Organic Geochemistry 82 (2015) 22–32

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Origin and sedimentary fate of plant-derived terpenoids in a small river catchment and implications for terpenoids as quantitative paleovegetation proxies

Sharmila J. Giri [a,b], Aaron F. Diefendorf [a,*], Thomas V. Lowell [a]

[a] Department of Geology, University of Cincinnati, PO Box 210013, Cincinnati, OH 45221-0013, USA
[b] Department of Marine Geosciences, Rosenstiel School of Marine & Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA

ARTICLE INFO

Article history:
Received 9 July 2014
Received in revised form 2 February 2015
Accepted 3 February 2015
Available online 14 February 2015

Keywords:
Plant terpenoids
Biomarkers
Paleovegetation
Rivers
Soil
Transport
Alteration
POM
DOM

ABSTRACT

Tricyclic diterpenoids and non-steroid or non-hopanoid pentacyclic triterpenoids are almost exclusively taxon-specific terrestrial plant biomarkers produced by conifers and angiosperms, respectively. Due to this source specificity and their prevalence in the geologic record, these compounds are often used to reconstruct paleovegetation. However, the physical and chemical processes that influence the dispersal and fate of terpenoids in sedimentary archives are poorly constrained. Modern fluvial systems can be used as ancient river analogs to provide information on the utility of terrestrial plant terpenoids as paleovegetation proxies by defining their potential flux and identifying the processes that control their transport to, and deposition and degradation in, sediments. To determine if the contribution of terpenoids from vegetation is reflected in forested soil and river sediments and to constrain the dispersal of these compounds in fluvial systems, di- and triterpenoid concentrations in Miners River drainage basin (Upper Peninsula of Michigan, USA) were quantified. In the basin, evergreen conifers are less abundant than deciduous angiosperms, but yet contribute substantially more terpenoids to soils and river sediments when scaled for leaf litter production and present vegetation cover. The composition and relative concentration of di- and triterpenoids in source vegetation do not match those in soils and river sediments, suggesting that some process or processes result in the preferential removal of diterpenoids. While the soil and river sediment terpenoid concentration, corrected for differential terpenoid inputs, can closely predict the basin wide vegetation cover in Miners River drainage basin, the extent to which terpenoids can be used a paleovegetation proxy in other modern or geologic sediments remains unclear.

1. Introduction

Rivers transport a substantial amount of organic carbon (0.4 Gt C/yr) and thus provide an integral pathway for terrigenous plant matter dispersal and integration from land to lacustrine and marine sediments (McKee et al., 2004; Pancost and Boot, 2004). Terrigenous plant biomarkers are useful tools for studying organic matter (OM) in rivers because they are source-specific and remain relatively unchanged during transport and post-depositional processes (ten Haven and Rullkötter, 1988; Otto and Simoneit, 2001; Otto et al., 2002, 2003; Pancost and Boot, 2004). Due to this source specificity and sedimentary resilience, they can also be used as paleovegetation proxies in geologic settings where pollen and macrofossils are absent (e.g. Bechtel et al., 2003; Diefendorf et al., 2014).

Tricyclic diterpenoids and non-steroidal pentacyclic triterpenoids are terrestrial plant biomarkers synthesized almost exclusively by higher plants (conifers and angiosperms). Plant terpenoids have multiple roles in plant function, including defense against insect herbivory and plants must biosynthesize a diverse array of compound structures to maintain toxicity (Langenheim, 1994). Most conifers in North America today (Pinaceae and Cupressaceae) produce tricyclic diterpenoids (DTs) in the abietane and pimarane classes (e.g. Otto and Simpson, 2005), while angiosperms commonly produce triterpenoids (TTs) in the lupane, oleanane and ursane classes (e.g. Diefendorf et al., 2012; Fig. 1). On average, terpenoids account for 1–2% of dry leaf mass (Langenheim, 1994), but terpenoid synthesis varies between conifers and angiosperms and between leaf lifespans (deciduous vs. evergreen). Angiosperms produce terpenoids in greater concentration than conifers of the same leaf lifespan and evergreen taxa produce a higher concentration of terpenoids than deciduous taxa. This difference between taxonomic groups and leaf lifespans

* Corresponding author. Tel.: +1 513 556 3787.
E-mail address: aaron.diefendorf@uc.edu (A.F. Diefendorf).

http://dx.doi.org/10.1016/j.orggeochem.2015.02.002
0146-6380/

suggests that angiosperms and evergreen taxa may be over represented in sedimentary deposits (Diefendorf et al., 2012).

Terpenoids are common constituents of plant tissue (leaves, bark or roots) transferred to soils and sediments as plant debris decomposes (Langenheim, 1994). Terpenoids are subject to early alteration and degradation and only a small portion is transferred to sedimentary archives (Hedges and Prahl, 1993; Hautevelle et al., 2006; Jacob et al., 2007; Bechtel et al., 2008; Diefendorf et al., 2014).

Due to the widespread distribution of DTs and TTs in sediments and their qualitative reflection of major taxonomic groups, they are commonly used to infer paleovegetation in terrestrial, lacustrine and marine sediments (e.g. Bechtel et al., 2003, 2005; Pancost and Boot, 2004; Schouten et al., 2007). For more accurate paleovegetation reconstruction, differences in terpenoid production between major taxonomic groups are required (Diefendorf et al., 2012, 2014).

Few studies have evaluated whether DT/TT ratios in modern river systems reflect the surrounding plant community (Medeiros and Simoneit, 2008; Medeiros et al., 2012) and understanding the distribution of these compounds in fluvial systems is important for interpreting the geologic terpenoid record. Various taphonomic processes likely result in the leaves of some species entering river systems over others (Spicer, 1981; Burnham, 1989; Greenwood, 1991; Burnham et al., 1992; Ellis and Johnson, 2013) and differential preservation potential and water solubility of DTs vs. TTs may bias the terpenoid composition in rivers, thereby transferring it to the sedimentary record. Preferential alteration and degradation in the sedimentary archive will add additional complications (Wakeham et al., 1980; Hedges and Prahl, 1993). To explore terpenoid composition and concentration changes between source and transport, we used a small modern river system. The focus on terpenoids in the sedimentary record has been studied elsewhere (e.g. Diefendorf et al., 2014) and we instead focus here on the transfer of terpenoids from source vegetation to soils and into river systems to evaluate any bias in transport and early alteration/degradation. This provides an analog for testing the utility of DT/TT ratios as a quantitative paleovegetation proxy before terpenoids enter long term sedimentary archives.

2. Site description

The study focused on Miners River drainage basin (7044 ha) in Alger County of the Upper Peninsula of Michigan (MI; USA). This watershed includes Miners River, a small stream (13.4 km long), which drains into Lake Superior (Fig. 2; Handy and Twentner, 1985). The area was chosen because it is an entirely freshwater system, draining through a mixed conifer–angiosperm forest. The river discharge is highest in late spring and early summer and ranges from 0.4 m^3/s in August to 3.0 m^3/s in April (Handy and Twentner, 1985). The stream substrate consists mainly of bedrock, cobble and gravel. Fine grain sediments are deposited and accumulate in areas of low flow (Handy and Twentner, 1985). Theiver drains through Miners Lake, a small teardrop-shaped lake with a maximum depth of 4.0 m and average depth of 1.9 m. Several small groundwater springs are found along the river and lake (Loope, 2004).

The Upper Peninsula of MI experiences a cool continental climate, which is heavily influenced by Lake Superior. The average annual temperature is 5 °C and average annual precipitation 85.9 cm, 32% of which falls as snow (Mechenich et al., 2006). The underlying Cambrian sandstone and surficial Pleistocene and Holocene deposits control the topography of the region (Handy and Twentner, 1985). The regional soils derive from underlying sandstone units and glacial material and vary in their OM abundance (ca. 1–50%). Miners River drains through soils from the Munising–Stuben Association, a dominantly loamy soil underlain with sand, as well as the Kalkaska Association, a sandy, moderately well drained soil. The sandy subsoil of the Munising–Stuben Association prevents deep rooting by plants (Berndt, 1977).

The river drainage basin commonly has an assemblage of taxa for a temperate broadleaf angiosperm and conifer needle afforestation. The present plant community composition has been extensively mapped within the boundaries of Pictured Rocks National Lakeshore (Hop et al., 2010). Plant community is patchy and varies depending on substrate type, moisture gradient and drainage patterns (Read, 1975). Hardwood deciduous angiosperm forests, comprising *Acer rubrum*, *Acer saccharum*, *Acer spicatum*, *Alnus viridis*, *Alnus* spp., *Betula alleghaniensis* and *Fagus grandifolia* are the predominant vegetation of the Munising–Stuben Association, while evergreen conifers, including *Picea glauca*, *Pinus resinosa*, *Thuja occidentalis* and *Tsuga canadensis*, are prevalent on the Kalkaska Association (Berndt, 1977). Overall, the drainage basin broadly represents a forest community that is 14% needle leaf conifers, 80% broad leaf angiosperms and 6% other vegetation (herbaceous plants, grasses, sedges, etc.; Hop et al., 2010). Due to extensive logging of conifers in the late 1800s, the vegetation of the Upper Peninsula changed quite drastically and the forest has transitioned from a predominantly coniferous forest to the present day mixed conifer–angiosperm one (Frederick et al., 1977).

