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Distribution and carbon isotope patterns of diterpenoids and triterpenoids in modern temperate C_3 trees and their geochemical significance

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Abstract

Tricyclic diterpenoids and pentacyclic triterpenoids are nearly exclusively produced by gymnosperms and angiosperms, respectively. Even though both classes of terpenoids have long been recognized as plant biomarkers, their potential use as phylogenetically specific $\delta^{13}C$ proxies remains largely unexplored. Little is known of how terpenoid abundance and carbon isotope composition vary either with plant phylogenetic position, functional group, or during synthesis. Here, we report terpenoid abundances and isotopic data for 44 tree species in 21 families, representing both angiosperms and gymnosperms, and both deciduous and evergreen leaf habits. Di- and triterpenoid abundances are significantly higher in evergreens compared to deciduous species, reflecting differences in growth strategies and increased chemical investment in longer-lived leaves. Carbon isotope abundances of terpenoid lipids are similar to leaf tissues, indicating biosynthetic isotope effects are small for both the MVA (−0.4‰) and MEP (−0.6‰) pathways. Leaf and molecular isotopic patterns for modern plants are consistent with observations of amber, resins and plant biomarkers in ancient sediments. The $\delta^{13}C$ values of ancient diterpenoids are higher than triterpenoids by 2–5‰, consistent with observed isotopic differences between gymnosperms and angiosperms leaves, and support the relatively small lipid biosynthetic effects reported here. All other factors being equal, evergreen plants will dominate the abundance of terpenoids contributed to soils, sediments and ancient archives, with similar inputs estimated for angiosperm and gymnosperm trees when scaled by litter flux.

1. INTRODUCTION

Terpenoids represent one of the largest and most diverse groups of vascular plant compounds. Terpenoids have long been used as biomarkers, or chemical fossils, for higher plants in geologic sediments and oils (ten Haven and Rullkötter, 1988; Otto and Simoneit, 2001; Otto et al., 2002, 2003; Nakamura et al., 2010), and at a broad level are diagnostic of major seed plant groups (i.e., angiosperms, gymnosperms). The pentacyclic triterpenoids are almost exclusively synthesized by angiosperms whereas the tricyclic diterpenoids are produced primarily by gymnosperms (Swain, 1965; SukhDev, 1989; Otto and Wilde, 2001; Cox et al., 2007). Variations in the abundances of these compounds can therefore be used to constrain the plant groups from which organic matter was derived, and have been invoked in reconstructions of paleoecology and paleoenvironments (Bechtel et al., 2003, 2005; Hautevelle et al., 2006; Schouten et al., 2007).

Carbon isotope composition of terpenoids is not significantly altered by post depositional alteration through aromatization (Freeman et al., 1994), which allows separate measurement of the $\delta^{13}C$ values of major plant groups despite the molecular alterations that accompany diagenesis (Freeman et al., 1990). Such insights are important when resolving the influence of mixed or changing floral inputs

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on the $\delta^{13}C$ values of terrestrial organic matter ($\delta^{13}C_{TOC}$) (Bechtel et al., 2003; Holdgate et al., 2009; Widodo et al., 2009) and $\delta^{13}C$ values of *n*-alkanes (Smith et al., 2007). Gymnosperm leaves, resins, wood, litter and leaf waxes are generally ~2–3‰ enriched in ^{13}C relative to those from angiosperms at similar sites (Brooks et al., 1997; Murray et al., 1998; Arens et al., 2000; Pataki et al., 2003; Diefendorf et al., 2010, 2011). Carbon isotope fractionation in modern leaves of C_3 trees (Δ_{leaf}) is tied to water availability, with angiosperms and gymnosperms exhibiting somewhat different isotopic sensitivity to precipitation (Diefendorf et al., 2010). Variability in precipitation, vegetation structure and major taxon composition likely underlies both spatial and temporal patterns in the $\delta^{13}C$ values of terrestrial organic carbon in the geologic past. The distribution and ^{13}C abundances of both di- and triterpenoids have potential to help separate taxonomic composition from other factors and thereby strengthen our interpretation of terrestrial plant response to climate or other paleoenvironment perturbations.

Di- and triterpenoids are produced by vascular plants to help defend against insects and pathogen attack (Langenheim, 1994). These isoprenoid lipids are synthesized (Fig. 1) from isopentenyl diphosphate (IPP). This important 5-carbon building block is produced in vascular plants by two pathways: in the cytosol via the mevalonic acid pathway (MVA) and in plastids by the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. Carbon isotope fractionation during the biosynthesis of terpenoids by both the MVA and MEP pathways has been characterized for various marine algae (Schouten et al., 1998), and for the cupressaceous conifer *Cryptomeria japonica* (Chikaraishi et al., 2004), but not for other trees.

Carbon isotope values of angiosperm and conifer derived plant resins in modern samples and fossil resin and amber consistently indicate that angiosperms have lower $\delta^{13}C$ values than conifers (Murray et al., 1998; McKellar et al., 2011), similar to the patterns observed in leaf $\delta^{13}C$ values (see above). Carbon isotope values of resins and ambers are similar in value to bulk $\delta^{13}C$ values, an unexpected observation given that lipids are typically ^{13}C-depleted relative to bulk $\delta^{13}C$ values (Stout, 1996; Murray et al., 1998; McKellar et al., 2011). Resin from living trees infested by insects is enriched in ^{13}C, as is fossil amber, which has led to the suggestion that the ^{13}C enrichment is a result of water stress (McKellar et al., 2011). Other mechanisms such as carbon flux changes between biosynthetic pathways could also be important.

In the cytosol, acetyl coenzyme-A (acetyl-CoA) is produced from pyruvate, and via the MVA path, three acetyl units are combined with the loss of one carbon to produce IPP. Three IPPs are combined to form the 15-carbon structure, farnesyl diphosphate (FPP). FPP can be converted to form sesquiterpenoid ringed structures, or it can be combined with a second FPP to form squalene, which can be cyclized to form 30-carbon ringed triterpenoids (Lange et al., 2000; Bouvier et al., 2005). In the plastid, the MEP pathway (Fig. 1) combines pyruvate and glyceraldehyde-3-phosphate to form IPP; four IPPs are subsequently combined to form geranylgeranyl diphosphate (GGPP). GGPP is converted to phytol and cyclized to form 20-carbon ringed diterpenoids (Lange et al., 2000; Bouvier et al., 2005). Evidence suggests IPP can be shared between pools in the cytosol and the plastids, potentially blending pathway influences on isotope values among di- and triterpenoids, should they occur in the same plant. Additionally, IPP is converted to volatile terpene compounds, such as isoprene, that are emitted by plants (Kesselmeier and Staudt, 1999).

Gymnosperms produce tri- or tetra-cyclic diterpenoids belonging to the abietane, beyerane, kaurane, labdane, phyllocladane, pimarane, and totarane classes (Fig. 2) (Erdtman, 1963; SukhDev, 1989; Langenheim, 1994; Otto and Wilde, 2001; Cox et al., 2007). Tetra-cyclic diterpenoid compounds beyerane and kaurane are observed in

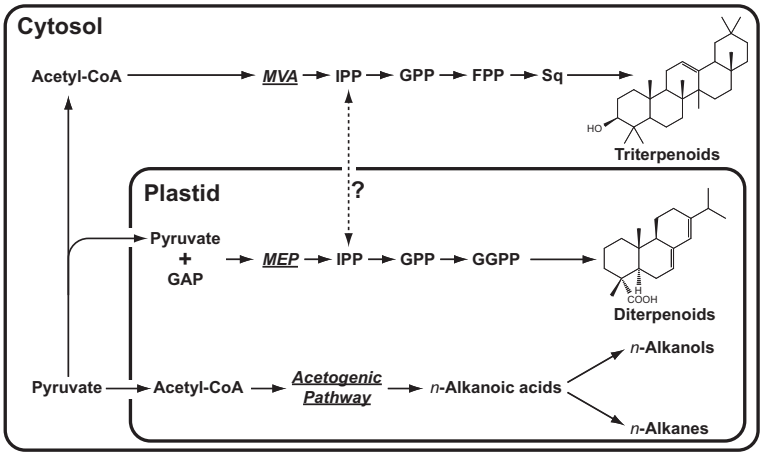

Fig. 1. Isoprenoid and *n*-alkyl lipid biosynthesis pathways in plants (see review by Bouvier et al., 2005). Abbreviations are as follows: acetyl-CoA: acetyl coenzyme-A; FPP: farnesyl diphosphate; GAP: glyceraldehyde 3-phosphate; GGPP: geranylgeranyl diphosphate; IPP: isopentenyl phosphate; MEP: 2-C-methyl-D-erythritol-4-phosphate pathway (also termed the 1-deoxy-D-xylulose-5-phosphate pathway; DOXP); MVA: mevalonic acid pathway; Sq: Squalene. Triterpenoids (e.g., β-amyrin) are produced in the MVA pathway within the cytosol and diterpenoids (e.g., abietic acid) are produced in the MEP pathway within the plastids. There is evidence that IPP is shared between the cytosol and the plastid (Bouvier et al., 2005).

