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Impact of aerobic reworking of biomass on steroid and hopanoid biomarker parameters recording ecological conditions and thermal maturity

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ABSTRACT

Steranes and hopanes are the biomarkers of eukaryotic sterols and bacterial hopanols. Extracted from sedimentary rock, they are widely used to assess burial temperatures and palaeoecological conditions. The relative proportion of steranes and hopanes is commonly applied as a measure of the flux of eukaryotic versus bacterial biomass into sediments, and the relative abundances of C_{27}, C_{28} and C_{29} steranes are proxies for shifts in eukaryote ecology. In Recent sediments, intact sterols provide additional information about particular eukaryotic origins. However, biological lipid distributions are not always recorded faithfully in sediments. Based on observations on modern algae and plants, and on 558 million year old fossil macroalgae from the Ediacaran of the White Sea, we suggest that these biomarker proxies can be severely altered by aerobic microbial reworking, to the extent that a complete loss of primary ecological information may occur. Network analysis on the biomarker data suggests that oxic degradation also affects isomer and homolog distributions of saturated and aromatic steroids, hopanes, cheilanthanes and *n*-alkanes, generating anomalies in apparent thermal maturity indicators and other proxies. In our dataset, between Ediacaran macroalgae that experienced the least and the most oxic degradation, the absolute concentration of biomarkers decreases 80-fold, and at the same time the proportion of steranes over hopanes decreases by a factor of 82, while the proportion of C_{29} steranes among total steranes decreases from 91% to 47%. Such redox dependent offsets may explain the recurrently erratic behaviour of numerous biomarker parameters. While these results impart constraints on the interpretation of biomarker distributions, they do provide a tool for evaluating the effects of oxygen exposure and microbial degradation on organic matter preservation in recent and ancient environments and may point towards a solution for the correction of such effects.

1. Introduction

Most of our knowledge of the history of life on Earth has been obtained from body fossils that yield a record of the evolution and diversification of some groups of organisms. However, this record is incomplete, as only a fraction of lifeforms are preserved as fossils. Particularly limited is our understanding of the base of the marine foodweb, namely the evolutionary trajectory, and the ecological importance of different photosynthetic primary producers in the oceans through time (Knoll et al., 2007). Algae and phototrophic bacteria without external skeleton are rarely fossilized, and even if they are, their relative abundance in the rock record does not reflect their contribution to primary production. This is due to an extreme taphonomic bias that affects the relative preservation of different types of organisms. However, there is a complementary record that is commonly used to obtain semi-quantitative information about biological sources in modern and ancient sedimentary settings: molecular fossils, or biomarkers.

Biomarkers are organic molecules that have a specific biological origin, and they have been found in sediments and sedimentary rocks going back to the Palaeoproterozoic (Vinnichenko et al., 2020). These molecules include biological lipids, pigments, fragments of macromolecules such as algal cell wall material, and the diagenetic products of these compounds. Some of the most commonly employed biomarkers derive from sterols, membrane lipids of most eukaryotes and a few bacteria, and hopanols produced by many bacteria. Alteration of sterols and hopanols by biological and chemical processes starts at the moment

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of death and continues throughout the history of the molecules, from transport through the water column and deposition in sediments, to deep burial in the rock record (Hedges and Keil, 1995; Peters et al., 2005). During these processes, biological sterols and hopanols are transformed into saturated hydrocarbon steranes (S) and hopanes (H), and aromatic steroids and hopanoids. The relative abundance of steranes and hopanes, expressed as the ratio S/(S + H), is regarded as a measure of the relative flux of eukaryotic versus bacterial organic matter to bottom sediments, whereas the proportions of steranes with different side chain alkylation, the 'sterane trifecta' cholestane (C_{27}), ergostane (C_{28}) and stigmastane (C_{29}), are commonly used as an indicator of changes in the organic matter flux of different groups of eukaryotes (e.g. Bull et al., 2002; Leeming et al., 1996).

The relative abundances of steranes and hopanes, and those of their alteration products, including different stereo- and structural isomers, are also routinely used in petroleum studies to obtain information about source rock characteristics when analysing oils from reservoirs, seeps and spills. For instance, sterane trifecta and S/(S + H) ratios are used for reservoir-source rock correlations and as age indicators for Palaeozoic, Mesozoic and Cenozoic oils (Grantham and Wakefield, 1988; Peters et al., 2005). Maturity parameters based on ratios between more and less thermally stable isomers are used to establish the thermal history of source rocks and their oil generation potential (Peters et al., 2005). Some isomer ratios in oils yield further information about source rock properties, such as the proportion of diasteranes to regular steranes, which depends on clay and carbonate content (Peters et al., 2005).

However, it has also been noted that most biomarker proxies are affected by more than one factor. For example, sterols in primary plant debris in soil can become so extensively recycled by the soil microbiome that S/(S + H) may drop from 1.0 to ≪ 0.1 (Moldowan et al., 1985; Peters et al., 2005; Ries-Kautt and Albrecht, 1989). By contrast, this effect is commonly considered minimal in marine environments, where both S/(S + H) and the relative abundance of C_{27}, C_{28} and C_{29} steranes are believed to be a reasonable reflection of the primary product. Previous work provides considerable understanding of the rates of sterol degradation in comparison to other biomarker classes; however, studies on relative rates of degradation of particular sterols in sediments have revealed contrasting and often opposite trends (Canuel and Martens, 1996; Colombo et al., 1997; Harvey and Macko, 1997; Hoefs et al., 2002; Rontani et al., 2012; Sun and Wakeham, 1998; Yunker et al., 2005). When comparing changes in sterol degradation in different sedimentary environments, such studies have to assume an invariable input of organic matter, thus any lateral/temporal changes in sterol input, which can be substantial, results in distorted perception of relative sterol degradation rates (Canuel and Martens, 1996; Colombo et al., 1997; Rontani et al., 2012; Yunker et al., 2005).

It is well known that traditional maturity indicators may be affected by factors other than thermal history (Brassell et al., 1984; Moldowan et al., 1986; Peakman et al., 1989; Sun et al., 2016; Ten Haven et al., 1986). In some instances, stronger isomerisation of steroids and hopanoids towards higher 'maturity' has been reported in apparently more oxic environments (Moldowan et al., 1986), whereas in other cases isomerisation appears to be elevated in anoxic, organic-rich samples (Sun et al., 2016). Other recent studies have shown that the ratio of ββ and βα hopanoid isomers in peats strongly depends on local pH (Huang et al., 2015; Inglis et al., 2018). However, the conditions that drive overall sterane and hopane isomerisation in such instances are generally poorly constrained.

To elucidate the control of redox and degradation on sterane and hopane biomarker parameters, we chose a system where the thermal maturity and source of primary organic matter remained constant: 14 specimens of organically preserved macroalgae from thermally immature siliciclastic rocks of late Ediacaran age (558 Ma) in the White Sea area (Fig. 1). Generally, bulk sediment extracts and petroleum represent mixtures of organic compounds of different sources, which display averaged biomarker distributions. By using individual fossils in this study, we increase our chances not only for clear interpretation of the data, but also for obtaining the most pronounced, non-averaged biomarker signals. The Ediacaran age ensures the absence of a significant terrestrial biomarker component, as well as an absence of bioturbation, as complicating factors. To place the results into context, we also performed a series of laboratory degradation experiments on extant macroalgae and higher plants under varying conditions.

2. Materials and methods

2.1. Laboratory materials

All solvents used in the study were 99.9 % grade (UltimAR®; Mallinckrodt Chemicals, St. Louis, MO, USA). Glassware, glass fibre filters and aluminium foil were cleaned by combustion at 300 °C for 9 h, and metal appliances were washed with methanol and DCM before use.

2.2. Ediacaran macroalgae

Biomarker analyses were conducted on 14 organically preserved

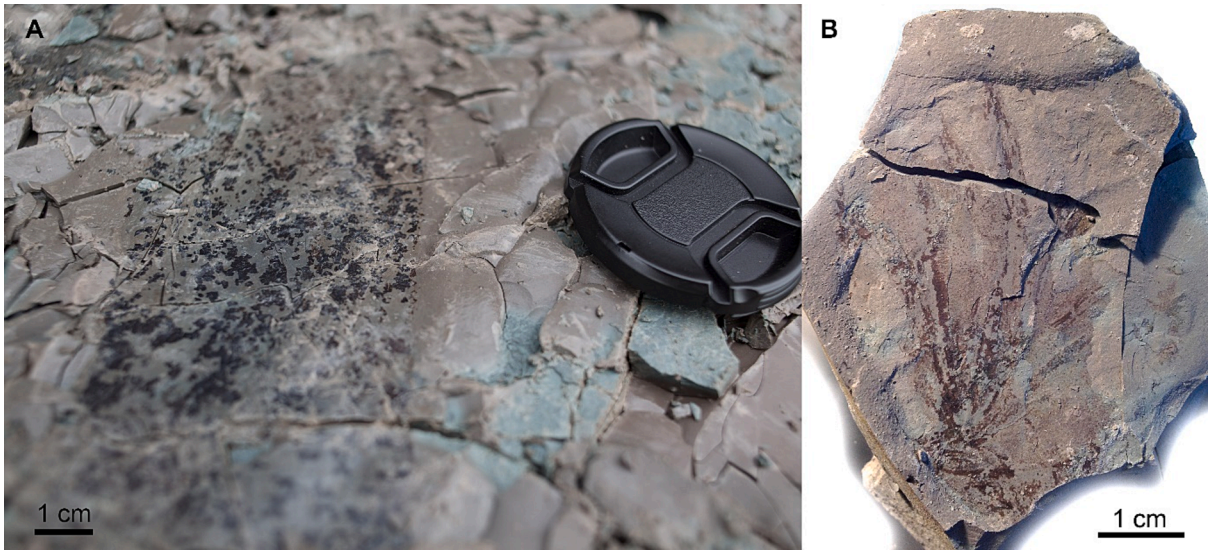


Fig. 1. Organically preserved Ediacaran macroalgae from the White Sea area. (A) *Archyfasma lamellata*; (B) ex gr. Vendotaenides.

macroalgae and a sample of clay that surrounded the fossils. Samples were collected specifically for biomarker analysis during fieldwork in 2015–2018, from the Lyamtsa locality of the Ediacara Biota in the White Sea area (Russia). Macroalgae were sampled from a 1.5 m thick layer of clay in the lower part of the Lyamtsa Beds (Ust-Pinega Formation, Redkino Regional Stage), exposed to the south of Lyamtsa Village. All samples were collected avoiding weathered zones and cracks and were immediately wrapped in pre-combusted aluminium foil (300 °C, 9 h) and packed in calico cotton bags under strict avoidance of contamination (Bobrovskiy et al., 2018b).

2.3. Extant plants and degradation experiments

Macroalgae were collected on the south-eastern coast of Australia, New South Wales. Green macroalgae *Ulva lactuca* and ocean water for the experiments were collected from a tidal zone. The water was then filtered through a 5 μm filter. Brown macroalgae *Ecklonia radiate* were collected from a shallow low-energy lagoon, formed in an area protected from waves by local coast configuration. We collected discoloured specimens that had started to become slimy and to lose their integrity. Freshly stranded *E. radiate* were collected from a nearby beach. Both fresh and degraded *E. radiate* were entangled in red macroalgae *Gracilaria edulis*, which we also analysed to account for any potential epiphyte lipids. *Lactuca sativa* (lettuce) specimens came from a supermarket. All specimens were washed with deionized (Millipore Elix 3UV, France) water, kept refrigerated overnight in glass jars and further processed one day after collection. All degradation experiments were carried out simultaneously in triplicate.

For degradation experiments, *U. lactuca* were organized into samples of around 0.6 g (wet weight) and put into glass jars. For anoxic experiments, three samples were further covered with glass fibre filters, followed by around 5 mm of sand and around 1 cm of clay. The sand was combusted at 600 °C prior to use; the sand and the clay used in the study contained no extractable organic matter, which was confirmed by GC–MS analyses. The jars were then filled with ocean water and closed to avoid evaporation. Anoxic conditions were achieved in the sediment within days, as was visible by the production of black iron monosulphide, and these conditions persisted throughout the length of the experiments (O_2 content $<$ 0.08 μmol/L, measured with a PreSens Microx 4 trace fiber optic oxygen meter, with a PreSens DP-PST7 factory-calibrated dipping probe). For oxic experiments, three jars with algae were filled with ocean water, closed with lids, and then mixed with a metal spatula once a week to saturate water with oxygen; the spatula was sterilised with methanol and dichloromethane (DCM) before use on each sample. Note that we cannot exclude the possibility that anoxic conditions developed in the vicinity of the decomposing plants in the early stages of the experiments, when organic matter was relatively abundant. All experiments were performed in the dark at room temperature (20 ± 3 °C) for 5 months.

For further analysis, samples that had been covered with sediment were frozen, the bottoms of the jars were broken off and plant material was carefully separated from the overlying glass fibre filters using tweezers and collected into glass vials for extraction. For the analysis of oxic experiments, all water from the jars was filtered through a glass fibre filter, and these filters were extracted together with the captured plant material.

L. sativa experiments were set up the same way as *U. lactua*, except that around 4 g of plant material (wet weight) was used for anoxic experiments without sediment cover, as anoxic conditions in the water were sustained owing to decomposition of the larger amounts of organic matter (O_2 content $<$ 0.05 μmol/L, measured with a PreSens Microx 4 trace fiber optic oxygen meter, with a PreSens DP-PST7 factory-calibrated dipping probe). For all experiments, plant material was recovered with tweezers for subsequent extraction. One experiment with lettuce covered by sediment was analysed in this study. In this setup, a lettuce leaf escaped from the sediment during methane production in the first week of the experiment and reached the top of the water layer. The leaf initiated rapid formation of a microbial layer around it. The lettuce was separated into three samples: two samples came from within the floating mat, and one came from within the water column (Fig. 2). One of the oxic lettuce experiments was excluded from the analysis as a lettuce leaf floated to the top of the water layer and became contaminated with mould.

