Origin, formation and environmental significance of des-A-arborenes in the sediments of an East African crater lake

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\textbf{A B S T R A C T}

Non-hopanoid pentacyclic triterpenoids occur widely in lake sediments, but their biological sources and diageneric pathways are not fully resolved. We tentatively identified a number of des-A-arborene isomers occurring in relatively high abundance in the 25,000 year (25 kyr) sedimentary record of Lake Chala, a deep crater lake in tropical East Africa. The mono-, di- and tri-unsaturated des-A-arborenes are transformation products of isoarborinol/arborinone. These precursors have an aquatic source and are most likely biosynthesized by algae or aerobic bacteria in the epilimnion. The relatively depleted \(^{13}\)C values (on average \(-32.3\%\pm1.3\%\)) of des-A-arbor-9(11)-ene are consistent with an aquatic source (algae or aerobic bacteria). In general, isoarborinol and its microbially induced transformation products are found in present and ancient tropical lacustrine settings (typically crater lakes) with permanently anoxic bottom waters and sediments. Based on molecular mechanics calculations of des-A-arborenes, it is clear that these transformation products are not in thermodynamic equilibrium, strongly indicating that their formation is microbially mediated. Subtle temporal and spatial differences in the microbial community might therefore not only dictate the variable relative contributions of different des-A-arborenes found in the sediments of Lake Chala over the last 25 kyr, but also explain the distribution of arborane derivatives in comparable crater-lake settings elsewhere.

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1. Introduction

Non-hopanoid pentacyclic triterpenoids preserved in lake and marine sediments are relatively well studied biomarkers and are predominantly used as a proxy for the terrestrial input of angiosperm higher plants (e.g., Rullkötter et al., 1982; Freeman et al., 1994; Sabel et al., 2005). The biological origin of sedimentary triterpenoids with an arborane carbon skeleton is, however, relatively unknown (e.g., Borrego et al., 1997; Volkman, 2005), so their use as biomarkers in paleoenvironmental reconstruction is limited. Arborane-type triterpenoids such as isoarborinol are known to occur in members of a few angiosperm families (Rutaceae: Vörbrüggen et al., 1963; Rubiaceae: Hui and Lam, 1965; Poaceae: Ohmoto et al., 1970; Euphorbiaceae: Hemmers et al., 1989), but their general scarcity in higher plants seems to be at odds with the widespread occurrence and relative abundance of arborane-type triterpenoids found in lake sediments (e.g., Albrecht and Ourisson, 1969; Jaffé and Hausmann, 1995). Furthermore, aromatic isoarborinol derivatives have been found in sediments predating the evolution of angiosperm plants (Hauke et al., 1992a, 1992b, 1995). Alternative sources for arborane triterpenoid hydrocarbons, such as algae or aerobic bacteria, have also been proposed (Ourisson et al., 1982; Hauke et al., 1992b, 1995; Borrego et al., 1997; Volkman, 2005), but it has proven difficult to confirm their biosynthesis by these alternative sources. Only recently, the aerobic marine heterotrophic bacterium Eudorella adriatica has been shown to produce arborane triterpenols (Banta et al., 2017).

During high-resolution stratigraphic analysis of n-alkanes (Sinninghe Damsté et al., 2011), n-alk-1-enes (van Bree et al., 2014) and des-A-lupane (van Bree et al., 2016) in the continuous and well-dated 25 kyr sedimentary record of Lake Chala, a deep equatorial crater lake on the border of Kenya and Tanzania, we also
encountered a series of des-A-arborenes. Des-A-arborenes are dia-
genetic products of pentacyclic triterpenoids with an arborane
skeleton such as isoarborinol and arborinone produced by micro-
bial cleavage of the A-ring (Trendel et al., 1989; Lohmann et al.,
90). In this paper we report on the origin, transformation path-
ways and potential applications of the pentacyclic triterpenoid
transformation products with an arborane skeleton in Lake Chala
and compare our setting with other known lacustrine deposits
containing arboranes. Molecular mechanics were applied to pro-
vide critical information regarding the relative stability of various
(stereo-)isomers of des-A-arborenes, and the likeliness of diage-
etic pathways of these compounds to more stable sedimentary
biomarkers (cf. van Duin et al., 1997; van Duin and Sinninghe
Damsté, 2003).

2. Material and methods

2.1. Study area

Lake Chala (3°19′S, 37°42′E; locally ‘Challa’, after a nearby vil-
lage) is a small, deep and relatively unproductive tropical freshwater
crater lake on the lower south-eastern slope of Mt. Kilimanjaro
(Fig. 1). It is located on the border of Kenya and Tanzania, ∼880 m
above sea level, with a surface area of ∼4.2 km², a catchment area
of ∼3.4 km² and a maximum depth of around 90 m. The bi-annual
passing of the Inter-Tropical Convergence Zone (ITCZ) results in
moderate “long rains” (March–May) and heavy “short rains” (Octo-
ber–December), and consequently a long dry season (June–
September). Subsurface groundwater inflow originating from rain-
fall on the mid-elevation slopes of Mt. Kilimanjaro is the main
water source into the lake (Payne, 1970), although temporary dis-
charge from a creek breaching the crater rim in the northwest can
occur during very heavy rains (Buckles et al., 2014). Daily wind-
driven water-column mixing is limited to 15–20 m year-round,
while seasonal deep mixing (June–September) extends to 40–
60 m. Consequently, the lower water column is permanently strat-
ified. Due to the limited density stratification, complete mixing
cannot be excluded, but its recurrence frequency is almost cer-
tainly decadal or longer, not inter-annual (Wolff et al., 2011, 2014).

2.2. Study materials

We studied Lake Chala’s finely laminated organic muds
retrieved mid-lake (Fig. 1) in 2003 and 2005 (see Verschuren
et al., 2009). The well-dated 20.82 m long master sequence covers
the last 25 kyr of continuous offshore lacustrine sedimentation
(see Blaauw et al., 2011). For this study, a total of 141 sediment
samples with 4 cm thickness were extracted and processed for bio-
marker analysis, generally at ~200 year intervals, with a higher
resolution of ~50 year intervals in the youngest 3.2 kyr, similar
to previous studies (van Bree et al., 2014, 2016). In addition, we
analyzed the compound-specific stable carbon isotopic composi-
tion of selected biomarkers in 14 sediment samples distributed
throughout the sequence.