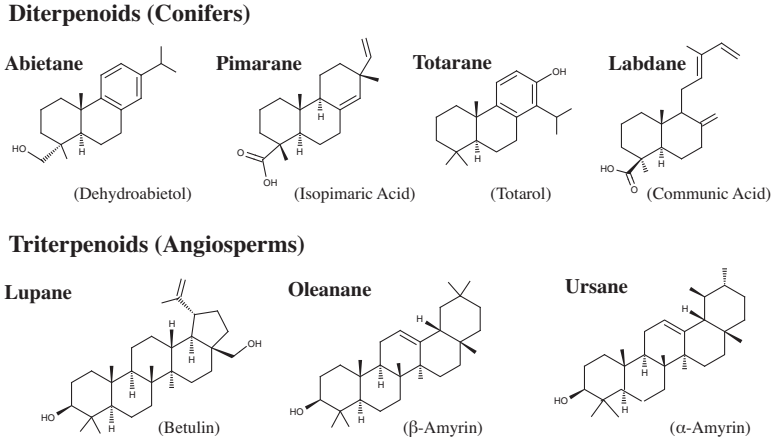

Fig. 1. DT and TT structures by compound class (classes in bold). Examples for compounds in each class are shown (names in parentheses).

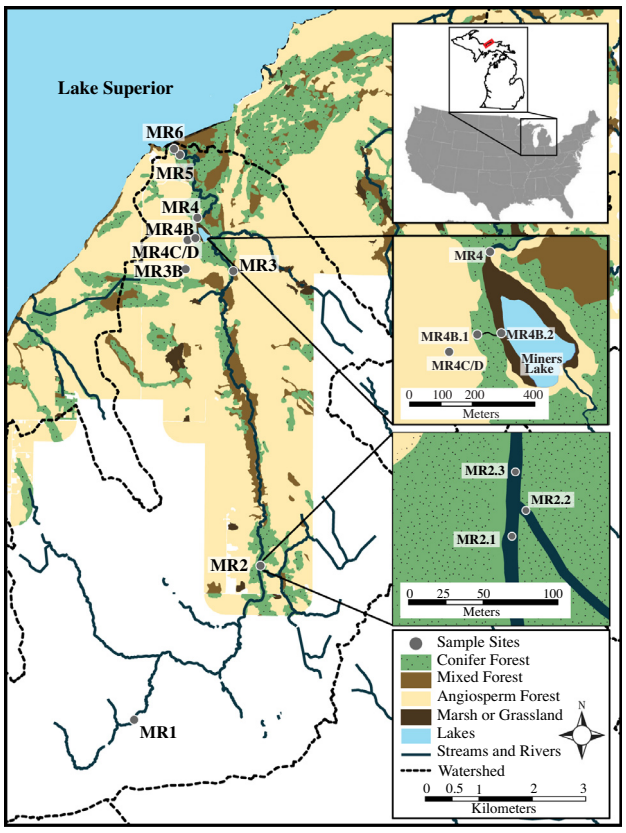

Fig. 2. Vegetation map of Miners River drainage basin and sampling locations. Vegetation data are only available for areas within the boundaries of Pictured Rocks National Lakeshore. Developed areas within the park boundaries are shown in white. Adapted from Hop et al. (2010).

3. Methods

3.1. Plant and sediment sampling

Plant samples, soil and river sediments were collected from six sites along the river (Fig. 2; Table 1), in June, 2012, when river discharge was the greatest (Mechenich et al., 2006). A total of 3.4 cm of rain fell during the week prior to sampling, but no rainfall events were recorded for the week of sampling (June 25th–30th, 2012; NOAA National Climatic Data Center). Surface stream discharge was measured at each site along the river using the cross sectional area of the stream and the surface water velocity (Table 1).

Mature leaves (ca. 10 g) were collected from all species at each site. Soil samples (ca. 200 g) were collected from the top 10 cm of the soil profile under trees sampled for fresh vegetation. Soils were categorized as either upland soils, collected from the older upper terrace, or valley floor soils, collected from the lower terrace. River sediment samples (ca. 200 g) were collected from areas of low river flow and included point bar and overbank deposits as potential recorders of sediments transported during highflow conditions or storm events. All samples were frozen (−5 °C) until analysis.

3.2. Particulate and dissolved OM sampling

Particulate OM (POM) samples (12–53 l) were collected in situ using a diaphragm pump and pre-combusted (460 °C, 8 h) glass microfiber filters (Whatman, GF/F, 142 mm diam., 0.7 μm pore size) and were frozen at −5 °C until analysis. Dissolved OM samples (10 l) were collected from each sampling site in polycarbonate bottles (Nalgene) and later filtered to isolate dissolved terpenoids. These samples were first filtered with a glass vacuum filtration unit with pre-combusted glass microfiber filters (Whatman, GF/F, 47 mm diam., 0.7 μm pore size) to remove POM and then acidified to pH ca. 2–3 with 1 M HCl (Kim et al., 2003). C_{18} solid phase extraction (SPE) disks (3M Empore, 47 mm diam., 0.5 mm thickness; 3M, St. Paul, MN, USA) were then used to isolate DOM by modifying the methods of Carrera et al. (1998, 2001) and Kim et al. (2003). Briefly, SPE disks were cleaned by eluting 20 ml MeOH and 20 ml dichloromethane (DCM) at ca. 30 ml/min. SPE disks were then conditioned with ca. 10 ml MeOH for 3–5 min and rinsed with ca. 10 ml water prior to sample adsorption at ca. 30 ml/min. SPE disks were rinsed with ca. 10 ml water to raise sample pH and then dried under vacuum for 5 min. the disks were frozen (−5 °C) until returned to the lab where dissolved lipids were desorbed with 60 ml MeOH and 60 ml DCM. Desorbed lipids were base hydrolyzed and analyzed as described below.

3.3. Total organic carbon (TOC)

Sediment samples were decarbonated with 1 M HCl and TOC values measured with a Flash 2000 NC soil analyzer (Table E-1). Precision and accuracy, calculated from standards run along with samples ($n = 22$), were 0.21% and 0.04%, respectively. TOC values for plant samples were approximated using data from Diefendorf et al. (2012) for evergreen conifers and deciduous angiosperms in the same families as those found in Pictured Rocks National Lake shore. TOC ranged from 48.0–51.9% for evergreen conifers ($n = 8$) and 44.4–50.1% ($n = 14$) for deciduous angiosperms (Diefendorf et al., 2012). Average TOC values were used for conifers (50.0% OC) and angiosperms (47.7% OC).

3.4. Extraction and hydrolysis

Plant, soil and sediment samples were freeze-dried and homogenized with a mortar and pestle. Powdered plant (ca. 100 mg), soil, river sediment (ca. 1–10 g) and POM (50% of a 147 mm filter) samples were extracted with an accelerated solvent extractor (Dionex ASE 350) with 2:1 (v/v) DCM/MeOH. Samples were subjected to three extraction cycles at 10.34 MPa (1400 psi) and 100 °C. The total lipid extract (TLE) was saponified to cleave ester groups with 2.5 ml 0.5 N KOH in MeOH/water (3:1, v/v) for 2 h at 75 °C, following the method reported by Diefendorf et al. (2012). Aliquots of saponified TLE were derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Sigma Aldrich, St. Louis, MO, USA) and pyridine at 70 °C for 15 min.

3.5. Terpenoid assignment and quantification

Lipids were analyzed using gas chromatography–mass spectrometry (GC–MS) and by flame ion detection (FID) with an Agilent 7890A GC instrument interfaced to an Agilent 5975C quadrupole mass selective detector (MSD), with electron ionization (70 eV). Lipids were separated on a fused silica column (Agilent J&W DB-5 ms; 30 m × 0.25 mm i.d., 25 μm film thickness) and a 5 m guard column (Restek Rxi, 5 m × 0.32 mm). The carrier gas was He at 1.5 ml/min. Following separation, column effluent was split equally between the MSD and the FID with a 2-way splitter (Agilent Microfluidics G3180B; He constant pressure makeup at 3.8 psi).

Aliquots of plant samples were injected in an Agilent Multimode Inlet in pulsed splitless mode and an injector temperature of 300 °C. Alternatively, aliquots of sediment and POM samples were injected in pulsed splitless mode with an injector temperature of 320 °C. The oven program for plant, sediments and POM samples was: 60 °C (1 min) to 130 °C at 20 °C/min, then to 320 °C (held 10 min) at 4 °C/min. DOM samples (16 μl) were

Table 1
Miners River sampling locations and stream data (nd, no data; L, location -used when multiple samples were taken at a site; V, vegetation samples; S, soil samples – refer to Supplemental Material Table E-1 for soil classifications as either upland soils or valley floor soils; RS, river sediment samples; TSS, total suspended samples). % Conifer and % angiosperm cover are visual approximations of the vegetation at each sampling site.