It should be noted that the anaerobic experiments cannot be considered to represent the same stage of degradation as aerobic experiments, because it is well known that anaerobic degradation is much slower than aerobic turnover. However, while neither of the experiments can be taken to represent the final result of degradation, the results can be used to observe trends and patterns in lipid degradation.

2.4. Preparation and extraction of samples

Extant plant material was dried at 50 °C overnight and extracted via ultrasonic agitation in solvents (methanol:dichloromethane (DCM) 3:2 for 15 min (x 2), DCM:*n*-hexane 2:3 for 15 min). The extract was fractionated into non-polar and polar fractions using micro-column chromatography over annealed (300 °C; 12 h) and dry packed silica gel (Silica Gel 60; 230– 600 mesh; EM Science). Non-polar molecules were eluted with 1.5 ml of *n*-hexane:DCM (1:1) and the polar fraction with 1 ml DCM:methanol (1:1). The polar fractions were saponified in 1 ml of 5 % KOH methanol:water 4:1 solution at 50 °C overnight, 1 ml of water was added and a neutral fraction was extracted by vortexing for 1 min with 1 ml of DCM:*n*-hexane 1:4 (X 3). Sterols were further converted to the trimethylsilyl (TMS) derivatives for gas chromatography–mass spectrometry analysis by reacting with BSTFA (Sigma Aldrich) overnight at 50 °C; BSTFA was then evaporated and the samples were redissolved in *n*-hexane.

All Ediacaran macroalgae were preserved as carbonaceous impressions within clay, so their organic matter could be gently removed from the rock surface with tweezers, sometimes applying a scalpel for initial separation. Hydrocarbons were extracted from the organic matter via ultrasonic agitation in solvents (methanol for 30 min, dichloromethane (DCM) for 15 min (x 2), DCM:*n*-hexane 1:1 for 15 min).

Extracts of fossils were fractionated into saturated, aromatic and polar fractions using micro-column chromatography over annealed and dry packed silica gel. Saturated hydrocarbons were eluted with 1 dead volume (DV) of *n*-hexane, aromatic hydrocarbons with 4.5 DV of *n*-hexane:DCM (4:1) and the polar fraction with 3 ml DCM:methanol (1:1). Extracts from fossils with low hydrocarbon content were only fractionated into saturated + aromatic and polar fractions to reduce the loss of analytes. An internal standard, 18-MEAME (18-methyleicosanoic acid methylester; Chiron Laboratories AS), was added to the saturated and aromatic fractions, whereas D4 (d_4-C_{29}-ααα*R*-ethylcholestane; Chiron Laboratories AS) was added to saturated and saturated + aromatic hydrocarbon fractions with low biomarker content. The samples were analysed and quantified by GC–MS.

2.5. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses of biomarker extracts were carried out on an Agilent 6890 gas chromatograph coupled to a Micromass Autospec Premier double sector mass spectrometer (Waters Corporation, Milford, MA, USA) at the Australian National University. The GC was equipped with a 60 m DB-5 MS capillary column (0.25 mm i.d., 0.25 μm film thickness; Agilent JW Scientific, Agilent Technologies, Santa Clara, CA, USA), and helium was used as the carrier gas at a constant flow of 1 ml min^{-1}. Samples were injected in splitless mode into a Gerstel PTV injector at 60 °C (held for 0.1 min) and heated at 260 °C min^{-1} to 300 °C. For full-scan, selected ion recording (SIR) and metastable reaction monitoring (MRM) analyses, the GC oven was programmed from 60 °C (held for 4 min) to 315 °C at 4 °C min^{-1}, with total run time of 100 min. All samples were injected in *n*-hexane to avoid deterioration of chromatographic signals

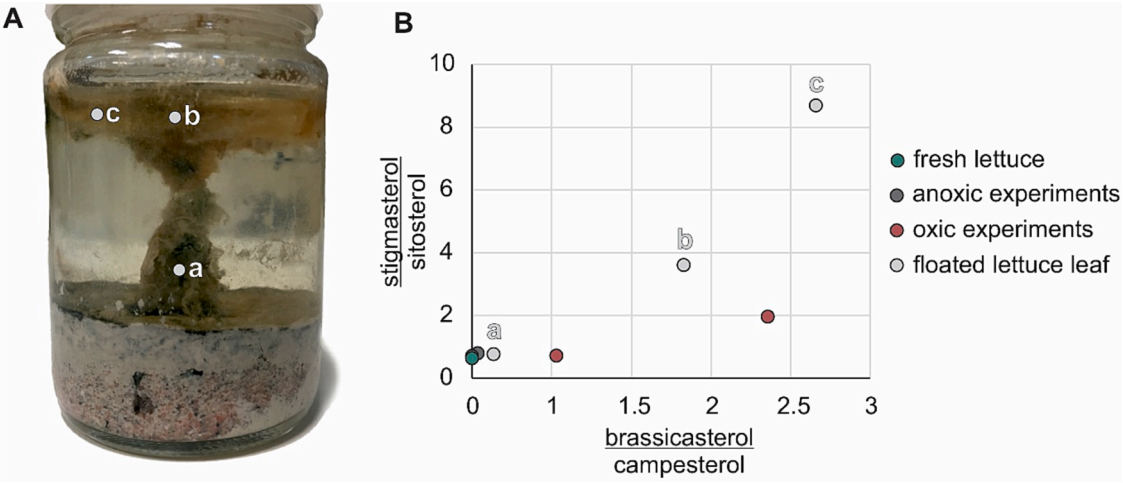


Fig. 2. A lettuce (*Lactuca*) leaf that floated to the top of the water layer and is surrounded by a microbial layer, with sampling points a, b and c **(A)**, and **(B)** a plot of the proportion of sterols with and without unsaturation in the side chain between C_{28} (brassicasterol/campesterol) and C_{29} (stigmasterol/sitosterol) sterols in the floated *Lactua* leaf, and augmented with data from other degradation experiments.

by $FeCl_2$ build-up in the MS ion source through use of halogenated solvents (Brocks and Hope, 2014). *n*-Alkanes were quantified in full-scan mode in *m/z* 85 trace, and ααα20R-stigmastane in *m/z* 217, relative to the 18-MEAME internal standard (*m/z* 340). Steranes and hopanes were quantified in MRM mode in $M^+ \rightarrow 217$ and $M^+ \rightarrow 191$ transitions, respectively, relative to ααα 20R-stigmastane. Monoaromatic steroids were quantified in SIR mode using the *m/z* 253 base ion, and triaromatic steroids using the *m/z* 231 base ion. To ensure accurate integration, TMS-ethers from modern plants were quantified in full-scan mode using *m/z* 129 for sterols, and *m/z* 191 and *m/z* 189 for hopanols and unknown terpanols (T); sterol and hopanol integrated areas were further corrected for differences in *m/z* response based on differences between single ion and total ion currents calculated for one of the samples. Peak areas for saturated and aromatic molecules are uncorrected for differences in GC–MS response.

2.6. Pyrolysis experiments

For pyrolysis, extracted material from Ediacaran macroalgae and extant plants was dried from the solvents and transferred to glass pyrolysis tubes (6 mm external diameter). The tubes were flushed with nitrogen gas, evacuated using a vacuum line, and flame sealed. The sealed tubes were then heated to 310 °C for 16 h for extant macroalgae and 72 h for Ediacaran organic matter. After cooling, the tubes were opened and pyrolysed material was extracted via ultrasonic agitation in solvents (DCM (×1) and *n*-hexane:DCM 1:1 (×2)), followed each time by centrifugation. The pyrolysate was fractionated into non-polar and polar fractions as described above. The non-polar fraction was dissolved in *n*-hexane and hydrogenated in a 4 ml vial by bubbling a gentle stream of hydrogen gas for 20 min under stirring with ~ 0.2 mg of platinum (IV) oxide on charcoal added as a catalyst, then the hydrogen gas was turned off, the vials were closed and stirred continuously for 16 h. The samples were separated from the catalyst via ultrasonic agitation in solvents (*n*-hexane (×2) and *n*-hexane:DCM 1:1 (×1)) followed each time by centrifugation. Samples were cleaned from any remaining catalyst by eluting with an excess of *n*-hexane over a small chromatographic column filled with activated silica gel.

2.7. Error quantification

As repeat injections of fossil extracts were not possible due to the limited abundance of sample material, standard deviations for the concentration of each biomarker were externally determined based on the clear relationship between the peak area and coefficient of variation determined for the GC–MS system: $C_v = 186.18x^{-0.31}$ ($R^2 = 0.82$), where *x* is the integrated peak area (Bobrovskiy et al., 2018a). The standard deviation was calculated as STD = C_v*signal area. For biomarker ratios, error propagation followed standard root mean square error propagation formulae. As βαα 20R-ergostane (5β) coelutes with two other compounds, ααα 20S-ergostane and an unknown sterane (tentatively identified as a βαα 20R-27-nor-sterane), its integration error should be expected to be much higher than the error for peaks that are not coeluting and are mainly controlled by signal/noise ratio. Thus, an integration error calculated on the whole Ediacaran White Sea area dataset based on deviation of 5β/5α ergostane values from 5β/5α of cholestanes and stigmastanes, where cholestane and stigmastane 5β/5α values were equal, was used to compute the coefficient of variation for the ergostane 5β/5α ratios. This error is further used to compute the coefficient of variation for the ergostane 5β/5α ratios, estimated to be $C_v = 0.34$ for the Ediacaran White Sea area extracts (Bobrovskiy et al., 2018a).

2.8. Laboratory system blanks

A comprehensive, accumulatory system blank was performed covering all analytical steps during extraction, fractionation and instrumental analysis of Ediacaran sediments and algae. For this purpose, pre-extracted powdered clay was used to monitor background contamination.

To confirm that the lipids extracted from plants in the decomposition experiments were only associated with the plants, we additionally analysed extracts of water and clay in the same containers. For this, 50 ml of water (from one experiment for each triplicate) was concentrated to 10 ml by evaporation at 50 °C, and extracted by swirling with 2 ml of DCM:hexane 1:4 solution for 1 min three times. Clay (from each anaerobic experiment on *U. lactua*) was extracted, and both water and clay extracts were further treated the same way as plant material. None of the extracts displayed any detectable steroids or hopanoids.

2.9. Data processing

As many biomarker parameters do not have normal data distributions, the Spearman rank correlation coefficient was used instead of the more classically applied Pearson coefficient. Spearman rank correlation matrix was built in PAST3 (Hammer et al., 2001), and the correlation matrix-based network was built in Gephi (Bastian et al., 2009) using the Force Atlas 2 layout algorithm.

3. Results

3.1. Biomarker composition of Ediacaran macroalgae

The White Sea area in Russia preserves thermally immature Ediacaran deposits (Bobrovskiy et al., 2018b) that formed in shallow-water marine environments at the edge of an intracratonic sea (Grazhdankin, 2003). Although the sea waters were well-mixed and sufficiently oxygenated to sustain macroscopic benthic bilaterians (Fedonkin et al., 2007), biomarkers are well-preserved throughout the entire section, including storm-deposited sandstone layers (Bobrovskiy et al., 2020; Bobrovskiy et al., 2018a). This can be attributed to the rapid development of anoxic conditions in the sediments, likely owing to the rapid growth of microbial mats that sealed the sediments from oxidant diffusion from the overlying water column. These conditions allowed for organic preservation of Ediacaran macrofossils (Bobrovskiy et al., 2019; Gehling, 1999) and, in some layers, abundant macroalgae as carbonaceous compressions (Bobrovskiy et al., 2018b; Leonov, 2007).

We analysed biomarkers extracted with organic solvents from 14 macroalgae (Table S1), all derived from multiple surfaces within a single 1.5 m-thick clay layer (in the clay, TOC = 0.063 %) in one outcrop, which included one specimen of the genus *Archyfasma* (sample OM12; Fig. 1A), two specimens of bush-like bifurcating ex.gr. Vendotaenides (OM13 and OM16; Fig. 1B), and eleven dark brown, unidentifiable organic films that are identical in thickness and colour to macroalgae, but distinct from organic films derived from bacterial colonies and Ediacara biota fossils from the same locality (Bobrovskiy et al., 2018a; Bobrovskiy et al., 2018b; Bobrovskiy et al., 2019).

The macroalgae, excluding *Archyfasma*, demonstrate continuous distribution of sterane trifecta values with stigmastanes (C_{29}) comprising 47 % to 91 % of total C_{27} to C_{29} steranes. Similarly, S/(S + H) ranges from 0.19 to 0.98, and 5β/5α sterane isomer values range from 0.49 to 3.93 (Figs. 3, 4, Table S1). Notably, all of these parameters strongly correlate with one another. Correlations between different biomarker parameters of the macroalgae extracts are summarized in Fig. 4 and Table S2. As 5β/5α varies from 3.93 to 0.49, the proportion of steranes over hopanes decreases by a factor of 82 (Spearman's $\rho = 0.84$, $p < 0.00001$), and the proportion of C_{29} steranes drops by a factor of ~ 2 ($\rho = 0.70$, $p < 0.008$) (Fig. 4B, Table S1). At the same time, the overall yield of steranes and hopanes extracted from the algal biomass ([S + H] in μg/g organic matter, OM) decreases by a factor of 80 ($R^2 = 0.99$, $\rho = 0.56$, $p < 0.04$ with S/H ratio) (Fig. 4B, Table S1). The only macroalga that does not follow this relationship, *Archyfasma,* demonstrates high S/(S + H) and 5β/5α values, but a relatively low stigmasteroid proportion (C_{27}: C_{28}: C_{29} = 37 %: 2 %: 61 %).

We also examined relationships between a wider range of biomarker parameters in the macroalgae extracts. To quantify how the variability of these parameters is connected, we built a correlation matrix with 37 ratios that provides a measure of correlation between each biomarker parameter pair (Table S2). A network diagram visualizes these relationships (Fig. 4A). The 'edges' (the connecting lines) in the network highlight individual correlations between the parameters, and the thickness of the edges, as well as the spacing of the nodes, reflects how strong the relationships are (quantified as Spearman's ρ). The network includes parameters that are traditionally interpreted as biological or ecological proxies (green nodes), thermal maturity indicators (red), redox proxies (yellow), and other parameters that are often employed in palaeoecological and petroleum studies with various interpretations (grey).