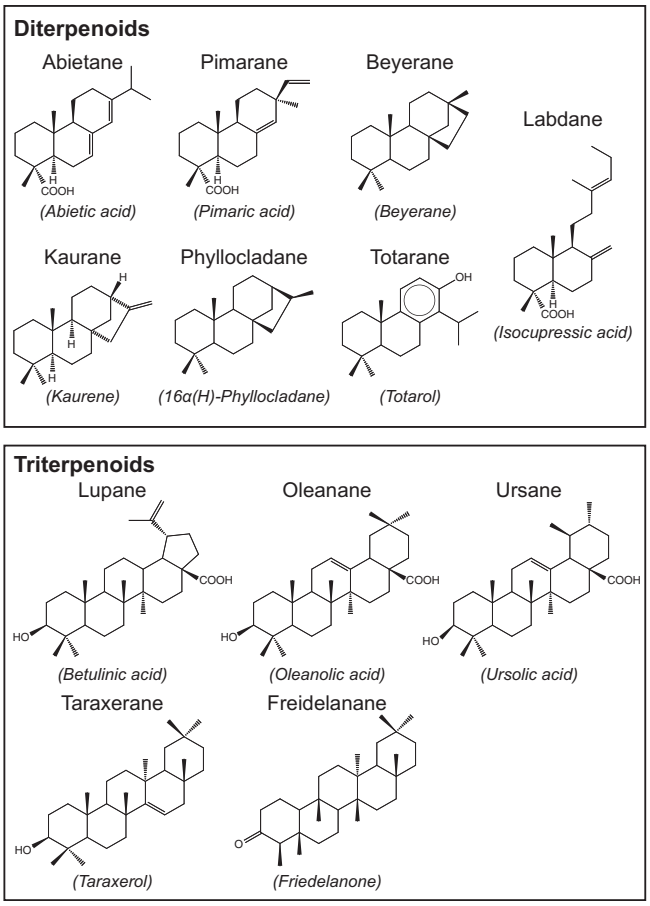

Fig. 2. Major classes of di- and tri-terpenoids are shown with example compounds found in each class (*italics*). The diterpenoids are either tri- or tetracyclic and the triterpenoids are all pentacyclic. Biological forms are commonly functionalized (e.g., acids, alcohols, ketones, aldehydes) although hydrocarbon structures of diterpenoids are also common. Terpenoids generally loose functionality and are preserved as hydrocarbons or aromatic compounds in ancient sediments.

angiosperms, albeit in low concentrations compared to gymnosperms (see below). Angiosperm triterpenoids are most commonly derived from the pentacyclic triterpenoids α- and β-amyrin, which are of the ursane and oleanane classes, respectively. Other important angiosperm compound classes include friedelane, lupane, and taraxerane (ten Haven and Rullkötter, 1988; Woolhouse et al., 1992).

Functional groups (i.e., acid, alcohol, ketone, and ester groups) can be lost from plant terpenoids during photochemical degradation, sedimentary transport, post-depositional diagenesis, or by thermal transformation (e.g., Laflamme and Hites, 1978; Wakeham et al., 1980; Rüllkotter et al., 1994). Defunctionalized terpenoids are found in both recent and ancient sediments as aliphatic and aromatic compounds (e.g., Simoneit, 2005) although occasionally structures are reported with functional groups still intact (Otto and Simoneit, 2001; Otto et al., 2003). The potential utility of terpenoid abundances to constrain sedimentary contributions of different plant groups remains largely unexplored (Moldowan et al., 1994; Bechtel et al., 2005; Taylor et al., 2006; Widodo et al., 2009; Nakamura et al., 2010). Such applications require knowledge of terpenoid concentration in plant tissues and how litter fluxes influence their inputs to the sedimentary environment. They also require understanding of their potential preservation or loss due to post depositional processes.

We measured terpenoid abundance and isotopic data for 44 angiosperm and gymnosperm (all conifers except *Ginkgo*) tree species, representing 21 families and including species with deciduous and evergreen leaf habits. We quantified terpenoid abundances and report these data both by taxa and as mean values for plant functional types. Drawing from ecological studies, we estimate terpenoid fluxes to sedimentary archives for warm temperate forests. In addition, we characterized carbon isotope fractionation (between leaves and lipids) for the tri- and diterpenoids for the same angiosperm and gymnosperm specimens.

2. METHODS AND SAMPLES

2.1. Modern plant samples

Fresh leaf, bark, and branch samples were collected from the sun-exposed side of mature trees (Table 1) at the Pennsylvania State University (University Park, PA, USA) during August and September of 2009 and from two locations in the Bighorn Basin (WY, USA). Characterization of the *n*-alkyl lipids from the leaves of these species

Table 1
Modern plant species, plant functional types, and tissues sampled.

Location[a]	PFT	Family	Species	Tissue sampled
PA	DA	Altingiaceae	*Liquidambar styraciflua*	L, Br
		Betulaceae	*Betula papyrifera*	L, Ba, Br
			Carpinus betulus	L
			Carpinus betulus 'fastigiata'	L
		Cannabaceae	*Celtis occidentalis*	L, Ba
		Cercidiphyllaceae	*Cercidiphyllum japonicum*	L
		Cornaceae	*Cornus florida*	L, Ba
			Nyssa sylvatica	L, Ba
		Fabaceae	*Gleditsia triacanthos* 'inermis'	L, Ba
		Fagaceae	*Fagus grandifolia*	L
			Quercus alba	L, Ba, Br
		Hamamelidaceae	*Hamamelis virginiana*	L
		Juglandaceae	*Carya ovata*	L, Ba
			Pterocarya fraxinifolia	L
		Lauraceae	*Sassafras albidum*	L
		Magnoliaceae	*Magnolia virginiana*	L, Ba
		Malvaceae	*Tilia cordata*	L, Ba, Br
		Platanaceae	*Platanus occidentalis*	L, Ba, Br
		Salicaceae	*Populus deltoides*	L, Ba, Br
			Salix babylonica	L, Ba, Br
		Sapindaceae	*Acer rubrum*	L, Ba, Br
			Aesculus glabra	L, Ba
			Koelreuteria paniculata	L
		Ulmaceae	*Ulmus americana*	L, Ba
			Zelkova serrata	L, Ba
	DG	Cupressaceae	*Metasequoia glypotstroboides*	L, Ba
			Taxodium distichum	L, Ba, Br
		Ginkgoaceae	*Ginkgo biloba*	L, Br
		Pinaceae	*Larix decidua*	L, Ba
	EA	Aquifoliaceae	*Ilex opaca*	L, Br
		Ericaceae	*Kalmia latifolia*	L
			Rhododendron maximum	L
	EG	Cupressaceae	*Cryptomeria japonica*	L, Ba
			Thuja occidentalis	L, Ba, Br
		Pinaceae	*Abies concolor*	L
			Cedrus atlantica 'Glauca'	L, Ba
			Picea abies	L, Br
			Pinus flexilis	L
			Pinus sylvestris	L, Ba, Br
			Pseudotsuga menziesii	L, Ba
WY-CF	DA	Salicaceae	*Populus angustifolia*	L, Ba, Br
			Salix alba	L, Ba, Br
WY-CG	EG	Cupressaceae	*Juniperus osteosperma*	L, Ba, Br
		Pinaceae	*Pinus contorta*	L, Ba, Br
			Pinus flexilis	L, Ba, Br

[a] Abbreviations are as follows: PA, Pennsylvania State University (40.7956°N 77.8639°W, 360 m); WY-CF, Wyoming Cabin Fork (43.98185°N 107.67353°W 1480 m); WY-CG, Wyoming Castle Gardens (42.931057°N 107.617792°W, 1870 m); PFT, plant functional type; DA, deciduous angiosperm; DG, deciduous gymnosperm; EA, evergreen angiosperm; EG, evergreen gymnosperm; Ba, Bark; Br, branch; L, leaf.

has previously been reported (Diefendorf et al., 2011). Samples (~10 g) were rinsed with distilled water, cut into <2 cm pieces, and stored at −20 °C. Samples were freeze-dried, powdered using a ball mill and kept frozen until analysis.