Site No		Site description	Latitude (°)	Longitude (°)	Water pH	Water temp (°C)	Stream discharge (m^3/s)	Volume water filtered (l)	TSS (mg/l)	Conifer cover (%)	Angiosperm cover (%)	Samples collected
MR1		Headwater Tributary	46.39801	-86.55407	6.33	nd	nd	22.7	1.1	76	24	V, S, RS, POM, DOM
MR2	L.1	Upstream River – confluence	46.42459	-86.52395	7.98	17.4	0.28	53	5.7	76	24	V, S, RS, POM, DOM
	L.2	Tributary			8.27	12.4	0.06	53	2.3			
	L.3	Downstream River – confluence			8.05	16.9	0.31	11	2.3			
MR3		Miners Falls	46.42433	-86.53143	8.22	18.3	0.23	10	2.0	50	50	V, S, POM, DOM
MR3B		Miners Falls Parking Lot	46.47451	-86.54327	nd	nd	nd	nd	nd	0	100	V, S
MR4		Miners Lake	46.48316	-86.54006	8.09	18.5	nd	15	1.5	94	6	V, S, POM, DOM
MR4B	L.1	Spring Source	46.48053	-86.54046	7.64	7.4	nd	12	0.0	94	6	S, RS, POM, DOM
	L.2	Spring Delta			8.32	8.7	> 0.01	12	2.2			
MR4C		Ridge Top Vegetation Survey	46.47999	-86.54173	nd	nd	nd	nd	nd	3	97	V, S
MR4D		Ridge Top Vegetation Survey	46.48142	-86.54353	nd	nd	nd	nd	nd	3	97	V
MR5		Abandoned Beaver Dam	46.49388	-86.54462	8.18	18.4	0.54	17	2.5	87	13	V, S, RS, POM, DOM
MR6		River Mouth	46.49475	-86.54531	8.29	19.5	0.49	15	2.4	85	15	V, S, RS, POM, DOM

injected in programmed temperature vaporization (PTV) mode, with an initial inlet temperature of 40 °C, and heated to 325 °C at 60 °C/s. The MSD range was *m/z* 30–700 at 3 scans/s and the ionization energy 70 eV. Compounds were assigned with authentic standards, library databases (2009 Wiley and 2008 NIST), published spectra and retention times. A few compounds had similar spectra to previously identified terpenoids, but retention times were not consistent and are thus reported as unknowns (Table E-2).

Plant, sediment and POM compounds were quantified using FID and the internal and external standards. Peak areas were normalized to either dodecan-2-ol (for plants and sediments), or undecanoic acid (for POM) and converted to mass with external standard response curves ranging in concentration from 0.5–100 μg/ml. External standards included isopimaric acid, α-amyrin, lupeol, uvaol, betulin, oleanolic acid, betulinic acid and ursolic acid. The coefficient of variation (CV) was calculated for the concentrations of all 8 external standards run as unknowns. The CV for the 8 external standards ranged from 0.11–0.32, meaning that the standard deviation was 11–32% of the mean concentration of each standard. Three of the external standards had a CV > 0.2. Plant and sediment terpenoid concentrations were normalized to TOC (μg/g OC; Section 3.3). TOC was not measured for POM and instead terpenoid concentrations were normalized to volume of water filtered (ng/l).

DOM terpenoid concentrations were quantified using the MSD with ion. Standard compound responses were assigned using the most abundant ions (Filley et al., 2008; Mueller et al., 2012). Extracted ion peak areas were converted to mass with ion response curves for external standards, ranging in concentration from 0.5–64 μg/ml. The CV ranged from 0.16–0.27 for the 8 external standards. Three of the external standards had CV > 0.2. DOM terpenoid concentration was normalized to volume of filtered water (ng/l).

3.6. Pictured Rocks National Lakeshore plant community composition

The vegetation cover in the drainage basin was estimated using plant community data for the portion of Miners River drainage basin that falls within the boundaries of Pictured Rocks National Lakeshore (USGS-NPS Vegetation Characterization Program; Fig. 2; Hop et al., 2010). Sampling Site 1 fell outside the park boundary but, based on field observations, the vegetation cover at this site is consistent with other angiosperm-dominated sampling sites within the park. USGS-NPS vegetation data were categorized as angiosperm, conifer, mixed conifer–angiosperm, grassland, and marshe. The latter two groups were categorized separately because they did not contain trees and were a minor part of the landscape. The total area of the mapped watershed, along with the area of all vegetation polygons of the same class (angiosperm, conifer, mixed, grassland or marsh) were measured with ImageJ (Rasband, 2012), assuming the canopy and sub-canopy vegetation cover in angiosperm and conifer polygons was 100% angiosperms or conifers. USGS-NPS vegetation inventory survey data for vegetation plots (VP) and accuracy assessment (AA) site canopy and sub-canopy data, collected by Hop et al. (2010), were combined and used to determine the proportion (%) vegetation cover in areas of mixed conifer–angiosperm cover. A total of 5 VP and 6 AA sites in the drainage basin fell within areas of mixed vegetation cover and these data were used to calculate the proportional cover of conifers and angiosperms within areas of mixed vegetation.

3.7. Calculation of terpenoid flux from modern vegetation

To determine the input of terpenoids from vegetation in the drainage basin, terpenoid synthesis was scaled for leaf litter production and % vegetation cover. This conversion is our first order approximation of annual rate of terpenoid input to modern sediments. The following equation was used to convert terpenoid synthesis to terpenoid flux:

$$\mathrm{DTflux} = (\mathrm{DLS})(\text{conifer litter production})(\%\ \text{conifer cover}) \quad (1)$$

$$\mathrm{TTflux} = (\mathrm{TLS})(\text{angiosperm litter production}) \times (\%\ \text{angiosperm cover}) \quad (2)$$

where DLS is the DT lipid synthesis and TLS the TT lipid synthesis. Litter production was not measured in the basin and was inferred

from cold temperate forest litter production from Vogt et al. (1986). Standard errors of all values were error propagated using the Monte Carlo method to determine the total standard deviation of the flux (Anderson, 1976).

3.8. Terpenoids as paleovegetation proxies

To determine if the present plant community was represented in surface sediments, the plant community composition of the basin was back calculated with substrate (soils, river sediments, POM and DOM) terpenoid concentrations, corrected for lipid synthesis, to adjust for differences in terpenoid synthesis by evergreen conifers and deciduous angiosperms (Diefendorf et al., 2012, 2014). The present plant community was estimated using the following equations modified from Bechtel et al. (2003) and Diefendorf et al. (2014):

$$\%\ \text{Conifer cover} = \frac{\frac{\sum \text{DT}}{\text{DLS}}}{\frac{\sum \text{DT}}{\text{DLS}} + \frac{\sum \text{TT}}{\text{TLS}}} \quad (3)$$

$$\%\ \text{Angiosperm cover} = \frac{\frac{\sum \text{TT}}{\text{TLS}}}{\frac{\sum \text{DT}}{\text{DLS}} + \frac{\sum \text{TT}}{\text{TLS}}} \quad (4)$$

where ΣDT is the mean substrate DT concentration of all measured DT compounds, ΣTT the mean substrate TT concentration of all measured TT compounds, and DLS and TLS are plant DT and TT synthesis. Standard errors for all values were error propagated using the Monte Carlo method to determine the total standard deviation of the percent vegetation cover (Anderson, 1976). It is important to note here that the data are not normally distributed and the mean plant community composition calculated in the Monte Carlo simulation is a first order estimate.

3.9. Statistical analysis

A regression model was used to determine how much of the variability in soil terpenoid concentration could be explained by needle/leaf terpenoid concentration (Mueller et al., 2013). Needle/leaf and soil terpenoid concentrations were natural log transformed to improve the normality of the data distribution and the analysis focused on 79 observations of average soil DT concentration and 80 observations of average soil TT concentration across all sampling sites, excluding 35 observations where DTs and 57 observations where TTs were not measured in soils or needles/leaves. Median DT and TT concentrations were compared among all sediment types (upland, valley floor and river sediments) with a Kruskal–Wallis test, and pair-wise Wilcoxon rank-sum tests were performed on median DT or TT concentrations for each combination of sediment type. POM and DOM DT and TT concentrations were also compared with Wilcoxon rank-sum tests. All statistical analyses were completed with JMP Software 10.0 or 11.0.

4. Results

DT and TT concentrations were quantified along the river for evergreen conifers, deciduous angiosperms, sediments, POM and DOM. DTs of the abietane, totarane, pimarane and labdane classes and TTs of the oleanane, ursane and lupane classes were most abundant and were measured for all sample types. Total DT and TT concentrations were reported and represent the sum of all compounds. One adjustment was made for total TT concentration: β-amyrin (oleanane class) and germanicol (oleanane class) coeluted with β-sitosterol in soil and river sediment samples and these compounds were therefore excluded from total plant TT values to make them comparable with other substrate TT concentrations.