3.2. Sterol degradation in extant macroalgae and plants

In the laboratory experiments, the green macroalga *Ulva lactuca*, and the higher plant *Lactuca sativa* (lettuce) were degraded under oxic and dysoxic to anoxic conditions (see Methods). Among algae, we specifically chose *Ulva lactuca* for the experiments as its relevant properties match those of the studied Ediacaran macroalgae: it consists of a large thin leaf, and it has a pronounced C_{29} sterol predominance. *Lactuca*

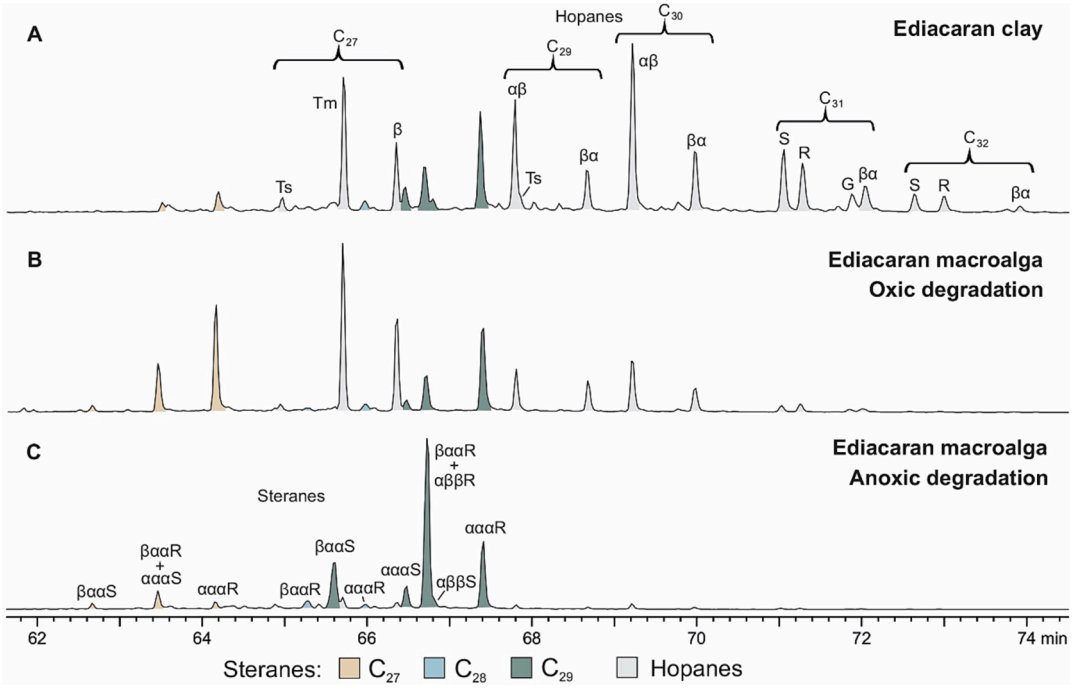


Fig. 3. MRM chromatograms showing the distribution of steranes and hopanes in Ediacaran macroalgae and enclosing sediment; each chromatogram is a sum of $C_{27\text{-}29}$ sterane and $C_{27\text{-}32}$ hopane MRM traces. **(A)** Ediacaran clay extract (34–01-MN); **(B)** extract of an Ediacaran macroalga (OM14) with relatively low S/(S + H) and 5β/5α ratios and low proportion of C_{29} steranes, patterns that were arguably caused by oxic degradation; **(C)** extract of an Ediacaran macroalga (BM1) with relatively high S/(S + H) and 5β/5α ratios and high proportion of C_{29} steranes, a pattern that may reflect degradation under largely anoxic conditions. G marks gammacerane peak.

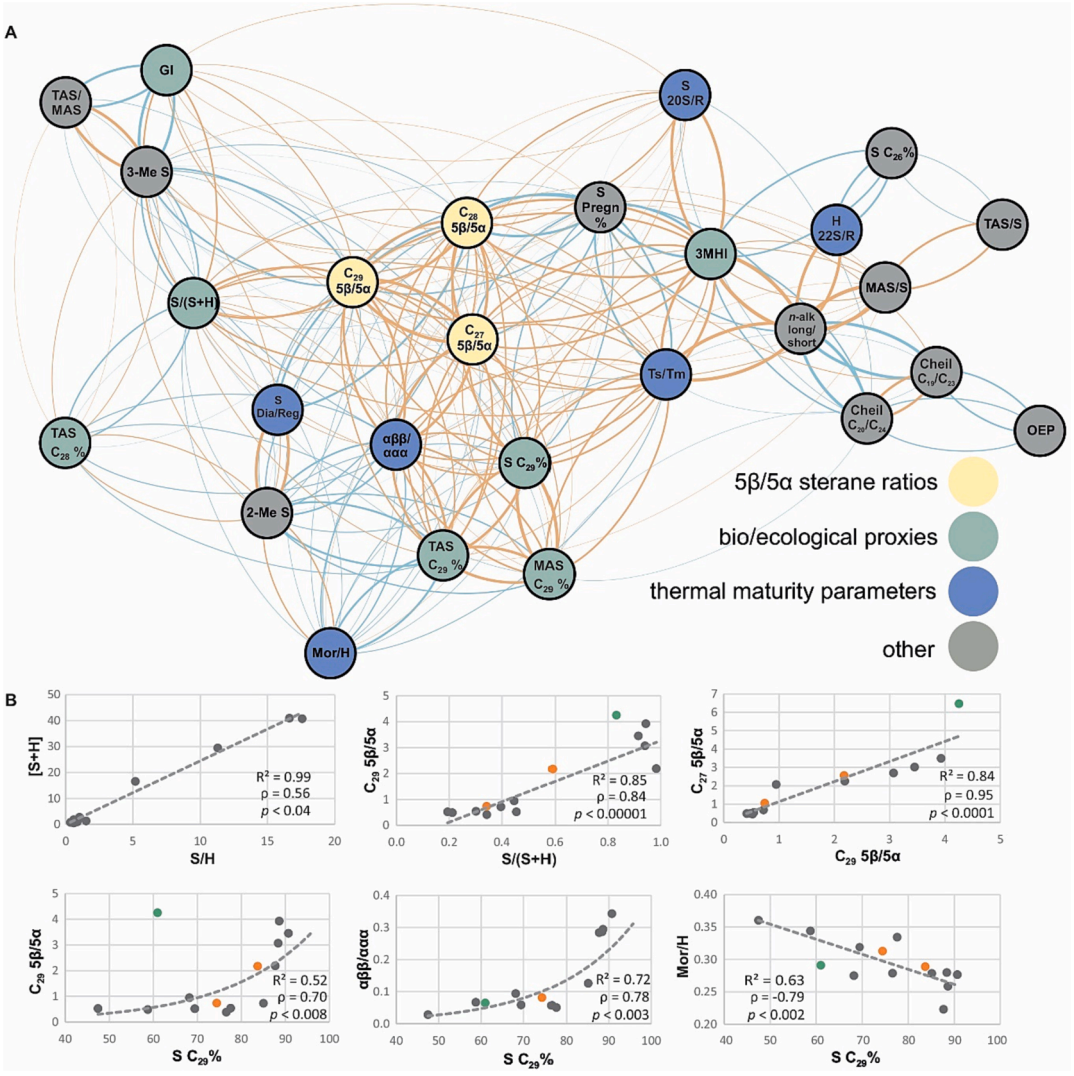


Fig. 4. Relationships between selected biomarker parameters of Ediacaran macroalgae. **(A)** Network analysis demonstrating the interlink between all major biomarker parameters. The structure is based on a Spearman's ρ correlation matrix, filtered by $p < 0.05$, $|\rho| > 0.6$; distance between nodes and edge line thickness reflect ρ correlation coefficient values between parameters, orange edges show positive correlation, light blue – negative correlation; nodes are coloured according to traditional interpretations of biomarker parameters: yellow – 5β/5α sterane ratios, reflecting diagenetic redox conditions; green – parameters traditionally interpreted as biological or ecological proxies; blue – ratios traditionally interpreted as thermal maturity parameters; grey – unknown or mixed interpretations; **(B)** xy plots showing correlations between selected biomarker parameters in macroalgae, green – *Archyfasma*, orange – ex. gr. Vendotaenides, grey – unidentifiable macroalgae. Biomarker parameters: **5β/5α** = (βαα20R + ααα20S)/ααα20R; **αββ/ααα** = C_{27} αββ(20S + 20R)/ααα20R; **S/(S + H)** = Σ($C_{27\text{-}29}$ steranes) / Σ($C_{27\text{-}29}$ steranes, $C_{27\text{-}35}$ hopanes), steranes: C_{27} = Σ(βα-20(S + R)-diacholestane, ααα- and βαα-20(S + R)-cholestane), C_{28} = Σ(βα-20(S + R)-diaergostane, ααα- and βαα-20(S + R)-ergostane), C_{29} = Σ(βα-20(S + R)-diastigmastane, ααα- and βαα-20(S + R)-stigmastane), ααα = 5α(H),14α(H),17α(H), βαα = 5β(H),14α(H),17α(H); hopanes: C_{27} = Σ(Ts, Tm, β), C_{29} = Σ(αβ, Ts, βα), C_{30} = Σ(αβ, βα), $C_{31\text{-}35}$ = Σ(αβ-22(S + R), βα), αβ = 17α(H)21β(H), βα = 17β(H)21α(H); C_{29} = Σ(αβ, Ts, βα), C_{30} hopane = Σ(αβ, βα), $C_{31\text{-}35}$ = Σ(αβ-22(S + R), βα); **3MHI** = 100*(Σ C_{31} 3-methylhopane) / (Σ C_{31} 3-methylhopane + Σ(αβ-22(S + R), βα) C_{30} hopane); **S C29%** = 100*C_{29} steranes / Σ($C_{27\text{-}29}$ steranes); **TAS C29%** = 100*C_{28} triaromatic steroids / Σ($C_{26\text{-}28}$ triaromatic steroids); **Ts/Tm** = Ts/Tm C_{27} hopanes; **GI** = gammacerane / C_{30} hopanes; **MAS C29%** = 100*C_{29} monoaromatic steroids / Σ($C_{27\text{-}29}$ monoaromatic steroids); **S 20S/R** = ααα20S/ααα20R C_{29} steranes; **MAS/S** = Σ($C_{27\text{-}29}$ monoaromatic steroids) / Σ($C_{27\text{-}29}$ steranes); **S C28%** = 100*C_{28} steranes / Σ($C_{27\text{-}29}$ steranes); **TAS C28 %** = 100*C_{27} triaromatic steroids / Σ($C_{26\text{-}28}$ triaromatic steroids); **Mor/H** = βα/αβ C_{30} hopane; **TAS/MAS** = Σ($C_{26\text{-}28}$ triaromatic steroids) / Σ($C_{27\text{-}29}$ monoaromatic steroids); **MAS C27%** = 100*C_{27} monoaromatic steroids / Σ($C_{27\text{-}29}$ monoaromatic steroids); **2-Me S** = ααα20R 2-methylstigmastane / ααα20R stigmastane; **Dia/Reg** = βα-20(S + R)-diacholestane / Σ(ααα- and βαα-20(S + R)-cholestane); **TAS C27%** = 100*C_{26} triaromatic steroids / Σ($C_{26\text{-}28}$ triaromatic steroids); **S C27%** = 100*C_{27} steranes / Σ($C_{27\text{-}29}$ steranes); **S Pregn%** = Σ($C_{21\text{-}22}$ steranes) / Σ($C_{27\text{-}29}$ steranes); **3-Me S** = ααα20R 3-methylstigmastane / ααα20R stigmastane; **TAS Pregn%** = Σ($C_{20\text{-}21}$ triaromatic steroids) / Σ($C_{26\text{-}28}$ triaromatic steroids); **MAS Pregn%** = Σ($C_{21\text{-}22}$ monoaromatic steroids) / Σ($C_{27\text{-}29}$ monoaromatic steroids).

sativa was chosen among higher plants for the same reasons. Oxygen limited degradation conditions were created either in water, reached by decomposition of large amounts of organic matter, or within sediments, where plants were sealed from the overlying water by a layer of clay (see Methods). *U. lactuca* and *L. sativa* have a strong C_{29} predominance among sterols, but the particular sterol species are different (Figs. 5, 6, Table S3): *U. lactuca* contains almost solely isofucosterol (C_{29} $\Delta^{5,24(28)}$, 99 % of total sterols, Fig. 6A), and *L. sativa* plant-typical stigmasterol (C_{29} $\Delta^{5,22}$, 35 %), sitosterol (C_{29} Δ^{5}, 57 %), and campesterol (C_{28} Δ^{5}, 8 %; Fig. 7A). Additionally, *L. sativa* contains a small proportion of unidentifed C_{30} terpenoids (T) with an unsaturated ring system (S/(S + T) = 0.90). Anoxic decomposition was accompanied by the formation of black iron monosulphides, whereas oxic decomposition resulted in partial discolouration, disintegration and a decrease in the amount of plant material.

