ratios gave angiosperm/gymnosperm estimates that agreed with fossil data. Although the diterpenoid and *n*-alkane-based paleovegetation proxy approach works for sites in the Bighorn Basin, we stress it needs to be evaluated for other time periods, paleogeographic and depositional settings, and major plant groups.

## 2. Material and methods

### 2.1. Geological and sedimentological setting

Samples were collected from outcrops of the Paleocene and lower Eocene Fort Union and Willwood formations in the Bighorn Basin, Wyoming, USA (Fig. 2). The basin is a Laramide structural

depression surrounded by mountains uplifted during the Paleocene and early Eocene (Bown, 1980). The Fort Union and Willwood formations are alluvial deposits with a total thickness of ca. 2 km, though thickness and lithology vary markedly across the basin (Gingerich, 1983; Wing and Bown, 1985; Kraus, 1992). The fluvial systems that deposited these two formations are reconstructed as having been anastomosed channels of moderate size, with dynamics controlled primarily by avulsion, i.e. the sudden switching of major channels through the process of crevasse-splay formation and growth (Bown and Kraus, 1987; Kraus, 1996; Kraus and Aslan, 1999). They created a mosaic of environments on the subsiding basin bottom, including active and abandoned channels, alluvial ridge and crevasse-splay deposits, and broad muddy floodplains that varied from well drained to poorly drained (Bown and Kraus, 1981, 1987; Wing, 1984; Kraus, 1996, 1998; Davies-Vollum and Kraus, 2001). Common lithologies include fluvial sandstones, mudstones, minor lignites and carbonaceous shales, and rare freshwater carbonates (Gingerich, 1983). Fort Union sediments are primarily gray-brown with interspersed lignitic and carbonaceous [i.e. organic carbon (OC)-rich] shales (Bown, 1980). The Willwood Formation is dominated by oxidized mudstone paleosols that are variegated red, purple and yellow, but there are also channel sandstones, laterally extensive carbonaceous shales and abandoned channel deposits containing plant fossils and dispersed organic material (Bown, 1980; Kraus and Riggins, 2007).

Samples were collected from eight stratigraphic levels spanning  $10 \times 10^6$  yr of the Paleocene and early Eocene, with the oldest level being ca. 63 Ma and the youngest ca. 53 Ma (Table 1). The age of each bed was calculated by linear interpolation between levels of known age and stratigraphic level within each section (Wing

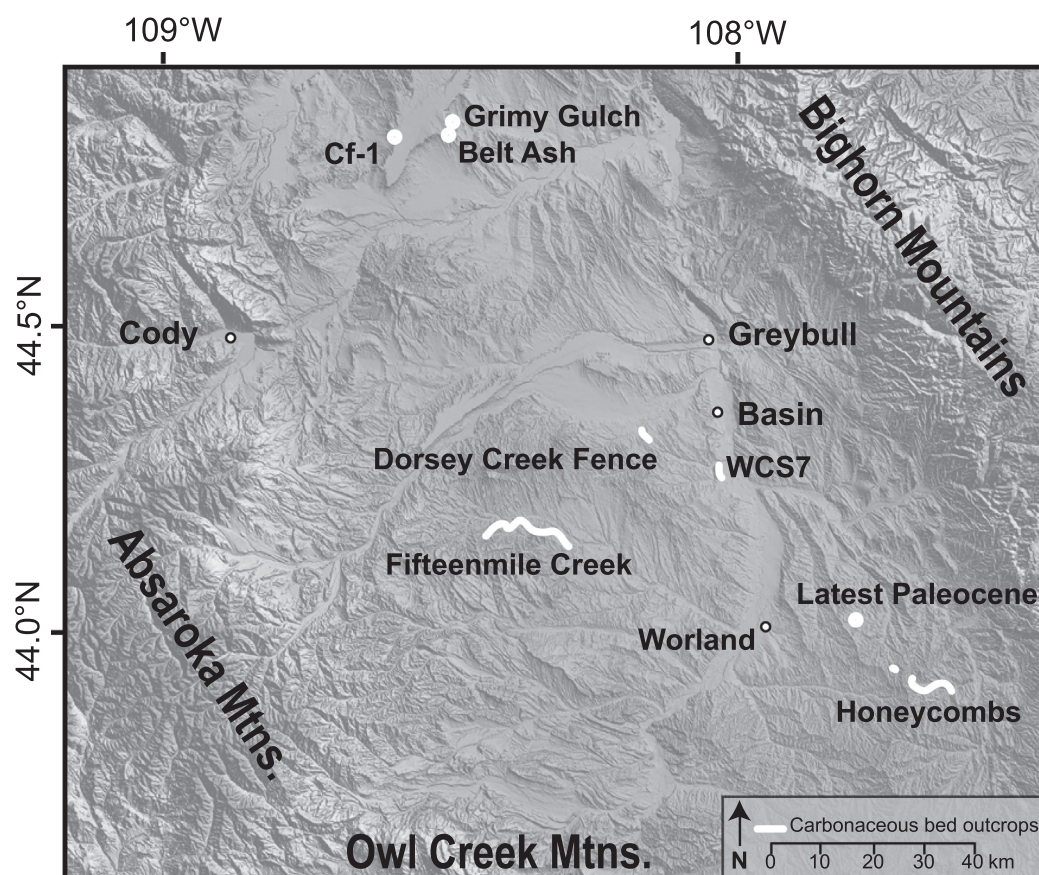


Fig. 2. Schematic of Bighorn Basin sampling locations. White dots and lines indicate sampling locations. Major towns are indicated with black outlined circles.

**Table 1**  
Site, stratigraphic position, age and paleovegetation.<sup>a</sup>

Bed name	Level (m) (section)	Age	Formation	Age (Ma)	Conifer paleovegetation (%) <sup>b</sup>
Fifteenmile Creek	700 m (ECS)	Eocene	Willwood	52.98	3–9 <sup>c</sup>
Dorsey Creek Fence	353 m (ECS)	Eocene	Willwood	54.37	≤5
WCS7	112 m (ECS)	Eocene	Willwood	55.34	≤5
Latest Paleocene	7.5 m below CIE	Paleocene	Fort Union	56.04	≤5
Honeycombs	13 m below CIE	Paleocene	Fort Union	56.1	≤14
Cf-1	429 m (WPB)	Paleocene	Fort Union	57.39	≤5
Belt Ash	351 m (SPB)	Paleocene	Fort Union	59.39	≤5
Grimy Gulch	56 m (SPB)	Paleocene	Fort Union	63	≤5

<sup>a</sup> ECS, Elk Creek Section (Schankler, 1980); CIE, Paleocene–Eocene Thermal Maximum Carbon Isotope Excursion (see text); WPB, West Polecat Bench; SPB, Southeast Polecat Bench.

<sup>b</sup> Paleovegetation estimated from previous floral studies in the Bighorn Basin (see text).

<sup>c</sup> Fossil plant leaf abundance from Davies-Vollum and Wing (1998) converted to relative biomass following Smith et al. (2007).

et al., 2000; Wing and Currano, 2013). Each sampled stratigraphic level is a laterally continuous (ca. 0.5–18 km) bed rich in OC, referred to as a Type I carbonaceous unit (Wing, 1984). Type I carbonaceous beds represent deposition on wet distal floodplains where organic matter (OM) was preserved because of a high water table, reducing conditions in the sediment and frequent depositional events (Wing, 1984; Davies-Vollum and Wing, 1998; Kraus, 1998; Davies-Vollum and Kraus, 2001). Lithological features and degree of preservation of plant remains characterize several sub-environments within each laterally extensive carbonaceous bed, generally ranging from wetter, more reduced and OC-rich environments distant from the channel to slightly better drained and environments more proximal to the channel (Wing, 1984; Davies-Vollum and Wing, 1998). Individual carbonaceous beds

are thought to represent deposition over a period of centuries to a few millennia (Davies-Vollum and Wing, 1998; Davies-Vollum and Kraus, 2001).