We also analyzed sediment trap, suspended particulate matter
(SPM), soil and litter samples (Sinninghe Damsté et al., 2009;
Buckles et al., 2014; van Bree et al., 2014, 2016). A sediment trap
deployed at 35 m water depth in oxic water (∼5 mg L−1) was
retrieved at ca. 4 week intervals between November 2006 and
December 2007 as described by Sinninghe Damsté et al. (2009).
On 10–11 September 2006, a suite of SPM samples was obtained
from throughout the water column, filtered and processed as
described in van Bree et al. (2014 and 2016). Soil (n = 8) and litter
(n = 5) samples were collected within the catchment area of Lake
Chala in 2005 and 2012, respectively (Fig. 1). These were stored
and processed as described by Sinninghe Damsté et al. (2009) and
van Bree et al. (2016).

2.3. Lipid extraction

Lipid extraction has been performed in the context of previous
studies (Sinninghe Damsté et al., 2011; van Bree et al., 2014, 2016).
In short, the freeze-dried and powdered sediments were extracted
with a Dionex™ Accelerated Solvent Extractor (ASE), using a
dichloromethane (DCM)/methanol (9:1, v/v) mixture at high tem-
perature (100 °C) and pressure (7.6 × 10⁶ Pa). Subsequently the
extracts were separated into an apolar and a polar fraction over
an activated Al₂O₃ column with hexane/DCM (9:1, v/v) and DCM/
MeOH (1:1, v/v), respectively. A subset of the apolar fractions were

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Fig. 1. Panel A shows the location of Lake Chala (3°19′S, 37°42′E) in East Africa, on the border of Kenya and Tanzania. Panel B shows lake bathymetry at 10 m intervals and locations of the suspended particulate matter (SPM) sampling (▲), the sediment trap (△) and the 25 kyr sediment record (●), as well as catchment soil (○) and litter (■) collection. The outer bold line is the crater rim confining the catchment area, and the inner bold line the shoreline at the time of coring in 2005. Modified after Moernaut et al. (2010) and van Bree et al. (2014, 2016).
separated with a small Ag⁺-impregnated silica column into a satu-
rated (hexane eluent) and an unsaturated hydrocarbon fraction (ethylen acetate eluent) prior to stable carbon isotopic composition analysis.

SPM and sediment trap samples were extracted previously (Sinninghe Damsté et al., 2009). Fluxes of apolar compounds (in mg m⁻² yr⁻¹) in the water column were calculated using the concentra-
tion of each of these components relative to total particle flux measured in the sediment trap. Litter and soil samples were extracted ultrasonically with DCM/MeOH (2:1, v/v) as described by van Bree et al. (2016). In short, the extracts were methylated with diazomethane in diethyl ether and separated over an acti-
vated Al₂O₃ column into apolar, ketone and polar fractions, using hexane/DCM (9:1, v/v), hexane/DCM (1:1, v/v) and DCM/MeOH (1:1, v/v) as eluents, respectively.

2.4. Lipid identification and quantification

As described previously (Sinninghe Damsté et al., 2011; van Bree et al., 2014, 2016), the apolar fractions of sediments, SPM, sedi-
ment trap, soil and litter extracts were analyzed by gas chro-
matography (GC) and GC–mass spectrometry (GC–MS). A selection of polar fractions from samples of sediment, sediment trap and SPM were methylated, silylated and screened for function-
alized triterpenoids.

GC was performed using a Hewlett-Packard (HP6890) instru-
ment equipped with an on-column injector, a flame ionization
detector (FID), a fused silica capillary column (25 m × 0.32 mm) coated with CP SIL-5 CB (film thickness 0.12 µm), and helium as carrier gas. The samples were injected at 70 °C with an oven pro-
gram of 20 °C min⁻¹ to 130 °C, 4 °C min⁻¹ to 320 °C, and held for 20 min. GC–MS was performed on a Finnigan Trace DSQ mass spec-
trometer operated at 70 eV with a mass range of m/z 40 to 800 and a cycle time of 1.7 s. The gas chromatograph was equipped with a fused silica capillary column as described above, with helium as carrier gas, and the same oven temperature program as for GC. Identification of the des-A-triterpenoids and other triterpenoid hydrocarbons is based on relative retention times, published mass spectra (including the NIST98 spectral library), and interpretation of observed fragmentation patterns. Quantification of compounds was performed by peak area integration of peaks in the FID chro-
matograms, including an internal standard. The concentration of apolar compounds are normalized to µg gTOC⁻¹.

2.5. Compound-specific carbon isotope analyses

We measured the compound specific δ¹³C signature of 20 unsaturated hydrocarbon fractions with an Agilent 6800 GC coupled to a ThermoFisher Delta V isotope ratio monitoring mass spec-
trometer. Samples were run in duplicate or triplicate, allowing an estima-
tion of the standard deviation of the measurements. The iso-
topic values were measured with reference to a calibrated external gas.
Instrument performance was monitored daily by injections of a mixture of a C₁₂ and a C₁₄ perdeuterated n-alkane with known isotopic composition. The δ¹³C values are reported in standard delta notation against the Vienna Pee Dee Belemnite (VPDB).

2.6. Molecular mechanics

We performed both quantum-chemistry-based Density Func-
tional Theory (DFT) and ReaxFF classical-force-field simulations to calculate the relative stabilities of various stereo-isomeric con-
figurations of the des-A-arboranes. DFT calculations are deemed more accurate, but require more computational power than ReaxFF. The ReaxFF method has been well established over the last decade to describe a wide range of systems including catalysis,
The distributions and abundances of these unsaturated des-A-arborenes in the sedimentary record vary considerably over time. The summed record of the des-A-arborenes (compounds 1–6) shows three periods of high concentrations, namely 23.1–19.2 kyr BP, 14.0–8.3 kyr BP and around 0.4 kyr BP (Fig. 3A and D). Des-A-arborenes, and compound 4 in particular, are at times dominant in the apolar fraction of Lake Chala’s lipid extracts. The overall average concentration is ~300 μg gTOC⁻¹ with peak values up to 3 mg gTOC⁻¹ for the summed des-A-arborenes. The relative proportion of mono-enes, dienes and trienes fluctuates over the past