After degradation of *U. lactuca* and *L. sativa* in the dark for five

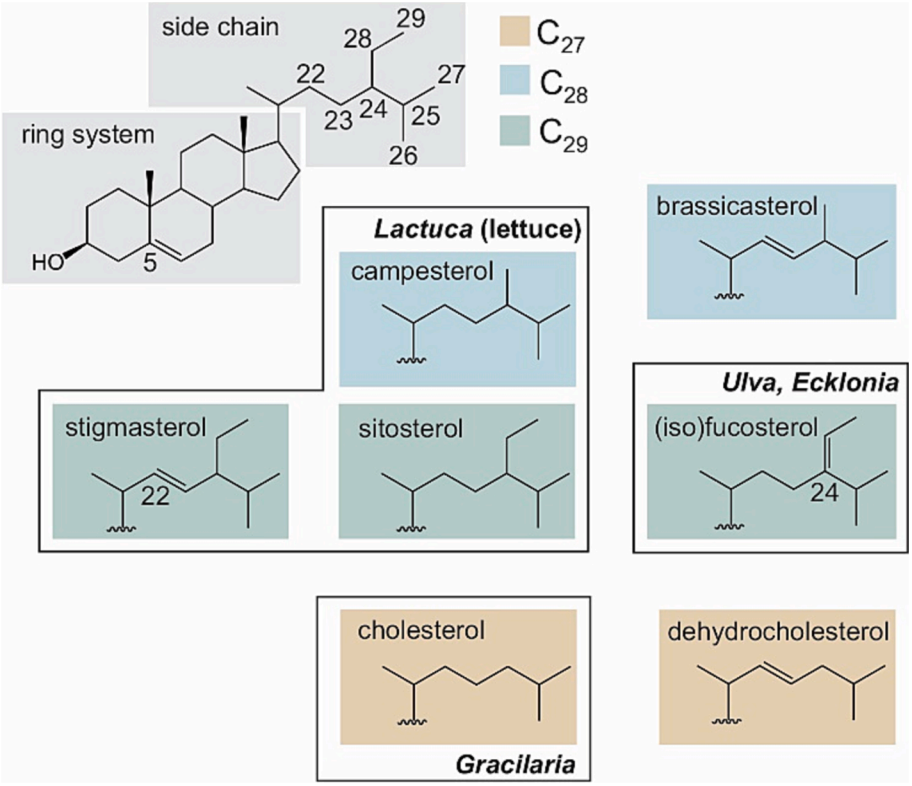


Fig. 5. Nomenclature of sterols of analysed macroalgae *Ulva, Ecklonia,* and *Gracilaria,* and the plant *Lactua* (lettuce).

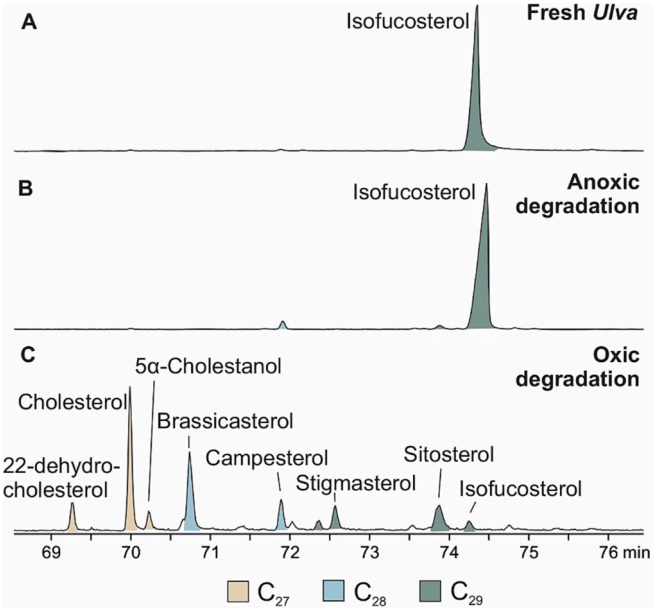


Fig. 6. Chromatograms showing the distribution of sterols in *Ulva* before and after degradation experiments (*m/z* 129). **(A)** Initial sterol composition of *Ulva*; **(B)** sterols of *Ulva* degraded in anoxic environments; **(C)** sterols of *Ulva* degraded in oxic environments.

months at room temperature, there was a major contrast between oxic and anoxic experiments (Table S3). Under anoxic conditions, the proportion between steroids and hopanoids (S/(S + H)) or C_{30} terpenoids (S/(S + T)) in the algae and plants did not change, and the sterane trifecta ratios stayed very close to the initial sterol composition of the specimens (Fig. 8A,B, 6B, 7B). During oxic degradation, however, the reduction of detectable steroids in macroalgae was in some experiments accompanied by a rise of GC-amenable hopanoids, driving the S/(S + H) ratio to lower values (down to 0.88) (Fig. 8A). At the same time, the decreasing sterol concentrations in both plants was accompanied by changes in the relative content of C_{27}, C_{28} and C_{29} sterols, with the proportion of C_{29} sterols dropping by a factor of up to 5.5 (from 99 % to 14–36 % in *U. lactuca* (Fig. 8A, 6C), and from 92 % to 36–85 % in *L. sativa* (Fig. 8B, 7C). C_{30} terpenoids present in *L. sativa* were initially S/(S + T) = 0.90, but their proportion increased during oxic degradation experiments (S/(S + T) = 0.27, Fig. 8B). We observed sterols, stanols and hopanols, but other diagenetic products of steroids or hopanoids (e.g. alkenes, ketones or acids) were only detected in trace amounts. It was not possible to obtain quantitative information about the sterol concentration in degraded plants due to the small mass and decomposed nature of the material. Instead, we quantified the absolute yield of recovered sterols as a first order estimate. However, loss of sterols might have occurred in anoxic experiments through diffusion of lipids into overlying clay. 30–90 % of lipids were recovered in anoxic experiments, and 1–5 % of lipids after oxic degradation, which is in accordance with previous estimates on the extent of sterol degradation under anoxic and oxic conditions (Hoefs et al., 2002; Sun and Wakeham, 1998).

In addition to the relative abundance of sterols with different carbon numbers, the degree and positions of unsaturation changed with the extent of oxic degradation. Isofucosterol, the major sterol in living *U. lactuca*, possesses Δ^5 and $\Delta^{24(28)}$ double bonds. Yet, this sterol was nearly absent after oxic degradation, and it was replaced by the C_{29} sterols sitosterol (Δ^5) and stigmasterol ($\Delta^{5,22}$) (Fig. 6C). In *L. sativa*, the proportion of stigmasterol to sitosterol increased, which was accompanied by the appearance of a double unsaturated $\Delta^{5,22}$ C_{28} sterol (brassicasterol), absent in living *L. sativa* (Figs. 2, 7). The increase in stigmasterol/sitosterol and brassicasterol/campesterol ratios, recording an increase in the relative abundance of Δ^{22} double bonds, progressed with time during the oxic degradation experiments along with a general decrease in C_{29} sterols relative to other sterols. This trend in increasing

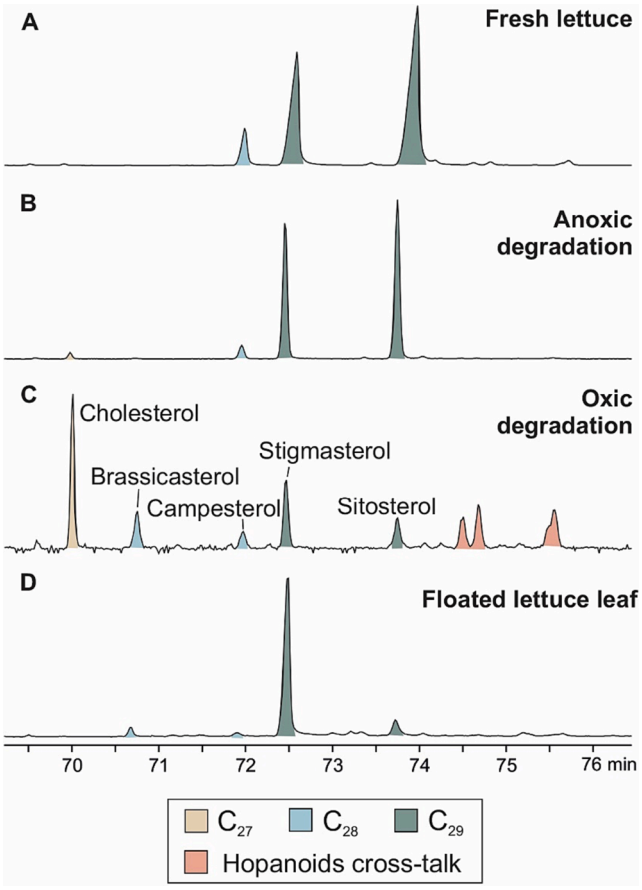


Fig. 7. Chromatograms showing the distribution of sterols in *Lactua* (lettuce) before and after degradation experiments (*m/z* 129). **(A)** Initial sterol composition of lettuce; **(B)** sterols of lettuce degraded in anoxic environments; **(C)** sterols of lettuce degraded in oxic environments; **(D)** sterols of a lettuce leaf that floated to the top of the water layer (sample C in the Supplementary Fig. 3).

Δ^{22} unsaturation was most pronounced in the leaf that floated at the water–air interface, even though the C_{29} sterol proportion in this leaf (88–95 %) stayed close to the initial value (Fig. 7D).

3.3. Algae from natural environments

To test whether the notable changes in sterol distributions during the laboratory degradation experiments are applicable to natural environments, we also analysed extant algae that had been degraded in natural shallow-marine environments. For this, we chose brown algae *E. radiate*. Red macroalgae *G. edulis*, entangling the brown algae were analysed separately to account for possible epiphyte lipids.

The fresh red macroalga *G. edulis* displayed a strong cholesterol (C_{27}) predominance among sterols (99.7 %, Fig. 9D, Table S3). However, in the *G. edulis* specimen entangling the degraded *E. radiate*, most cholesterol (over 85 %) was replaced with 22-dehydrocholesterol, thus showing the same trend of increasing Δ^{22} unsaturation as the C_{28} and C_{29} sterols in the laboratory degradation experiments. At the same time, only traces of C_{28} (3.7 %) and C_{29} (0.7 %) sterols were found, indicating that only small amounts of alien lipids (i.e. potentially not derived from the macroalga) were introduced during degradation (Fig. 9E, Table S3). The fresh, brown macroalga *E. radiate* yielded 96 % fucosterol ($\Delta^{5,24(28)}$ C_{29} sterol; Fig. 9A, Table S3), but the fucosterol content in the degraded specimens dropped to 54–65 %, accompanied by a rise of C_{27} and C_{28} sterols, following the same trend as observed in our degradation experiments (Fig. 9B,C, Table S3).

3.4. Lipid incorporation into macromolecular organic matter

As the extracts of extant plants were saponified, our experiments captured extractable ester- and sulfur-bound steroids and hopanoids. However, incorporation of lipids into macromolecular cell material could be a factor that altered the composition of extractable molecules of both Ediacaran macroalgae and extant plants. To estimate the proportion of lipids bound to macromolecular organic matter, we performed pyrolysis experiments on selected extracted specimens. Pyrolysis of the largest specimen of Ediacaran macroalgae (sample BM2) only yielded biomarkers close to the MRM detection limit, about 300 times lower than in the extractable fraction of the same specimen. Likewise, we pyrolyzed the non-extractable residue of one *U. lactuca* specimen (sample *Ulva*-4) after oxic degradation experiments, and of one degraded *E. radiate* (sample *Ecklonia*-2). In both pyrolysates, the concentration of generated steroids was ~ 6 orders of magnitude lower than in the saponified extracts. These findings suggest that only a minor proportion of macroalgal lipids was incorporated into the kerogen of Ediacaran macroalgae, and virtually no sterols were incorporated into the insoluble fraction of extant macroalgae analysed in this study.

4. Discussion

4.1. Syngeneity of biomarkers in fossil macroalgae

GC–MS MRM, SIR and full-scan analyses of the comprehensive accumulatory laboratory system blank confirmed that the hydrocarbons detected in fossil macroalgae were not introduced by laboratory processes. Monitoring of the blank yielded no *n*-alkanes, hopanes or steranes, even when measured using the most sensitive GC–MS MRM methods. The only contaminants detected in the blanks were trace amounts of phthalates — plasticizers present in small amounts in solvents used for extraction of biomarkers.

The results of exterior-interior analysis (Jarrett et al., 2013) on rock samples with fossil macroalgae showed that the surfaces of these samples were not contaminated with hydrocarbons (Bobrovskiy et al., 2018b). Moreover, the macroalgae extracts demonstrate the same exceptionally low thermal maturity as other fossils from the White Sea area (Bobrovskiy et al., 2018a; Bobrovskiy et al., 2018b; Bobrovskiy et al., 2022). The high βα/αβ and low Ts/Tm hopane isomer ratios, virtual absence of diasteranes, αββ and ααα20S sterane isomers, are characteristic of the most immature sediments, which demonstrates that the hydrocarbons are significantly below the oil-generation window (i.e. the kerogen never thermally generated and expelled liquid hydrocarbons (Peters et al., 2005); for abbreviations of biomarkers and formulas see Fig. S2 and Table S1 captions). Such a low thermal maturity is never observed in contaminant petroleum products and migrated oils, which by their very nature must have a maturity within the oil generation window. Thus, contamination of the samples with petroleum products or by migrated hydrocarbons did not occur.

Following previous publications on biomarkers extracted from Ediacaran fossils (Bobrovskiy et al., 2018a; Bobrovskiy et al., 2018b; Bobrovskiy et al., 2022), there has been a suggestion that these biomarkers are contaminants derived from modern sewage that percolated into the outcrops from overlying agricultural land (Love and Zumberge, 2021), which has been for the first time explained in detail by one of the Reviewers of the current paper (see Supplementary Material). According to this view, the up to 100 m high cliffs of the Ediacaran sediments cropping out along the coast of the White Sea are overlain by agricultural land, and biomass of mammal faeces percolated through tens of meters of clay deposits, contaminating Ediacaran fossils and imprinting the elevated 5β/5α sterane isomer pattern found in some of these fossils.