Within each carbonaceous bed, samples were collected from five sites spaced at intervals of hundreds of m along the outcrop of each bed and at each site one to three different lithologies were collected from unweathered rock dug from a short vertical section through the bed (Table 1; Supplementary material, Table E-1). For each sample we measured the proportion (wt.%) of total OC (TOC;  $n = 75$ ). A subset of samples was selected for biomarker analysis ( $n = 43$ ).

The oldest bed was the Grimy Gulch lignite. The flora at this location was studied by Hickey (1980; LJH loc. 7861). Three lithologies were sampled from the same laterally extensive



carbonaceous bed: carbonaceous mudstone, lignite and carbonaceous shale. The bed is at the 56 m level in the Southeast Polecat Bench section, placing it in Chron C27r, 14 m below the well known Torrejonian fauna from Rock Bench Quarry (Secord et al., 2006). The precise age of the Grimy Gulch lignite is difficult to determine because of a major unconformity at ca. 30 and 90 m in the southeast Polecat Bench section (Secord et al., 2006). We used an age of ca. 63 Ma based on its position in C27r and below Torrejonian mammals (Gradstein et al., 2012). The Grimy Gulch fossil flora includes the gymnosperms *Taxodium olrikii* (Cupressaceae), *Fokieniopsis catenulata* (Cupressaceae) and *Podocarpus* sp. (Podocarpaceae; incredibly rare at this site). Angiosperm species include (Hickey, 1980) “*Ficus*” *planicostata* (probably Lauraceae) and *Wardiaphyllum daturaefolium*, *Debeya* sp., *Paleoaster inquierenda*, “*Vitis*” *stantonii* and “*Rhamnus*” *cleburnii* (family affinities uncertain).

The second oldest level was a ca. 1.5 m thick laterally extensive carbonaceous mudstone and lignitic shale at ca. 331 m in the Southeast Polecat Bench section. The bed is in the lower part of fossil vertebrate locality SC-261, which produces a Tiffanian (Ti4a) fauna (Secord et al., 2006). This carbonaceous bed is laterally equivalent to the carbonaceous bed containing the Belt Ash, which has been radiometrically dated at 59.39 Ma, making it slightly older than the nearby fossil flora at Skeleton Coast, which we interpolate as having an age of 59.3 Ma (351-m level; 59.0 Ma; Wilf et al., 2006). Fossil plants from Skeleton Coast include *Cercidiphyllum genatrix* (Cercidiphyllaceae), *Browniea serrata* (Cornales), *Platanus raynoldsii* (Platanaceae), *Davidia antiqua* (Cornales) and Juglandaceae spp. No conifer fossils were observed at Skeleton Coast (Wilf et al., 2006; Currano, 2008). Mean annual temperature estimates for the late Tiffanian are  $10.5 \pm 2.9$  °C (Currano et al., 2008).

The third sampling level was at the 429 m level of the West Polecat Bench section, in a widespread carbonaceous bed not far above the lowest Clarkforkian (Cf-1) mammalian fauna (Secord et al., 2006). The estimated age of this bed is ca. 57.39 Ma. Three megafossil collections (USNM41836–41838) preserved in slightly coarser near-channel sediments just above this carbonaceous shale contain a flora of mixed broadleaved angiosperms and deciduous conifers, with the angiosperms including “*Ampelopsis*” *acerifolia* (probably Cercidiphyllaceae), *Averrhoites affinis*, *Beringiophyllum cupanioides* (Cornales), *B. serrata* and *Macginitiea gracilis* (Platanaceae), the conifers being *Glyptostrobus europaeus* and *Metasequoia occidentalis* (both Cupressaceae). Ordinarily, conifers are more abundant in finer grained carbonaceous shales than in the overlying coarser sediments (Wing, 1984), but here the carbonaceous shale does not contain identifiable fossils.

The fourth sampling level is a widespread, fine grained carbonaceous bed in the uppermost Fort Union Formation of the southern Bighorn Basin. This bed, informally referred to as Carbshale 1, or the Honeycombs Carbshale (Davies-Vollum and Wing, 1998), is in faunal zone Cf-3, ca. 13 m below the base of the Willwood Formation, which coincides with the start of the carbon isotope excursion (CIE) and the onset of the Paleocene–Eocene thermal maximum (PETM; Wing et al., 2005; Smith et al., 2007). We interpolate the age of this level to be ca. 56.1 Ma. Paleotemperature estimates for Cf-3 are  $15.7 \pm 2.4$  °C (Wing et al., 2000). The flora of this widespread floodplain swamp deposit includes the sphenopsid *Equisetum* sp. (Equisetaceae), angiosperms such as “*Ampelopsis*” *acerifolia*, *Corylites* sp. (Betulaceae), *Fagopsiphyllum groenlandicum* (Fagaceae), “*Ficus*” *planicostata*, Hamamelidaceae sp. 1, *Phoebe* sp. (Lauraceae), *P. raynoldsii* (Platanaceae), *Zingiberopsis isonervosa* (Zingiberaceae) and the conifer *M. occidentalis*.

The fifth sampling level is a carbonaceous bed in the uppermost Fort Union Fm., 7.5 m below the PETM CIE. It has an interpolated age of 56.04 Ma. This carbonaceous layer is composed of a number of lenticular deposits of siltstone and very fine sandstone, with

small scale, cross stratification resulting from current flow, and probably represents deposition on a wet floodplain proximal to a fluvial channel or crevasse splay system. The fossil flora of the bed, collected from several sites, includes angiosperms such as *Corylites* sp., *Cranea wyomingensis* (Betulaceae), *C. genatrix* (Cercidiphyllaceae), *F. groenlandicum* (Fagaceae), “*Ficus*” *planicostata*, *M. gracilis*, *P. raynoldsii*, the conifer *M. occidentalis* and *Ginkgo adiantoides*.

The sixth sampling level is a mostly fine-grained carbonaceous bed in the Willwood Fm. that outcrops for several km along the South Fork of Elk Creek in the east-central part of the basin (Wing et al., 1995; Davies-Vollum and Wing, 1998; Clyde et al., 2007; Currano, 2009). It is at the 112 m level of the Elk Creek section, within faunal zone Wa-2, and has an estimated age of 55.34 Ma. The composition of the flora varies laterally from place to place along the bed, but overall is dominated by the angiosperms *Alnus* sp. (Betulaceae) and *A. affinis* (likely Sapindales). Also abundant are *G. europaeus* and *Equisetum* sp. (Equisetaceae) (Wing, 1980; Wing et al., 1995). Floral composition and sedimentary features suggest the floodplain was submerged much of the time during the deposition of this bed (Wing, 1984). The mean annual temperature estimated from the Elk Creek flora using leaf margin analysis is  $16.4 \pm 2.7$  °C (Wing et al., 2000).

The seventh sampling level is a laterally extensive carbonaceous bed south of Dorsey Creek and the Greybull River in the central Bighorn Basin. It is located at the 353 m level of the Elk Creek section, within Wa-4 (Wing, 1980; Clyde et al., 2007). The estimated age for the site is 54.37 Ma, based on interpolating between paleomagnetic reversals identified by Clyde et al. (2007). Fossil plants at this level include the angiosperms *Alnus* sp., “*Ampelopsis*” *acerifolia*, *C. genatrix*, Hamamelidaceae sp., Lauraceae sp., Juglandaceae sp. and *Populus cinnamomoides* (Salicaceae). The conifers *G. europaeus* and *M. occidentalis* are moderately abundant, as are *Equisetum magnum* and the aquatic fern *Salvinia preauriculata* (Wing, 1980; Wing et al., 1995; Currano, 2008). Fossil plants document the coolest climates of the early Eocene (MAT  $10.8 \pm 3.3$  °C) in the basin (Wing et al., 1991, 2000).