2.2. Plant lipid extraction and fractionation

Powdered samples (~200 mg) were extracted using an accelerated solvent extractor (Dionex ASE 200) with dichloromethane (DCM)/methanol (MeOH) (65:35, v/v) over three extraction cycles at 6.9 MPa (1100 psi) and 100 °C. The total lipid extract (TLE) was concentrated with nitrogen in a Zymark TurboVap LV. The TLE was base saponified, to cleave ester bound fatty acids and alcohols, with 3 ml of 0.5 N KOH MeOH/H_2O (3:1, v/v) for 2 h at 75 °C. After cooling, ~2.5 ml of NaCl in water (5%, w/w) was added to the saponified lipid extract (SLE) and acidified with 6 N HCl to a pH of ~1. The acidic solution was extracted with hexanes/DCM (4:1, v/v), neutralized with $NaHCO_3/H_2O$ (5%, w/w), followed by water removal over Na_2SO_4. The solvent was concentrated to ~1 ml with nitrogen gas and transferred to an 8 ml vial by rinsing with

DCM and ethyl acetate. The SLE was subsequently gently dried under nitrogen, dissolved in DCM/MeOH (1:1, v/v), and stored at −5 °C until analysis.

The SLE was separated into four polarity fractions with 0.5 g of aminopropyl-bonded silica gel in 6 ml glass reaction tubes under pressure (~14 kPa) (Sessions, 2006). Hydrocarbons were eluted with 4 ml hexanes (100%) in the first fraction, ketones were eluted with 8 ml hexanes/DCM (6:1, v/v) in the second fraction, alcohols were eluted with 8 ml of DCM/acetone (9:1, v/v) in the third fraction, and acids were eluted in a fourth fraction with 8 ml of DCM/85% (w/w) formic acid (49:1 v/v). Prior to analyses, aliquots of alcohol and acid fractions were converted to trimethylsilyl (TMS) derivatives by reaction with 50 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 μl of pyridine at 70 °C for 20 min. All fractions were analyzed by gas chromatography (GC), GC/mass spectrometry (MS), and isotope ratio monitoring GC/MS (irm-GCMS).

2.3. Identification and quantification

Lipids were identified in each fraction by GC–MS using a Hewlett–Packard (HP) 6890 GC connected to a HP 5973 quadropole MS with electron-impact ionization. A fused silica capillary column (Agilent J&W DB-5; 30 m, 0.25 mm, 25 μm) was used with helium as the carrier gas. The split/splitless injector was operated in pulsed splitless mode with a temperature of 320 °C. For the hydrocarbon and ketone fractions, the column flow rate was 2.0 ml/min and the oven program started with an initial temperature of 60 °C for 1 min, followed by a ramp to 320 °C at 6 °C/min, and a final hold of 20 min. For the alcohol and acid fractions, the column flow rate was 1.5 ml/min and the oven program started with an initial temperature of 60 °C for 1 min, followed by a ramp to 140 °C at 15 °C/min, then to 320 °C at 4 °C/min, and a final hold time of 20 min. The electron impact MS was operated with a scanning mass range of *m/z* 50–700 at 3 scans per second and an ionization energy of 70 eV. Compounds were identified using published spectra and retention times, authentic standards (α-amyrin, Sigma Aldrich, St. Louis, USA; ursolic acid, MP Biomedicals, Solon, USA; friedelin, Sigma Aldrich, St. Louis, USA), NIST 98 spectral library, and fragmentation patterns.

Prior to quantification of lipids, a known aliquot of each fraction was spiked with internal standards hexadecane and 1,1′-binaphthyl in the hydrocarbon and ketone fractions, and with phthalic acid and 2-dodecanol in the alcohol and acid fractionations. Compounds were quantified on a HP 5890 GC with a flame ionization detector (FID) using GC conditions as described above. Compound peak areas were normalized to those for 1,1′-binaphthyl or phthalic acid and converted to mass quantities using response curves for 31 surrogate standard compounds analyzed in concentrations ranging from 0.1 to 120 μg/ml. Surrogate standard compounds are from Sigma Aldrich (St. Louis, USA) or MP Biomedicals (Solon, USA), are greater than 98% purity, and include *n*-alkanes (C_{14} to C_{20}, C_{25}, C_{27}, C_{29}, C_{34}, C_{38}), *n*-alkanoic acids (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{21}, C_{22}, C_{28}), 3,4-dimethoxybenzoic acid, *n*-alkanols (C_{17}, C_{18}, C_{22}, C_{28}), α-amyrin, cholesterol, friedelin, pristane, squalene, and ursolic acid. Accuracy and precision of measurements are 5.5% (1σ, $n = 56$) and 5.7% (1σ, $n = 56$), respectively, and were determined by treating additional analyses of external standards as unknowns. Plant lipid abundances were normalized to the mass of dry leaf material extracted (e.g., μg/g dry).

2.4. Compound-specific carbon isotope analyses

Compounds were separated on a Varian model 3400 GC equipped with a split/splitless injector operated in splitless mode. A fused silica capillary column (Agilent J&W DB-5; 30 m, 0.32 mm, 25 μm) was used with operating conditions as above. Following GC separation, compounds were combusted over nickel and platinum wire with O_2 in He (1%, v/v) at 1000 °C with the resulting CO_2 monitored using a Finnegan Mat 252 and isotopic abundances determined relative to a reference gas calibrated with Mix A (*n*-C_{16} to *n*-C_{30} alkanes, Arndt Schimmelmann, Indiana University). Carbon isotope values of samples (SA) are reported in delta notation relative to the standard Vienna Pee Dee Belemnite (VPDB) as $\delta^{13}C = [(^{13}R_{SA}/^{13}R_{VPDB}) - 1]$ where $^{13}R = {}^{13}C/{}^{12}C$. Derivatized compounds (acids and alcohols) were corrected for the addition of carbon from the reaction with BSTFA by isotope mass balance. The $\delta^{13}C$ value of added carbon was characterized with phthalic acid (−27.21‰, Arndt Schimmelmann, Indiana University) and was −45.01‰ ± 0.46 (1σ, $n = 8$). Within run precision and accuracy of all samples was determined with co-injected internal standards (*n*-C_{12}, *n*-C_{38}, *n*-C_{41}, 1,1′-binaphthyl) and is 0.16‰ (1σ, $n = 103$) and −0.02‰ ($n = 103$), respectively. Total uncertainty in the derivatized samples is ±0.49‰ based on sum of squares from precision in sample measurements and precision in the TMS carbon.

2.5. Bulk carbon isotope analyses

$\delta^{13}C$ of bulk organic matter and weight percent total organic carbon (wt.% TOC) were determined via continuous flow (He; 120 ml/min) on a Costech elemental analyzer (EA) by oxidation at 1020 °C over chromium (III) oxide and silvered cobalt (II, III) oxide followed by reduction over elemental copper at 650 °C. CO_2 was subsequently passed through a water trap and then a 5 Å molecular sieve GC at 50 °C to separate N_2 from CO_2. CO_2 was diluted with helium in a Conflo III interface/open split prior to analysis. $\delta^{13}C$ values were measured on a Thermo Finnegan Delta Plus XP irm-MS. $\delta^{13}C$ values were corrected for sample size dependency and then normalized to the VPDB scale with a two-point calibration (Coplen et al., 2006) and internal standards. Error was determined by analyzing independent standards as samples across all EA runs. Accuracy was ±0.02‰ ($n = 54$) and precision was ±0.02‰ ($n = 88$; 1σ).

3. RESULTS

3.1. Modern plant terpenoid abundances

Di- and triterpenoid abundances measured in modern leaves ($n = 44$ species), bark ($n = 28$), and branches

($n = 16$) are presented in Table 1, Electronic annex EA-1, and EA-2. Notably, only 5 of 44 specimens are from Wyoming, and therefore we combined all specimens into one dataset; we denote the locations of samples in figures. Molecular abundance data for Pennsylvania and Wyoming specimens are not significantly different in any comparison, even though growing conditions are significantly different between the two locations. Gymnosperms contain substantial quantities of hydrocarbons, alcohols and acids (EA-1 and EA-2). Within the angiosperms, we found abundant alcohols and acids, and minor amounts of ketones. Small amounts of diterpenoid hydrocarbons are present as beyeranes (~0.1 μg/g) in the leaves of *Celtis occidentalis* and *Carya ovata* and as kauranes in *Rhododendron maximum* (61.7 μg/g). In our statistical comparisons of compound abundances by PFTs and phylogeny, we do not include these angiosperm diterpenoids because their abundance is at least 50-fold lower than either triterpenoids in angiosperms and at least 20-fold lower than diterpenoids in gymnosperms (Table 2).