The land above the cliffs is, however, covered by taiga forest, and the polar fractions of the rock extract contain no detectable plant terpenoids, sterols or animal faeces-indicators; the only found polar steroids and terpenoids are represented by small amounts of secondary oxidation

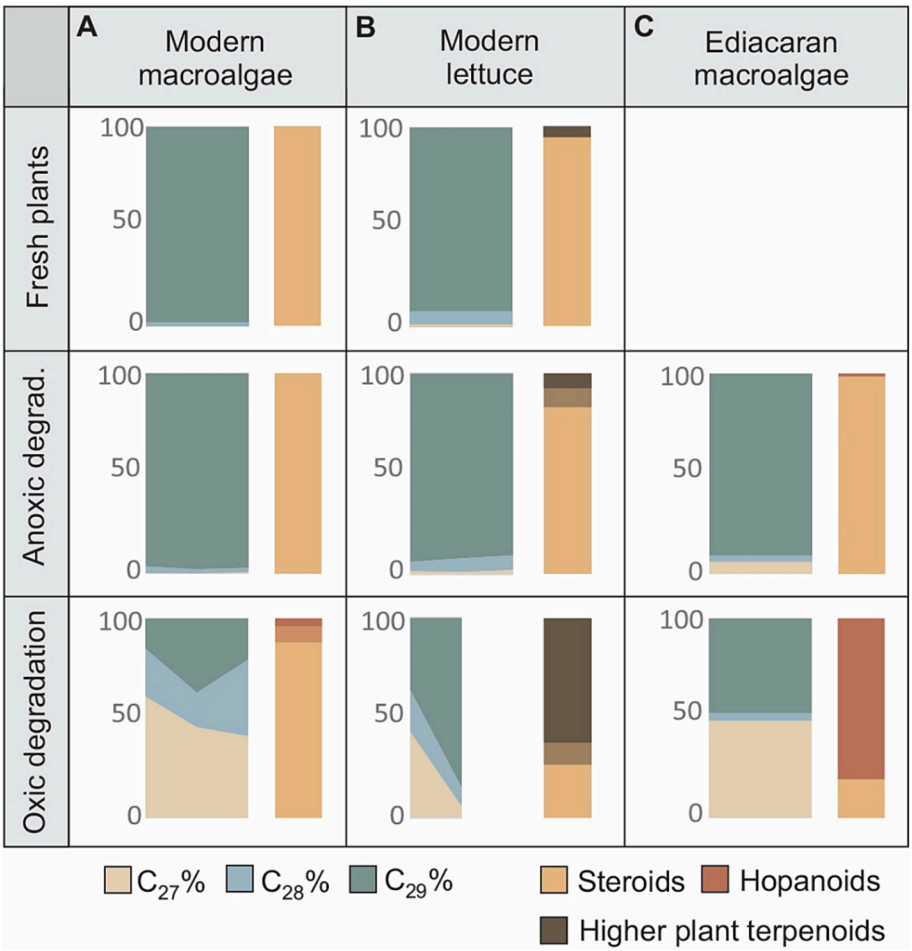


Fig. 8. Contrast between the biomarker distributions of organic matter degraded in more anoxic and oxic settings. **(A)** distribution of terpenoids in fresh and degraded extant green macroalga *Ulva*; oxic and anoxic conditions are represented by three experiments each; **(B)** distribution of terpenoids in fresh and degraded lettuce; anoxic conditions are represented by three experiments, oxic conditions by two experiments; **(C)** the end members of the correlations of the 5β/5α ratio with S/(S + H) and percentage of C_{29} steranes in the Ediacaran macroalgae dataset (excluding *Archyfasma*), representing algae degraded in most anoxic and most oxic conditions as inferred from 5β/5α ratios; note that the assignment of biomarker distributions to oxic and anoxic settings for the fossil algae is an interpretation. Half-shaded areas on S/(S + H) and S/(S + T) plots indicate the range of observed values in different experiments.

products of hydrocarbons. The extracts of the fossils contain steranes and aromatic steroids - geological hydrocarbons that form from sterols and stanols during heating upon burial. Furthermore, the relative content of sterane isomers that form during heating (diasteranes, αββ and 20S) indicates that molecules in the extracts of the Ediacaran fossils were heated to the same degree as biomarkers in the surrounding rocks, and so must share the same 560-million-year-old burial history.

In addition to the crucial difference between sterols and steranes, the contamination scenario requires several additional unlikely assumptions. Firstly, the contaminants would need to selectively target fossils only, leaving microbial mats on the same sedimentary surface perfectly pristine (Bobrovskiy et al., 2018a; Bobrovskiy et al., 2022). Secondly, the concentration of contaminants per surface area would need to be strictly proportional to the size of the fossils, as observed for *Dickinsonia* (Bobrovskiy et al., 2018a). Thirdly, different types of contaminants would need to taint specific fossils in a targeted way, such that all putative animal fossils have a cholesteroids predominance (approaching 100 %), typical of animals, while fossil algae have a strong stigmasteroids predominance (approaching 100 %), typical of green algae (Bobrovskiy et al., 2018a; Bobrovskiy et al., 2018b; Bobrovskiy et al., 2022). Thus, we conclude that the Ediacaran sediments analysed in this study are unaffected by Recent contamination.

4.2. Potential controls on the biomarker distributions in the Ediacaran macroalgae

The strong correlations between biomarker concentrations ([S + H]) and biomarker parameters in the extracts of the Ediacaran macroalgae implies that there is a single dominant factor controlling their distributions. It is highly unlikely that the initial lipid composition between the analysed algae (except *Archyfasma*) was notably different because of the homogenous sample set of algal macrofossils and the continuous covariations of ecological and diagenetic parameters (Fig. 4).

Covariation of a set of geochemical parameters may generally be explained either by a process, or connected processes, that affected all samples to varying extent, or by mixing two components with contrasting initial compositions. Two component mixing scenarios for the macroalgal biomarker data will be considered first.

4.2.1. Testing component mixing scenarios

The correlations between numerous biomarker ratios in extracts of fossil macroalgae may be explained by different two component mixing scenarios: (1) Mixtures of varying abundances of indigenous and contaminant hydrocarbons; contamination has been ruled out in the previous section, but in principle also fits under the discussion below. (2) Mixing of lipids from macroalgae and varying proportions of epiphytes. (3) Mixing of macroalgal lipids with lipids of the background

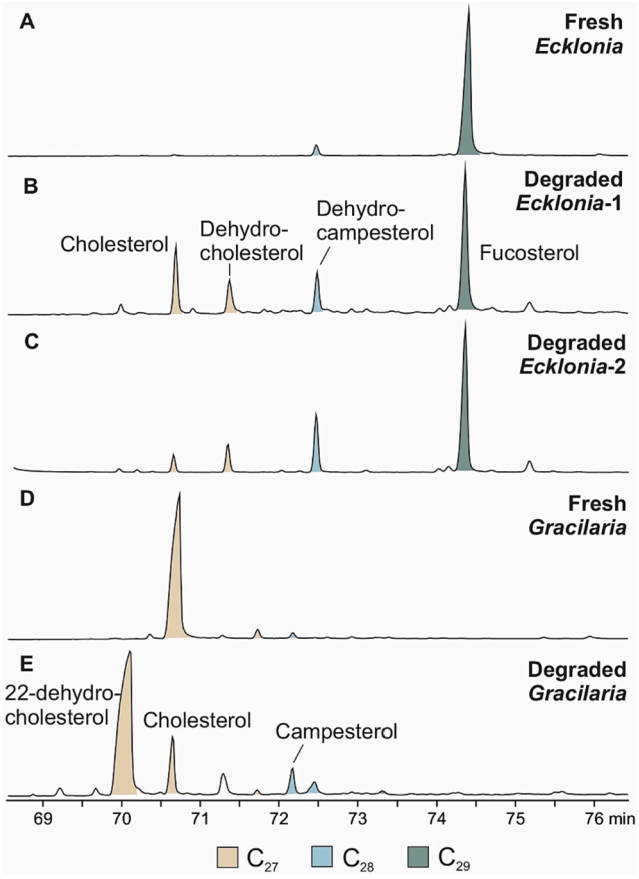
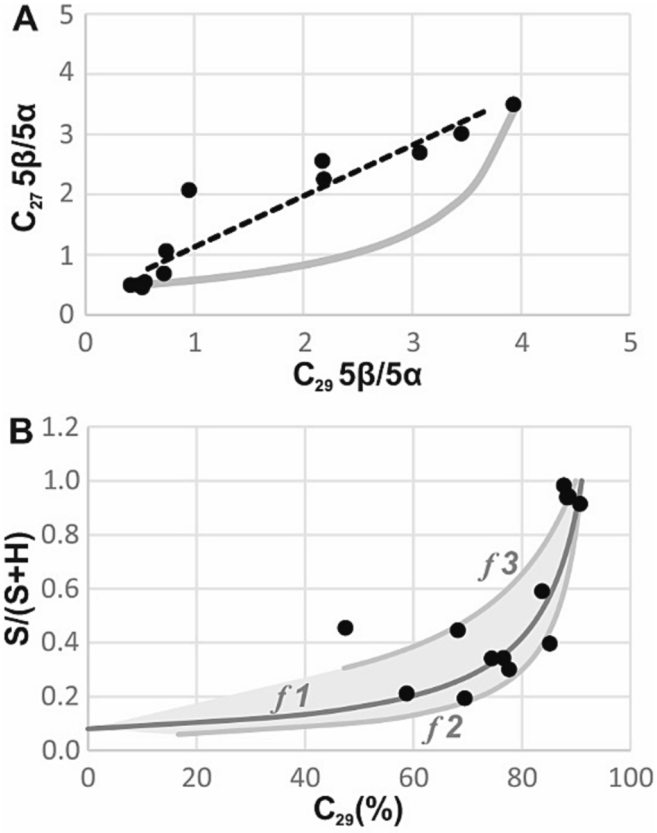


Fig. 9. Chromatograms showing the distribution of sterols in fresh and degraded brown (*Ecklonia*) and red algae (*Gracilaria*) (*m/z* 129). The two degraded *Ecklonia* specimens were visually distinct, representing different pathways or stages of decomposition in terms of discolouration and structural integrity.

biomass. (4) Postdepositional mixing of biomarkers derived from the macroalgal biomass and from the surrounding clay – for instance, by diffusion of biomarkers into the algal organic matter, or by inclusion of some clay in the extracts through incomplete separation of the fossil algae from rock surfaces.

Two component mixing scenarios can be tested using biomarker data from the Ediacaran macroalgae. Biomarker compositions of two putative endmembers are estimated by extrapolation of extreme values in biomarker parameters in macroalgal extracts. Based on the data, putative endmember 1 (EM1) would have low relative proportion of C_{29} steroids, low S/(S + H) and 5β/5α sterane ratios, and lower values of maturity parameters. Endmember 2 (EM2), in contrast, would have a high relative proportion of C_{29} steroids, high S/(S + H) and 5β/5α ratios, and relatively elevated apparent maturities.

However, looking at the exact trajectories in x-y plots, it is clear that the observed correlations cannot be explained by mixing of EM1 and EM2. For instance, physical mixing of macroalgal material (EM2) with organically lean surrounding clay (EM1) would significantly decrease the concentration of extracted biomarkers and simultaneously decrease the S/(S + H) ratio, as observed in the data (Fig. 4). However, the surrounding clay can be excluded, as EM1 requires a C_{29} sterane abundance of < 47 %, while the actual clay has a very high C_{29} sterane content of ~ 81 % (Fig. 2, Table S1). More generally, two component mixing between the two endmembers would create a strongly curved mixing hyperbola function for the correlation between 5β/5α and the proportion between C_{27}, C_{28} and C_{29} steranes (Fig. 10A). However, the observed correlation is clearly linear (R^2 = 0.9, ρ = 0.95, p < 0.01; Fig. 10A), which is conclusively inconsistent with a two-compound mixing system and more



	Heterotrophs		Macroalgae	
	C_{27}:C_{28}:C_{29}	S/(S+H)	C_{27}:C_{28}:C_{29}	S/(S+H)
f1	100:0:0	0.08	6:3:91	1.00
f2	83:0:17	0.08	6:3:91	1.00
f3	50:3:47	0.31	7:3:90	1.00

Fig. 10. Simulations of two component mixing scenario and heterotrophic reworking as a control on the correlations among biomarker parameters in Ediacaran macroalgae. **(A)** Linear correlation (black dotted line) between the C_{27} and C_{29} 5β/5α sterane ratios (black filled circles) and the two-component mixing curve between the Ediacaran macroalgae with lowest and highest 5β/5α ratios. Two component mixing scenarios do not replicate the data. **(B)** Numerical model that describes the replacement of macroalgal lipids with lipids of heterotrophic degraders. The grey curves show three different numerical models (*f1* to *f3*) for the evolution of the S/(S + H) and C_{29}(%) biomarker parameters and juxtapose the results with data from the Ediacaran macroalgae (black filled circles). Model parameters (biomarker composition of hypothetical heterotrophs and initial composition of macroalgae) used for the three replacement scenarios are provided in the table. Model *f*1 provides the best fit to the data with the mean square error of 0.019.

reminiscent of a gradual chemical process.

4.2.2. *Thermal maturity and clay catalysis*

All algae were collected from the same 1.5 m thick sedimentary layer within the same outcrop with no faults, so differences in thermal maturity can be excluded as a factor controlling the biomarker distributions. It is also unlikely that biomarker ratios were affected by mineral surface effects, such as clay catalysis, as the biomarkers were extracted from coherent organic films and their contact with mineral surfaces was

presumably minimal. Moreover, distributions of biomarkers that had much higher chance of being subject to mineral surface effects, such as those extracted from the surrounding clay, fall within the range observed in macroalgal extracts and are far from the endmembers of this range.