The youngest sampling level is a laterally extensive carbonaceous bed informally called the Fifteenmile Creek carbonaceous shale, which is exposed for 18 km east–west and 3 km north–south along the south side of Fifteenmile Creek (Davies-Vollum and Wing, 1998). The bed is at the 700 m level of the Elk Creek section, 13 m below a bentonitic ash dated at  $52.59 \pm 0.12$  Ma (Smith et al., 2004) and is above vertebrate sites documenting a Wa-7 fauna (Schankler, 1980). It has an interpolated age of 52.98 Ma. Fossil plants at the level include a heterogeneous mix of dicots (angiosperms), gymnosperms and ferns, with lateral variability in composition reflecting the original spatial mosaic of vegetation on the ancient floodplain (Davies-Vollum and Wing, 1998). The most abundant angiosperms are *Alnus* sp., “*Dombeya*” *novi-mundi* (Malvaceae) and *Platycarya castaneopsis* (Juglandaceae). Additional angiosperms include *P. cinnamomoides* and species of Lauraceae and Magnoliaceae. The conifer *G. europaeus* is also common, as are many species of ferns (Wing et al., 1995; Davies-Vollum and Wing, 1998; Currano, 2009). The unit was deposited during the Early Eocene Climatic Optimum under a mean annual temperature of  $22.2 \pm 2$  °C (Currano, 2009).

## 2.2. Extraction and separation

Samples were rinsed with dichloromethane (DCM), broken into ca. 1 cm pieces, oven-dried and powdered in a ball mill. Powdered samples (25–120 g) were extracted by Soxhlet with DCM/MeOH (9:1, v/v) for 24 h. Each total lipid extract (TLE) was concentrated via rotary evaporation and purified via asphaltene precipitation with hexane/DCM (4:1, v/v) at  $-5$  °C for 12 h. It was separated into

an apolar and a polar fraction with flash column chromatography using 1.55 g silica gel in 6 ml glass columns with hexane/DCM (9:1, v/v) and DCM/MeOH (1:1), respectively. The apolar fraction was separated into saturated and unsaturated fractions on 5% Ag-impregnated silica gel (w/w). The saturated and unsaturated hydrocarbons were eluted with 4 ml hexane and 4 ml EtOAc, respectively.

### 2.3. Biomarker assignment and quantification

Lipids were assigned using gas chromatography–mass spectrometry (GC–MS) with a Hewlett–Packard (HP) 6890 GC instrument coupled to a HP 5973 quadrupole MS instrument, with electron ionization. A fused silica column (Agilent J&W DB-5; 30 m, 0.25 mm, 25  $\mu$ m) was used with He as carrier gas. A split/splitless injector was operated in pulsed splitless mode at 320 °C with a column flow of 1.5 ml/min. The oven temperature program was: 60 °C (1 min) to 140 °C at 15 °C/min, then to 320 °C (held 20 min) at 4 °C/min. A scanning range of  $m/z$  50–700 at 3 scans/s was used, with ionization energy 70 eV. Compounds were assigned using one or more of: authentic standards, NIST 98 spectral library, published spectra, interpretation of fragmentation patterns, and retention times (Diefendorf, 2010).

Prior to quantification of lipids, a known aliquot of each fraction was spiked with internal standards (dodecane and 1,1'-binaphthyl). Compounds were quantified using a HP 5890 GC instrument with flame ionization detection (FID) and GC conditions as above. Peak areas were normalized to those of 1,1'-binaphthyl and converted to abundance using external standards (14 surrogate standard compounds) analyzed in a concentration ranging from 0.2 to 100  $\mu$ g/ml. Uncertainty in measurements was determined by treating additional analyses of the external standards as unknowns and measuring the coefficient of variation (CV; a measure of the dispersion and calculated from the standard deviation divided by the mean multiplied by 100) of the estimated concentration. The CV for each surrogate standard ranged from 4.8% to 8.2% of the mean with an average CV of 6.7%. Compound abundances were normalized to TOC content for each sample (e.g.  $\mu$ g/g C).

Maturity was assessed with the homohopane ( $C_{31}$ ) maturity index for the isomerism at C-22 (Peters et al., 2005). The 22S and 22R isomer abundances were measured from 17 $\alpha$ , 21 $\beta$ -homohopane using GC–MS and the  $m/z$  191, 205 and 426 chromatograms. Homohopane maturity indices were calculated using the 22S/(22S + 22R) ratio for each ion to rule out effects from coelution of closely eluting compounds; no significant differences were detected between ratios based on the three extracted ions. Regular sterane/17 $\alpha$ -hopane ratios were determined from the  $C_{27}$ ,  $C_{28}$  and  $C_{29}$   $\alpha\alpha\alpha$ (20S + R) and  $\alpha\beta\beta$ (20S + R) steranes and the  $C_{29}$ ,  $C_{30}$ ,  $C_{31}$ , and  $C_{32}$  17 $\alpha$ -hopanes using  $m/z$  217 for steranes and  $m/z$  191 for hopanes (Moldowan et al., 1985).

## 3. Results and discussion

### 3.1. Organic geochemical characterization of sediments

All sediments analyzed were deposited in wet floodplain environments, but variation in grain size, TOC and sedimentary features indicated a range of sub-habitats relating to duration of flooding and proximity to paleochannels (Table 1). Given the variability in sub-habitats, we would expect some heterogeneity in the floral assemblages (Davies-Vollum and Wing, 1998) as well as some variation in the organic geochemistry analyses (Fig. 3).

The ratio of pristane (Pr) to phytane (Ph) is commonly applied as a proxy for depositional redox conditions of marine or aquatic oils (Peters et al., 2005); however, it has many limitations. For

example, Pr and Ph can have multiple sources, including prominently from the phytol side chain of chlorophyll in phototrophic organisms and bacteriochlorophyll in purple sulfur bacteria, although other sources (tocopherols, archaeal lipids) are possible (Goossens et al., 1984; Koopmans et al., 1999). In practice, for petroleum, Pr/Ph values < 1 are typically taken to indicate a reducing environment and values > 3 an oxic depositional environment (Hughes et al., 1995; Peters et al., 2005). Notably, values associated with terrestrially dominated OM can be significantly higher, reaching > 10 in high wax non-marine oils (Brooks et al., 1969; Powell and McKirdy, 1973). For Bighorn Basin samples (Fig. 3), Pr/Ph ranged from 1.3 to 6.6, with the highest values for the organic-rich carbonaceous shales and lignites, reflecting the dominance of terrestrial OM in these facies, as indicated by overall elevated values for the carbon preference index (CPI; Fig. 3).

All samples had homohopane values < 0.2 (Fig. 3), indicating they were immature (Peters et al., 2005). This is consistent with prior studies of the Bighorn Basin burial history (Roberts et al., 2008). Thermal maturity indices do not appear to systematically vary with age or with geographic location within the Bighorn Basin.

Bacterial degradation of OM was likely more favorable within some sub-habitats than others. Hopanes, bacterial biomarkers produced as the degradation products of bacteriohopanepolyols (Rohmer et al., 1984) can provide a relative indication of the amount of bacterial OM preserved in sediments. Hopanes were common at all sites and were most abundant at the Fifteenmile Creek site (Fig. 3), a backswamp environment. The ratio of steranes (biomarkers for eukaryotic life, i.e. algae and higher plants) to 17 $\alpha$ -hopanes was low (between 0 and 0.34; Supplementary material, Fig. E-1), indicating that the OM contains a significant component of bacterial biomass (Volkman, 2005). The sterane/17 $\alpha$ -hopane ratio varied, but no clear lithologic pattern existed, as might have been expected with changing OM source and/or microbial reworking among depositional environments (Bechtel et al., 2003).