We sum abundances of all compound types (hydrocarbon, ketone, alcohol, and acid fractions) within a compound class (Table 2 and EA-3) for the different plant functional types. Among the gymnosperms, diterpenoid abundances in needles vary considerably by compound class and by leaf habit (Fig. 3). The pimaranes, abietanes, and labdanes are the most abundant forms in evergreen species (2136, 1357, 1402 μg/g) and are minor constituents in the deciduous gymnosperm species (363, 266, 141 μg/g). Minor amounts of beyerane and kaurane compounds are present in most gymnosperms, as well as in the angiosperm species noted previously. Triterpenoids in the ursane class are highly abundant in evergreen and deciduous angiosperm leaves (11,317 and 614 μg/g, respectively). Compounds in the lupane, oleanane, and friedelane classes are more abundant in the evergreen specimens, while compounds in the taraxerane class are present at low abundance in all angiosperms.

Terpenoid abundances (Fig. 4) are highest in specimens from the families Aquifoliaceae, Cornaceae, Cupressaceae, Ericaceae, and Pinaceae. Terpenoid abundances were low or not detected in Altingiaceae, Fabaceae, Ginkgoaceae, Lauraceae, and Magnoliaceae. Total terpenoid abundances are highest in all tissues of the evergreen gymnosperms and in the leaves of evergreen angiosperms (Fig. 5). Triterpenoid abundances are significantly higher in leaves from evergreen angiosperms than in their deciduous counterparts (Wilcoxon rank-sum test, $p = 0.007$). We see the same pattern for the diterpenoid abundances among gymnosperm specimens (Wilcoxon rank-sum test, $p = 0.022$). Statistical comparisons between other plant tissues could not be made due to the small sample sizes.

Terpenoids will be incorporated into soils or sedimentary archives in a manner that reflects their abundance in leaves and other tissues, scaled by litter production in different types of plants. Therefore, to estimate relative proportions of plant groups from mixtures of terpenoids in sediment samples, we must take into account the differential concentrations of compounds and the differential production of litter by deciduous versus evergreen plants and angiosperms versus gymnosperms. We therefore convert our terpenoid abundances to potential compound fluxes using leaf and woody litterfall data for broadleaf evergreen, broadleaf deciduous, needleleaf evergreen, and needleleaf deciduous forests from the global database of Vogt et al. (1986). The warm temperate forest types are most similar to our study areas, although it should be noted that mean values for litterfall fluxes from angiosperm and gymnosperm trees and by leaf life-span are not statistically different for most forest types, including tropical forests (Vogt et al., 1986). Because of this general consistency, we suggest relative fluxes are potentially useful for ancient applications, assuming that ancient plants were similar to their living relatives in the amount and distribution of terpenoids. Because bark is a minor component in woody litter compared to branches (Li et al., 2005) and because the Vogt

Table 2
Leaf total di- and triterpenoid abundances by compound class and plant functional type.

Compound	PFT[a]	DA	EA	DG	EG
Diterpenoid abundances (μg/g leaf)					
Abietane	Mean	0.0	0.0	266.3	1356.8
	SE	0.0	0.0	200.1	418.9
	N	27	3	4	11
Beyerane	Mean	0.0	0.6	0.0	49.2
	SE	0.0	0.6	0.0	49.2
	N	27	3	4	11
Kaurane	Mean	0.0	20.6	0.0	4.1
	SE	0.0	20.6	0.0	3.3
	N	27	3	4	11
Labdane	Mean	0.0	0.0	140.8	1401.9
	SE	0.0	0.0	140.8	683.5
	N	27	3	4	11
Pimarane	Mean	0.1	0.1	22.9	2136.1
	SE	0.1	0.1	15.3	995.4
	N	27	3	4	11
ΣDiterpenoids	Mean	0.1	21.3	430.0	4948.0
	SE	0.1	21.3	353.0	1517.3
	N	27	3	4	11
Triterpenoid abundances (μg/g leaf)					
Lupane	Mean	258.8	1676.6	0.0	0.0
	SE	113.9	1038.4	0.0	0.0
	N	27	3	4	11
Ursane	Mean	614.2	11317.4	0.0	0.0
	SE	466.0	2726.5	0.0	0.0
	N	27	3	4	11
Oleanane	Mean	289.8	1806.6	0.0	0.0
	SE	89.6	453.2	0.0	0.0
	N	27	3	4	11
Fredoleanane	Mean	9.9	438.1	0.0	0.0
	SE	9.9	438.1	0.0	0.0
	N	27	3	4	11
Teraxerane	Mean	0.0	0.0	0.0	0.0
	SE	0.0	0.0	0.0	0.0
	N	27	3	4	11
ΣTriterpenoids	Mean	1172.8	15238.7	0.0	0.0
	SE	514.3	4188.1	0.0	0.0
	N	27	3	4	11

[a] Abbreviations are as follows: PFT, plant functional type; DA, deciduous angiosperms; EA, evergreen angiosperms; DG, deciduous gymnosperms; EG, evergreen gymnosperms.

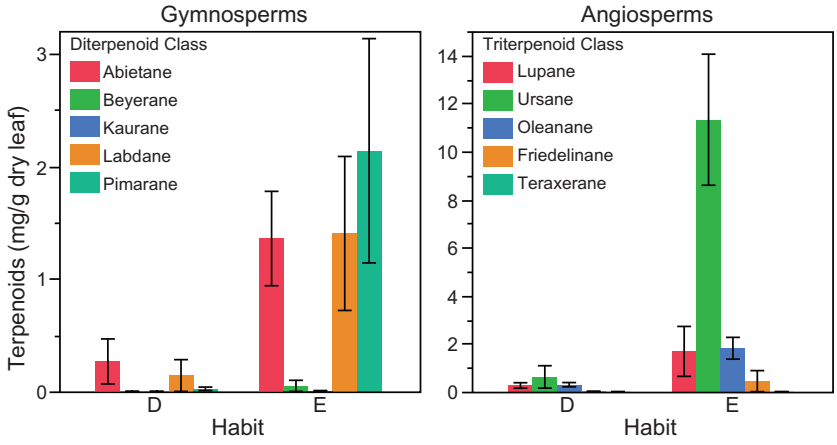

Fig. 3. Mean terpenoid abundances within leaves (mg/g dry leaf) for each classes separated by phylogeny and leaf habit (D, deciduous; E, evergreen). Note the differences in scale. Error bars represent 1 standard error. See Table 2 for means, total number of samples, and standard error.

et al. (1986) dataset does not differentiate between branch and bark litter, we use only branch terpenoid abundance data. Triterpenoid fluxes carried by evergreen angiosperm litter (combined leaf and branch) are higher than for deciduous angiosperms by a factor of 16 (Table 3 and Fig. 6). The potential diterpenoid contribution to soils from evergreen gymnosperm litter is 19-fold higher than from deciduous gymnosperm litter.

3.2. Carbon isotope patterns in modern plant terpenoids

Measured $\delta^{13}C$ values of di- and triterpenoids from deciduous and evergreen species are presented in EA-4. We were not able to measure all terpenoid $\delta^{13}C$ values due to co-elution or low abundances of some terpenoids. We determined values for one to nine individual structures from each of 10 angiosperm species and 15 gymnosperm species. The isotopic difference between plant tissue carbon and compounds, which reflects isotopic fractionation during lipid biosynthesis, is reported in ε notation:

$$\varepsilon_{\text{terpenoid}} = \left[\frac{\delta^{13}C_{\text{terpenoid}} + 1000}{\delta^{13}C_{\text{leaf}} + 1000} - 1\right] \times 10^3 \quad (1)$$

In this study, we represent isotope fractionation during carbon fixation using Δ_{leaf} values:

$$\Delta_{\text{leaf}} = \frac{\delta^{13}C_{\text{atm}} - \delta^{13}C_{\text{leaf}}}{1 + (\delta^{13}C_{\text{leaf}}/10^3)} \quad (2)$$

This unfortunately mixes notation and nomenclature, but it allows our bulk leaf data to be compared to results in the ecological literature (typically presented as Δ_{leaf} values; e.g., Diefendorf et al., 2010 and references therein) and our lipid data to that in the (see Diefendorf et al., 2010 and references therein) geochemical literature, which is typically presented as ε values (e.g., Hayes, 2001; Chikaraishi et al., 2004). As a general comparison between notations, Δ values approximate the opposite sign of ε values when differences between delta (δ) values are small. We provide all measurements in δ notation in EA-4.