Fig. 2. Distribution of des-A-triterpenoids in the Lake Chala sediment record. Panels A and B show partial GC-FID traces of the apolar fractions of hydrocarbon extract from representative sediment horizons at 272–276 cm and 432–436 cm depth, deposited ~3.0 and ~5.3 kyr BP, respectively. Panel C shows a summed mass chromatogram (m/z 163 + 177 + 189 + 203 + 218 + 309) illustrating the distribution of des-A-triterpenoids from the apolar lipid fraction of a sample taken at 996–1000 cm depth, which is dated ~12.1 kyr BP; # indicates plant-derived des-A-triterpenoids (van Bree et al., 2016). Panel D shows the mass spectra of compounds 1 and 2 (mono/di-unsaturated des-A-triterpenes with an arborane skeleton), 3 and 4 (des-A-arbor-9(11)-ene), 5 (des-A-arbora-5,7,9-triene), and 6 (des-A-arbora-5(10),9(11)-diene); these identifications are tentative. Possible structures are shown in Fig. 5.

Fig. 3. Distribution of des-A-arborenes (in μg gTOC⁻¹) in Lake Chala sediments over the past 25 kyr. Panel A shows the summed concentration of des-A-arborenes (in μg gTOC⁻¹) in Lake Chala sediments. Panel B shows the relative abundances, revealing a large proportion of mono-enes (sum of 1, 2, 3 and 4; green), and smaller proportions of the triene (5; yellow) and diene (6; orange). Panel C shows the fractional abundance of the mono-enes. Panels [D], [E], and [F] zoom in on these plots over the past 3 kyr, the section investigated at high temporal resolution. In panel [G] the des-A-arborene concentrations (in μg gTOC⁻¹) of compounds 1, 2, 4, 5 and 6 over the last 25 kyr are plotted separately. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
25 kyr (Fig. 3B). The mono-enes are dominated by compound 4, but in few cases by compound 3 (Fig. 3C). The mono-enes are dominant in most samples (average 83%, median 91%). The fractional abundance of the diene (6) is highly variable and ranges between 0 and 100% (average 15%, median 8%). The triene (5) is typically present as a minor (<5%) component (average 1.8%, median 0.8%), but has peak occurrences of up to 35%, and is entirely absent in some intervals even though the sedimentary record has been sampled at very high resolution (e.g., between 2.7 and 3.7 kyr BP; n = 14). When the dienes and trienes are relatively abundant, the overall concentration of unsaturated des-A-arborenes is always relatively low (Fig. 3).

The stable carbon isotopic composition of des-A-arborene compound 4 was determined successfully in 14 out of 20 sediment horizons (Fig. 4), and ranges from −29.8‰ to −33.8‰ (−32.3‰ ± 1.3‰ on average). Reliable determination of the stable carbon isotopic composition of other des-A-arborenes was unsuccessful due to co-elution or low abundances.

3.2. (Des-A-)triterpenoids in soil and litter surrounding the lake, in settling particles and suspended particulate matter

No des-α-triterpenoids were identified in the hydrocarbon extracts from soils and litter, and no functionalized arborane-type triterpenoids were detected in other fractions. Van Bree et al. (2016) reported the presence of functionalized triterpenoids with oleanane, ursane and lupane skeletons in the soil and litter, but arborane or fernane-type triterpenoids were not encountered. In the extracts of particulate matter recovered with the sediment trap, only low concentrations of des-A-urs-13(18)-ene and des-A-lupane were detected, next to traces of functionalized triterpenoids such as α-amyrinol. Hydrocarbon concentrations in the SPM were too low for identification of specific triterpenoid compounds.

3.3. Molecular mechanics: DFT simulation

DFT molecular mechanics calculations were performed to calculate the stabilities of the isomers of mono/di/tri-unsaturated des-A-arborenes (Table 1; Fig. 5). If isomerization of double bonds via relatively stable tertiary carbocations (de Leeuw et al., 1989) occurs, five double bond isomers, i.e. $\Delta^{8(11)}$, $\Delta^{7}$, $\Delta^{8}$, $\Delta^{9}$, and $\Delta^{9(11)}$ may result from the original $\Delta^{8(11)}$ des-A-arborene. Isomerization of the $\Delta^{9(11)}$-isomer via the tertiary carbocation at C-9 can produce $\Delta^{7}$- and $\Delta^{9}$-isomers. The $\Delta^{8}$-isomer can give rise to $\Delta^{7}$-isomers via the tertiary carbocation at C-8, whereas the $\Delta^{9}$-isomer can evolve into the $\Delta^{8(10)}$-isomer via the tertiary carbocation at C-10. Chiral centers in des-A-arbora-monoenes occur at C-8, C-9, and C-10, and, hence, stereoisomers may occur as well. As the double bond isomers of des-A-arbora-monoenes can have 2 or 4 stereo-isomers each, there are 16 possible configurations (see Fig. 5 for roman numeral codes). DFT calculations indicate that generally, the $\Delta^{8(11)}$-isomers are most stable (II1a-b), followed by $\Delta^{7}$ (IIlg), $\Delta^{9(11)}$ (IIe-f) and $\Delta^{9}$ (IIJk). More specifically, DFT molecular mechanics calculations indicate that $\Delta^{8(11)}$-8$\beta$-H,10$\beta$-Me (IIJa) is the most stable des-A-arbora-monoene (Table 1, Fig. 5). The DFT calculations predict stabilities for $\Delta^{8(11)}$-8$\beta$-H,10$\beta$-Me (IIJa), $\Delta^{9(11)}$-10$\beta$-Me (IIJe), $\Delta^{8(11)}$-Me (IIIf), $\Delta^{9}$-9$\beta$-H,10$\beta$-Me (IIlg), and $\Delta^{9(11)}$-8$\beta$-Me (IIlk) that are less stable but are within 2 kcal/mol, compared to that of $\Delta^{8(11)}$-8$\beta$-H,10$\beta$-Me (IIJa). Hence, if thermodynamic equilibrium exists for the des-A-arborenes, these compounds should be present in significant amounts (Table 1).

