Biotic and abiotic degradation of the sea ice diatom biomarker IP 25 and selected algal sterols in near-surface Arctic sediments

Rontani, J-F

http://hdl.handle.net/10026.1/11288

10.1016/j.orggeochem.2018.01.003
Organic Geochemistry
Elsevier

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.
Biotic and abiotic degradation of the sea ice diatom biomarker IP_{25} and selected algal sterols in near-surface Arctic sediments

Jean-François Rontani a*, Simon T. Belt b, Rémi Amiraux a

a Aix Marseille University, Université de Toulon, CNRS/INSU/IRD, Mediterranean Institute of Oceanography (MIO) UM 110, 13288 Marseille, France

b Biogeochemistry Research Centre, School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, Devon PL4 8AA, UK

* Corresponding author. Tel.: +33-4-86-09-06-02; fax: +33-4-91-82-96-41.
ABSTRACT

The organic geochemical IP$_{25}$ (Ice Proxy with 25 carbon atoms) has been used as a proxy for Arctic sea ice in recent years. To date, however, the role of degradation of IP$_{25}$ in Arctic marine sediments and the impact that this may have on palaeo sea ice reconstruction based on this biomarker have not been investigated in any detail. Here, we show that IP$_{25}$ may be susceptible to autoxidation in near-surface oxic sediments. To arrive at these conclusions, we first subjected a purified sample of IP$_{25}$ to autoxidation in the laboratory and characterised the oxidation products using high resolution gas chromatography–mass spectrometric methods. Most of these IP$_{25}$ oxidation products were also detected in near-surface sediments collected from Barrow Strait in the Canadian Arctic, although their proposed secondary oxidation and the relatively lower abundances of IP$_{25}$ in other sediments probably explain why we were not able to detect them in material from other parts of the region. A rapid decrease in IP$_{25}$ concentration in some near-surface Arctic marine sediments, including examples presented here, may potentially be attributed to at least partial degradation, especially for sediment cores containing relatively thick oxic layers representing decades or centuries of deposition. An increase in the ratio of two common phytoplanktonic sterols – epi-brassicasterol and 24-methylenecholesterol – provides further evidence for such autoxidation reactions given the known enhanced reactivity of the latter to such processes reported previously. In addition, we provide some evidence that anaerobic biodegradation processes also act on IP$_{25}$ in Arctic sediments. The oxidation products identified in the present study will need to be quantified more precisely in downcore records in the future before the effects of degradation processes on IP$_{25}$-based palaeo sea ice reconstruction can be fully understood. In the meantime, a brief overview of some previous investigations of IP$_{25}$ in relatively shallow Arctic marine sediments suggests that overlying climate conditions were likely dominant over degradation...
processes, as evidenced from often increasing IP$_{25}$ concentration downcore, together with positive relationships to known sea ice conditions.

**Keywords.** IP$_{25}$; Sterols; Arctic sediments; Degradation; Autoxidation; Aerobic and anaerobic biodegradation; Palaeo sea ice reconstruction.
1. Introduction

Over the past decade, the Arctic sea ice diatom biomarker IP$_{25}$ (Ice Proxy with 25 carbons atoms; Belt et al., 2007) has emerged as a useful proxy for the past occurrence of seasonal (spring) sea ice when detected in Arctic marine sediments (for a review see Belt and Müller, 2013). Consistent with its origin (i.e., sea ice-associated or sympagic diatoms; Brown et al., 2014), IP$_{25}$ is a common component of surface sediments across the Arctic (Müller et al., 2011; Stoynova et al., 2013; Xiao et al., 2013, 2015; Navarro-Rodriguez et al., 2013; Ribeiro et al., 2017; Köseoğlu et al., 2018), while its variability in downcore abundance is generally believed to reflect temporal changes to spring sea ice cover, especially when its concentration profile is considered alongside those of other biomarkers indicative of open-water or ice-edge conditions (e.g., Müller et al., 2009, 2011; Belt et al., 2015), through a combined IP$_{25}$-phytoplankton biomarker index (PIP$_{25}$) (Müller et al., 2011), or a multivariate biomarker approach (Köseoğlu et al., 2018). To date, however, the majority of IP$_{25}$-based studies have focused either on surface sediment analysis or on long-term (multi-centennial or longer) records. Thus, surface sediment analyses have addressed aspects of proxy calibration, generally by comparison of IP$_{25}$ and other biomarker content with satellite-based measurements of sea ice conditions (Müller et al., 2011; Navarro-Rodriguez et al., 2013; Stoynova et al., 2013; Xiao et al., 2013, 2015), while temporal studies have concentrated mainly on the reconstruction of sea ice conditions on a multi-centennial scale during the Holocene (e.g., Vare et al., 2009; Belt et al., 2010; Müller et al., 2012; Berben et al., 2014, 2017; Hörner et al., 2016, 2017; Stein et al., 2017), recent glacial/interglacial intervals (Müller et al., 2014; Hoff et al., 2016), the Mid-Pleistocene Transition (Detlef et al., 2018), and even longer timeframes extending back to the Pliocene/Pleistocene boundary and the late Miocene (Stein and Fahl, 2013; Knies et al., 2014; Stein et al., 2016). One key attribute of IP$_{25}$ as a sea ice proxy is its apparent relative stability in sediments. Indeed, the identification of IP$_{25}$ in
sediments several million years old (Knies et al., 2014; Stein et al., 2016) has been attributed, in part, to such stability, and is supported by laboratory-based investigations, where it has been shown to be significantly less reactive towards degradation processes such as photo-oxidation and autoxidation, at least compared to other common phytoplanktonic lipids (Rontani et al., 2011, 2014). As such, sedimentary signals have been interpreted as reflecting climatic (sea ice) conditions rather than diagenetic artefacts, although the possibility of some diagenetic over-printing of the environmental signal has been noted (e.g., Belt and Müller, 2013; Polyak et al., 2016). In contrast, temporal investigations covering recent decades or centuries are less common, although some studies from North Iceland (Massé et al., 2008; Andrews et al., 2009), East Greenland (Alonso-García et al., 2013; Kölling et al., 2017), the Barents Sea (Vare et al., 2010; Köseoğlu et al., 2018), northern Baffin Bay (Cormier et al., 2016) and the Chukchi-Alaskan margin (Polyak et al., 2016) have been reported. Such studies are somewhat different from those carried out on surface sediments (typically 0–1 cm) or longer timeframe investigations generally conducted on material from gravity/piston cores since, in some cases, at least, they likely result from analysis of material that spans the oxic/anoxic (redox) sediment boundary. However, such boundary layers are not generally identified (reported), even though they are likely found in the upper few centimetres of box cores or multi-cores, which reflect accumulation over decades or recent centuries for many Arctic Shelf regions (e.g., Stein and Fahl, 2000; Darby et al., 2006; Mudie et al., 2006; Maiti et al., 2010; Vare et al., 2010). On the other hand, in the central Arctic Ocean, such a layer may reflect substantially longer-term accumulation due to much lower sedimentation rates (e.g., Stein et al., 1994a,b).