$\varepsilon_{\text{terpenoids}}$ values (Fig. 7, Table 4 and EA-5) for the MVA pathway range between −2.5‰ and 1.0‰ with an average of −0.4‰ ($n = 10$). In the MEP pathway, values range between −5.2‰ and 1.1‰ with a mean of 0.6‰ ($n = 10$). ε values are not statistically different between the MVA and MEP-derived compounds (Wilcoxon rank-sum test, $p = 0.910$), nor between evergreen and deciduous leaves (Wilcoxon rank-sum test, $p = 0.362$ and $p = 0.255$, respectively). Diterpenoids (MEP) in bark tissue shows a relatively large range in values (Table 4), although the means for leaves and bark tissues are not statistically different (Kruskal–Wallis test, $p = 0.486$). Lipid ε values for gymnosperms and angiosperms can be compared with those of squalene, which is also produced by the MVA path in all plants (Fig. 7, Table 4 and EA-6). Mean fractionation values for squalene are not statistically different between angiosperms and gymnosperms (Wilcoxon rank-sum test, $p = 0.103$), although the gymnosperm sample set is small ($n = 3$). There is little correlation between isotopic and abundance data when evaluated for all individual molecules. When available data for triterpenoids are pooled, mean isotopic and abundance values also do not correlate. However, for the diterpenoids, a relationship emerges, with more negative fractionation factors associated with higher lipid abundance (ε, ‰ $= 0.87 - 3 * 10^{-4} *$ (diterpenoid abundance, μg/g); $r^2 = 0.75$, $n = 10$, $p = 0.001$). This correlation is strongly pulled by the data for *C. japonica*, which has very high abundances of diterpenoids and significantly more negative fractionation factors than any other species evaluated. When data for *C. japonica* diterpenoids are excluded, the correlation weakens considerably ($r^2 = 0.38$, $p > 0.05$).

4. DISCUSSION

4.1. Terpenoid abundances, litter production and geologic implications

For both angiosperms and gymnosperms, significantly more terpenoids are observed in leaves, bark and branches

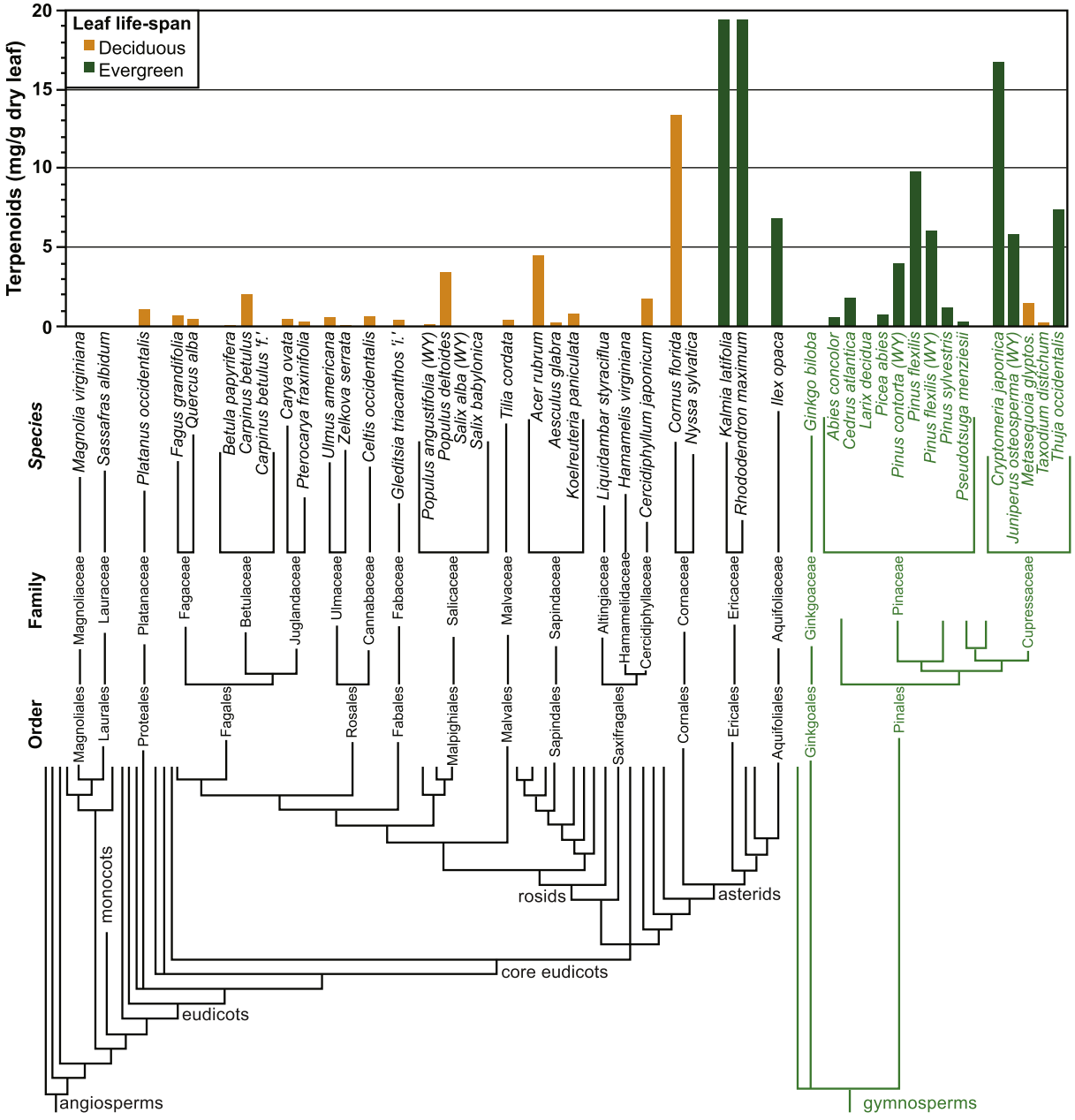

Fig. 4. Terpenoid abundances among leaves (mg/g dry leaf) by phylogenetic groups. Angiosperm phylogeny is constructed following The Angiosperm Phylogeny Group (2009) and gymnosperm phylogeny following Rai et al. (2008). Data is provided in EA-1 and EA-2.

of evergreen plants compared to deciduous species (Figs. 3–5). Evergreen trees are known to contain greater amounts of terpenoids in leaf tissues, and it is thought these compounds help prevent herbivory and increase survival (Coley, 1983; Coley et al., 1985). The importance of terpenoids in plant defense is well known and is even suggested to account for the diversity of terpenoid structures (Langenheim, 1994). Although observed patterns of terpenoid abundance are related to leaf life spans, we caution that the contrast between evergreen and deciduous species may also be influenced by phylogeny (Villar et al., 2006). In our data set most families are represented by either deciduous or evergreen species, though Cupressaceae, which has both, shows the same pattern of higher terpenoid abundance in the evergreen species (Fig. 4).

The quantity of terpenoids received by soils and sediments reflects their abundance in leaves and other tissues scaled by production of these tissues in different types of plants. In order to interpret terpenoid abundances in ancient sediments in terms of the relative abundances of the plants that produced them we must account for both differential concentration in plant tissues and differential production of litter by different taxonomic groups and functional types. In warm temperate forests, terpenoid litter flux is

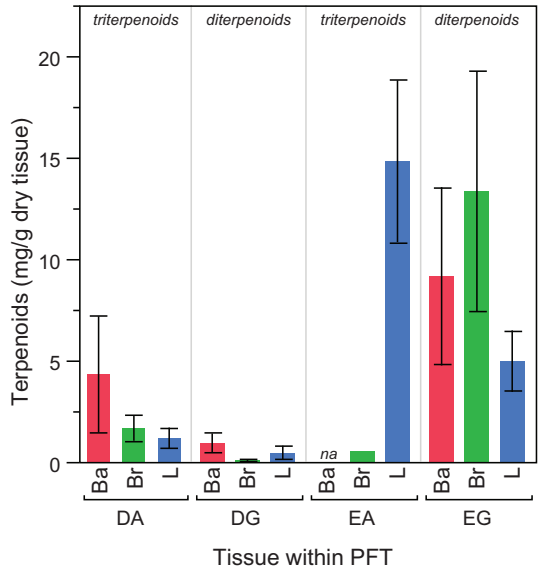

Fig. 5. Mean terpenoid abundances for all tissues (mg/g dry tissue) separated by plant functional type (phylogeny, leaf habit). Error bars represent 1 standard error. See Table 3 for means, total number of samples, and standard error. These values were used to scale to forest production levels (see text and Table 3). Diterpenoids present in angiosperms were not included in the above figure because abundances are minor compared to gymnosperms. Abbreviations are as follows: Ba, bark; Br, branch; L, leaf; DA, deciduous angiosperm; DG, deciduous gymnosperm; EA, evergreen angiosperm, EG, evergreen gymnosperm; na, not available. All values are provided in Table 3.