Isomerization of des-A-arboradienes (IIa to IIId) can only occur via relatively stable aliphatic tertiary carbocations (de Leeuw et al., 1989). Hence, only four possible des-A-arboradienes may be formed starting from the $\Delta^{8(11)}$-isomer: $\Delta^{8(11)}$-8$\beta$H (IIa), $\Delta^{8(11)}$-10$\beta$-H,8$\beta$H (IIJa), $\Delta^{9(11)}$-8$\beta$H (IIJe), and $\Delta^{9}$-9$\beta$-H,10$\beta$-Me (IIJf; Fig. 5). The DFT calculations indicate that $\Delta^{8(11)}$-8$\beta$H (IIJa) is the most stable isomer and would dominate the mixture (~99.9%) if at equilibrium. We also calculated the inter-conversion energetics of the most stable monoene (IIJa), diene (IIa) and B-ring aromatized $\Delta^{9(11)}$ des-A-arboratriene (IV), considering molecular hydrogen as reference for hydrogenation and dehydrogenation reactions. DFT calculations predict an exothermic conversion process of diene IIa to monoene IIJa ($\Delta E_1 = -14.10$ kcal/mol), and endothermic conversions for both diene IIa to triene IV ($\Delta E_2 = 1.13$ kcal mol$^{-1}$), and monoene IIJa to triene IV ($\Delta E_3 = 15.50$ kcal mol$^{-1}$).

4. Discussion

4.1. Arborane vs fernane precursors

One important issue regarding des-A-arborenes is the possibility that they may have been formed by transformation of fernane-type triterpenoids, since des-A-fernes are enantiomers of des-A-arborenes (Jaffé and Hausmann, 1995). Arborane and fernane skeletons (Fig. 5: 1a-Id) differ only in stereochemistry due to chair and boat conformations of the B-ring when they are synthesized (Ohimoto et al., 1970). Therefore, its precursor skeleton is no longer identifiable when aromatized or when the A-ring is removed. Fernane-type triterpenoids have been reported from different biological sources including plants, lichens and bacteria (e.g., Ohimoto et al., 1970; Bottari et al., 1972; Howard et al., 1984; Hemmers et al., 1989; Shiojima and Ageta, 1990; González et al., 1991; Hauke et al., 1992b; Paull et al., 1998; Maier et al., 2009). Although a fernane-type origin cannot be ruled out completely, it is highly unlikely in Lake Chala, firstly due to the presence of trace amounts of isoarborinol and absence of functionalized fernenes in the modern water column and surface sediments; and secondly due to the similarity of Lake Chala with other lacustrine environments from where large amounts of arborane-triterpenoids have been reported (Section 4.2). Therefore, we are most likely dealing with compounds derived from functionalized arboranes, more specifically isoarborinol and/or arborinone.

4.2. Comparison with other lacustrine records

Isoarborinol and its derivatives are often found in lake settings similar to Lake Chala. Sometimes large quantities of isoarborinol/
arborinone are found in small (sub-) tropical freshwater lakes with a permanently anoxic hypolimnion (Table 2). This includes modern lakes such as Lake Valencia (Venezuela; Jaffé and Hausmann, 1995) and Lake Albano (Italy; Hanisch et al., 2003), but also paleolake deposits such as the Eocene Lake Messel (~47.8 ± 0.2 Ma; Albrecht and Ourisson, 1969; Kimble et al., 1974; Hauke et al., 1992a), Eckfeld Maar (~44.3 Ma; Sabel et al., 2005), the Bouxwiller shales (Arpino et al., 1971; Hauke et al., 1995), the Ménat shales (Hauke et al., 1992a, 1995), and the Miocene Clarkia deposits (Logan and Eglinton, 1994; Huang et al., 1995). Isoarborinol/arborinone is usually found in highly productive (eutrophic to hypereutrophic) lakes, while Lake Chala is oligotrophic. Typically, isoarborinol is found in tropical or warm and humid climates, but it has also been detected in the Pleistocene sediments of the Arctic Lake El'gygytgyn (D'Anjou et al., 2013). Lake Valencia and Lake Chala are thought to reflect the bacterial OSC signature of the parent compound and can be used to distinguish between alternative plant or microbial sources. The relative δ13C depletion found here is comparable to that reported for arborane compounds in other lake settings. In a study of Eocene shales (Hauke et al., 1992a), des-A-arbora-5,7,9-triene (IV) has a δ13C value of ~28.7‰, contrasting with significantly less depleted δ13C values (~23‰ to ~25‰) of lupane or ursane-type triterpenoids. Such a clear offset between the δ13C values of terrestrial triterpenoid derivatives and arborane derivatives is also seen in Lake Chala (Fig. 4), where compound 4 is significantly more depleted in 13C (~29.8‰ to ~33.8‰; ~32.3‰ ± 1.3‰ on average) than the plant-derived des-A-lupanes (~25.1‰ to ~29.4‰; ~27.4‰ ± 1.1‰ on average; van Bree et al., 2016). Isoarborinol is reported with δ13C values of ~28.0‰ to ~28.4‰ in Miocene Lake Clarkia (Logan and Eglinton, 1994; Huang et al., 1995), and ~24.7‰ to ~27.9‰ in Lake Valencia (Xu and Jaffé, 2008). The significant 13C depletion of compound 4 in Lake Chala sediments indicates that here an aquatic source is much more likely than a terrestrial higher plant source of arborane-type triterpenoids.

### 4.3. Microbial origin of isoarborinol/arborinone

There are various potential biological sources of the des-A-arborene precursors isoarborinol and arborinone. Traditionally they are attributed to a terrestrial higher plant source (e.g., Albrecht and Ourisson, 1969; Ohimoto et al., 1970; Vliet et al., 1994; Jacob et al., 2005), but they have also been assigned to phytoplankton and bacteria (Ourisson et al., 1982; Hauke et al., 1992b, 1995; Jaffé and Hausmann, 1995; Volkman, 2005). Oxidosqualene cyclases (OSCs), the enzymes responsible for the biosynthesis of C3-oxigenated cyclic triterpenoids, are predominantly occurring in the eukaryotic domain, but a limited number of bacterial OSCs has also been identified (Banta et al., 2017). The only confirmed bacterial production of arborane triterpenes is by the aerobic marine heterotrophic bacterium Eudoraea adriatica (Banta et al., 2017). However, metagenomic sequences from both marine and lacustrine environments indicate the presence of the bacterial OSC responsible for the biosynthesis of arborane triterpenols, suggesting the possibility of other bacterial sources for these types of triterpenoids.