4.2.3. Redox conditions and (microbial) degradation

Sterane 5β/5α ratios show the strongest correlations with most biomarker ratios and are at the centre of the network diagram (Fig. 4). Thus, by determining plausible controls on 5β/5α, we gain access into the network diagram and the major factors forcing the correlations. The formation of 5β and 5α sterane isomers in sediments is relatively well understood. The most commonly observed, abiological mechanism for the formation of 5β and 5α sterane isomers from sterol precursors goes through Δ^5 sterenes, which isomerise into Δ^4 sterenes at a constant proportion of $\Delta^5 / \Delta^4 = 0.64$ (reviewed in Peakman et al., 1992). As Δ^5 sterenes are the precursors of 5β steranes, and Δ^4 yield 5α isomers, the most common 5β/5α value in sedimentary rocks is ~ 0.6 (Peakman et al., 1992). This is also the value characteristic of steranes extracted from siliciclastic rocks of the Ediacaran White Sea area (5β/5α = 0.65 ± 0.26, $n = 54$), including the location where the fossil macroalgae were collected (Bobrovskiy et al., 2018a), and is close to the lowest ratios observed in the analysed macroalgae (Fig. 4B, Table S1). However, 5β/5α values in the macroalgae (excluding *Archyfasma*) are up to 4.3, indicating that an additional mechanism is driving isomerization.

It is well established that excess concentrations of 5β steroids are derived through microbially-mediated conversion of sterols to 5β-stanols (De Leeuw and Baas, 1986; Gaskell and Eglinton, 1975; Nishimura, 1982; Peakman et al., 1992; Reed, 1977). Evidence from both field and laboratory studies indicates that production of 5β-stanols in natural environments reflects the depositional conditions in which microbial transformation proceeds. Hydrogenation in both anoxic and oxygenated environments might yield 5α-stanols, whereas the production of 5β-stanols is restricted to anoxic conditions and is tied to anaerobic microbial reduction of sterols (Björkhem and Gustafsson, 1971; De Leeuw and Baas, 1986; Gaskell and Eglinton, 1975; Gaskell and Eglinton, 1976; Kok et al., 2000; Meyers and Ishiwatari, 1993; Nishimura, 1982; Peakman et al., 1992; Reed, 1977; Taylor et al., 1981).

Apart from natural environments, 5β-stanols are known to be present in faeces of selected mammals, including humans. These mammals host a microbiome capable of reducing sterols to 5β-stanols in their guts (Bull et al., 2002; Eyssen et al., 1973; Leeming et al., 1996). 5β-stanols have therefore been widely used for monitoring of domestic sewage pollution and in archaeology to detect latrines, producing an overwhelming amount of literature on faeces-derived 5β-stanols compared to reports on sedimentary 5β-stanols (e.g. Bull et al., 2002; Leeming et al., 1996; Prost et al., 2017). Although the generation of 5β-stanols in sedimentary environments is acknowledged in many studies, the presence of these molecules in sediments has established itself nearly universally, but misleadingly, solely as an indicator of mammal faecal material (e.g. Argiriadis et al., 2018; Bethell et al., 1994; Grimalt et al., 1990; Nichols et al., 1993; Sherwin et al., 1993; Takada et al., 1994; Vachula et al., 2019). As mammals and their gut microbiomes appeared in Earth history long after the Ediacaran, elevated sterane 5β/5α ratios in the fossil macroalgae most likely reflect rapid hydrogenation of sterols by anaerobic bacteria in the anoxic microenvironments of their decomposing biomass (Björkhem and Gustafsson, 1971; Bobrovskiy et al., 2018a; De Leeuw and Baas, 1986; Gaskell and Eglinton, 1975; Gaskell and Eglinton, 1976; Kok et al., 2000; Meyers and Ishiwatari, 1993; Nishimura, 1982; Peakman et al., 1992; Reed, 1977; Taylor et al., 1981).

The considerable knowledge about the 5β/5α ratio gathered by the scientific community now provides an opportunity to interpret the conditions that controlled the decay of the Ediacaran macroalgae. Although all algae were collected from the same 1.5 m thick sedimentary layer, it is likely that they experienced different oxygen exposure times. Some algae may have been buried alive, experiencing the lowest exposure to oxygen during decay through rapid onset of anoxia. Other algae may have experienced extensive aerobic decay at the sediment water interface before eventual burial. Aerobic decay would have generated 5β/5α = 0.64, and subsequent burial and anoxic conditions would have switched the degradation of remaining sterols to an anaerobic process, potentially causing bacterial hydrogenation and generation of elevated 5β/5α values. Overall, the main driving force for the biomarker variations in the Ediacaran macroalgae would have been oxygen exposure.

The 5β/5α ratio also correlates with the relative abundance of C_{29} steranes among trifecta steranes (S C29% in Fig. 4), suggesting that the extent of oxygen exposure also affects the relative abundance of different sterol species. From the highest to the lowest 5β/5α values, C_{29} percentages drop linearly from 91 to 47 %. Such a strong effect of degradation on sterane pseudohomolog distributions has, to our knowledge, not been observed or suggested before. However, the strong linear correlations mandate that all analysed macroalgae, except *Archyfasma*, initially contained little or no hopanoids and had similar steroid compositions, close to those in the specimens with highest S/(S + H) and 5β/5α values (C_{27}: C_{28}: C_{29} = 6 %: 3 %: 91 %). Likely during oxic degradation, as S/(S + H) dropped, the sterane trifecta ratio changed towards C_{27}: C_{28}: C_{29} = 49 %: 4 %: 47 % (Fig. 4B, 8C).

S/(S + H) and sterane trifecta ratios are widely employed in palaeoecological reconstructions, and significant diagenetic changes in these parameters observed in Ediacaran macroalgae might have broad consequences for interpreting the distributions of these biomarkers in ancient deposits. S/(S + H) ratio in macroalgae follows the known oxic degradation pattern of aerobic decomposition of eukaryotic biomass, where steroids are replaced by hopanoids of heterotrophic bacteria (Moldowan et al., 1985; Peters et al., 2005; Ries-Kautt and Albrecht, 1989). However, the mechanism responsible for changes in sterane trifecta ratios in the Ediacaran macroalgae is currently unknown.

We consider two hypotheses. First, macroalgal sterols might have been replaced by lipids of heterotrophs. As suggested by models in Fig. 10B, a small proportion of microscopic eukaryotes might have been present among the heterotrophs (<10 % of eukaryotes with a strong, possibly close to 100 % C_{27} predominance, according to the best fitting scenario *f1*), which contributed *de novo* synthesized sterols to the total algal extracts. Onset of anoxia would have ended the activity of aerobic eukaryotes and *de novo* sterol production. Thus, the duration of oxygen exposure would have controlled the extent of algal sterols replacement with the lipids of heterotrophs, thus explaining the positive correlation of S/(S + H) and C_{29}% with 5β/5α ratios. In the second hypothesis that could explain our data, heterotrophic organisms might have mediated the degradation of algal sterol side chains, gradually converting C_{29} steroids to C_{27} steroids, and/or degrading C_{29} steroids at a faster rate than other steroids. Complete degradation of steroidal side chains by bacteria is well studied for the purposes of drug production (e.g. reviewed in Olivera and Luengo, 2019). However, a number of eukaryotes, including animals (Bradshaw et al., 1989; Harvey et al., 1987; Malik et al., 1988; Teshima and Kanazawa, 1971; Teshima et al., 1979) and protists (Nes et al., 1975; Tomazic et al., 2011; Tomazic et al., 2014) also possess the ability to dealkylate sterols at the C-24 position. Whereas a specific genetic toolkit employed for this in animals is currently unknown, it has been suggested that ciliates and choanoflagellates most likely acquired the gene responsible for C-24 dealkylation from bacteria via parallel gene transfer (Tomazic et al., 2011; Tomazic et al., 2014). Therefore, as no bacteria are known to produce 24-alkylated sterols (Wei et al., 2016), it is possible that some bacteria have the ability to dealkylate the side chain at the C-24 position of exogenous eukaryotic sterols as a source of energy and/or carbon. This possibility currently remains hypothetical and needs to be tested using genomic and experimental data on extant bacteria.

4.2.4. Oxic degradation effects on maturity parameters and other biomarker ratios

The network diagram shows that variations in 23 biomarker ratios in the Ediacaran macroalgae extracts are significantly interlinked (Fig. 4, Table S2). The 5β/5α ratios form the core of the network. In the following discussion we accept the hypothesis that the 5β/5α ratio in Ediacaran macroalgae reflects the extent of oxygen exposure (see discussion in the previous section). If correct, then oxygen exposure is likely also responsible for a large proportion of the variability in the other biomarker parameters.

In the correlation network, some biomarker parameters conventionally interpreted as maturity proxies show positive correlations with each other, but also with sterane 5β/5α ratios, S/(S + H) and sterane C_{29}%. Isomerisation of steroids and hopanoids to thermodynamically more stable structures, and thus higher apparent 'thermal maturity', is greatest in specimens that have experienced the lowest oxygen exposure (Fig. 4). Some isomerization reactions depend on the presence of double bonds and other functional groups. However, it is plausible that compounds with a higher degree of functionalization are also more prone to selective removal during oxygen exposure (Lengyel et al., 2012; Škorňa et al., 2014). Thus, with faster onset of anaerobic conditions, a greater proportion of intact functionalized lipids may survive and can then isomerize into thermodynamically more favourable configurations (Brassell et al., 1984). Additionally, radical or cationic intermediates that form during isomerization may be quenched by oxygen, retarding isomerization during the oxic burial phase. However, these mechanisms remain purely speculative. Based on the network analysis, proxies that trend towards higher 'maturity' with decreasing oxygen exposure (earlier onset of reducing conditions) include Ts/Tm hopanes, moretanes/hopanes, αββ/ααα steranes, and 20S/R steranes, as well as the ratio of aromatic steroids over saturated steranes (MAS/S and TAS/S) (note that the value of moretanes/hopanes generally decreases with increasing maturity and thus shows negative correlations). Remarkable is the increasing aromatization of steroids with increasing sterane 5β/5α ratios, i.e. under more reducing conditions, and the same trend has been observed for cholesteroids in Ediacaran fossils of *Dickinsonia* (Bobrovskiy et al., 2018a). The formation of triaromatic steroids from sterols is formally an oxidation process, so a higher degree of aromatization may be expected during oxic degradation. However, the opposite is observed, consistent with the hypothesis that oxygen exposure reduces double formation and survival, thus reducing aromatization. Alternatively, the aromatization of sterols might be a microbial process that is favoured under reducing conditions.

A second class of conventional maturity and source parameters in the macroalgae shows the opposite trend, i.e. their apparent maturity decreases under more reducing conditions. This includes the proportion of steranes with a cleaved side chain (C_{20} and C_{21} pregnanes) relative to intact C_{27}-C_{29} steranes ('S Preg%'), and the proportion of short-chain homologs among *n*-alkanes (expressed in the network diagram as the inverse '*n*-alk long/short') and cheilanthanes ('Cheil C_{19}/C_{23}' and 'Cheil C_{20}/C_{24}'). All of these ratios show negative correlations with 5β/5α ratios in the fossil macroalgae. Cleavage of C–C bonds in all of these compounds may be related to degradation under elevated oxygen exposure, for instance through attack of double bonds by reactive oxygen species (Lengyel et al., 2012; Škorňa et al., 2014). Cheilanthanes are ubiquitous components of oils and bitumens, although their biological source is unknown (Brocks and Pearson, 2005; Ourisson, 1994). C_{19}/C_{23} and C_{20}/C_{24} cheilanthane ratios are highest in oils and bitumens with organic matter derived from terrestrial environments (French et al., 2014; Noble et al., 1986; Peters et al., 2005). Terrestrial organic matter suffers long oxygen exposure times within soil and during riverine transport, providing one possible explanation why C_{19}/C_{23} and C_{20}/C_{24} cheilanthane ratios from such environments are elevated. If this is correct, then these ratios will also be elevated in bitumens from non-terrestrial but well-oxygenated marine environments, a proposition that is testable.

The 3-methylhopane index (3MHI) and gammacerane index (GI) are also a part of the network. 3-methylhopanoids are thought to be mainly derived from methanotrophic bacteria (Summons et al., 1994; Zundel and Rohmer, 1985), whereas gammacerane is a likely diagenetic product of tetrahymanol (Ten Haven et al., 1989), a membrane lipid found abundantly in some protozoa (Mallory et al., 1963; Takishita et al., 2017) and in a wide range of bacteria (Banta et al., 2015). However, as both the 3MHI and GI ratios are normalised to hopanes, in our sample set these ratios are most likely primarily affected by the variation in hopane abundances, and might be connected to the network via S/(S + H). In other words, lower 3MHI and GI may simply be caused by increased production of hopanols by aerobic bacteria during degradation of the algae, for instance through longer exposure to oxygen before burial. They do not necessarily represent lower activity of methanotrophs and ciliates.

4.3. Sterol degradation in extant macroalgae and plants

4.3.1. Observations

To investigate whether the apparent control of oxygen exposure on biomarkers in Ediacaran macroalgae is reflected in modern environments, we performed a series of laboratory degradation experiments on extant macroalgae and plants, and also investigated the behaviour of sterols during degradation of algae in natural environments. Under both natural and laboratory conditions, sterol distributions and relative abundances of macroalgae changed notably under the influence of oxygen, and the changes show some resemblance with the Ediacaran algae. As in the Ediacaran algae, the proportion of hopanoids over steroids increased during oxic degradation, and the content of C_{29} steroids dropped relative to other steroids (Fig. 8). One difference between the data on Ediacaran macroalgae and our degradation experiments, is that no 5β-stanols were produced in the latter. It is known that 5β-stanols are only found in anoxic settings, but that does not imply that they are necessarily formed in *all* anoxic sediments (Björkhem and Gustafsson, 1971; De Leeuw and Baas, 1986; Gaskell and Eglinton, 1975; Gaskell and Eglinton, 1976; Kok et al., 2000; Meyers and Ishiwatari, 1993; Nishimura, 1982; Peakman et al., 1992; Reed, 1977; Taylor et al., 1981). Naturally, some factors other than Eh must also have a control on the presence of bacteria that mediate the conversion, which were not met in the experiments, e.g. initial presence of required types of bacteria.