4.1. Plant terpenoid concentrations

Terpenoids in the needle/leaf tissue of conifers and angiosperms present at each sampling site were analyzed (Fig. 3; Table E-3). Concentration of DTs in evergreen conifers ranged from 3431–16237 μg/g OC (n = 15; Table E-3a). *T. occidentalis* had the widest diversity and produced compounds of the pimarane, abietane and totarane classes and was the only species to produce sempervirol. Communic acid (labdane class) was also present, but below the limit of quantitation. *P. resinosa*, *P. glauca* and *T. canadensis* produced fewer DTs than *T. occidentalis* and did not produce totarane DTs. Total DT concentrations from *T. occidentalis* and *P. resinosa* were an order of magnitude higher than those from *P. glauca* and *T. canadensis*. Levopimaric acid and methyl sandaracopimarate (quantified in other substrates) were not found in any of the needles collected throughout the basin. TTs in deciduous angiosperms ranged from 79–769 μg/g OC (n = 14; Table E-3b). Oleanane, ursane and lupane class TTs were found in *Alnus* spp. and *A. rubrum*, while *A. saccharum*, *A. spicatum* and *B. alleghaniensis* contained only oleanane and ursane TTs, and *F. grandifolia* had only ursane and lupane TTs. Two compounds, the unknown TT with $M^{+\cdot}$ at m/z 586 and uvaol were not found in any leaves collected along the river, but were found in valley floor soils and POM. Evergreen conifers and deciduous angiosperms also differed substantially in the concentration of DTs and TTs. The total mean of all DTs was 37× higher than TTs (Fig. 3).

4.2. Modern plant contribution to sediments

Estimating the potential flux of DTs and TTs from plants to soils required correcting plant lipid synthesis for both modern

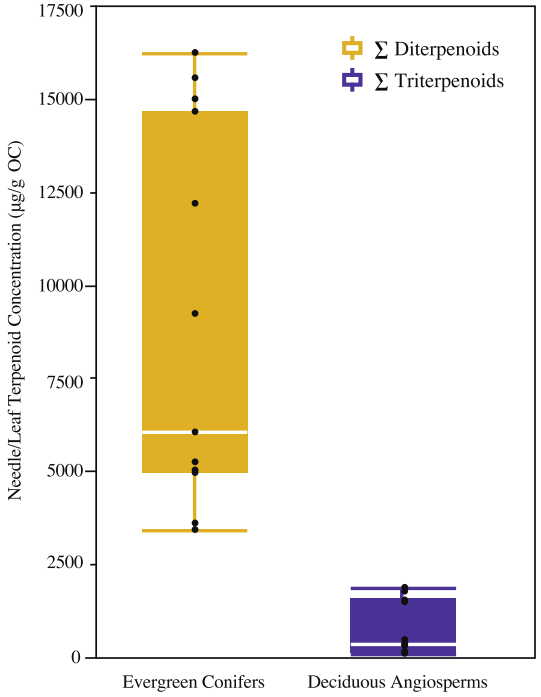

Fig. 3. DT and TT synthesis for evergreen conifers and deciduous angiosperms. Box and whisker plots show median, minimum and maximum values and the upper and lower quartiles. All samples are shown and values are given in Table E-3. Median values and one standard deviation are: evergreen conifers 6052 ± 4817 μg/g OC (n = 15); deciduous angiosperms 145 ± 189 μg/g OC (n = 14).

vegetation cover and litter production (Eqs. 1 and 2). Vegetation data collected by Hop et al. (2010) for the portion of the drainage basin within Pictured Rocks National Lakeshore were used to estimate the present plant community composition (80% angiosperms, 14% conifers and 6% grassland or marsh; Fig. 2; Hop et al., 2010). Litter production between evergreen needleleaf conifers (3144 ± 194 kg/ha/yr) and deciduous broadleaf angiosperms (3854 ± 213 kg/ha/yr) does not significantly differ in cold temperate forests (Vogt et al., 1986). When cover and litter production were accounted for, conifers contributed 6.3× more DTs (1.9 ± 0.4 kg/ha/yr) to the sediments than angiosperm TTs (0.3 ± 0.1 kg/ha/yr; Table 2; Fig. 4).

4.3. Sediment terpenoid concentration

Terpenoids were found in all soils and sediments throughout the drainage basin ($n = 23$; Fig. 5; Table E-4). Combined soil and sediment DT concentrations ranged from 0–968 μg/g OC (Table E-4a). Methyl sandaracopimarate was found only at Sites 1, 2, 4, 4B, 5 and 6, and was interestingly the only hydrocarbon biomarker that occurs in the drainage basin. No conifers were present at Site 3B but DTs were detected in the soil (114 μg/g OC; Table E-4a). Needle DTs were on average 1-2 orders of magnitude higher than soil DTs (Fig. 6). Needle DT concentration explained ca. 23% (R^2 0.23, $p < 0.001$) of the variability in soil DT concentration in a least-squares linear regression analysis. Combined soil and sediment TT concentration ranged from 20–875 μg/g OC (Table E-4b). Almost all TT compounds in the basin were in higher concentration in soils than in leaves, with the exception of α-amyrin, which was almost always higher in leaves than in soils (Fig. 6). There was no significant correlation between leaf and soil TT concentrations.

The distribution and concentration of terpenoids in the basin were examined further between three different sediment types: upland soils, valley floor soils and river sediments (Fig. 5; Table E-4). Upland soil DTs ranged from 0–114 μg/g OC, valley floor soils from 108–968 μg/g OC and river sediments from 115–578 μg/g OC. Upland soil mean DT concentrations (38 ± 38 μg/g OC, 1σ) were statistically different from valley floor (404 ± 68, 1σ; Wilcoxon rank-sum test, $p = 0.01$) and river sediments (390 ± 70, 1σ; $p = 0.03$), but the valley floor soil mean DT concentrations were not statistically different from river sediments. Upland soil TTs ranged from 20–101 μg/g OC, valley floor soils from 47–875 μg/g OC and river sediments from 88–501 μg/g OC. Mean TT concentrations in upland soils (60 ± 24 μg/g OC) were marginally lower than valley floor soils (263 ± 80 μg/g OC) and river sediments (312 ± 72 μg/g OC; Wilcoxon rank-sum test, $p = 0.06$).

The concentrations of terpenoids in soils and sediments did not vary along the river despite some variation in vegetation (Fig. 6; Table 1). DT to TT concentrations in upland and river sediments were not significantly different, but were different in valley floor soils (Wilcoxon rank-sum test, $p = 0.05$; Fig. 5). There were no

Table 2
Scaled plant influx of DT and TT by plant functional type (PFT).

PFT	Mean terpenoid concentration (μg/g dry leaf)	SE[c]	Litter production[d] (kg/ha/yr)	SE[c]	Vegetation cover[e](%)	SE[c]	Flux (kg/ha/yr)	ΣSD[f]
EC[a]	4336	622	3144	194	14	1.6	1.9	0.4
DA[b]	113	24	3854	213	80	1.6	0.3	0.1

[a] EC, evergreen conifer.
[b] DA, deciduous angiosperm.
[c] SE, standard error.
[d] Data from Vogt et al. (1986),
[e] Data from Hop et al. (2010); standard error was calculated with the confidence interval for the vegetation map.
[f] ΣSD is the total standard deviation calculated with the Monte Carlo method using the standard deviations of mean terpenoid concentrations, litter production and % vegetation cover.

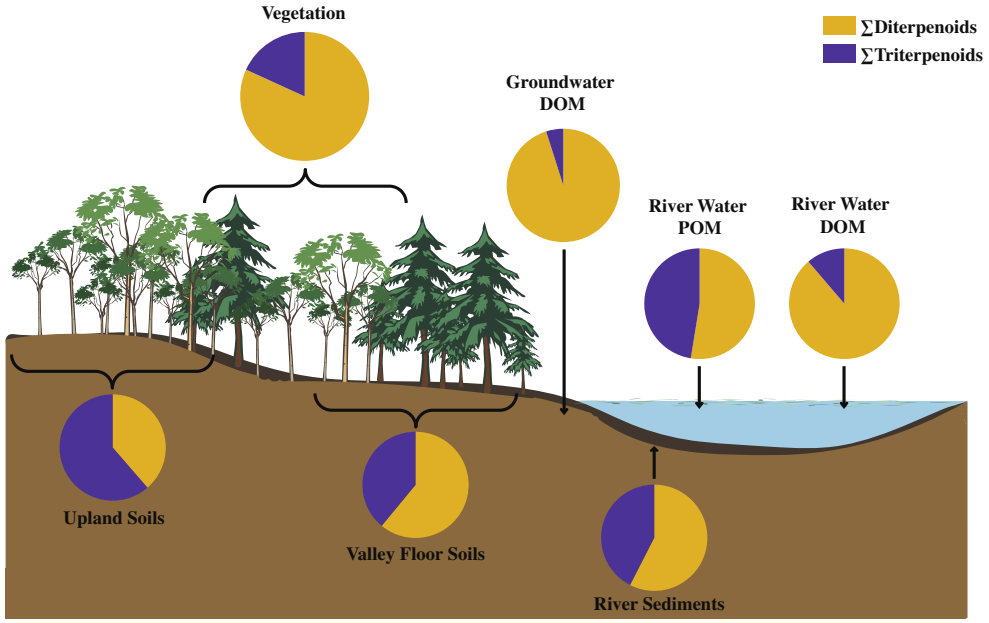

Fig. 4. Cross section (not to scale) of Miners River and DT/TT ratios for all sample types.