### 3.2. *n*-Alkane abundance

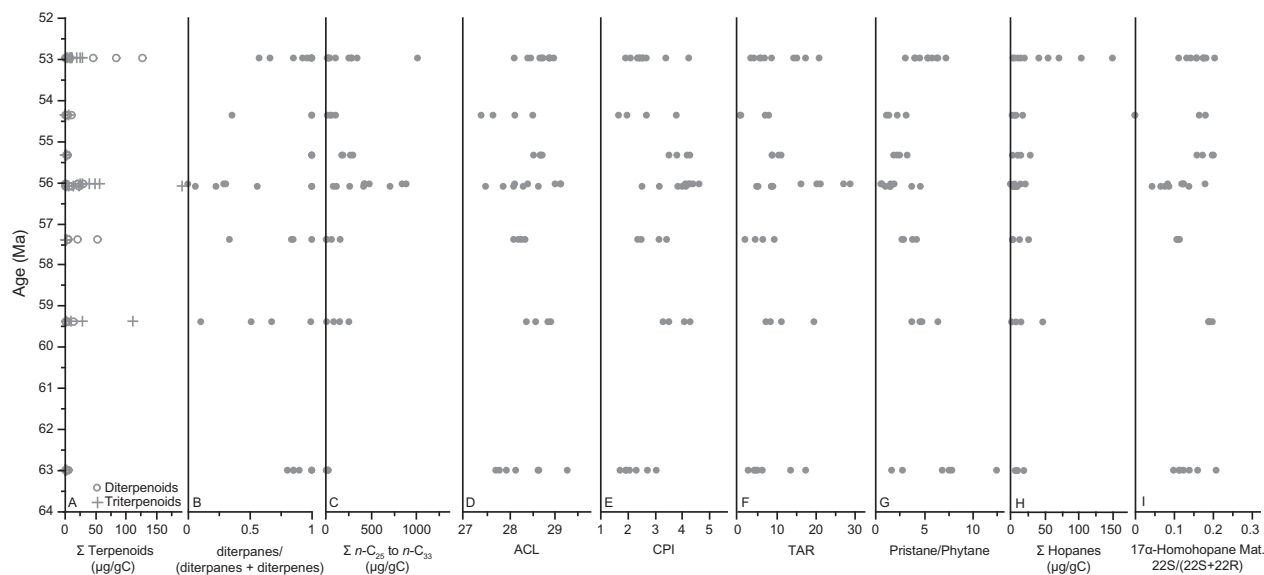
The *n*-alkane concentration (Fig. 3) was highest for long chain *n*-alkanes (e.g.  $C_{27}$ – $C_{33}$ ) derived from vascular plant leaf wax. The most abundant was *n*- $C_{31}$ , with a concentration ranging from 1 to 210  $\mu$ g/g C (mean 36  $\mu$ g/g C). Long chain *n*-alkane abundance was highest for samples from Fifteenmile Creek and the July 4th Quarry. Mid-chain *n*-alkanes ( $C_{19}$ ,  $C_{21}$ ,  $C_{23}$ ,  $C_{25}$ ; Ficken et al., 2000), produced by submerged and floating aquatic plants, were typically lower in abundance (15  $\mu$ g/g C) than long chain *n*-alkanes. Short chain *n*-alkanes typical of algae ( $C_{15}$ ,  $C_{17}$ ,  $C_{19}$ ) had the lowest abundance (5  $\mu$ g/g C). This preference for longer chain *n*-alkanes is characteristic of terrestrial sediments, where the dominant source of *n*-alkanes is higher plant leaf wax, with only a minor contribution from aquatic sources.

To identify any major or systematic changes in long chain *n*-alkanes, we calculated average chain length (ACL) values using the equation of Eglinton and Hamilton (1967):

$$ACL = \frac{(25n-C_{25} + 27n-C_{27} + 29n-C_{29} + 31n-C_{31} + 33n-C_{33} + 35n-C_{35})}{(n-C_{25} + n-C_{27} + n-C_{29} + n-C_{31} + n-C_{33} + n-C_{35})} \quad (1)$$

where *n*-alkane abundances are converted to respective chain length numbers (Fig. 3). ACL values ranged between 27.4 and 29.3, within the range of 26 and 34 observed for modern tree species (Diefendorf et al., 2011).

To determine if there was an odd chain length preference in the long chain *n*-alkanes, the CPI was measured using the Marzi et al. (1993) relationship: where values > 1 are characterized by higher



**Fig. 3.** Biomarker values for Bighorn Basin samples. Points are individual samples and panels are as follows: (A) Total di- and triterpenoids (normalized to g of OC), (B) diterpenoid saturation index [diterpanes/(diterpanes + diterpenes)], (C) total *n*-alkanes (odd chain length from C<sub>25</sub> to C<sub>33</sub>), (D) average chain length (ACL), (E) carbon preference index (CPI), (F) ratio of terrestrial to aquatic *n*-alkanes (TAR), (G) ratio of pristane (Pr) to phytane (Ph), (H) total bacterial hopanes and (I) 17 $\alpha$ -homohopane (C<sub>31</sub>) 22S and 22R maturity index.

$$\text{CPI} = \frac{(n\text{-}C_{23} + n\text{-}C_{25} + n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31}) + (n\text{-}C_{25} + n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31} + n\text{-}C_{33} + n\text{-}C_{35})}{2 * (n\text{-}C_{24} + n\text{-}C_{26} + n\text{-}C_{28} + n\text{-}C_{30} + n\text{-}C_{32})} \quad (2)$$

odd *n*-alkane abundances than even. CPI values ranged from 1.5 to 4.3 (Fig. 3). These are at the lower end of the range for modern plants (Diefendorf et al., 2011), but are nonetheless typical for terrestrial sediments where some oxidation of odd long chain *n*-alkanes has occurred (Freeman and Colarusso, 2001).

Sampled shales, lignites and mudstones are overbank facies associated with aggrading fluvial systems (Jones and Hajek, 2007); as a result, the contribution of aquatic OM likely varied. This was supported by variable abundances of short chain *n*-alkanes in the sediments. To qualitatively explore the relative input of terrestrial to aquatic OM, we measured the ratio of higher plant *n*-alkanes (C<sub>27</sub>, C<sub>29</sub>, C<sub>31</sub>) to aquatic *n*-alkanes (C<sub>15</sub>, C<sub>17</sub>, C<sub>19</sub>) using the terrestrial to aquatic ratio (TAR) following Bourbonniere and Meyers (1996):

$$\text{TAR} = \frac{(n\text{-}C_{27} + n\text{-}C_{29} + n\text{-}C_{31})}{(n\text{-}C_{15} + n\text{-}C_{17} + n\text{-}C_{19})} \quad (3)$$

where high values are indicative of a higher plant input. Relative TAR changes through time can be meaningful (Peters et al., 2005), although absolute TAR values are obfuscated by various processes, including higher concentrations of *n*-alkanes in terrestrial plants than in aquatic plants (Meyers, 1997) and the potential of reworked alkanes. All sites had TAR > 1 (Fig. 3), consistent with greater *n*-alkane input from higher plants, with values > 20 at the July 4th Quarry. Although TAR values were > 1, they were highly variable and indicate that aquatic OM was variable in these fluvial sediments.

### 3.3. Terpenoid lipid abundance and paleovegetation

Plant-derived terpenoids were present in all but one of the samples (Fig. 3, Table E-1) and were found in the saturated and

unsaturated hydrocarbon fractions, but none were detected in the ketone, alcohol or acid fractions (Diefendorf, 2010). The most abundant diterpenoids were in the pimarane class (Fig. 1) including 18-norisopimarane and 18-norpimarane. Diterpenoids in the abietane class were common and abundant, especially dehydroabietane, 19-norabieta-8,11,13-triene and abietane. Triterpenoids included representatives of the oleanane and ursane classes, as well as A-ring degraded pentacyclic triterpenoids, des-A-lupane, des-A-ursane and des-A-oleanane (Table E-1). Tetracyclic diterpenoids, including phyllocladane, beyerane and kaurane were present, but in low abundance (Table E-1), except at Fifteenmile Creek, where kaurane concentration was as high as 33 µg/g C. Tetracyclic diterpenoids are poor taxon-specific biomarkers because they can derive from both angiosperms and conifers, and have therefore been omitted from analyses and comparisons below.