### 4.3.1. Stable carbon isotopic composition

One way of differentiating between terrestrial and aquatic source of lipid biomarkers is the carbon isotopic composition of specific lipids (Fig. 4). In Lake Chala, δ13C values of compound 4 range from ~29.8‰ to ~33.8‰ (~32.3‰ ± 1.3‰ on average). No isotopic fractionation is expected to occur during transformation of triterpenoids (Freeman et al., 1994), therefore these δ13C values are thought to reflect the δ13C signature of the parent compound and can be used to distinguish between alternative plant or microbial sources. The relative δ13C depletion found here is comparable to that reported for arborane compounds in other lake settings. In a study of Eocene shales (Hauke et al., 1992a), des-A-arbora-5,7,9-triene (IV) has a δ13C value of ~28.7‰, contrasting with significantly less depleted δ13C values (~23‰ to ~25‰) of lupane or ursane-type triterpenoids. Such a clear offset between the δ13C values of terrestrial triterpenoid derivatives and arborane derivatives is also seen in Lake Chala (Fig. 4), where compound 4 is significantly more depleted in 13C (~29.8‰ to ~33.8‰; ~32.3‰ ± 1.3‰ on average) than the plant-derived des-A-lupanes (~25.1‰ to ~29.4‰; ~27.4‰ ± 1.1‰ on average; van Bree et al., 2016). Isoarborinol is reported with δ13C values of ~28.0‰ to ~28.4‰ in Miocene Lake Clarkia (Logan and Eglinton, 1994; Huang et al., 1995), and ~24.7‰ to ~27.9‰ in Lake Valencia (Xu and Jaffé, 2008). The significant 13C depletion of compound 4 in Lake Chala sediments indicates that here an aquatic source is much more likely than a terrestrial higher plant source of arborane-type triterpenoids.

### Table 1

DFT molecular calculation results of double bond and stereoisomers of des-A-arborene, -diene and –triene. ΔE_{eq} marked with an * are significantly present in equilibrium compositions calculated at 298 K, and their equilibrium composition (%) is calculated for mono-enes and dienes separately. ΔE was calculated compared to compounds IIc and IIIe, marked with an #.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Double bond position</th>
<th>Stereisomer configuration</th>
<th>DFT (kcal/mol)</th>
<th>ΔE_{eq} (kcal/mol)</th>
<th>Equilibrium composition (%)</th>
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<tr>
<td>IIc</td>
<td>Δ(^{5(10)}\sub{11})</td>
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<td>–</td>
<td># 0.00</td>
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<td>8(\text{H})</td>
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<td>Δ(^{5,7,9}\sub{})</td>
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<td>–</td>
<td>-587639.18</td>
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4.3.2. Distribution patterns of des-A-arborenes

Besides the depleted δ13C values, also the distribution of des-A-arborenes in Lake Chala sediments is different from those of known higher plant-derived biomarkers. Indeed, the concentration of des-A-arborenes in the sediment is often higher than those of bona fide terrestrial higher plant biomarkers such as n-alkanes and the plant-derived des-A-triterpenoids. The concentration of compound 4 is not correlated with that of des-A-lupane, des-A-ursenes and...
### Characteristics of Lake Chala compared to other (paleo-)lakes with a sedimentary record of various arborane-type triterpenoid compounds.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Location (paleo-)climate</th>
<th>Salinity</th>
<th>Age</th>
<th>Lake characteristics</th>
<th>Arborene-compounds reported</th>
<th>Interpretation precursor origins</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chala</td>
<td>Kenya/Tanzania Tropical</td>
<td>Fresh</td>
<td>0–25 kyr BP (Quaternary)</td>
<td>Volcanic crater lake with an area of 4.5 km². Average depth 81 m, maximum depth ~90 m. Oligotrophic. Permanently anoxic hypolimnion.</td>
<td>des-A-arbor-9(11)-enes; des-A-arbora-5(10)(9)(11)-diene; des-A-arbora-5,7,9-triene; isoarborinol (trace)</td>
<td>Microbial</td>
<td>This study</td>
</tr>
<tr>
<td>Messel oil shales</td>
<td>Germany Tropical</td>
<td>Fresh</td>
<td>47.8 ± 0.2 Ma (Eocene)</td>
<td>Volcanic crater lake with an area of 1.7 km². Depth between 10 s and 100 s of m. Hypereutrophic with a permanently anoxic hypolimnion. Small crater lake (~1 km²). Depth of 160–210 m. Eutrophic, with a permanently anoxic hypolimnion.</td>
<td>isoarborinol; arborinone; des-A-arbora-5,7,9-triene; 24,25-dinorarbora-1,3,5(10)(9)(11)-tetraene; 24,25-dinorarbora-1,3,5,7,9-pentaene</td>
<td>Microbial</td>
<td>C</td>
</tr>
<tr>
<td>Eckfeld Maar</td>
<td>Germany Tropical</td>
<td>Fresh</td>
<td>44.3 ± 0.4 Ma (Eocene)</td>
<td>Volcanic crater lake with an area of 1.7 km². Depth between 10 s and 100 s of m. Hypereutrophic with a permanently anoxic hypolimnion. Small crater lake (~1 km²). Depth of 160–210 m. Eutrophic, with a permanently anoxic hypolimnion.</td>
<td>isoarborinol; arborinone; des-A-arbora-5,7,9-triene; 24,25-dinorarbora-1,3,5(10)(9)(11)-tetraene; 24,25-dinorarbora-1,3,5,7,9-pentaene</td>
<td>Microbial</td>
<td>D</td>
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<tr>
<td>Albano</td>
<td>Italy Mediterranean</td>
<td>Fresh</td>
<td>Holocene (Quaternary)</td>
<td>Volcanic crater lake with an area of 6 km². Average depth of 77 m, 175 m maximum. Trophic state is meso- to eutrophic, with a permanently anoxic hypolimnion.</td>
<td>isoarborinol; arborinone</td>
<td>Phytoplankton, other microbial origin possible</td>
<td>E</td>
</tr>
<tr>
<td>Clarkia lacustrine</td>
<td>Idaho, USA Tropical</td>
<td>Fresh</td>
<td>15.4–16 Ma (Miocene)</td>
<td>Drainage basin dammed by flood basalts. Deeper than 12 m. Trophic state is meso- to eutrophic, with a permanently anoxic hypolimnion.</td>
<td>isoarborinol; arborinone</td>
<td>Terrestrial (original studies), but microbial possible</td>
<td>F G</td>
</tr>
<tr>
<td>Kupfer- scheifer</td>
<td>Germany Lagoonal Permian</td>
<td>Fresh</td>
<td>Permian</td>
<td>Permanently anoxic hypolimnion.</td>
<td>des-A-arbor-8-ene; B-ring aromatized fernenes; des-A-arbora-5,7,9-triene; 24,25-dinorarbora-1,3,5(10)(9)(11)-tetraene; 24,25-dinorarbora-1,3,5,7,9-pentaene</td>
<td>Pre-angiosperm deposits, therefore interpreted as gymnosperm terrestrial origin (H; I), but possible microbial source. B-ring aromatized compounds are fernane-type (optical rotation), while the triene is from isoarborinol (NMR)</td>
<td>H I J K</td>
</tr>
<tr>
<td>Bouxwiller shales</td>
<td>France Lagoonal</td>
<td>Fresh</td>
<td>Eocene</td>
<td>Lake with an area &lt;2.5 km², and less than 12 m deep. Oligotrophic. no permanently anoxic hypolimnion.</td>
<td>isoarborinol; arborinone arbor-9(11)-en-3β-ol methyl ester</td>
<td>Terrestrial</td>
<td>K L MN</td>
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<tr>
<td>Caçô</td>
<td>Brazil Tropical</td>
<td>Fresh</td>
<td>0–20 kyr BP (Quaternary)</td>
<td>Lake with an area &lt;2.5 km², and less than 12 m deep. Oligotrophic. no permanently anoxic hypolimnion.</td>
<td>des-A-arbora-5,7,9-triene</td>
<td>Microbial origin in pre-angiosperm deposits. Derived from fernenes (O) or derived from isoarborinol (K)</td>
<td>K O</td>
</tr>
<tr>
<td>Orbagnoux Basin</td>
<td>France Lagoonal Kimmeridgian (Late Jurassic)</td>
<td>Fresh</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Lake Location (paleo-)