substantially higher in leaves with longer life-spans within each taxonomic group (Fig 6 and Table 2). Importantly, the fluxes of lipids to the litter for taxonomic groups of similar leaf life-spans (i.e., EA compared to EG or DA compared to DG) are not equal, with lipid fluxes for angiosperms greater than gymnosperms in both cases. The greater angiosperm flux is primarily caused by the higher concentration of terpenoids in angiosperms relative to gymnosperms rather than litter flux differences. If ancient plants had similar tendencies, then our findings suggest sediment terpenoids will over represent angiosperm taxa slightly more than gymnosperms, provided the influence of transport and preservation are similar for both compound types. More importantly, lipids from taxa with long leaf life-spans will dominate contributions to the sedimentary archive. Di- and triterpenoid ratios can be a useful proxy for gymnosperm/angiosperm ratios (Bechtel et al., 2003, 2005; Schouten et al., 2007; Widodo et al., 2009), provided paleobotanical evidence for leaf life-spans of major taxa are available. For example, in paleofloras that include a mixture of evergreen and deciduous trees, like many mid-latitude Paleogene floras (Wing, 1987), the evergreen taxa would likely be over-represented in the sedimentary terpenoid record. This could influence estimates of the relative abundances of taxonomic groups from terpenoids if plant phenology and taxonomic group covaried, for example if all the gymnosperms were deciduous. Studies of fossil floras also need to account for the possibility that different terpenoid compounds degrade at different rates (Nakamura

Table 3
Terpenoid abundances for leaves and branches scaled to litter production levels.

PFT[a]	Tissue	Diterpenoid mean (μg/g)	SD	N	Triterpenoid mean (μg/g)	SD	N	Litter (kg ha^{-1} yr^{-1})[b]	SD	N	ΣDLP (kg ha^{-1} yr^{-1})[c]	ΣSD[d]	ΣTLP (kg ha^{-1} yr^{-1})[c]	ΣSD[d]
DA	Br				1636	1928	10	891	262	7				
DA	L				1163	2675	27	4236	575	12			6	11
DG	Br	62	87	2				1107	722	7				
DG	L	430	706	4				4432	936	16	2	3		
EA	Br				531		1	3690	512	2				
EA	L	21	37	3	14,801	6904	3	6484	2737	5			98	60
EG	Br	13,310	14,514	6				1107	722	7				
EG	L	4948	5032	11				4432	936	16	37	29		

[a] Abbreviations are as follows: PFT, Plant functional type; DA, deciduous angiosperm; DG, deciduous gymnosperm; EA, evergreen angiosperm; EG, evergreen gymnosperm; Br, branch; L, leaf; N, number of samples; SD, standard deviation; DLP, diterpenoid litter production; TLP, triterpenoid litter production.
[b] Leaf and woody litter production for temperate forests from Vogt et al. (1986).
[c] ΣDLP and ΣTLP are the sum of all diterpenoids or triterpenoids, respectively, for leaves and branches scaled by forest litter production for each tissue.
[d] ΣSD is the total standard deviation of the terpenoid abundances for each PFT and is calculated from error propagation of the individual standard deviations for terpenoid and litter production within each tissue.

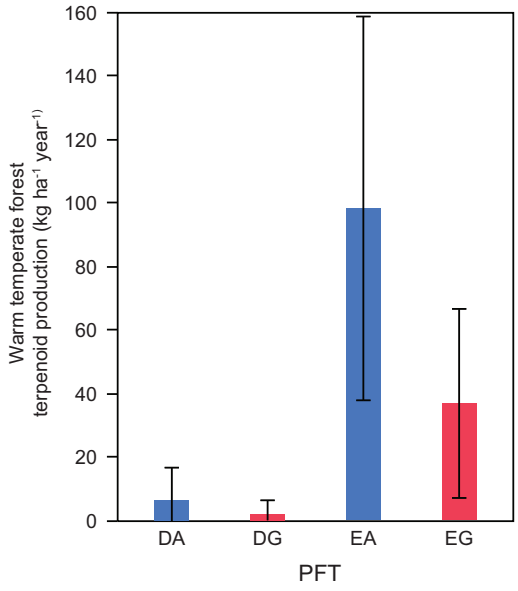

Fig. 6. Terpenoid production estimates for warm temperate forests (kg ha^{-1} yr^{-1}) using terpenoid abundance data for branches and leaves (Figure ure5) and scaling to the forest level using data from Vogt et al. (1986). Flux estimates are based on triterpenoids for angiosperms and on diterpenoids for the gymnosperms. Errors are propagated standard deviations (root sum of squares) for terpenoid abundances and forest ranges to capture the natural variability within each population. Abbreviations are as follows: DA, deciduous angiosperm; DG, deciduous gymnosperm; EA, evergreen angiosperm, EG, evergreen gymnosperm. All values are provided in Table 3.

et al., 2010), or have different potential for transport to sedimentary settings (Medeiros and Simoneit, 2008).

4.2. Carbon isotope data

Carbon isotope fractionation during terpenoid lipid synthesis, as measured relative to leaf tissue, is small in both the MVA and MEP pathways (Fig. 7 and Table 4) as indicated by small differences in the $\delta^{13}C$ values between terpenoids and bulk tissues. ε values are significantly less negative than observed for *n*-alkyl lipids (Diefendorf et al., 2011) where the primary source of fractionation occurs during the oxidation of pyruvate to acetyl CoA (DeNiro and Epstein, 1977). This step is also present in the MVA pathway, yet triterpenoids have $\varepsilon_{terpenoid}$ values that are ~4–6‰ ^{13}C enriched relative to *n*-alkyl lipids in the same plant tissue. In addition, the similarity in ε values between both the MVA and MEP pathways is notable given the differences in biochemical intermediates, enzymes, and locations of biosynthesis within the cell (Bouvier et al., 2005). This could be accounted for by MVA and MEP pathways exchanging or 'sharing' IPP, which has been documented previously (Bartram et al., 2006).

The evergreen conifer, *C. japonica* (Cupressaceae) contains both triterpenoids (minor) and diterpenoids (abundant). Its lipids were also studied by Chikaraishi et al. (2004), who reported $\delta^{13}C$ values for squalene, β-sitosterol, and sesquiterpenes that were −1.7‰ to −3.1‰ lower than whole leaf tissue values. In addition, they reported phytol and diterpenoids were depleted in ^{13}C relative to leaf tissue by −3.6‰ to −5.9‰ and that *n*-alkyl lipids were depleted by −2.4‰ to −9.9‰. Our lipid isotopic results are similar

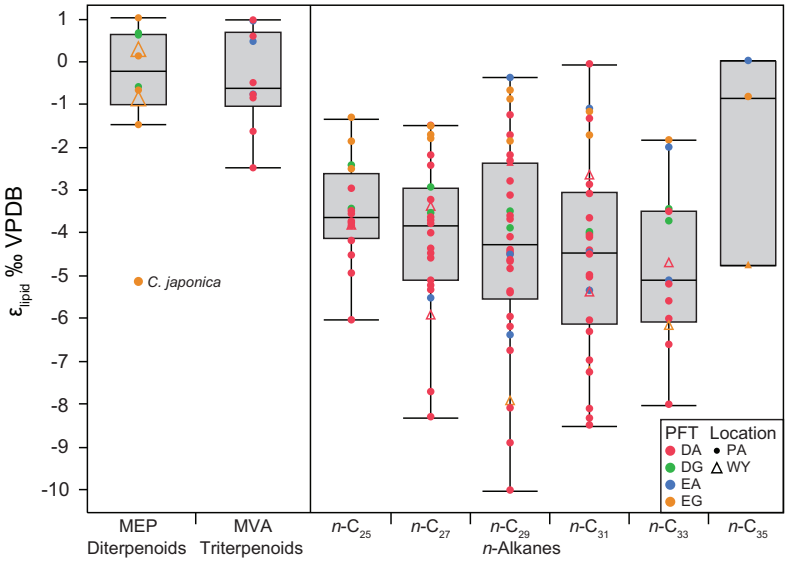

Fig. 7. Box and whisker plots for ε_{lipid} values (‰, VPDB) of the diterpenoids synthesized in the MEP pathway (gymnosperms), the triterpenoids in the MVA (angiosperms), and the *n*-alkanes in the acetogenic pathway (angiosperms and gymnosperms). Within each phylogeny, leaf habits were not statistically different from each other. Box and whisker plots show the median, upper and lower quartiles, and maximum and minimum values, with outlier values shown as black dots. Abbreviations are as follows: A, angiosperm; G, gymnosperm; D, deciduous, E, evergreen; PA, Pennsylvania; WY, Wyoming. All values are provided in Table 4, EA-5, and EA-6. *n*-Alkane values are reported in Diefendorf et al. (2011).