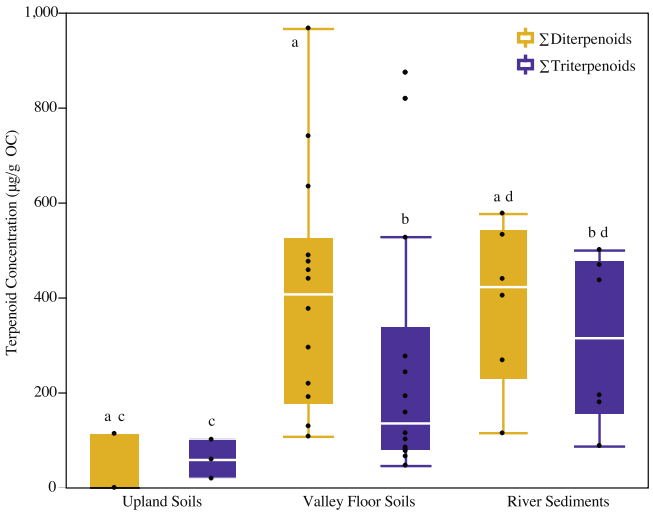

Fig. 5. Distribution of basin-wide DT and TT abundances by sediment type. Box and whisker plots show median, minimum and maximum values and the upper and lower quartiles. All values from samples are given in Table E-4. Median values and one standard deviation area; upland soils 0 ± 66 µg/g OC for DT and 60 ± 41 µg/g OC (n = 3) for TTs; valley floor soils 409 ± 253 µg/g OC for DT and 137 ± 278 µg/g OC (n = 14) for TTs; river sediments 422 ± 173 µg/g OC for DT and 316 ± 177 µg/g OC (n = 6) for TTs. Boxes with the same letters are not statistically different.

significant differences for DT or TT concentrations in these soils and sediments between upstream (Sites 1, 2 and 3) and downstream sampling sites (Sites 4, 5, and 6). The average DT/TT ratio for the upland, valley floor and river was 0.6, 1.5, and 1.3, respectively (Fig. 4).

4.4. POM and DOM terpenoid concentrations

DTs and TTs were found in both river POM and DOM fractions (Fig. 7; Tables E-5 and 6). POM DTs ranged from 39–609 ng/l and TTs from 104–366 ng/l in river water (n = 8; Table E-5). The average DT/TT ratio for POM was 1.1. DT and TT concentrations in the POM fraction of river water were not statistically different (DTs 195 ± 66 ng/l; TTs 176 ± 27 ng/l; Fig. 7).

DOM DTs ranged from 44–194 ng/l and TTs from 9–17 ng/l in river water (n = 8). In groundwater, DOM concentrations were 14 ng/l for DTs and 1 ng/l for TTs (n = 1; Table E-6). The average DT/TT ratio was 7.8 for river water DOM and 14 for groundwater (Fig. 4). Basin-wide DT concentration in the DOM fraction of river water (101 ± 15 ng/l) was significantly higher than TT concentration (13 ± 1 ng/l; Wilcoxon rank-sum test, $p < 0.001$). DT concentration in DOM was not significantly different from DT concentration in POM (Wilcoxon rank-sum test). TTs were significantly less abundant in DOM than POM (Wilcoxon rank-sum test, $p < 0.001$; Fig. 7).

4.5. Estimates of plant community composition from terpenoids

To test whether substrate terpenoid concentrations reflected the composition of overlying vegetation, DT and TT concentrations in soils, sediments, POM and DOM were corrected for plant lipid synthesis (Eqs. 3 and 4). Valley floor and river sediments had similar concentrations of DTs and TTs and we calculated the vegetation to be 4% conifer and 96% angiosperms for valley floor soils and 3% conifer and 97% angiosperm for river sediments (Table 3). Basin-wide plant community composition was also calculated with terpenoid concentrations in the POM and DOM fractions of river and was estimated to be 3% conifers and 97% angiosperms for the POM fraction of river water and 18% conifers and 82% angiosperms from the DOM fraction of river water.

5. Discussion

Terpenoids are often used to reconstruct plant paleoecology (Schouten et al., 2007; Bechtel et al., 2003, 2005). However, few quantitative studies of plant, soil and sediment terpenoid concentrations have been carried out (Medeiros and Simoneit, 2008; Medeiros et al., 2012). In order to evaluate the potential of using

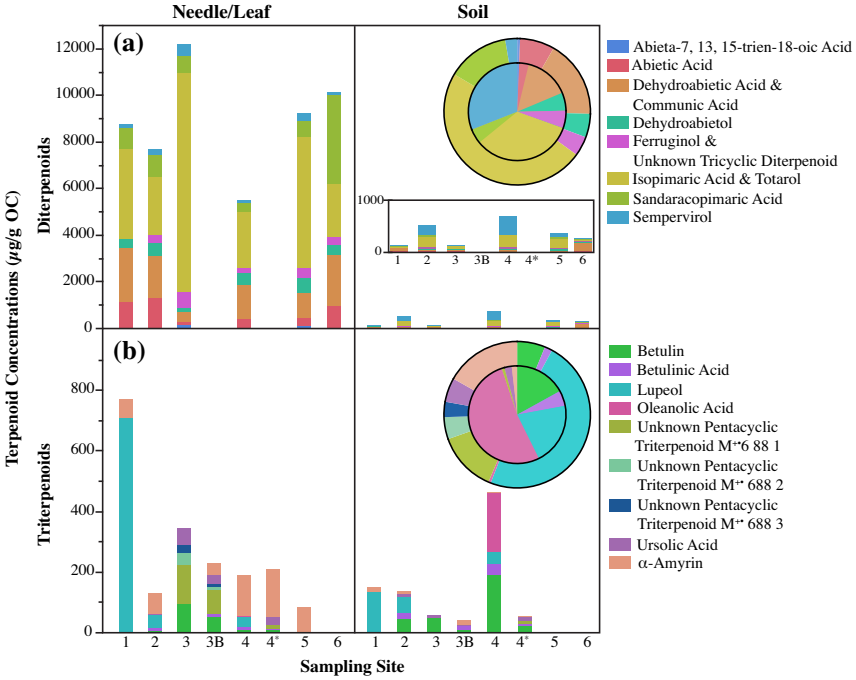

Fig. 6. Composition and concentrations in needle/leaf and soil (combined upland and valley floor soils) of (a) DTs and (b) TTs by site. Sites are arranged from upstream (Site 1) to downstream (Site 6). Site 4* corresponds to the combined leaf and soil concentrations from Sites 4B, 4C and 4D. Average values of all leaves and soils collected at each site are represented for each compound. Soil DTs are enlarged to show differences in composition. Pie charts depict the average composition of needle/leaf (outside) and soil (inside) terpenoids.

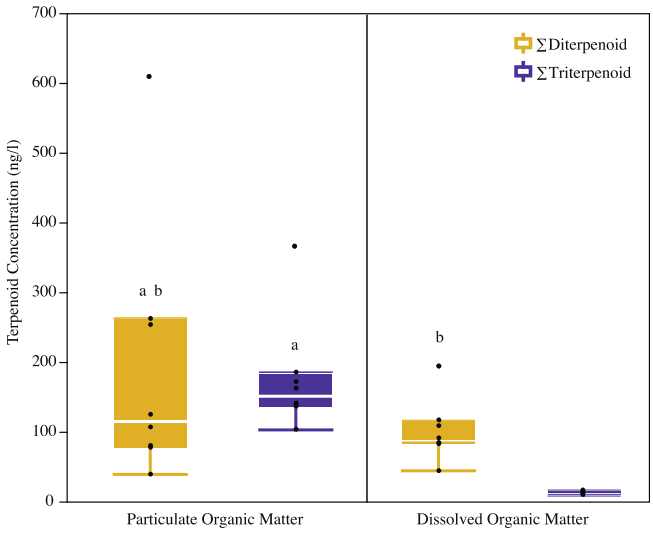

Fig. 7. Distribution of DT and TT abundances in river water particulate and DOM. Box and whisker plots show median, minimum and maximum values and the upper and lower quartiles. All samples are shown and values are given in Tables E-5 and E-6. Median values and one standard deviation are: POM 116 ± 187 ng/l for DTs and 152 ± 81 ng/l for TTs (n = 8); DOM 88 ± 44 ng/l for DTs and 12 ± 3 ng/l for TTs (n = 8). Boxes with the same letters are not statistically different.

terpenoids as a paleovegetation proxy, the factors that influence their dispersal and depositional fate need to be better constrained. The following sections discuss patterns of DT and TT concentrations in a modern river basin and the implications for using these compounds in the geologic record.