The degree of saturation was not the same in di- and triterpenoids. All the triterpenoids were unsaturated (triterpenes), with the exception of the A-ring degraded compounds, which were saturated. In contrast, the samples contained diterpanes, mixtures of diterpanes and diterpenes, or, in one sample, only diterpenes. To quantify differences among samples, we calculated the diterpenoid saturation index (diterpane/[diterpane + diterpene]; Bechtel et al., 2003). Index values ranged from 0 to 1, with a mean of 0.76, indicating that most samples had more saturated than unsaturated diterpenoids (Fig. 3). Diterpenoid saturation is suggested to reflect conditions during early diagenesis (Wakeham et al., 1980), with compounds becoming progressively unsaturated in oxic environments and more saturated in anoxic environments. We found a range in the extent of diterpenoid saturation even among samples collected at the same site, likely reflecting variation in sediment sub-habitats. Haberer et al. (2006) speculated that lithology may

result in different degradation modes; for example, acid-catalyzed diagenetic reactions, possibly associated with high clay mineral content or lignite lithology, can enhance diterpenoid degradation (Rubinstein et al., 1975; Sieskind et al., 1979). In the Bighorn Basin, diterpenoid saturation index values ranged widely and did not correlate systematically with lithology (Wilcoxon nonparametric test). Interestingly, many samples that contained only saturated diterpanes also contained unsaturated triterpenes. Assuming that both were deposited contemporaneously, this suggests that redox conditions did not control extent of saturation for both the di- and triterpenoids.

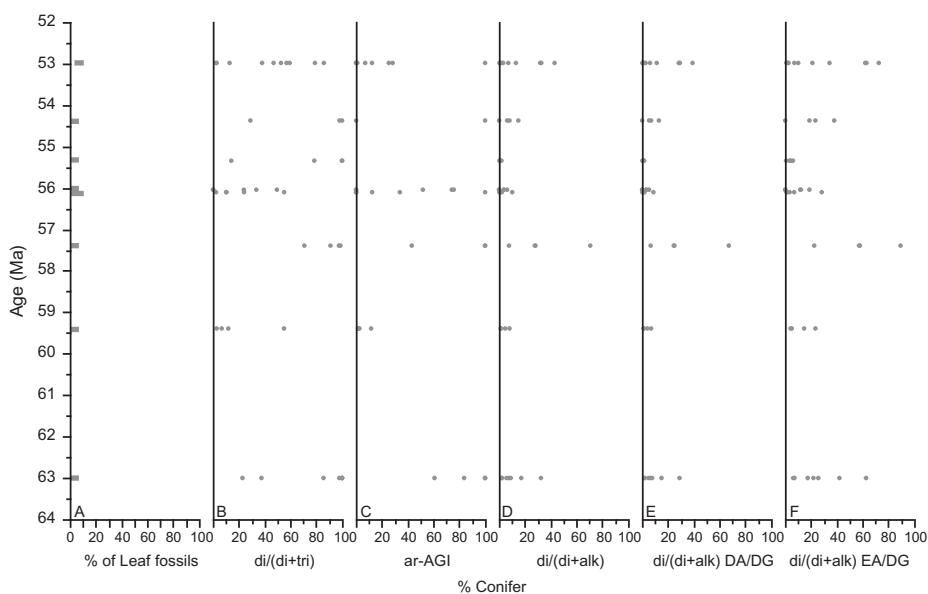
The Latest Paleocene samples had the highest concentrations of long chain *n*-alkanes and triterpenoids. More qualitatively, they also contained the most fossil cuticles. Therefore, we speculate that conditions that enhance cuticle preservation may also enhance survival of *n*-alkanes and triterpenoids. For example, triterpenoid biomarkers from mangroves are preserved in association with cuticle fossils, while coexisting diterpenoids are oxidatively degraded (Killops and Frewin, 1994). For other lithologies, it remains unclear why triterpenoids were largely unsaturated, while diterpenoid compounds exhibited a wide range in saturation. Diterpanes were preserved in high abundance and generally with a low degree of unsaturation, suggesting they may be selectively protected after becoming saturated in reducing environments and during subsequent burial and outcrop exposure.

We characterized the changes in the relative abundance of plant biomarkers by terpenoid class. Di- and triterpenoid abundances were summed (Fig. 3) for all structures except tetracyclic diterpenoids, which have angiosperm and conifer origins (cf. Diefendorf et al., 2012). Diterpenoid abundance ranged from 0 to 87.59  $\mu\text{g/gC}$  and was highest at the Fifteenmile Creek site. Triterpenoid abundance ranged from 0 to 192  $\mu\text{g/gC}$  and was highest at July 4th Quarry. Because leaf fossil data indicate communities at all sites comprised 85–100% angiosperms, we expected triterpenoid concentration would be significantly higher than diterpenoid concentration.

Angiosperms tend to produce more total terpenoids per unit of biomass, a fact that exacerbates the disparity between leaf fossil

data and the low abundance of triterpenoids relative to diterpenoids. More quantitatively, we modified di- to triterpenoid ratio calculations (Bechtel et al., 2003; Nakamura et al., 2010) to account for biomarker production estimated from modern vegetation (Diefendorf et al., 2012). All di- and triterpenoid-based paleovegetation proxies significantly overestimated conifer abundance compared with the megafossil estimates (Fig. 4, Table 2). A similar observation has been noted by Bechtel et al. (2008) where di/(di + tri) ratios (not corrected for terpenoid production) were positively correlated with pollen-based estimates, but the terpenoids significantly overestimated the conifer abundance. The authors proposed a plausible argument, whereby pollen represented the flora within the catchment, whereas the terpenoids represented the very local peat-forming flora. It should also be noted that tetracyclic diterpenoids were included as conifer biomarkers in their study, possibly leading to some overestimation of conifers given that these biomarkers are also produced by angiosperms (e.g. Diefendorf et al., 2012). Nevertheless, the similarities between Bechtel et al. (2008) and this study are striking and suggest that diterpenoids overexpress conifers in the floral community.

The influence of longer leaf lifespan on terpenoid and *n*-alkane concentrations in modern plants has recently been identified in modern calibration studies (Diefendorf et al., 2011, 2012). Previous calibrations measured terpenoid and leaf wax concentrations in 44 tree species from 21 families representing both angiosperms and gymnosperms grown at a single site to minimize the impact of climate on lipid concentration. These studies indicated that *n*-alkanes are significantly higher in angiosperms than conifers (Diefendorf et al., 2011) and that evergreen plants produce greater concentrations of both *n*-alkanes and terpenoids than deciduous leaves (Fig. 5; Diefendorf et al., 2012). Thus, it is important to differentiate between evergreen (E) and deciduous (D) leaves in the angiosperms (A) and gymnosperms (G; specifically conifers in this study) when using these lipids as paleovegetation indicators or when studying compound-specific carbon or hydrogen isotopes. This poses a challenge in geologic studies because it is difficult to constrain leaf lifespan from fossils. Therefore, we evaluated the potential influence of leaf lifespan on expected production-



**Fig. 4.** Fossil and biomarker-based estimates of the proportion (%) of flora that is conifer. Points are individual samples and panels are as follows: (A) Fossil-based estimates, (B) estimated conifer abundance based on di- to triterpenoids, (C) estimated conifer abundance using the aromatic angiosperm/gymnosperm index (ar-AGI; Nakamura et al., 2010), (D) estimated conifer abundance using the new approach outlined herein with diterpenoid and *n*-alkane concentrations, (E) same approach as D, except estimates were corrected for deciduous lifespans and (F) estimates corrected for evergreen angiosperm and deciduous gymnosperm lifespans. Abbreviations: di, diterpenoids; tri, triterpenoids; ar-AGI, aromatic angiosperm/gymnosperm index; alk, alkanes; DA, deciduous angiosperms; DG, deciduous angiosperms; EA, evergreen angiosperms.