Microbial (bacterial) origin

Climate origins in pre-angiosperm deposits.

des-A-arborane-5,7,9-triene; 25-norhopa-5,7,9-triene; 25-norarbora(ferna)-1,3,5,7,9-pentaene;
isoarbora(ferna)-triene; 24,25-dinorarbora(ferna)-1,3,5,7,9-pentaene;

Lake characteristics

Location

Puertollano oil shale

Elgygytgyn

Kinneret

Lake characteristics

Location

Spain

Russia

Israel

Lake characteristics

Puertollano oil shale

Elgygytgyn

Kinneret

Age

~300 Ma (Carboniferous; Late Stephanian)

275–475 kyr BP (Quaternary)

Holocene (Quaternary)

Arborene-compounds reported


Lake characteristics

Salinity

Fresh

Fresh

Fresh

Microbial (bacterial) origin

Arborene-compounds reported


Lake characteristics

Salinity

Fresh

Fresh

Fresh

Interpretation precursor origin

Microbial (bacterial) origin

Table 2

In this regard, the occurrence of diatoms is also the dominant group of phytoplankton in Lake Chala. The occurrence of isoarborinol derivatives in other lacustrine deposits predating the development of angiosperm higher plants in the Cretaceous (Hauke et al., 1995; Borrego et al., 1997) is interpreted as a strong argument for a non-higher plant source of the precursor compounds.

Although most studies conclude that a microbial aquatic origin of arborane triterpenoids is most probable, in some settings a terrestrial origin is possible. For example, in Lake Caçó the arborane triterpenoids are of terrestrial origin (Jacob et al., 2007). Although Lake Caçó is a small, tropical, oligotrophic, freshwater lake like Chala, it is relatively shallow and has no permanent anoxic hypolimnion and is in that respect different to the small crater lakes where arborane triterpenoids are most likely of microbial aquatic origin (Table 2). In other lakes and shales, the interpretation of arborane derivatives as of terrestrial or aquatic origin is more ambiguous. For example, when there is known input of fernane or arborene-type triterpenoids or when the depositional setting is different (e.g., the Kupferschiefer and Orbagnoux sites; see Table 2), or when the possibility of a microbial aquatic origin has not been studied. For example, des-A-arborane-8,9-nes from the Permian Kupferschiefer sediments have been interpreted as terrestrial, due to the large co-occurring quantities of bisaccate conifer pollen in the sedimentary record during anoxic freshwater depositional conditions (Bechtel and Püttermann, 1992). Using optical rotation techniques, Hauke et al. (1995) demonstrated that the arborenes/ferrenes IV in these Permian sediments where in fact isoarborinol-derived transformation products. Therefore, co-occurrence of pollen and arboranes by no means excludes a microbial aquatic origin, but this was not discussed in the original study. Based on interpretation of isoarborinol $^{13}$C values, a terrestrial origin has also been suggested for arborane compounds in the Miocene Clarika deposits (Logan and Eglinton, 1994; Huang et al., 1995). However, isoarborinol from Clarika is more depleted in $^{13}$C ($-28.0\%$ to $-26.4\%$) than are terrestrial des-A-lupane ($-25.1\%$) and oleanane-triterpenoids ($-26.2\%$; Logan and Eglinton, 1994; Huang et al., 1995). Due to their biosynthetic pathway, triterpenoids in plants are $5–8\%$ enriched in $^{13}$C compared to $^{12}$C.
straight-chain compounds (Diefendorf et al., 2012). When Clarkia δ^{13}C values of isoarborinol (~28.0% to ~28.4%) are compared to those of n-alkanes (~27.4% to ~32.8%) taking into account this triterpenoid enrichment, it is not evident that the isoarborinol is of terrestrial origin. An aquatic source of isoarborinol in Lake Clarkia is therefore more likely.