5.1. Terpenoid synthesis and potential fluxes to sedimentary reservoirs

At Pictured Rocks National Lakeshore, the DTs in evergreen conifers are 37× higher than TTs in deciduous angiosperms (Fig. 3). A similar study of extant trees in Pennsylvania (PA) found that conifer DT concentrations were only ca. 6× higher than those of deciduous angiosperm TTs (Diefendorf et al., 2012). β-Amyrin and germanicol concentrations were not included in our total TT concentrations and were thus removed from the total TT concentrations reported by Diefendorf et al. (2012) to make the data directly comparable between sites. After limiting comparisons to similar compounds, we found that both MI and PA evergreen conifers in the families Cupressaceae and Pinaceae produced similar amounts of DTs (8671 ± 1244 μg/g OC in MI; n = 15 and 9483 ± 4097 μg/g OC in PA; n = 8). Although species composition within each family was not the same, DT concentration was not significantly different for *T. occidentalis* sampled at both sites (12756 ± 1237 μg/g OC in MI; n = 6 and 14478 μg/g OC in PA; n = 1). TTs were considerably lower in concentration for angiosperm families Betulaceae, Fagaceae and Sapindaceae from MI (237 ± 51 μg/g OC; n = 14) than PA (1545 ± 717 μg/g OC; n = 8; Wilcoxon rank-sum test, p = 0.06). This pattern was also observed in species sampled at both sites (769 μg/g OC in MI, n = 1; 5370 μg/g OC in PA; n = 1 for *A. rubrum* and 282 ± 81 μg/g OC in MI, n = 3; 1430 μg/g dry leaf mass in PA, n = 1 for *F. grandifolia*).

This differential production of TTs in deciduous angiosperms between the Upper Peninsula of MI and PA sampling locations may be controlled by many factors not explored here. Most notably, insect predation, a major driver for the biosynthesis of these compounds, may differ between sampling locations (Langenheim, 1994) and was not constrained here. Additionally, total terpenoid concentration may be influenced by differences in light intensity (Langenheim, 1994). Differences in vegetation density and sun exposure between sites may therefore account for this variability in triterpenoid production. MI samples were collected from a dense forest, whereas PA samples were collected from the sun-exposed side of trees from individual trees. Regardless of the magnitude of the variability, evergreen conifers appear to produce a higher concentration of DTs than deciduous angiosperms produce TTs, suggesting that DTs may possibly be over-represented in sedimentary systems when this production bias is not accounted for.

To correct for the difference in terpenoid biosynthesis between conifers and angiosperms, plant terpenoid concentrations were adjusted for litter production and basin wide tree canopy cover (Table 2; Eqs. 1 and 2). Litter production likely depends on tree species and abundances of these species in a particular forest, but can be approximated using an average value for similar forested ecosystems, to understanding broad patterns of terpenoids in the sedimentary record. Because leaf litter production was not specifically measured in the river drainage basin and accounts for ca. 80% of total litter fall (Vogt et al., 1986), our adjustment here represents a first order approximation for potential needle/leaf DTs and TTs fluxes to the drainage basin and serves as an estimate of the ratio that should be expected in soil, river sediments or water samples. Using this approach, we find that the basin wide average estimated flux of DTs is 6.3× higher than that of TTs (Fig. 4).

5.2. Comparison of terpenoids between leaves and soils

Plant terpenoid flux corresponds to one seasonal loss of needle/leaf biomass, while sediment terpenoid abundances represent an accumulation of lipids over an unknown period of time and most descriptive paleovegetation studies still assume that the concentration and composition of plant lipids in soils reflect the overlying

Table 3
Basin-wide vegetation cover (%) estimated from Miners River drainage basin sediments, POM and DOM.

	DLS[a] (μg/g OC)	SE[b]	TLS[c] (μg/g OC)	SE[b]	ΣDTs[d] (μg/g OC or ng/l)	SE[b]	ΣTTs[e] (μg/g OC or ng/l)	SE[b]	Conifer cover[f] (%)	Angiosperm cover[f] (%)	SD[g]	Conifer cover[h] (%)	Angiosperm cover[h] (%)	ΣSD[i]
Vegetation	8671	1244	237	51										
Valley Floor Soils					401	68	262	74	61	39	8	4	96	3
River Sediments					390	71	312	72	56	44	10	3	97	2
POM					195	66	176	29	53	47	10	3	97	1
DOM					101	15	13	1	89	11	2	18	82	5

[a] DLS = DT lipid synthesis.
[b] SE = standard error.
[c] TLS = TT lipid synthesis.
[d] ∑DTs = total diterpenoid concentration.
[e] ∑TTs = total diterpenoid concentration.
[f] Not corrected for terpenoid lipid synthesis.
[g] SD = standard deviation.
[h] Corrected for terpenoid lipid synthesis.
[i] ∑SD is the total standard deviation calculated with the Monte Carlo method using the standard deviations of DLS, TLS, ∑DTs and ∑TTs.

vegetation. Recently, a quantitative statistical analysis of modern lipids collected from leaves, roots and soils demonstrated, with least squares regression analysis, that ca. 70% of modern sediment lipid composition and concentration is explained by the lipids from leaves and roots (Mueller et al., 2013). While that study did not consider processes that remove lipids from sedimentary reservoirs, it offers a starting point from which soil lipid concentration can be quantitatively and causally related to vegetation (e.g. Mueller et al., 2013).

Using a similar approach to Mueller et al. (2013), we found that only 23% of soil terpenoid concentrations of individual terpenoids are explained by the needle/leaf concentrations using least squares regression analysis. This indicates that, at these sites, leaf terpenoid concentrations of individual terpenoids are poor predictors of sediment terpenoids. These weak correlations between needle/leaf and soil terpenoid concentrations might be explained by (i) other sources of terpenoids, thereby explaining compounds in sediments that are not observed in leaves, and (ii) degradation processes that selectively remove some terpenoids over others. Bark, branches (Diefendorf et al., 2012), root bark (Wu et al., 2012) and herbaceous understory plants are indeed important sources of terpenoids (Table E-7). However, woody litter fall tends to account for only a small portion (ca. 20%) of the total litter fall (Vogt et al., 1986) and herbaceous plants account for only 6% of the basin cover (Hop et al., 2010), but could account for the few compounds found only in sediments. Nonetheless, the largest contribution of terpenoids to our sediments is likely from the overlying needles/leaves and this suggests that other processes influence the composition and concentration of soil terpenoids.

Due to this mismatch of terpenoid compounds and concentrations between plants and sediments, we used a sum of all DT and TT concentrations to reconstruct vegetation cover, rather than comparing specific compounds between source and sink (Bechtel et al., 2003). Using this approach, we find that total soil DT concentrations are 1-2 orders of magnitude lower than total needle concentrations, whereas total soil and total leaf TT concentrations are similar (Fig. 6). A loss of both di- and triterpenoids would be expected (Otto and Simpson, 2005; Kanerva et al., 2008), but the differential loss of DTs relative to TTs is surprising and the opposite of the pattern observed in geologic sediments (Diefendorf et al., 2014). This is most apparent when comparing DT/TT ratios in soils and sediments to the estimated ratio based on the surrounding vegetation (Fig. 4). This suggests that regardless of the source, DTs are preferentially lost at a very early stage.

Several mechanisms can be invoked to explain these differences in terpenoid concentration between needles/leaves and sediments. Due to the long residence time of lipids in sedimentary reservoirs (Eglinton et al., 2002; Lavrieux et al., 2012), Mueller et al. (2013) demonstrate that compounds from previously logged species influence soil lipid concentration. We acknowledge that logging has considerably changed the plant community composition of Picture Rocks National Lakeshore. Based on the shift from a predominantly conifer forest 150 yr ago to a mixed conifer–angiosperm forest today (Frederick et al., 1977), we would expect sediment DT concentrations to be higher than estimated for the plant community and this is not consistent with our observations. It is more likely that the differences are related to terpenoid reabsorption or catabolism prior to leaf abscission (Gershenzon, 1994; Langenheim, 1994), post-depositional diagenesis (Wakeham et al., 1980), or enhanced solubility and leaching of DTs (Section 5.3). It is also possible that other factors are important, such as basin sediment composition, sediment redox state (Hedges and Keil, 1995), exposure to light (Simoneit, 2005), or chemical/physical differences that result in preferential preservation or removal of DTs or TTs (Nakamura et al., 2010; Diefendorf et al., 2014).

5.3. Terpenoid partitioning in river water

The transport of lipids by DOM has rarely been studied (Gomez-Belinchon et al., 1988; Jaffé et al., 1995; McCallister et al., 2006; Xu and Jaffé, 2007), but Jaffé et al. (1995) found that up to 40% of total lipids in tropical river water were in the DOM fraction. Here, we find similar DT concentrations in the river water DOM and POM fraction. The composition of the terpenoids is broadly similar in both fractions, but levopimaric acid is significantly lower than would be expected in the DOM fraction compared with dehydroabietic acid (Tables E-5 and E-6). In contrast, TTs are almost exclusively transported in the POM (Fig. 7). It is important to note that our samples were collected in June during the growing season during high flow conditions. It is possible that these ratios may change through the growing season, reflecting changes in water flow, but also when deciduous trees lose their leaves in the fall.

Although DT and TT concentrations cannot be directly compared between plants, soils and river water samples, a few patterns are important to note. First, DT/TT ratios are consistent with the OC sources of DOM and POM from other studies, POM being derived mainly from soil in other river systems (Hatten et al., 2010). The DT/TT ratio in the river POM fraction is consistent with the DT/TT ratio of valley floor soils and river sediments, indicating that the POM is not directly sourced from vegetation, but derives rather from the surrounding sediments and integrates OM across the entire drainage basin (Fig. 4).