Table 4
Average ε values for compound classes within the MVA and MEP pathways in tree leaves.

Pathway[a]	Compound class	PFT or phylogeny	ε_{lipid} ‰ VPDB	SD	*N*
MVA	Triterpenoid	DA	−0.6	1.2	7
MVA	Triterpenoid	EA	0.2	0.9	3
MEP	Diterpenoid	DG	0.3	0.7	3
MEP	Diterpenoid	EG	−0.9	2.0	7
MVA	Triterpenoid	A	−0.4	1.2	10
MEP	Diterpenoid	G	−0.6	1.8	10
MVA	Squalene	DA	−0.1	1.5	20
MVA	Squalene	DG	0.9	0.7	2
MVA	Squalene	EA	2.1	2.4	2
MVA	Squalene	EG	2.1	na	1
MVA	Squalene	A	0.1	1.7	22
MVA	Squalene	G	1.3	0.8	3

[a] Abbreviations are as follows: MVA, mevalonic acid pathway; MEP, 2-C-methyl-D-erythritol-4-phosphate pathway; PFT, plant functional type; A, angiosperm; D, deciduous; E, evergreen; G, gymnosperm; SD, standard deviation; *N*, number of species.

for both the linear lipids (Diefendorf et al., 2011) and terpenoids (this study); our *C. japonica* diterpenoid $\varepsilon_{terpenoid}$ values average −5.2‰ (SD = 0.8‰, 4 diterpenoids measured; EA-5). Although our findings are consistent with the prior work, it is notable that diterpenoids in *C. japonica* are significantly more depleted in ^{13}C than terpenoids in all other plants in this study, including three other species in the same family (Fig. 7). An explanation for the divergence in $\varepsilon_{terpenoid}$ values for *C. japonica* is not currently available. This species has similar terpenoid abundances to other conifers at this site, has similar total extractable terpenoids as other conifers (Yatagai and Sato, 1986), and has similar volatile terpenoid emissions to other conifers (Bao et al., 2008). It is unclear what factors or mechanisms might account for the greater ^{13}C depletion in the lipids of this species.

The smaller averaged ε values for lipids synthesized via the MVA and MEP pathways relative to those from the acetogenic path are consistent with observations reported in other studies. For example, in *Phaseolus lunatus* (lima been; Jux et al., 2001; Bartram et al., 2006), $\delta^{13}C$ values of volatile sesquiterpenoids (MVA; $\delta^{13}C = -37.4‰$) and ocimene (MEP; $\delta^{13}C = -28.5‰$) are both enriched relative to acetogenic lipids (hexenyl aetate, $\delta^{13}C = -40.2‰$). In algae, palmitic acid (acetogenic pathway) has $\delta^{13}C$ values that are 2–5‰ lower than phytol (MEP pathway; Schouten et al., 1998). In *Quercus rubra*, β-carotene (MEP pathway) has an ε value of ∼−2.6‰, which is less negative than fractionation factors determined for fatty acids (∼−4.1‰; acetogenic pathway; Sharkey et al., 1991).

Isoprene, a volatile terpenoid produced in large quantities by most plants and synthesized in the MEP pathway, can be formed from *de novo* synthesis of photosynthetic carbon or from alternate sources of carbon including Calvin cycle intermediates, glycolysis, starch reserves (Affek and Yakir, 2003; Schnitzler et al., 2004; Ghirardo et al., 2010), and from xylem-transported starches (Kreuzwieser et al., 2002). Under periods of water stress, when stomata are closed, stored biochemical resources become the primary source of carbon for isoprene synthesis (e.g., Kreuzwieser et al., 2002; Affek and Yakir, 2003). Given that starch reserves are typically ^{13}C enriched relative to bulk leaf tissue (e.g., Park and Epstein, 1961), starch carbon could potentially explain the higher $\varepsilon_{terpenoid}$ values compared to *n*-alkyl lipids. Alternatively, or perhaps additionally, production and release of large quantities of even a slightly ^{13}C-depleted volatile isoprene could leave a residual pool of IPP that is relatively ^{13}C-enriched, effectively decreasing isotopic differences between terpenoid lipid and bulk leaf carbon.

Another possibility is related to the timing of di- and triterpenoid synthesis. If terpenoids were produced when photosynthesis is not active, such as at night, they could be produced from stored carbon resources, such as starch, that are ^{13}C enriched relative to bulk tissue. This mechanism has been suggested to explain ^{13}C enriched wood and other plant tissues (Cernusak et al., 2009; Wegener et al., 2010). Similarly, terpenoids have a defensive role, and plants could be required to produce large quantities in a short period of time, possibly exceeding recently fixed carbon supplies. If so, then synthesis would be likely to tap starch or other stored carbon reservoirs within the plant that are enriched in ^{13}C.

It is likely that multiple factors control the $\delta^{13}C$ values of terpenoids within plants. Future studies that focus on the sources of carbon and the timing of di- and triterpenoid synthesis will help to clarify the role of different agents. Such studies would potentially benefit from ^{13}C-labeling methods similar to previous studies of isoprene (e.g., Kreuzwieser et al., 2002; Affek and Yakir, 2003). A recent study has identified the role of insects in causing ^{13}C-enrichment in modern tree resins and fossiliferous ambers (McKellar et al., 2011). Future work will need to determine if this is related to carbon flux changes that are potentially initiated by insect attack.

4.3. Terpenoid carbon isotope patterns in ancient sediments

There are consistent differences in the carbon isotopic composition of di- and triterpenoids and *n*-alkanes relative to one another in modern plants and ancient terrestrial environments as shown in Fig. 8 (Schoell et al., 1994; Simoneit et al., 1995; Schouten et al., 2007). In all of the geological examples from continental settings, $\delta^{13}C$ values for

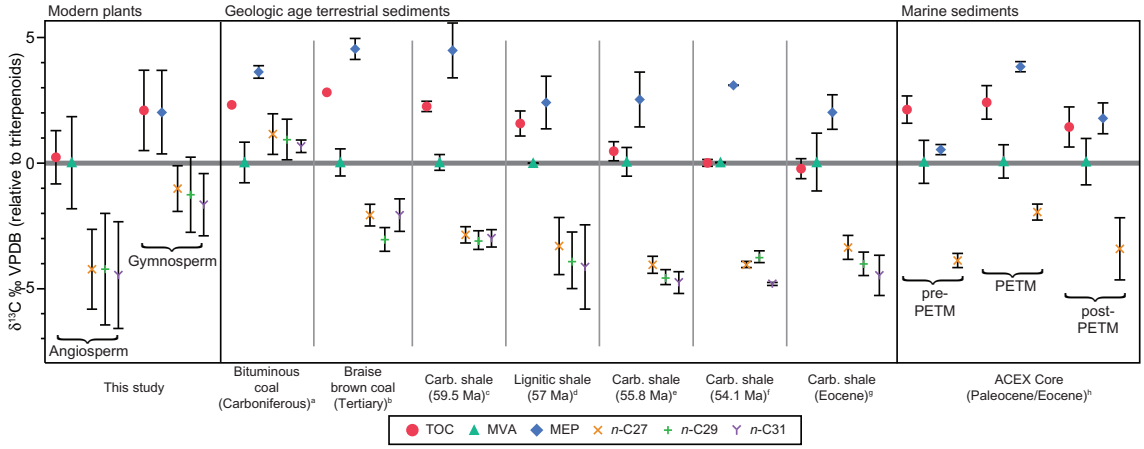

Fig. 8. $\delta^{13}C$ values of di- and triterpenoids, *n*-alkanes, and $\delta^{13}C_{TOC}$ values from modern plants and geologic examples. For comparison, $\delta^{13}C$ values have been plotted relative to triterpenoid (MVA) values at each site (grey line) thus easing comparison between times with different atmospheric $\delta^{13}C$ values. Geologic samples are as follows: (a) Carboniferous bituminous coal from Ukraine (Simoneit et al., 1995), (b) Tertiary Braise brown coal from China (Schoell et al., 1994), (c–g) Bighorn Basin (WY, USA) Paleocene/Eocene samples (Diefendorf, 2010), and (h) marine sediments from the ACEX core from before, during and after the Paleocene–Eocene Thermal Maximum (PETM; Schouten et al., 2007). Error bars represent the standard deviation for all species measured (this study) and the standard deviations for all compounds measured (Schoell et al., 1994; Simoneit et al., 1995; Schouten et al., 2007).

diterpenoids are typically higher than triterpenoids by ~3‰. This is consistent with the 2–3‰ difference observed between modern angiosperm and gymnosperm leaves (Brooks et al., 1997; Murray et al., 1998; Arens et al., 2000; Pataki et al., 2003; Diefendorf et al., 2010, 2011) and resins (Murray et al., 1998). In most of the ancient samples, triterpenoids are ~5–6‰ enriched in ^{13}C relative to *n*-alkanes (also likely from angiosperms; Fig. 8; Diefendorf et al., 2011), consistent with the small fractionation factors for terpenoid synthesis observed in this study.