4.3.4. Aerobic production of des-A-arborenes precursors

The mere presence of ring-A degraded arborene-type triterpenoids in sediments indicates that their functionalized precursors must be produced by aerobic organisms in a (sub)oxic environment, because des-A-triterpenoids can only be formed when there is an oxygenated function present at C-3 (Lohmann et al., 1990). Triterpenoids with an oxygenated function at C-3 are formed by OSCs, which require oxidosqualene as the substrate. The biosynthesis of oxidosqualene requires molecular oxygen (Ourisson et al., 1982; Summons et al., 2006; Fischer and Pearson, 2007). This constrains the depth of production of the des-A-arborenes precursor(s) to the upper portion of the water column in Lake Chala, since the water column below 45–55 m is anoxic throughout the year. Oxygen penetration in the water column varies seasonally (Wolff et al., 2011, 2014; Buckles et al., 2014; van Bree et al., 2018), and during some stratified periods, anoxic conditions extend upwards to ~15 m depth. However, production of arborane triterpenoids in the modern-day water column of Lake Chala seems to be low. Isoarborinol is only present in trace amounts in settling particles and SPM, and des-A-arborenes were not detected at all. This makes the identification of a microbial production zone more challenging, although it is reasonably certain they are produced in the oxic or suboxic water column due to the aforementioned C-3 oxygenation. As the extent of the epilimnion and chemocline depth is seasonally variable, we do expect seasonal changes in the microbial production of isoarborinol, which may in turn be reflected in the variable concentration of arborane compounds in the sediment record (Fig. 3; see Section 4.5).

4.4. Transformation pathways of isoarborinol: abiotic diagenesis vs microbial transformations

A suite of transformation products of isoarborinol/arborinone occurs in the sedimentary record of Lake Chala, including mono-unsaturated (1–4), di-unsaturated (5) and tri-unsaturated (monoaromatic) (6) des-A-arborenes. One of the remaining questions is how these transformations from functionalized arborane triterpenoids to the des-A compounds take place, and if this is an abiotic or microbially mediated process. Molecular mechanics calculations can help solve this question. If abiotic transformations occur, such as the loss of ring A and possible isomerization through tertiary carbocations, one may expect that the mixture of des-A-arborenes is either in thermodynamic equilibrium or approaches it with depth.

4.4.1. Microbial A-ring removal: diene formation

Des-A-arboradienes are probably the first product formed after A-ring removal. The process of A-ring removal is thought to be mediated by microbial activity under anoxic or reducing conditions (Lohmann et al., 1990; Jaffé and Hausmann, 1995; Jacob et al., 2007; Huang et al., 2008) and may yield four possible isomers of des-A-arboradienes: IIa to IIId (Fig. 5), if we assume that double bond isomerization can take place through allyl-stabilized tertiary carbocations after loss of ring A. In Lake Chala sediments we detected only one diene in high abundance (compound 6) and only minor traces of three other dienes in some of the sedimentary horizons. Compound 6 is, therefore, likely 8βH-des-A-arbora-5(10),9(11)-diene IIa, which is the most likely microbial product of (iso)arborinol and/or arborinone after A-ring loss and by far the most stable diene isomer based on molecular mechanics calculations (Table 1). Hence, we cannot conclude that IIa or IIb is the dominant des-A-arbora-diene produced after the loss of ring A, or if this dominant diene results from other dienes upon double bond isomerizations through tertiary carbocations.

Fig. 6. Schematic diagenetic pathway of (iso)arborinol. For clarification, only Ia (iso)arborinol is depicted, but the pathways are valid for all possible parent compounds Ia–Id. Hydrogenation of the 9(11) double bond is unlikely based on DFT calculations, but is depicted for completeness. Hydrogenation of IIa to IIia is exothermic (green values), and the dehydrogenation of IIa and IIia to IV is endothermic (red values). Tertiary C⁺ isomerization is indicated where possible. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
4.4.2. Mono-unsaturated des-A-arborenes: abiotic isomerisation vs microbially induced variation

Des-A-arbor-monoenes are formed by hydrogenation of des-A-arboradienes. As the $\Delta^{(10)}$ double bond hydrogenation (from IIA → IIIa) is exothermic (Fig. 6), this process is likely to happen spontaneously. This could explain the dominance of compound 4 in Chala sediments (Fig. 3C), which most likely has the IIIa ($8\beta$H,10$\beta$Me-$\Delta^{(11)}$) configuration. In the sedimentary record, we observe substantial variation in the distributions of mono-unsaturated des-A-arborenes but no distinct trend with time (Fig. 3C and F). When we compare the sedimentary distribution of these isomers with their modeled distribution at thermodynamic equilibrium (Table 1), we see that the des-A-arbora-monoene isomers present in Lake Chala sediments are not in thermodynamic equilibrium, nor is there a trend towards thermodynamic equilibrium over time. This also indicates that even over a time span of 25 kyr, there is no significant abiotic transformation between the des-A-arborene isomers. This implies that the four different monounsaturated des-A-arborenes are more likely formed microbially, either directly after the loss of ring A or after microbial hydrogenation, and that the isomerization of double bonds via terary carbocations can be excluded as a transformation process. This implies further that spatial or temporal variations within the microbial community could be reflected in the different monoene distributions observed.

The $\Delta^{(11)}$ arbor-monoene has four possible stereoisomers: $8\beta$H,10$\beta$Me (IIa), $8\alpha$H,10$\alpha$Me (IIib), $8\beta$H,10$\alpha$Me (IIlc), and $8\alpha$H,10$\beta$Me (IIld). Two des-A-arbora-monoenes (compounds 3 and 4) in Lake Chala sediments have mass spectra similar to des-A-arbora-monoenes published previously (Jaffé and Hausmann, 1995; Jacob et al., 2007). Double bond positions in arborane-type compounds are however difficult to differentiate based on mass-spectrometric fragmentation patterns alone, as they are similar for isomers of various pentacyclic triterpenoids and their diagenetic derivatives (cf. Shiojima et al., 1992; Lavrieux et al., 2011). Therefore, mass spectrometry alone will not be able to distinguish all four stereoisomers. The order of elution of the various des-A-arborene isomers is also unknown. Since there is no thermodynamic equilibrium observed, it is not possible to use molecular mechanics calculations to determine the individual stereochimical isomers of the $\Delta^{(11)}$-ene isomers.