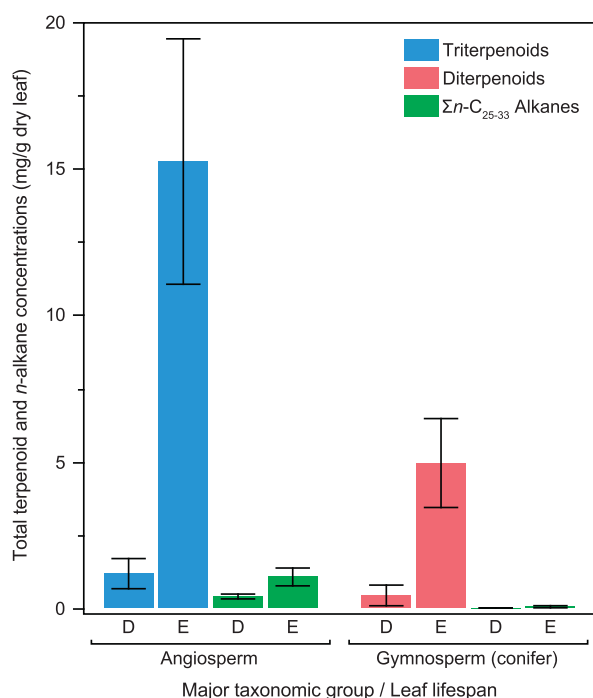


**Table 2**  
Estimated proportion (%) of conifer flora from terpenoids and leaf wax *n*-alkanes (n.d., not determined).

Age (Ma)	Level	Paleovegetation <sup>a</sup> (macrofossils)	di/(di + tri) <sup>b</sup>	di/(di + tri) DA, DG	di/(di + tri) EA, DG	ar-AGI	di/(di + alkanes)	di/(di + alkanes) DA, DG	di/(di + alkanes) EA, DG
52.98	Fifteenmile Creek	3–9%	44% (±9)	62% (±11)	88% (±6)	22% (±12)	13% (±5)	12% (±5)	28% (±9)
54.37	Dorsey Creek Fence	≤5%	76% (±23)	85% (±14)	98% (±2)	50% (±50)	7% (±3)	6% (±3)	20% (±8)
55.34	WCS7	≤5%	73% (±20)	82% (±16)	97% (±3)	n.d.	1% (±0)	1% (±0)	4% (±1)
56.04	Latest Paleocene	≤5%	21% (±10)	38% (±16)	58% (±24)	40% (±17)	3% (±1)	2% (±1)	8% (±4)
56.1	Honeycombs	≤14%	20% (±9)	38% (±12)	83% (±8)	29% (±19)	3% (±2)	2% (±2)	8% (±5)
57.39	Cf-1	≤5%	90% (±6)	96% (±3)	100% (±0)	81% (±19)	34% (±13)	31% (±13)	57% (±14)
59.39	Belt Ash	≤5%	19% (±12)	34% (±16)	80% (±8)	4% (±2)	4% (±2)	3% (±1)	12% (±5)
63	Grimy Gulch	≤5%	78% (±13)	87% (±8)	99% (±1)	86% (±9)	11% (±4)	9% (±4)	26% (±8)
		Average	50% (±6%)	64% (±5)	88% (±4)	40% (±7)	10% (±2)	9% (±2)	21% (±4)

<sup>a</sup> See Table 1 for source of paleovegetation based on macrofossils.

<sup>b</sup> Standard error in parentheses.



**Fig. 5.** Total terpenoid and *n*-alkane concentrations by major taxonomic group and leaf lifespan (D, deciduous; E, evergreen). Standard errors are denoted with black bars. Data compiled from Diefendorf et al. (2011, 2012).

adjusted diterpenoid/triterpenoid ratios from modern calibration data (Diefendorf et al., 2012) by making estimates of both evergreen and deciduous lifespans. To illustrate, di- and triterpenoid concentration lines were plotted (Fig. 6) for plant communities consisting of 85% angiosperm and 15% conifer floras using biomarker production data for four plant functional type combinations (DA, DG, EA, EG; Diefendorf et al., 2012). The 15% conifer community was used as a conservative upper limit, and our findings emphasize the much higher than expected diterpenoid abundances. These vegetation lines are a first approximation, as they assume similar biochemistry of ancient and extant vegetation, do not account for floral heterogeneity at a given sampling location (Davies-Vollum and Wing, 1998) and were not adjusted for molecular preservation and taphonomic differences. Most samples plot well below the minimum vegetation lines in Fig. 6a, suggesting community composition does not account for low triterpenoid abundance (see further discussion of leaf lifespan in Section 3.4).

In modern plants, *n*-alkanes are far more abundant in angiosperm leaves than gymnosperm taxa (Diefendorf et al., 2011). Here, we use *n*-alkanes as a molecular signature reflecting

dominantly angiosperm contribution in mixed floral communities (Diefendorf et al., 2011). The sum of the odd *n*-C<sub>25</sub> to *n*-C<sub>33</sub> alkanes was plotted relative to the triterpenoids to compare angiosperm-to-angiosperm sourced biomarker abundances (Fig. 6b). Using the same approach as above, biomarker production lines were constructed for deciduous and evergreen angiosperms. All sediment samples plotted significantly below the leaf lifespan production lines. However, unlike the prior comparison where samples were randomly scattered, there was a small, but significant correlation ( $R^2$  0.1,  $p$  0.02) between *n*-alkanes and triterpenoids. When the three samples with the highest terpenoid concentration were removed, the explanatory power increased substantially ( $R^2$  0.45,  $p$  < 0.0001). Nevertheless, the results suggest that triterpenoids were significantly lost relative to *n*-alkanes. We suggest that abundance ratios based on *n*-alkanes and diterpenoids have potential utility as a paleovegetation proxy (Figs. 4 and 6, Table 2).

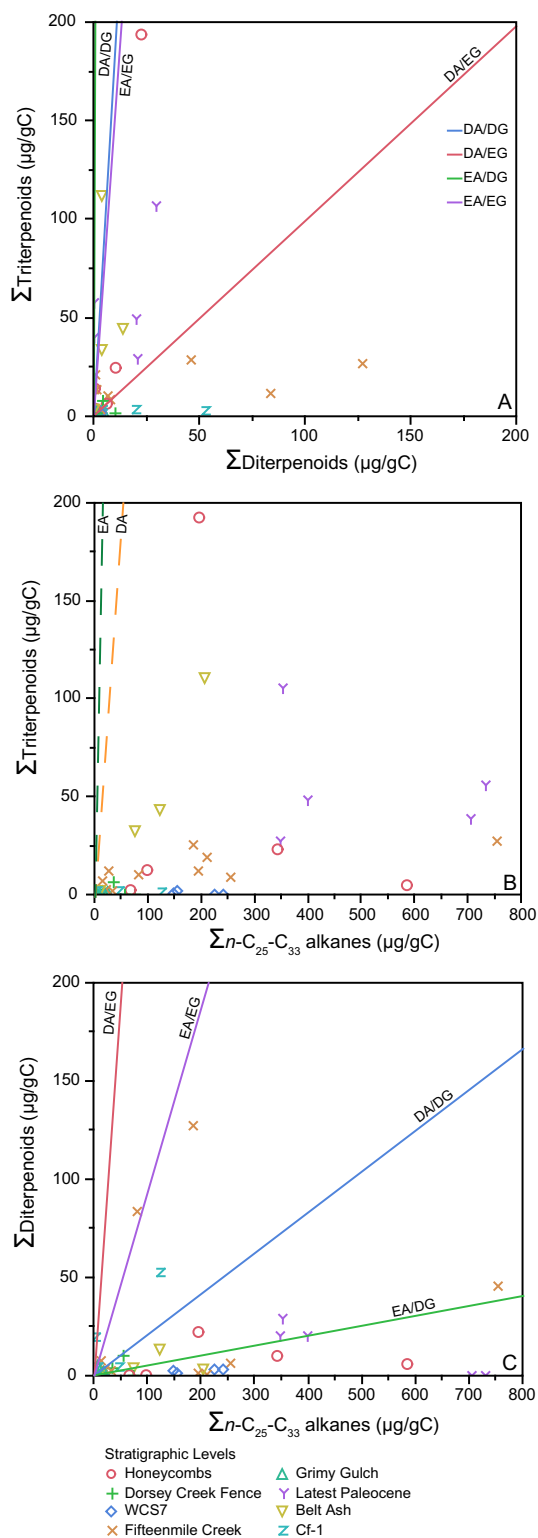
### 3.4. Paleovegetation reconstruction using diterpenoids and *n*-alkanes