The observations on the degraded red and brown algae collected from the tidal zone at the coast of SE Australia provide some limited insights into the mechanism responsible for the changes in sterol carbon numbers. As discussed earlier for the Ediacaran macroalgae, the drop in the percentage of C_{29} sterols in our degradation experiments may be caused by the *de novo* generation of C_{27} and C_{28} sterols by heterotrophs, preferential consumption of C_{29} sterols by heterotrophs, and/or targeted dealkylation of sterol side chains by either heterotrophic eukaryotes or bacteria. In red algae *G. edulis* entangling undegraded macroalgae *E. radiate*, which were collected at the coast, sterols are mainly represented by cholesterol (>99 %, Fig. 9D). *G. edulis* entangling degraded *E. radiate*, show only traces of C_{28} (3.7 %) and C_{29} (0.7 %) sterols although most of their cholesterol was replaced by 22-dehydrocholesterol (Fig. 9R). Detectable generation of C_{28} and C_{29} sterols by epiphytes or heterotrophs on these algae evidently did not occur. By contrast, during degradation of the brown alga *E. radiate*, high proportions of secondary sterols emerged (Fig. 9; 0 %: 4 %: 96 % in the fresh alga, 34 %: 12 %: 54 % in the most degraded specimen). The differences in sterols that arose during degradation of the red and brown algae could be caused by differences in heterotrophic communities, or epiphytes that might start growing on algae once they are dead. However, given that the algae were physically entangled, it also cannot be excluded that the new sterols in the degraded algae were generated by side-chain modification of existing algal sterol, and additional research is required to distinguish between these scenarios.

Another notable observation on plants degraded under laboratory

and natural conditions is the increase of the relative proportion of 22-unsaturated sterols relative to their saturated counterparts (Fig. 2). During the degradation of lettuce, the simultaneous emergence of C_{28} and C_{29} $\Delta^{5,22}$ sterols, in concentrations proportional to C_{28} and C_{29} Δ^{5} sterols of the undegraded plant, points to a mechanism where saturated precursors are dehydrogenated at C-22 independent of side chain alkylation. The same process seems to have affected the red alga *G. edulis*, where 85 % of cholesterol in the undegraded alga was replaced by 22-dehydrocholesterol (Fig. 9D,E). By contrast, fucosterol of the undegraded brown alga *E. radiate* did not become dehydrogenated at C-22 (Fig, 9A-C), possibly because fucosterol already possesses a double bond in the side chain in 24(28) position. These changes might not by directly caused by exogenous activity but could result from the metabolic activity of the plants themselves during senescence or as a response to bacterial attack (Aboobucker and Suza, 2019; Griebel and Zeier, 2010). While these effects have been observed in higher plants (Aboobucker and Suza, 2019; Griebel and Zeier, 2010), they may in principle also occur in algae, as suggested by our data on *G. edulis*. Brassicasterol and 22-dehydrocholesterol are among the most common sterols in modern phytoplankton (Volkman, 2003; Volkman, 2006). Based on our observations, large proportions of these sterols in sediments and the water column might also be derived from secondary dehydrogenation of sterols that initially have saturated side chains.

4.3.2. Comparison to previous studies on sterol degradation

The degradation of sterols has been investigated in many published studies. It has been found that sterols are among the most labile lipids under oxic conditions (e.g. Harvey and Macko, 1997; Hoefs et al., 2002) but are quite resilient in anoxic settings (Harvey and Macko, 1997). Among studies focused on the relative degradation rates of individual sterols, a few performed sterol incubation experiments, but degradation rates for individual sterols in oxic conditions were unfortunately not quantified (Harvey and Macko, 1997; Sun and Wakeham, 1998). Others investigated sterol distributions in Recent sediments, but the results of such studies are commonly ambiguous because they cannot account for changes in biological input (Canuel and Martens, 1996; Colombo et al., 1997; Rontani et al., 2012; Yunker et al., 2005) or redistribution effects caused by physical sorting of sediment particles in turbid flow deposits (Hoefs et al., 2002). Despite these difficulties, some general trends in oxic sterol degradation patterns observed in Recent sediments are found across the studies, and seem to be consistent with the patterns found in our degradation experiments and the Ediacaran macroalgae, such as the overall pattern of increased reactivity of C_{28} brassicasterol $<$ C_{28} campesterol $<$ C_{29} sitosterol (Rontani et al., 2018; Rontani et al., 2012; Yunker et al., 2005). On the other hand, C_{29} isofucosterol was sometimes found to be more stable than any of these sterols (Yunker et al., 2005), in contrast to our degradation experiments on *Ulva* and also based on theoretical expectations of a labile 24(28) double bond (e.g. Kamal-Eldin et al., 1998); such observations on Recent sediments could either arise from an unaccounted source of isofucosterol, absent in a control ('non-degraded') samples, or from yet unknown factors playing a role in sterol degradation.

4.4. Implications and perspectives for modern and ancient marine settings

We show that nearly all biomarker proxies investigated in our study, including those traditionally applied as ecological, biological or maturity parameters, are also affected during the oxic degradation of marine organic matter. This effect may have a major impact on the interpretations of biomarker distributions in both modern and ancient settings. Using the example of biomarker extracts of Ediacaran macroalgae, we suggest a way to recognize the effects of oxic degradation on biomarker distributions. Basic data treatments such as correlation matrix analysis, network analysis, and principal component analysis can be the first step in identifying factors that may influence the distributions of multiple biomarkers in analyzed samples. In order to determine what these factors may be, it is important to find variables that are not controlled by multiple factors. For example, hopanes in marine sedimentary rocks usually originate from a limited number of biological precursors, and therefore anomalies in their isomerization can be related to the biological and chemical alteration of the biomarkers, and distributions of redox-sensitive elements can be used to monitor redox effects on biomarker diagenesis. Using such tools, it may be possible not only to recognize but also quantify the effects of oxic degradation on biomarker distributions, opening the possibility to approximate the composition of past ecosystems more closely.

5. Conclusions

Combined evidence from extant plants and Ediacaran macroalgae illustrates that the primary distributions of steroids can be severely altered during oxic degradation. Conversely, anoxic degradation may lead to the preservation of biomarker distributions that reflect the original lipid composition more closely. Under oxic conditions, the proportion of steroids to hopanoids, and of C_{29} steroids to other steroids, can decrease sharply, and the degree of organic matter degradation becomes the main control on these biomarker ratios (Fig. 8).

Sterol distributions in recent sediments, and core biomarker proxies such as the proportion of steranes and hopanes, and sterane homolog distributions, can be altered by aerobic heterotrophic degradation to the extent of complete loss of primary ecological information. Furthermore, conventional 'thermal maturity' biomarker parameters can be affected during early diagenesis by microbial and, possibly, chemical alteration of the biomarker precursors. While biomarkers may generally preserve information about the composition of primary producer communities, the heterotrophic overprint requires consideration before such information can be reliably extracted.

Likely, sediments deposited under permanently anoxic waters can retain biomarker distributions closest to the initial averaged biomass composition. However, it is important to note that any organic matter transported into the basin from terrestrial or shallow-water environments, or any slow-sinking biomass exported from the oxygenated mixed zone, may still have experienced significant oxygen exposure and aerobic alteration. For example, over 95 % of sterols can be degraded within the oxygenated layer of anoxic basins (Gagosian et al., 1979; Wakeham and Beier, 1991), so assuming that organic matter deposited in anoxic basins is unaffected by oxic degradation is not necessarily correct (Mackenzie et al., 1982). Our results suggest that the effects of oxic degradation on biomarker distributions should be considered in oils and bitumens from any setting.

The data presented here show that statistical analysis of biomarker distributions may be a powerful tool for identifying the effects of oxic degradation. Apart from correlations with other biomarker parameters, additional consideration of inorganic geochemistry, isotope systematics, and general understanding of the depositional environments and sources of organic matter may serve as indicators of degradation effects in each given situation. The major impact of aerobic degradation on lipid distributions observed in this study uncovers an underestimated control on biomarker parameters that have traditionally been interpreted as proxies for biological community composition, environmental setting, source rock lithology and thermal maturity. A redox control on such parameters may explain incongruent results between different proxies often observed in organic geochemical studies, and the correlations described here may eventually allow quantification and correction of oxic degradation effects. Future studies also need to explore how the affected biomarker ratios evolve with true increasing thermal maturity, and how other parameters such as mineralogy, organic matter type, and the proportion of kerogen-bound molecules modify the response to oxic degradation. It is striking that the modification of algal sterol side chains during aerobic degradation is comparable in modern and 558 Ma old systems. Further work needs to identify the heterotrophic communities involved in the degradation and alteration of the algal sterols, including

the search for genes responsible for potential side chain dealkylation and dehydrogenation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

The Supplementary Tables and Research Data file contains Table S1 with values for biomarker parameters measured for Ediacaran macroalgae, Table S2 with Spearman ρ correlation matrix on biomarker parameters of the macroalgae, Table S3 with distributions of steroids, hopanoids and higher plant terpenoids in extant algae and higher plants analysed in this study, as well as Research Data – chromatograms used in the manuscript figures in numerical format and biomarker concentrations in Ediacaran macroalgae. The Supplementary Material file contains a dialogue about biomarker interpretations with one of the reviewers and provides an extended discussion on the topics of biomarker syngeneity, isomerisation and methodological approaches.

Supplementary material to this article can be found online at https://doi.org/10.1016/j.gca.2023.11.024.

References

Aboobucker, S.I., Suza, W.P., 2019. Why do plants convert sitosterol to stigmasterol? Front. Plant Sci. 10 (354).

Argiriadis, E., Battistel, D., McWethy, D.B., Vecchiato, M., Kirchgeorg, T., Kehrwald, N. M., Whitlock, C., Wilmshurst, J.M., Barbante, C., 2018. Lake sediment fecal and biomass burning biomarkers provide direct evidence for prehistoric human-lit fires in New Zealand. Sci. Rep. 8 (1), 12113.

Banta, A.B., Wei, J.H., Welander, P.V., 2015. A distinct pathway for tetrahymanol synthesis in bacteria. Proc. Natl. Acad. Sci. 112 (44), 13478–13483.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks, Third international AAAI conference on weblogs and social media.

Bethell, P., Goad, L., Evershed, R., Ottaway, J., 1994. The study of molecular markers of human activity: the use of coprostanol in the soil as an indicator of human faecal material. J. Archaeol. Sci. 21 (5), 619–632.

Björkhem, I., Gustafsson, J.-Å., 1971. Mechanism of microbial transformation of cholesterol into coprostanol. Eur. J. Biochem. 21 (3), 428–432.

Bobrovskiy, I., Hope, J.M., Ivantsov, A., Nettersheim, B.J., Hallmann, C., Brocks, J.J., 2018a. Ancient steroids establish the Ediacaran fossil *Dickinsonia* as one of the earliest animals. Science 361 (6408), 1246–1249.

Bobrovskiy, I., Hope, J.M., Krasnova, A., Ivantsov, A., Brocks, J.J., 2018b. Molecular fossils from organically preserved Ediacara biota reveal cyanobacterial origin for *Beltanelliformis*. Nat. Ecol. Evol. 2 (3), 437–440.

Bobrovskiy, I., Krasnova, A., Ivantsov, A., Luzhnaya, E., Brocks, J.J., 2019. Simple sediment rheology explains the Ediacara biota preservation. Nat. Ecol. Evol. 3 (4), 582–589.

Bobrovskiy, I., Hope, J.M., Golubkova, E., Brocks, J.J., 2020. Food sources for the Ediacara biota communities. Nat. Commun. 11 (1), 1261.

Bobrovskiy, I., Nagovitsyn, A., Hope, J.M., Luzhnaya, E., Brocks, J.J., 2022. Biomarker evidence for guts, gut contents, and feeding strategies of Ediacaran animals. Curr. Biol. 32 (24), 5382–5389.e5383.

Bradshaw, S.A., O'Hara, S.C., Corner, E.D., Eglinton, G., 1989. Assimilation of dietary sterols and faecal contribution of lipids by the marine invertebrates Neomysis integer, Scrobicularia plana and Nereis diversicolor. J. Mar. Biol. Assoc. U. K. 69 (4), 891–911.

Brassell, S., McEvoy, J., Hoffmann, C., Lamb, N., Peakman, T., Maxwell, J., 1984. Isomerisation, rearrangement and aromatisation of steroids in distinguishing early stages of diagenesis. Org Geochem. 6, 11–23.

Brocks, J.J., Hope, J.M., 2014. Tailing of chromatographic peaks in GC–MS caused by interaction of halogenated solvents with the ion source. J. Chromatogr. Sci. 52 (6), 471–475.

Brocks, J.J., Pearson, A., 2005. Building the biomarker tree of life. Rev. Mineral. Geochem. 59 (1), 233–258.

Bull, I.D., Lockheart, M.J., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2002. The origin of faeces by means of biomarker detection. Environ. Int. 27 (8), 647–654.

Canuel, E.A., Martens, C.S., 1996. Reactivity of recently deposited organic matter: Degradation of lipid compounds near the sediment-water interface. Geochim. Cosmochim. Acta 60 (10), 1793–1806.

Colombo, J., Silverberg, N., Gearing, J., 1997. Lipid biogeochemistry in the Laurentian trough—II. Changes in composition of fatty acids, sterols and aliphatic hydrocarbons during early diagenesis. Org Geochem. 26 (3–4), 257–274.

De Leeuw, J., Baas, M., 1986. Early-stage diagenesis of steroids. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, pp. 101–123.

Eyssen, H.J., Parmentier, G.G., Compernolle, F.C., de Pauw, G., Piessens-Denef, M., 1973. Biohydrogenation of Sterols by Eubacterium ATCC 21,408—Nova Species. Eur. J. Biochem. 36 (2), 411–421.

Fedonkin, M.A., Simonetta, A., Ivantsov, A.Y., 2007. New data on Kimberella, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. Geol. Soc. Lond. Spec. Publ. 286 (1), 157–179.

French, K.L., Sepulveda, J., Trabucho-Alexandre, J., Gröcke, D., Summons, R.E., 2014. Organic geochemistry of the early Toarcian oceanic anoxic event in Hawsker Bottoms, Yorkshire, England. Earth Planet. Sci. Lett. 390, 116–127.

Gagosian, R.B., Lee, C., Heinzer, F., 1979. Processes controlling the stanol/stenol ratio in Black Sea seawater and sediments. Nature 280 (5723), 574–576.