The source of DOM is thought to be predominantly from soil leachates (Lu et al., 2013) and this emphasizes the importance of solubility in controlling chemical composition of DOM. Here, we find that the DT/TT ratio in groundwater is significantly higher than the expected DT/TT ratio from the vegetation. This suggests that terpenoid solubility may be an important control on the concentration of DTs in groundwater DOM (Fig. 4). It is not unexpected that solubility would be greater for DTs than TTs. DTs are smaller molecules and have a higher ratio of polar functional groups in the biological compounds, making them more hydrophilic (Schwarzenbach et al., 1993). In the context of other studies, our DT concentrations are 6 orders of magnitude lower (44.2–194.2 ng/l) than river waters downstream of papermills (0.008–0.19 mg/l; Volkman et al., 1993) than here and emphasizes the potential high solubility of DTs. The implications of this enhanced DT solubility in the geologic record are discussed below.

5.4. Implications for terpenoids as paleovegetation proxies

Using the concentration of needle/leaf terpenoids to estimate the flux of terpenoids to sediments suggests that evergreen conifers are likely over represented in the sedimentary record (Table 3). As noted above, not all plant tissues were sampled here, but the striking difference in DT and TT biosynthesis between evergreen conifer needles and deciduous angiosperm leaves necessitates correcting terpenoid concentration in geologic studies to make quantitative estimates of plant community composition (Eqs. 3 and 4; Diefendorf et al., 2012, 2014).

In the drainage basin investigated, if differential terpenoid synthesis is ignored, valley floor soils and river sediment terpenoid concentrations would overestimate conifer cover by ca. 40–50% and underestimate angiosperm cover by ca. 30–40%. When soil and sediment terpenoid concentrations are normalized for plant terpenoid biosynthesis, valley floor and river sediment terpenoid concentrations underestimate conifers and overestimate angiosperms by ca. 10–15% (Table 3). Despite the alteration in the composition and DT/TT ratio from the source vegetation to soils and sediments, our results suggest that conifer and angiosperm cover can broadly be determined using the concentrations of DTs and TTs in sediments, if normalized to DT and TT synthesis.

These findings emphasize the importance of using total DT and TT concentrations to reconstruct vegetation cover, rather than using specific compounds.

Basin-wide vegetation patterns were also estimated using Miners River water POM and DOM (Table 3). POM and DOM terpenoid concentrations are not directly measurable in the geologic record, but are useful for determining how OM is transported and incorporated to sediments that integrate large basins (i.e. nearshore marine sediments, lacustrine sediments and deltas). Terpenoid concentration in the POM fraction of river water also estimates modern vegetation cover in our drainage basin within ca. 10–15%. The calculated conifer and angiosperm ratios for POM are consistent with estimates of vegetation from valley floor and river sediments, suggesting that POM is derived from surrounding soils, transported and deposited in river sediments. Basin-wide vegetation patterns are reflected in OM transported by Miners River and also in soils and sediments collected from the drainage basin. Terpenoid concentrations in river water DOM estimate the present plant community within 1–5%. While the DOM fraction of river water comes closest to estimating the present plant community, it is not directly measurable in the geologic record.

Although the effects of enhanced DT solubility on downstream sediment concentrations are minimal in entirely freshwater systems, like Miners River, these findings have significant implications for deltaic deposits and nearshore marine sediments. Changes in water chemistry are known to cause POM and DOM to rapidly flocculate and precipitate at the saltwater intrusion of an estuary (Sholkovitz, 1976). Because DTs are transported in similar concentration in POM and DOM fractions (Fig. 7), there are two ways in which DTs can be transported and deposited in estuarine and near-shore marine sediments, relative to TTs. These sediments may therefore over represent the basin-wide conifer population, if other basins behave similarly to Miners River drainage basin. Contrary to our predictions, other studies indicate that DTs are rare in offshore sediments (Medeiros and Simoneit, 2008; Medeiros et al., 2012) and other processes may result in the preferential removal of diterpenoids from downstream sediments.

6. Conclusions

Despite being the dominant plant taxa in Miners River drainage basin, deciduous angiosperms contribute substantially lower quantities of terpenoids to soils and river sediments than evergreen conifers, even when differential production of DTs in conifer species is taken into account. Thus, using sediment terpenoid ratios alone (i.e. no corrections for production differences between major taxonomic groups) to reconstruct vegetation greatly over represents conifer populations. Sediment DT concentrations are considerably lower than the expected flux from vegetation, suggesting that these compounds are preferentially lost between source vegetation and soils and sediments.

When soils and river sediments are corrected for plant terpenoid synthesis, basin-wide terpenoid concentration is useful in predicting the present plant community composition within 10–15% in Miners River drainage basin. This suggests that terpenoids entering the sedimentary archive are good indicators of the surrounding vegetation. However, post depositional alteration and degradation most likely alter the composition in lacustrine and marine sediments (Diefendorf et al., 2014). While we have resolved some of the uncertainties in using these compounds to infer patterns of paleovegetation, more work is required to identify the processes that affect their transport from source vegetation to sedimentary archive in other depositional environments.

Acknowledgments

We thank C. Hassan, J. Marsh, D. Sberna and K. McClanahan for laboratory assistance, K. Walters for mapping and sample collection, and P. Wilcox and J. Marsh for sample collection and assistance in the field. We also thank A. Townsend-Small and B. Ransohoff for providing the resources for TOC measurements, D. Conover for aid with plant identification and L. Loope for help in obtaining sampling permits and advice on accessing sampling sites. Finally, we thank K. Mueller, K. Freeman, B. Crowley and J. Shann, as well as M. Lavrieux and R. Smittenberg for insightful comments and revisions to drafts of the manuscript. Support of the research was provided by the American Chemical Society Petroleum Research Fund (PRF #51787-DNI2 to A.F.D.) and Sigma Xi Grants-in-Aid (G20111015158353 to S.J.G.).

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.orggeochem.2015.02.002.

Associate Editor—**P. Schaeffer**

References

Anderson, G.M., 1976. Error propagation by the Monte Carlo method in geochemical calculations. Geochimica et Cosmochimica Acta 40, 1533–1538.

Bechtel, A., Gratzer, R., Sachsenhofer, R.F., Gusterhuber, J., Lücke, A., Püttmann, W., 2008. Biomarker and carbon isotope variation in coal and fossil wood of Central Europe through the Cenozoic. Palaeogeography, Palaeoclimatology, Palaeoecology 262, 166–175.

Bechtel, A., Sachsenhofer, R.F., Markic, M., Gratzer, R., Lücke, A., Püttmann, W., 2003. Paleoenvironmental implications from biomarker and stable isotope investigations on the Pliocene Velenje lignite seam (Slovenia). Organic Geochemistry 34, 1277–1298.

Bechtel, A., Sachsenhofer, R.F., Zdravkov, A., Kostova, I., Gratzer, R., 2005. Influence of floral assemblage, facies and diagenesis on petrography and organic geochemistry of the Eocene Bourgas coal and the Miocene Maritza-East lignite (Bulgaria). Organic Geochemistry 36, 1498–1522.

Berndt, L.W., 1977. Soil Survey of Delta County and Hiawatha National Forest of Alger and Schoolcraft Counties, Michigan. Department of Agriculture Soil Conservation Service Report.

Burnham, R.J., 1989. Relationships between standing vegetation and leaf litter in a paratropical forest: implications for paleobotany. Review of Palaeobotany and Palynology 58, 5–32.

Burnham, R.J., Wing, S.L., Parker, G.G., 1992. The reflection of deciduous forest communities in leaf litter – implications for autochthonous litter assemblages from the fossil record. Paleobiology 18, 30–49.

Carrera, G., Fernandez, P., Vilanova, R., Grimalt, J.O., 1998. Analysis of trace polycyclic aromatic hydrocarbons and organochlorine compounds in atmospheric residues by solid-phase disk extraction. Journal of Chromatography A 823, 189–196.

Carrera, G., Fernández, P., Vilanova, R.M., Grimalt, J.O., 2001. Persistent organic pollutants in snow from European high mountain areas. Atmospheric Environment 35, 245–254.

Diefendorf, A.F., Freeman, K.H., Wing, S.L., 2012. Distribution and carbon isotope patterns of diterpenoids and triterpenoids in modern temperate C_3 trees and their geochemical significance. Geochimica et Cosmochimica Acta 85, 342–356.

Diefendorf, A.F., Freeman, K.H., Wing, S.L., 2014. A comparison of terpenoid and leaf-fossil vegetation proxies in Paleocene and Eocene Bighorn Basin sediments. Organic Geochemistry 71, 30–42.

Eglinton, T.I., Eglinton, G., Dupont, L., Sholkovitz, E.R., Montluçon, D., Reddy, C.M., 2002. Composition, age, and provenance of organic matter in NW African dust over the Atlantic Ocean. Geochemistry, Geophysics, Geosystems 3, 1–27.

Ellis, B., Johnson, K.R., 2013. Comparison of leaf samples from mapped tropical and temperate forests: Implications for interpretations of the diversity of fossil assemblages. Palaios 28, 163–177.