As noted above, *n*-alkanes are highly abundant in angiosperm leaves, and here we explore the utility of using them as a substitute for triterpenoids in a molecular paleovegetation proxy. The Bechtel et al. (2003) terpenoid relationship was modified by substituting *n*-alkanes for triterpenoids:

$$\% \text{ Gymnosperm vegetation} = \frac{\left( \frac{\sum \text{Diterp.}}{\text{DLP}} \right)}{\left( \frac{\sum \text{Diterp.}}{\text{DLP}} + \frac{\sum n\text{-C}_{25} \text{ to } n\text{-C}_{33} \text{ alkanes}}{\text{ALP}} \right)} \quad (4)$$

where the abundances of diterpenoids and *n*-alkanes (*n*-C<sub>25</sub> to *n*-C<sub>33</sub>) are corrected for diterpenoid (DLP) and *n*-alkane lipid production (ALP) values (Fig. 5). Lipid production values were determined from modern calibration studies (Diefendorf et al., 2011, 2012) and took into account alkane and terpenoid concentrations in modern plants, accounting for leaf lifespan, and litter flux to sediments. There are many assumptions in using this type of proxy, as is inherently true for all biomarker-based proxies (cf. Hedges and Prahl, 1993). The primary assumption is that modern vegetation data are a good estimator of ancient floral lipid production. For example, if the fossil species are significantly different from the modern species used to generate the lipid biomarker production values, the approach will likely fail. Nonetheless, species in the modern datasets (Diefendorf et al., 2011, 2012) were selected to represent taxa prominent in Paleocene and Eocene Bighorn Basin fossil assemblages. Another potential confounding factor is the incorporation of resin into sediments, as terpenoids are a major component of resins. Modern terpenoid production estimates used here were determined from leaf and branch tissues (Diefendorf et al., 2012) and did not specifically include any additional resin sources. However, for gymnosperms, only the Araucariaceae and Pinaceae families





**Fig. 6.** Terpenoid and total *n*-alkane concentrations for Bighorn Basin samples coded by sample locality (see Tables 1 and E-1). Expected lines denote highest possible conifer end members based on megafossils (15% conifer/85% angiosperm) and estimates of biomarker production derived from modern plants (Diefendorf et al., 2011, 2012). (A) Diterpenoid (conifer) and triterpenoid (angiosperm) concentrations are plotted; no systematic trends are noticeable. (B) Total *n*-alkanes (angiosperm) and triterpenoid (angiosperm) concentrations; overall, *n*-alkanes are higher in concentration than triterpenoids in the same samples; almost all samples plot significantly to the right from expected values. (C) Total *n*-alkanes and diterpenoid abundances; samples plot close to the predicted deciduous angiosperm (DA)/deciduous gymnosperm (DG) and evergreen angiosperm (EA)/DG lines. Almost all samples plot below evergreen angiosperm (EG) lines.

produce large quantities of resin (e.g. Langenheim, 1990). These families were not found in the Bighorn Basin during the Paleogene, but some resin has been observed in Bighorn Basin carbonaceous shales, including those sampled here. Resin may be a minor bias if it was completely transformed to amber in sediments. The transformation from resin to amber is important because terpenoids are not solvent extractable once converted to amber. Nevertheless, resin may be an important source of terpenoids in some studies and will need to be carefully evaluated. We would speculate that resin results in an overestimation of the source taxa in sediments when important resin producing taxa are present. This is not likely the case here given that the *n*-alkane based proxy agrees with the fossil-based estimates, indicating that resin-derived diterpenoids were minor and because resin producing species were not present at this time in the Bighorn Basin.

As mentioned in the previous section, leaf lifespan is an important predictor of terpenoid and *n*-alkane concentrations. Constraining leaf lifespan is challenging from fossils alone, but by using the nearest living relatives, some constraints on leaf lifespan can be made. In the Bighorn Basin, Eocene gymnosperms such as *G. europaeus* were most likely deciduous, similar to their extant relatives. Angiosperm taxa leaf lifespans are more difficult to constrain. The dominant families (Betulaceae, Malvaceae and Juglandaceae) in the Eocene have mostly deciduous modern relatives; the abundant Lauraceae and Magnoliaceae families are notable for extant evergreen species. These latter two families have a low abundance at the Fifteenmile Creek site (Curran, 2009) and the Cabin Fork site (Smith et al., 2007). Some studies have made the simplifying assumption that angiosperms were primarily deciduous during the Eocene (Wilf, 2000).

Estimated proportions of angiosperm and gymnosperm were calculated twice (DA/DG, EA/DG), by adjusting lipid production for short and long (i.e. deciduous and evergreen) leaf lifespans (Table 2) using the modern calibration data from Diefendorf et al. (2011, 2012). The proportion estimate of angiosperms averaged 9% (range 1–31%) for DA/DG communities and 21% (4–57%) for EA/DG communities. Values for the DA/DG comparison most closely approached the megafossil estimate of ca. 5% conifer, and both estimates were superior than traditional triterpenoid-based methods, even when accounting for biomarker production differences. If confirmed by future studies for other floras, at other times and in other locations, alkane/diterpenoid ratios promise a useful, and potentially quantitative, proxy for paleovegetation estimates.

### 3.5. Terpenoid diagenesis and preservation

This study highlights differential di- and triterpenoid preservation, where triterpenoid concentrations are significantly lower than expected based on fossils. This observation was noted by Bechtel et al. (2008), who observed that diterpenoids over predicted conifers compared with pollen. These observations suggest that triterpenoid diagenesis is enhanced relative to diterpenoids or that diterpenoids are somehow preferentially preserved. Although the primary goal of this study was to evaluate terpenoids as paleovegetation indicators, it is possible to provide a comparison with the numerous terpenoid studies that focused on diagenesis to explore some differences in preservation between di- and triterpenoids (e.g. Simoneit, 2005; Hauteville et al., 2006).

Biological terpenoids are synthesized initially as functionalized compounds including acid, alcohol, ketone and/or ester groups that may or may not be attached to sugars (glycolipids). In conifers, it is often common to find diterpenes along with functionalized compounds in fresh leaves (e.g. Simoneit et al., 1986; Diefendorf et al., 2012). Alteration of terpenoids can begin as early as on the living leaf and can continue in the leaf litter through photochemical or photomimetic reactions (Corbet et al., 1980). Terpenoid

diagenesis continues through oxidative or reductive processes, depending on water exposure, rate of sediment burial, and through post-depositional diagenesis and thermal transformation leading to numerous terpenoid structures with varying degree of saturation (Spyckerelle et al., 1977; Laflamme and Hites, 1978; Corbet et al., 1980; Wakeham et al., 1980; Chaffee et al., 1984; Trendel et al., 1989; Stout, 1992; ten Haven et al., 1992; Rullkötter et al., 1994; Murray et al., 1997). Surprisingly, some functionalized compounds escape diagenesis and are preserved in a nearly unaltered functionalized state for millions of years (Rullkötter et al., 1994; Otto and Simoneit, 2001; Otto et al., 2003).