Gaskell, S.J., Eglinton, G., 1975. Rapid hydrogenation of sterols in a contemporary lacustrine sediment. Nature 254 (5497), 209–211.

Gaskell, S.J., Eglinton, G., 1976. Sterols of a contemporary lacustrine sediment. Geochim. Cosmochim. Acta 40 (10), 1221–1228.

Gehling, J.G., 1999. Microbial mats in terminal Proterozoic siliciclastics. Ediacaran Death Masks. PALAIOS 14 (1), 40–57.

Grantham, P., Wakefield, L., 1988. Variations in the sterane carbon number distributions of marine source rock derived crude oils through geological time. Org Geochem. 12 (1), 61–73.

Grazhdankin, D., 2003. Structure and depositional environment of the Vendian Complex in the southeastern White Sea area. Stratigr. Geol. Correl. 11 (4), 313–331.

Griebel, T., Zeier, J., 2010. A role for β-sitosterol to stigmasterol conversion in plant–pathogen interactions. PlJ 63 (2), 254–268.

Grimalt, J.O., Fernandez, P., Bayona, J.M., Albaiges, J., 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environ. Sci. Tech. 24 (3), 357–363.

Hammer, Ø., Harper, D.A., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4 (1), 9.

Harvey, H.R., Macko, S.A., 1997. Kinetics of phytoplankton decay during simulated sedimentation: changes in lipids under oxic and anoxic conditions. Org Geochem. 27 (3–4), 129–140.

Harvey, H.R., Eglinton, G., O'Hara, S.C., Corner, E.D., 1987. Biotransformation and assimilation of dietary lipids by Calanus feeding on a dinoflagellate. Geochim. Cosmochim. Acta 51 (11), 3031–3040.

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar. Chem. 49 (2–3), 81–115.

Hoefs, M.J., Rijpstra, W.I.C., Damsté, J.S.S., 2002. The influence of oxic degradation on the sedimentary biomarker record I: Evidence from Madeira Abyssal Plain turbidites. Geochim. Cosmochim. Acta 66 (15), 2719–2735.

Huang, X., Meyers, P.A., Xue, J., Gong, L., Wang, X., Xie, S., 2015. Environmental factors affecting the low temperature isomerization of homohopanes in acidic peat deposits, central China. Geochim. Cosmochim. Acta 154, 212–228.

Inglis, G.N., Naafs, B.D.A., Zheng, Y., McClymont, E.L., Evershed, R.P., Pancost, R.D., 2018. Distributions of geohopanoids in peat: Implications for the use of hopanoid-based proxies in natural archives. Geochim. Cosmochim. Acta 224, 249–261.

Jarrett, A.J.M., Schinteie, R., Hope, J.M., Brocks, J.J., 2013. Micro-ablation, a new technique to remove drilling fluids and other contaminants from fragmented and fissile rock material. Org Geochem. 61, 57–65.

Kamal-Eldin, A., Määttä, K., Toivo, J., Lampi, A.-M., Piironen, V., 1998. Acid-catalyzed isomerization of fucosterol and Δ 5-avenasterol. Lipids 33 (11), 1073–1077.

Knoll, A.H., Summons, R.E., Waldbauer, J.R., Zumberge, J.E., 2007. The Geological Succession of Primary Producers in the Oceans. 133-163.

Kok, M.D., Rijpstra, W.I.C., Robertson, L., Volkman, J.K., Damstéé, J.S.S., 2000. Early steroid sulfurisation in surface sediments of a permanently stratified lake (Ace Lake, Antarctica). Geochim. Cosmochim. Acta 64 (8), 1425–1436.

Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res. 30 (12), 2893–2900.

Lengyel, J., Rimarčík, J., Vagánek, A., Fedor, J., Lukeš, V., Klein, E., 2012. Oxidation of sterols: Energetics of C-H and O–H bond cleavage. Food Chem. 133 (4), 1435–1440.

Leonov, M.V., 2007. Macroscopic plant remains from the base of the Ust'-Pinega formation (Upper Vendian of the Arkhangelsk Region). Paleontol. J. 41 (6), 683–691.

Love, G.D., Zumberge, J.A., 2021. Emerging Patterns in Proterozoic Lipid Biomarker Records. Cambridge University Press, Cambridge.

Mackenzie, A.S., Brassell, S.C., Eglinton, G., Maxwell, J.R., 1982. Chemical fossils: the geological fate of steroids. Sci 217 (4559), 491–504.

Malik, S., Kerr, R.G., Djerassi, C., 1988. Biosynthesis of marine lipids. 19. Dealkylation of the sterol side chain in sponges. Journal of the American Chemical Society 110(20), 6895-6897.
Mallory, F.B., Gordon, J.T., Conner, R.L., 1963. The isolation of a pentacyclic triterpenoid alcohol from a protozoan. J. Am. Chem. Soc. 85 (9), 1362–1363.
Meyers, P.A., Ishiwatari, R., 1993. The Early Diagenesis of Organic Matter in Lacustrine Sediments. In: Engel, M.H., Macko, S.A. (Eds.), Organic Geochemistry: Principles and Applications. Springer, US, Boston, MA, pp. 185–209.
Moldowan, J.M., Seifert, W.K., Gallegos, E.J., 1985. Relationship between petroleum composition and depositional environment of petroleum source rocks. AAPG Bull. 69 (8), 1255–1268.
Moldowan, J.M., Sundararaman, P., Schoell, M., 1986. Sensitivity of biomarker properties to depositional environment and/or source input in the Lower Toarcian of SW-Germany. Org Geochem. 10 (4–6), 915–926.
Nes, W.R., Alcaide, A., Mallory, F.B., Landrey, J.R., Conner, R.L., 1975. Dealkylation of 24-ethylsterols byTetrahymena pyriformis. Lipids 10 (3), 140–144.
Nichols, P.D., Leeming, R., Rayner, M.S., Latham, V., Ashbolt, N.J., Turner, C., 1993. Comparison of the abundance of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia. Journal of Chromatography A 643 (1), 189–195.
Nishimura, M., 1982. 5β-isomers of stanols and stanones as potential markers of sedimentary organic quality and depositional paleoenvironments. Geochim. Cosmochim. Acta 46 (3), 423–432.
Noble, R.A., Alexander, R., Kagi, R.I., Nox, J.K., 1986. Identification of some diterpenoid hydrocarbons in petroleum. Org Geochem. 10 (4), 825–829.
Olivera, E.R., Luengo, J.M., 2019. Steroids as environmental compounds recalcitrant to degradation: Genetic mechanisms of bacterial biodegradation pathways. Genes 10 (7), 512.
Ourisson, G., 1994. Biomarkers in the Proterozoic record. In: Bengston, S. (Ed.), Early Life on Earth. Nobel Symposium, Vol. 84. Columbia University Press, New York, pp. 259–269.
Peakman, T.M., Haven, H.L.T., Rechka, J.R., De Leeuw, J.W., Maxwell, J.R., 1989. Occurrence of (20R)- and (20S)-Δ8(14) and Δ14 5α(H)-sterenes and the origin of 5α(H),14β(H),17β(H)-steranes in an immature sediment. Geochim. Cosmochim. Acta 53 (8), 2001–2009.
Peakman, T.M., De Leeuw, J.W., Rijpstra, W.I.C., 1992. Identification and origin of Δ8(14)5α- and Δ14 5α-sterenes and related hydrocarbons in an immature bitumen from the Monterey Formation, California. Geochimica Et Cosmochimica Acta 56 (3), 1223–1230.
K. Peters C. Walters J. Moldowan The Biomarker Guide Biomarkers and Isotopes in Petroleum Systems and Earth History Volume 2 2nd ed. 2005 Cambridge University Press New York.
Prost, K., Birk, J.J., Lehndorff, E., Gerlach, R., Amelung, W., 2017. Steroid Biomarkers revisited–improved source identification of faecal remains in archaeological soil material. PLoS One 12 (1), e0164882.
Reed, W.E., 1977. Biogeochemistry of Mono Lake, California. Geochim. Cosmochim. Acta 41 (9), 1231–1245.
Ries-Kautt, M., Albrecht, P., 1989. Hopane-derived triterpenoids in soils. Chem. Geol. 76 (1–2), 143–151.
Rontani, J.-F., Belt, S.T., Amiraux, R., 2018. Biotic and abiotic degradation of the sea ice diatom biomarker IP25 and selected algal sterols in near-surface Arctic sediments. Org Geochem. 118, 73–88.
Rontani, J.F., Charriere, B., Forest, A., Heussner, S., Vaultier, F., Petit, M., Delsaut, N., Fortier, L., Sempéré, R., 2012. Intense photooxidative degradation of planktonic and bacterial lipids in sinking particles collected with sediment traps across the Canadian Beaufort Shelf (Arctic Ocean). Biogeosciences 9 (11), 4787–4802.
Sherwin, M., Van Vleet, E., Fossato, V., Dolci, F., 1993. Coprostanol (5β-cholestan-3β-ol) in lagoonal sediments and mussels of Venice, Italy. Marine Pollution Bulletin 26 (9), 501–507.
Škorňa, P., Lengyel, J., Rimarčík, J., Klein, E., 2014. Investigation of oxidation attack sites in sterols: Thermodynamics of hydrogen atom transfer. Comput. Theor. Chem. 1038, 26–32.
Summons, R.E., Jahnke, L.L., Roksandic, Z., 1994. Carbon isotopic fractionation in lipids from methanotrophic bacteria: relevance for interpretation of the geochemical record of biomarkers. Geochim. Cosmochim. Acta 58 (13), 2853–2863.
Sun, M.-Y., Wakeham, S., 1998. A study of oxic/anoxic effects on degradation of sterols at the simulated sediment–water interface of coastal sediments. Org Geochem. 28 (12), 773–784.
Sun, X., Zhang, T., Sun, Y., Milliken, K.L., Sun, D., 2016. Geochemical evidence of organic matter source input and depositional environments in the lower and upper Eagle Ford Formation, south Texas. Org Geochem. 98, 66–81.
Takada, H., Farrington, J.W., Bothner, M.H., Johnson, C.G., Tripp, B.W., 1994. Transport of sludge-derived organic pollutants to deep-sea sediments at Deep Water Dump Site 106. Environ. Sci. Tech. 28 (6), 1062–1072.
Takishita, K., Chikaraishi, Y., Tanifuji, G., Ohkouchi, N., Hashimoto, T., Fujikura, K., Roger, A.J., 2017. Microbial eukaryotes that lack sterols. J. Eukaryot. Microbiol. 64 (6), 897–900.
Taylor, C.D., Smith, S.O., Gagosian, R.B., 1981. Use of microbial enrichments for the study of the anaerobic degradation of cholesterol. Geochim. Cosmochim. Acta 45 (11), 2161–2168.
Ten Haven, H., De Leeuw, J., Peakman, T., Maxwell, J., 1986. Anomalies in steroid and hopanoid maturity indices. Geochim. Cosmochim. Acta 50 (5), 853–855.
Ten Haven, H., Rohmer, M., Rullkötter, J., Bisseret, P., 1989. Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments. Geochim. Cosmochim. Acta 53 (11), 3073–3079.
Teshima, S.-I., Kanazawa, A., 1971. Bioconversion of the dietary ergosterol to cholesterol in Artemia salina. Comp. Biochem. Physiol. 603–607.
Teshima, S.-I., Kanazawa, A., Miyawaki, H., 1979. Metabolism of β-sitosterol in the mussel and the snail. Comparative Biochemistry and Physiology Part b: Comparative Biochemistry 63 (3), 323–327.
Tomazic, M.L., Najle, S.R., Nusblat, A.D., Uttaro, A.D., Nudel, C.B., 2011. A Novel Sterol Desaturase-Like Protein Promoting Dealkylation of Phytosterols in <span class=“named-content genus-species” id=“named-content-1”>Tetrahymena thermophila. Eukaryot. Cell 10 (3), 423–434.
Tomazic, M.L., Poklepovich, T.J., Nudel, C.B., Nusblat, A.D., 2014. Incomplete sterols and hopanoids pathways in ciliates: Gene loss and acquisition during evolution as a source of biosynthetic genes. Mol. Phylogen. Evol. 74, 122–134.
Vachula, R.S., Huang, Y., Longo, W.M., Dee, S.G., Daniels, W.C., Russell, J.M., 2019. Evidence of Ice Age humans in eastern Beringia suggests early migration to North America. Quat. Sci. Rev. 205, 35–44.
Vinnichenko, G., Jarrett, A.J.M., Hope, J.M., Brocks, J.J., 2020. Discovery of the oldest known biomarkers provides evidence for phototrophic bacteria in the 1.73 Ga Wollogorang Formation, Australia. Geobiology(00), 1-16.
Volkman, J.K., 2003. Sterols in microorganisms. Appl. Microbiol. Biotechnol. 60 (5), 495–506.
Volkman, J.K., 2006. Lipid Markers for Marine Organic Matter. In: Volkman, J.K. (Ed.), Marine Organic Matter: Biomarkers, Isotopes and DNA. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 27–70.
Wakeham, S.G., Beier, J.A., 1991. Fatty acid and sterol biomarkers as indicators of particulate matter source and alteration processes in the Black Sea. Deep Sea Research Part A. Oceanographic Research Papers 38, S943–S968.
Wei, J.H., Yin, X., Welander, P.V., 2016. Sterol synthesis in diverse bacteria. Front. Microbiol. 7 (990).
Yunker, M.B., Belicka, L.L., Harvey, H.R., Macdonald, R.W., 2005. Tracing the inputs and fate of marine and terrigenous organic matter in Arctic Ocean sediments: A multivariate analysis of lipid biomarkers. DSR 52 (24), 3478–3508.
Zundel, M., Rohmer, M., 1985. Prokaryotic triterpenoids: 1. 3β-Methylhopanoids from Acetobacter species and Methylococcus capsulatus. Eur. J. Biochem. 150 (1), 23–27.