Filley, T.R., McCormick, M.K., Crow, S.E., Szlavecz, K., Whigham, D.F., Johnston, C.T., van Den Heuvel, R.N., 2008. Comparison of the chemical alteration trajectory of *Liriodendron tulipifera* L. leaf litter among forests with different earthworm abundance. Journal of Geophysical Research – Biogeosciences 113, G01027.

Frederick, D.J., Rakestraw, L., Eder, C.R., Van Dyke, R.A., 1977. Original forest vegetation of Pictured Rocks National Lakeshore and comparison with present conditions. Michigan Academician 9, 433–443.

Gershenzon, J., 1994. The cost of plant chemical defense against herbivory: a biochemical perspective. In: Bernays, E.A. (Ed.), Insect-Plant Interactions, vol. V. CRC Press, Boca Raton, pp. 105–173.
Gomez-Belinchon, J.I., Llop, R., Grimalt, J.O., Albaigés, J., 1988. The decoupling of hydrocarbons and fatty acids in the dissolved and particulate water phases of a deltaic environment. Marine Chemistry 25, 325–348.
Greenwood, D.R., 1991. The taphonomy of plant macrofossils. In: Donovan, S.K. (Ed.), The Processes of Fossilization. Belhaven Press, Great Britain, pp. 141–169.
Handy, A.H., Twentner, F.R., 1985. Water Resources of Pictured Rocks National Lakeshore, Michigan. US Geologic Survey Water Investigation Report, 85–4013.
Hatten, J.A., Goñi, M.A., Wheatcroft, R.A., 2010. Chemical characteristics of particulate organic matter from a small, mountainous river system in the Oregon Coast Range, USA. Biogeochemistry 107, 43–66.
Hautevelle, Y., Michels, R., Lannuzel, F., Malartre, F., Trouiller, A., 2006. Confined pyrolysis of extant land plants: a contribution to palaeochemotaxonomy. Organic Geochemistry 37, 1546–1561.
Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Marine Chemistry 49, 81–115.
Hedges, J.I., Prahl, F.G., 1993. Early diagenesis: consequences for applications of molecular biomarkers. In: Engel, M., Macko, S.A. (Eds.), Organic Geochemistry. Plenum Press, pp. 237–253.
Hop, K., Menard, S., Drake, J., Lubinski, S., Dieck, J., 2010. National Park Service Vegetation Inventory Program: Pictured Rocks National Lakeshore, Michigan. Natural Resource Report NPS/GLKN/NPR-2010/201.
Jacob, J., Disnar, J.-R., Boussafir, M., Spadano Albuquerque, A.L., Sifeddine, A., Turcq, B., 2007. Contrasted distributions of triterpene derivatives in the sediments of Lake Caçó reflect paleoenvironmental changes during the last 20,000 yr in NE Brazil. Organic Geochemistry 38, 180–197.
Jaffé, R., Wolff, G.A., Cabrera, A., Carvajal Chitty, H., 1995. The biogeochemistry of lipids in rivers of the Orinoco Basin. Geochimica et Cosmochimica Acta 59, 4507–4522.
Kanerva, S., Kitunen, V., Loponen, J., Smolander, A., 2008. Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biology and Fertility of Soils 44, 547–556.
Kim, S., Simpson, A.J., Kujawinski, E.B., Freitas, M.A., Hatcher, P.G., 2003. High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C_{18} solid phase disk. Organic Geochemistry 34, 1325–1335.
Langenheim, J.H., 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of Chemical Ecology 20, 1223–1280.
Lavrieux, M., Bréheret, J.-G., Disnar, J.-R., Jacob, J., Le Milbeau, C., Zocatelli, R., 2012. Preservation of an ancient grassland biomarker signature in a forest soil from the French Massif Central. Organic Geochemistry 51, 1–10.
Loope, L., 2004. An Aquatic Monitoring Plan for Pictured Rocks National Lakeshore. National Park Service Report.
Lu, Y., Bauer, J.E., Canuel, E.A., Yamashita, Y., Chambers, R.M., Alt, R.J., 2013. Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. Journal of Geophysical Research: Biogeosciences 118, 1–15.
McCallister, S.L., Bauer, J.E., Ducklow, H.W., Canuel, E.A., 2006. Sources of estuarine dissolved and particulate organic matter: a multi-tracer approach. Organic Geochemistry 37, 454–468.
McKee, B.A., Aller, R.C., Allison, M.A., Bianchi, T.S., Kineke, G.C., 2004. Transport and transformation of dissolved and particulate materials on continental margins influenced by major rivers: benthic boundary layer and seabed processes. Continental Shelf Research 24, 899–926.
Mechenich, C., Kraft, G.J., Szcytko, S.W., Mechenich, D.J., 2006. Assessment of Coastal Water Resources and Watershed Conditions at Pictured Rocks National Lakeshore. National Park Service Report NPS/NRWD/NRTR-2006/231.
Medeiros, P.M., Sikes, E.L., Thomas, B., Freeman, K.H., 2012. Flow discharge influences on input and transport of particulate and sedimentary organic carbon along a small temperate river. Geochimica et Cosmochimica Acta 77, 317–334.
Medeiros, P.M., Simoneit, B.R.T., 2008. Multi-biomarker characterization of sedimentary organic carbon in small rivers draining the Northwestern United States. Organic Geochemistry 39, 52–74.
Mueller, K.E., Polissar, P.J., Oleksyn, J., Freeman, K.H., 2012. Differentiating temperate tree species and their organs using lipid biomarkers in leaves, roots and soil. Organic Geochemistry 52, 130–141.
Mueller, K.E., Eissenstat, D.M., Müller, C.W., Oleksyn, J., Reich, P.B., Freeman, K.H., 2013. What controls the concentration of various aliphatic lipids in soil? Soil Biology & Biochemistry 63, 14–17.
Nakamura, H., Sawada, K., Takahashi, M., 2010. Aliphatic and aromatic terpenoid biomarkers in Cretaceous and Paleogene angiosperm fossils from Japan. Organic Geochemistry 41, 975–980.
Otto, A., Simoneit, B.R.T., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz formation, Saxony, Germany. Geochimica et Cosmochimica Acta 65, 3505–3527.
Otto, A., Simoneit, B.R.T., Rember, W.C., 2003. Resin compounds from the seed cones of three fossil conifer species from the Miocene Clarkia flora, Emerald Creek, Idaho, USA, and from related extant species. Review of Palaeobotany and Palynology 126, 225–241.
Otto, A., Simpson, M.J., 2005. Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from Western Canada. Biogeochemistry 74, 377–409.
Otto, A., White, J.D., Simoneit, B.R., 2002. Natural product terpenoids in Eocene and Miocene conifer fossils. Science 297, 1543–1545.
Pancost, R.D., Boot, C.S., 2004. The palaeoclimatic utility of terrestrial biomarkers in marine sediments. Marine Chemistry 92, 239–261.
Rasband, W.S., 2012. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. <imagej.nih.gov/ij/, 1997–2012>.
Read, R.H., 1975. Vascular plants of Pictured Rocks National Lakeshore, Alger County, Michigan. Michigan Botanist 14, 3–43.
Schouten, S., Woltering, M., Rijpstra, W.I.C., Sluijs, A., Brinkhuis, H., Sinninghe Damsté, J.S., 2007. The Paleocene-Eocene carbon isotope excursion in higher plant organic matter: differential fractionation of angiosperms and conifers in the Arctic. Earth and Planetary Science Letters 258, 581–592.
Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 1993. Organic solvent – water partitioning: the octanol – water partition constant. In: Environmental Organic Chemistry. John Wiley & Sons Inc., New York, pp. 124–156.
Sholkovitz, E.R., 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochimica et Cosmochimica Acta 40, 831–845.
Simoneit, B.R., 2005. A review of current applications of mass spectrometry for iomarker/molecular tracer elucidation. Mass Spectrometry Reviews 24, 719–765.
Spicer, R.A., 1981. The sorting and deposition of allochthonous plant material in a modern environment at Silwood Lake, Silwood Park, Berkshire, England. U.S. Geological Survey Professional Paper 1143, 1–77.
ten Haven, H.L., Rullkötter, J., 1988. The diagenetic fate of taraxer-14-ene and oleanene isomers. Geochimica et Cosmochimica Acta 52, 2543–2548.
Vogt, K.A., Grier, C.C., Vogt, D.J., 1986. Production, turnover, and nutrient dynamics of above and belowground detritus of world forests. Advances in Ecological Research 15, 303–347.
Volkman, J.K., Holdsworth, D.G., Richardson, D.E., 1993. Determination of resin acids by gas chromatography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania. Journal of Chromatography A 643, 209–219.
Wakeham, S.G., Schaffner, C., Giger, W., 1980. Polycyclic aromatic hydrocarbons in recent lake sediments—II. Compounds derived from biogenic precursors during early diagenesis. Geochimica et Cosmochimica Acta 44, 415–429.
Wu, J., Zhou, Y., Wang, L., Zuo, J., Zhao, W., 2012. Terpenoids from root bark of *Celastrus orbiculatus*. Phytochemistry 75, 159–168.
Xu, Y., Jaffé, R., 2007. Lipid biomarkers in suspended particles from a subtropical estuary: assessment of seasonal changes in sources and transport of organic matter. Marine Environmental Research 64, 666–678.