In other lacustrine settings, only des-A-arborenes with a $\Delta^{(11)}$ double bond have been described. In oil shales of the Duaringa Basin, Boreham et al. (1994) tentatively identified two des-A-arbor-9(11)-ene isomers with identical mass spectra but different retention times. $10\alpha$Me-des-A-arbor-9(11)ene was tentatively identified in sediments of Lake Valencia (Jaffé and Hausmann, 1995) and Lake Caçó (Jacob et al., 2007). The identification of des-A-arborenes in all studies is tentative, and the stereochemistry at C-10 is either not discussed (Boreham et al., 1994) or inferred to be $10\alpha$Me (Jaffé and Hausmann, 1995; Jacob et al., 2007), although $10\beta$Me is the most stable isomer based on molecular mechanics calculations (Table 1). It can be expected that the most abundant compound 4 has the most stable configuration, which is $8\beta$H,10$\beta$Me-des-A-arbor-9(11)-ene (IIa), in contrast to IIlc ($8\beta$H,10$\alpha$Me) that was postulated by Jaffé and Hausmann (1995). Further studies, based on synthesized des-A-arborenes, are required to elucidate the stereochemistry of these compounds.

4.4.3. Microbially induced des-A-arbor-triene formation

The mono-aromatic des-A-arborene IV occurs throughout the sedimentary record of Lake Chala (compound 5, Fig. 3C). The transformation of dienes II and mono-enes III to the B-ring aromatized compound IV is strongly endothermic (Fig. 6), and although IV features an aromatic ring, and is therefore expected to be more stable than non-aromatic des-A-arborenes, molecular mechanics calculations indicate it is not. The strongly endothermic transformation must thus involve enzymatic and/or inorganic catalytic processes. The idea that micro-organisms can induce molecular aromatization is not new. For example, Wakeham et al. (1980) and Lohmann et al. (1990) stress the importance of microorganisms in the degradation of triterpenoid precursors to polycyclic aromatic hydrocarbons within sediments. In Lake Chala, IV is already present in the profoundal surface sediments. This whole transformation process from A-ring removal to B-ring aromatization, therefore, must take place relatively rapidly, within 40 years. This is much faster than observed in Lake Valencia sediments (Jaffé and Hausmann, 1995), where only small amounts of the B-ring aromatized des-A-arbo were detected at 45 cm depth, indicating that it took ~180 years to form small amounts of this compound. This difference could be due to different microbial communities in the water column and sediments.

4.5. Application in paleoenvironmental reconstruction

As the transformation of isoarborinol and arborinone to the different des-A-arborenes depends on enzymatic action, it is likely that the microbial community is influenced by subtle spatial variation in the water column environment and thereby the structures and relative abundances of des-A-arborenes in the sediment. These differences in microbial communities will be most pronounced between different lake systems, but also within one system the microbial communities will differ seasonally and along a depth gradient. Although these microbial changes are likely to occur on short spatial and temporal scales, sediments can record these changes cumulatively in the form of long-term trends, and hence examining absolute and relative changes in sedimentary des-A-arborene degradation products through time provide insight in some physical aspects of Lake Chala. As only low (trace) amounts of isoarborinol are found in Lake Chala at present, it is reasonable to assume that the vast majority of triterpenoids originally present have been subjected to microbial transformation. Therefore, the accumulation of des-A-arborenes in the sediment record can be used as a proxy for the production rates of isoarborinol/arborinone in the water column. These parent compounds are most likely synthesized by aerobic bacteria residing just above the chemocline of the lake (Section 4.3).

To elucidate possible paleoclimatological and paleoenvironmental controls on the concentration of des-A-arborenes in Lake Chala sediments, this record is compared with two independent proxy records (Fig. 7) of climatic moisture balance (the precipitation/evaporation ratio), namely the seismic-reflection record of lake level fluctuation (Moernaut et al., 2010) and the branched isoprenoid tetraether (BIT) index of past rainfall variations (Verschuren et al., 2009; Bickel et al., 2016). The concentration of des-A-arborenes is somewhat higher during inferred wetter periods, and lower during inferred drier periods (Fig. 7). While concrete evidence for a mechanistic link explaining this correlation is not provided by this study, we can infer that des-A-arborene production must have been higher during times of more pronounced water column stratification and/or higher precipitation than today. Variations over time between the different des-A-arborene compounds could indicate changes in the activity of different microbial assemblages producing and degrading isoarborinol or arborinone. It would be interesting to compare the proliferation of microbial communities in different lake systems where des-A-arborenes and their precursors have been found, as this could be informative to understand the subtleties in microbial transformation patterns of isoarborinol/arborinone. Future metagenomic studies of the modern lake system of Lake Chala and its sediments may reveal the connection between microbial communities and isoarborinone production and its microbial transformations.
5. Conclusions

The sedimentary record of Lake Chala contains a variety of transformation products of pentacyclic triterpenoids with an arborane skeleton. The functionalized triterpenoids are synthesized by an unverified microbial source in the water column, most likely algae or aerobic bacteria. After production in the (sub-)oxic water column, microbially mediated A-ring loss of isoarborinol/arborinone occurs in the suboxic/anoxic water column, producing $8^bH$-des-A-arbor-5(10),9(11)-diene, which is hydrogenated forming predominantly $8^bH$-10$^b$Me-des-A-arbor-9(11)-ene. The process of aromatization from mono- and di-unsaturated des-A-arborenes to des-A-arbor-5,7,9-triene is endothermic, and therefore most likely microbially mediated. Although their precise biological source remains unknown, des-A-arborenes are specific biomarkers with high preservation potential in the anoxic sediments of Lake Chala, and in the anoxic sediments of similar small, tropical crater lakes with a permanently anoxic hypolimnion. The varying proportion of des-A-arborenes in these systems could be a reflection of subtle differences in the physical parameters of the water column along the depth gradient, resulting in changing microbial communities and activity. Differences in arborane-type triterpenoids and their distribution patterns between different lakes, both extant and in the geological past, are likely due to different microbial communities producing and transforming isoorborinol and/or arborinone and des-A-arborenes with one, two and three double bonds.

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Supplementary material

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