The bituminous coal from the Carboniferous (Simoneit et al., 1995) is an exception to this pattern. In the coal, *n*-alkane values are ~1‰ higher than triterpenoid values and 3–4‰ lower than values for diterpenoid lipids. The origin of triterpenoid lipids in a sample of this age is enigmatic, given that angiosperm triterpenoids are rare in sediments or oils until the Jurassic and become increasingly abundant in Cretaceous and younger samples (Moldowan et al., 1994), tracking an hypothesized increase in angiosperm abundance (Wing and Boucher, 1998). The triterpenoids and *n*-alkanes in these Carboniferous coals (Simoneit et al., 1995) may be derived from pre-angiosperm seed plants (Moldowan et al., 1994; Taylor et al., 2006).

Carbon isotope values of total organic carbon in the terrestrial sediments are variable, but in almost all of the geologic samples, $\delta^{13}C_{TOC}$ values fall between the measured values for triterpenoids that represent MVA (angiosperm) inputs and those from MEP (gymnosperm). This pattern is consistent with a largely terrestrial origin for TOC, and with the 2–3‰ isotopic enrichment generally observed for gymnosperm relative to angiosperm leaf biomass (Diefendorf, 2010). We note that other factors can influence $\delta^{13}C_{TOC}$ values in fine-grained terrestrial archives, especially within wet depositional settings that tend to preserve higher amounts of organic matter. For example, bacterial organic matter is likely an important source of carbon in some samples as indicated by high concentrations of hopanes (Diefendorf, 2010) and would potentially lower $\delta^{13}C_{TOC}$ values to some degree.

Climate could influence the leaf-lipid isotopic differences (ε_{lipid}), potentially resulting in divergent bulk organic carbon and molecular isotopic records or potential differences in the isotopic records provided by *n*-alkanes, triterpenoid and diterpenoids. Such differences might arise, for example, with less seasonal temperature and/or precipitation that led to longer growth seasons, and longer leaf life-spans, resulting in increased alkane and terpenoid production. Changes in mean annual or seasonal water availability could be accompanied by either changes in the timing of lipid synthesis or shifts in the relative use of stored intermediates or newly produced carbohydrates as carbon substrates for IPP production. This influence is similar to that postulated to account for differences in ε_{lipid} values for *n*-alkanes between temperate C3 trees (which exhibit values of ~−4‰ to −5‰) and tropical species with significantly lower ε_{lipid} values (Diefendorf et al., 2011).

The geochemical record provides an example of divergent isotopic records during a large climate change event. In the Arctic, $\delta^{13}C$ values for terpenoids from before, during, and after the Paleocene–Eocene Thermal Maximum (PETM) are highly variable, and record different magnitudes of the carbon isotope excursion associated with the hyperthermal event (Schouten et al., 2007). These patterns have been suggested to reflect different climate sensitivity in water-use efficiency for the parent vegetation. However the ε_{lipid} data presented here for terpenoids and previously for *n*-alkanes (Diefendorf et al., 2011) provide a new context for considering the biomarker isotope patterns observed in Arctic sediments. In particular, the pre-PETM terpenoid isotope values stand out as anomalous compared to modern patterns as well as the signatures for these compounds observed in PETM and post-PETM samples. In the latter, triterpenoids (MVA, angiosperms) have isotopic values that are ~4‰ higher than *n*-C_{27} alkanes, which is what

we would expect if *n*-alkanes are derived primarily from angiosperms (Diefendorf et al., 2011). Further, diterpenoids from the PETM and post-PETM samples are all ^{13}C enriched relative to triterpenoids, consistent with the general enrichment of gymnosperm leaves relative to angiosperms in modern vegetation. In contrast, the pre-PETM isotopic values for triterpenoids and diterpenoids differ only slightly (~0.5‰). In addition, $\delta^{13}C_{TOC}$ values are higher than all lipid $\delta^{13}C$ values in the pre-PETM sample. The causes of (a) similar triterpenoid and diterpenoid-lipid isotope values and (b) higher than expected TOC values are not clear, but they suggest the pre-PETM experienced differences in the source, transport or preservation of organic matter compared to PETM or post-PETM samples. We note the pre-PETM samples are characterized by the highest BIT index values, which also suggests differences in the supply or preservation of terrestrial organic matter input compared to PETM and post-PETM sediments (Sluijs et al., 2006).

Post-PETM and PETM $\delta^{13}C$ values between di- and triterpenoids and *n*-alkanes in the Arctic samples (Fig. 8) are similar to relationships observed in modern vegetation. Both times exhibit about 6‰ differences between diterpenoids and *n*-alkanes, consistent with differences in modern gymnosperms and angiosperms. We note the isotopic difference between *n*-alkanes and triterpenoids is ~2‰ for the PETM, and ~4‰ for the post-PETM, and both values are within ranges observed in modern vegetation. Alternatively, the increasing offset between the *n*-alkanes and triterpenoids could also indicate (1) modest deposition of remobilized ^{13}C enriched pre-PETM alkanes in the PETM or (2) a change in ε_{lipid} patterns within angiosperm lipids during the hyperthermal. For all of the samples in the Schouten et al. (2007) study, gymnosperm derived terpenoids are higher in abundance than angiosperm derived terpenoids. Thus gymnosperm lipids are overrepresented compared to pollen-based estimates (Sluijs et al., 2006; Schouten et al., 2007) even when accounting for differences in terpenoid production between deciduous angiosperms and gymnosperms, possibly caused by many factors outlined in Schouten et al. (2007).

We suggest shifting plant lipid sources in the surrounding landscape, a change in allocation of carbon resources (and ε_{lipid} values) within plants, and/or changing modes of transport potentially contributed to the shifting lipid carbon isotope differences relative to one another observed in the Arctic samples. Although our study cannot independently address these important factors, they demonstrate how abundance and isotopic data in modern plants can be used to identify potentially anomalous patterns in the past. These findings highlight the need for greater understanding of terpenoid $\delta^{13}C$ values under different ecologic, transport and depositional systems.

5. CONCLUSIONS

Evergreen trees produce significantly higher abundances of terpenoids than deciduous species, regardless of major taxonomic group, consistent with prior studies showing that leaf lifespan is important in determining the chemical investment by plants in protecting their leaves from herbivores. Terpenoid abundance patterns can be generalized and scaled using litter flux data from extant forests. Terpenoid relative abundances in geological samples can thus be interpreted in terms of relative abundances of different plant types on ancient landscapes, provided the leaf life-spans of the fossil flora can be constrained. Using litter flux and terpenoid abundance data from living forests, we would predict that angiosperms and gymnosperms produce similar amounts of terpenoids in proportion to their biomass in warm temperate forests. These compounds can potentially provide insights to the relative abundances of angiosperms and gymnosperms in past environments, provided differences in preservation and transport of the molecules are taken into account.

Carbon isotope fractionation during terpenoid synthesis is similar for the MVA and MEP pathways in modern plants, and 3–5‰ higher than fractionation in the acetogenic pathway (Diefendorf et al., 2011). As a result, average terpenoid $\delta^{13}C$ values are similar or slightly lower than those of bulk leaf tissue. We speculate that this pattern may be related to the use of stored starch compounds within the plant for terpenoid synthesis or loss of volatile isoprenes that leave a ^{13}C-enriched residual IPP pool.

By studying terpenoids, it is possible to track $\delta^{13}C$ values of gymnosperms and angiosperms independently, lending insight to how these plant groups responded to past climatic change and how their distribution influenced temporal and spatial changes in $\delta^{13}C_{TOC}$ (e.g., Holdgate et al., 2009). Carbon isotope signals from angiosperms and gymnosperms in ancient ecosystems are also important for understanding physiological responses to warm and high-CO_2 climates, which will be important for anticipating the results of increasing anthropogenic greenhouse gas emissions (e.g., Tylianakis et al., 2008).

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2012.02.016.

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