Diterpenoids in this study were characterized by aromatic, olefinic and saturated hydrocarbons. In oxidizing environments, diterpenoids are decarboxylated and/or dehydrated and then are progressively aromatized (Laflamme and Hites, 1978; Wakeham et al., 1980; Chaffee and Johns, 1983; Simoneit et al., 1986; Otto et al., 1997, 2002; Otto and Simoneit, 2001; Stefanova et al., 2005). This leads to compounds observed here such as dehydroabietane, norabietatriene, retene and tetrahydroretene. Diterpenoids can also undergo hydrogenation in reducing conditions to form diterpanes (e.g. Otto and Simoneit, 2001). This includes compounds observed here such as abietane, isopimarane, pimarane and fichtelite. It is important to note that many of the diterpenoids studied here were saturated (Fig. 3). Although it is not uncommon to observe saturated and aromatic diterpenoids in the same samples (e.g. Bechtel et al., 2002; Tuo and Philp, 2005), it does suggest that diterpenoids were altered through both reductive and oxidative pathways in the same samples. Although much is known about potential pathways for terpenoid diagenesis, very little is known about the difficulty of hydrogenating these compounds in terrestrial sediments.

In this study, triterpenoids were characterized by olefinic and aromatic hydrocarbons and by des-A-triterpanes. Olefinic triterpenoids form through dehydration and produce stable olefins regardless of redox conditions (ten Haven et al., 1992). This would include compounds observed here, such as olean-12-ene, olean-18-ene and olean-13(18)-ene. Subsequent hydrogenation under reducing conditions can occur, although triterpanes were not detected. In oxic environments, triterpenoid diagenesis progresses from biologic terpenoids to fully aromatized compounds that are very stable (Spyckerelle et al., 1977; Laflamme and Hites, 1978; Wakeham et al., 1980; Rullkötter et al., 1994; Murray et al., 1997; Jacob et al., 2007). Many mono-, tri- and tetra-aromatic triterpenes were observed in these samples, suggesting oxidizing conditions during diagenesis and subsequent stabilization. Cleavage of the A-ring, possibly via microbial degradation or photochemical cleavage, is common and produces des-A-triterpenoids (Corbet et al., 1980; Trendel et al., 1989). Des-A-lupane, des-A-oleanane, and des-A-ursane were observed in these sediments, although des-A-lupane was by far the most abundant and most common of them. Jacob et al. (2007) suggested that des-A-lupane may have a unique degradation pathway compared with the other two compounds. Biologic terpenoids with a lupane structure often have a double bond on the isopropyl group, possibly favoring reduction. This is in contrast to the oleanane and ursane structures that have internal double bonds that favor aromatization. Therefore, des-A-lupane may be relatively resistant to diagenesis. However, given that aromatization was a key pathway in the triterpenoid alteration, it is surprising that we did not observe any aromatic des-A-oleanenes or des-A-ursenes, as these would be expected under oxidizing conditions (Trendel et al., 1989; Stout, 1992; Murray et al., 1997; Jacob et al., 2007). We speculate that the minor concentration of des-A-oleanane and des-A-ursane may point to reducing microenvironments that favored hydrogenation of olefinic intermediates produced after A-ring cleavage, before compounds could be aromatized, leading to stable saturated

compounds. Apart from the des-A-oleanane and des-A-ursane compounds, it is likely that triterpenoids studied here were altered primarily under oxidizing conditions.

The presence of olefinic and aromatic terpenoids in the sediments is consistent with an oxidative diagenetic pathway. However, the presence of diterpanes and the des-A-triterpanes may suggest that these compounds were reduced in microenvironments that favored stabilization via hydrogenation. It is not surprising that oxidative and reducing environments existed, given the complex nature of fluvial depositional environments. Terpenoids enter the geosphere most likely via leaves, resin and bark and are subsequently altered in soils and sediments. Paleobotanical studies suggest the leaf does not fall far from the tree (Burnham et al., 1992), but leaves and terpenoids can be selectively transported in rivers (Medeiros and Simoneit, 2008; Medeiros et al., 2012; Ellis and Johnson, 2013). Therefore, in many cases, terpenoids may land quickly in reducing environments such as swamps or be rapidly buried. Rising and falling water levels, especially in overbank deposits and swamps, could provide changes in redox conditions, as noted in other studies (e.g. Jacob et al., 2007), thereby producing both oxidized and reduced terpenoids. We also speculate that diterpanes could be transferred into reducing microenvironments as resins, thereby shielding diterpenoids from oxidation, until they were reduced and stabilized as diterpanes. It is also important to note that functionalized diterpenoids are often observed in geologic samples (Otto et al., 1997; Otto and Simoneit, 2001, 2002; Otto et al., 2002, 2003, 2005), although that is not to say that functionalized triterpenoids cannot be preserved (Rullkötter et al., 1994; Otto et al., 2005). However, it does suggest that diterpenoids might have enhanced preservation over triterpenoids in geologic sediments. Interestingly, functionalized terpenoids were not detected in these samples despite preservation of *n*-alkanoic acids and *n*-alkanols (Diefendorf, 2010).

An alternative explanation for the patterns observed above could be weathering during exhumation of Bighorn Basin sediments. The weathering of OM in these sediments is significant and extends to ca. 30 m below the surface (Clyde et al., 2013). Therefore, recent weathering may have caused oxidation of samples collected from outcrops. A recent study found strong evidence that oxidation during weathering has a considerable effect on biomarker preservation (Marynowski et al., 2011). Thus, if some factor protects diterpenoids, such as preservation in resins, it is conceivable that diterpenoids would be preferentially protected, even during recent weathering. Nevertheless, the severe loss of triterpenoids relative to diterpenoids and *n*-alkanes highlights the importance of molecular preservation and its influence on biomarker-based community reconstructions (Hedges and Prahl, 1993). Future studies should test differences in the rate of terpenoid degradation under various redox and thermal conditions and determine the importance of recent weathering on biomarkers to improve paleovegetation studies.

#### 4. Conclusions

Plant-derived terpenoids from Paleocene and Eocene sediments in the Bighorn Basin (WY) were evaluated to determine their potential utility as quantitative indicators of paleovegetation. We found di- and triterpenoids uniquely distributed with respect to their saturation, with diterpenoids preserved as saturated, olefinic and aromatic compounds, and triterpenoids preserved only as olefinic and aromatic hydrocarbons (except for A-ring cleaved compounds). More importantly, we found a significant bias in terpenoid preservation, whereby triterpenoids were in significantly lower concentration than both diterpenoids and the proportion of angiosperms estimated from megafossils. Comparisons between triterpenoids and *n*-alkanes supported the loss of triterpenoids. Selective loss of

triterpenoids biases paleovegetation proxies via an over-representation of gymnosperm signatures. We propose an alternative proxy that uses diterpenoid and *n*-alkanes as conifer and angiosperm biomarkers, respectively. Using these compound classes and adjusting for production (which varies with taxa and leaf lifespan), this ratio provides estimates of vegetation in agreement with fossil leaf assemblage data. Future studies will need to test and identify mechanistic explanations for the selective loss of triterpenoids in geologic studies. It will also be important to verify if the proxy works in other depositional environments, such as marine and highly reducing environments, where terpenoids are often preserved. If the proxy is validated, it will provide a biomarker-based approach for providing estimates of paleovegetation for locations where pollen and megafossils are poorly preserved in the geologic record. This would significantly enhance our ability to constrain carbon isotope values in bulk sediments and the carbon cycle (cf. Diefendorf et al., 2010) and to explore the influences of changing paleoecology feedbacks on the hydrologic cycle.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.orggeochem.2014.04.004>.

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