The rate and extent of degradation of sedimentary organic compounds is strongly dependent on the molecular structure of the substrate, protective effects offered by association of organic matter with particle matrices, and the length of time accumulating particles are
exposed to molecular oxygen in sedimentary pore waters (Henrich, 1991; Hartnett et al., 1998). The main degradative processes in the oxic layer of sediments are aerobic biodegradation and autoxidation. Numerous organisms, including bacteria, fungi and micro- and macrofauna, are responsible for the aerobic biodegradation of organic carbon in sediments (Fenchel et al., 1998) and almost all of these organisms have the enzymatic capacity to perform a total mineralization of numerous organic substrates (Kristensen, 2000).

Although autoxidation of organic matter involving spontaneous free radical reaction of organic compounds with \( \text{O}_2 \) has been rather under-considered in the marine realm, it is now known that autooxidative processes can act very intensively on vascular plant debris in Arctic sediments (Rontani et al., 2017). This high autooxidation efficiency likely reflects the enhanced photooxidation of senescent vascular plants in the region (thus yielding high amounts of hydroperoxides), together with high lipoxygenase activity (a potential source of radicals; Fuchs and Spiteller, 2014). Indeed, the latter mechanism has recently been observed in sinking particles dominated by ice algae (Amiraux et al., 2017) and in particles discharged from the Mackenzie River (Galeron et al., 2017).

The principal aim of the current study, therefore, was to investigate whether we could provide evidence for oxidative degradation processes acting on \( \text{IP}_{25} \) in near-surface Arctic sediments and thus, potentially, on any resultant palaeo sea ice reconstructions. To achieve this, we first carried out laboratory-based oxidation of purified \( \text{IP}_{25} \) and carried out product identification using high resolution mass spectral analysis. Since \( \text{IP}_{25} \) was shown previously to be relatively resistant to oxidation (Rontani et al., 2014), more powerful oxidizing conditions were used. We then investigated the occurrence of the same oxidation products in sediment samples taken from box cores retrieved from three regions of the Canadian Arctic. To complement the \( \text{IP}_{25} \)-based findings, we also measured the ratios of two common algal sterols – epi-brassicasterol and 24-methylenecholesterol – to provide further evidence of
different oxidative pathways under oxic and anoxic conditions. Geochemical analysis of the box cores revealed variable redox boundary depths, which provided further context for interpreting the biomarker data.

2. Experimental

2.1. Sediment and sea ice algal sampling

Investigations of in situ degradation processes were performed on sediment material and sea ice algal aggregates. Sediment material was obtained from three locations within the Canadian Arctic Archipelago (CAA) on board the CCGS Amundsen in 2005 and 2007 (Vare et al., 2009; Belt et al., 2010; 2013). Sampling locations correspond to Barrow Strait (STN 4), Viscount Melville Sound (STN 308) and the western Amundsen Gulf (STN 408) (Fig. 1). In each case, box cores were collected, sectioned on board, with sub-samples (1 cm resolution) then being freeze-dried before storage between −20 °C and +4 °C prior to analysis. Regular monitoring of IP$_{25}$ concentration in these sediments stored under such conditions (since their collection) has not revealed any significant degradation (i.e. < 10%; Cabedo-Sanz et al., 2016). Previous reports of sedimentation rates from the study area (e.g., 0.15 cm/yr for the Barrow Strait (STN 4) core (Belt et al., 2010)) and preliminary additional $^{210}$Pb data (S. Schmidt, personal communication) suggest that box cores (ca. 20 cm) from the region typically represent decades to centuries of accumulation. A sample of floating sea ice algal aggregates was obtained from Resolute Passage (western Barrow Strait) in 2012 as described by Brown et al. (2014).

Redox boundary layers in each of the box cores were identified using the change (reduction) in Mn content as described previously (Vare et al., 2009; Brown, 2011 and References cited therein). Using this approach, redox boundaries were identified at ca. 2 cm in the box core from Barrow Strait (STN 4) and at ca. 11 cm and ca. 8 cm in box cores from
Viscount Melville Sound (STN 308) and the western Amundsen Gulf (STN 408), respectively (L. Vare, personal communication).

2.2. IP$_{25}$ isolation

A sample of IP$_{25}$ (ca. 99%) was obtained by extraction of a multi-kg quantity of sediment from Barrow Strait in the Canadian Arctic (STN 4; Fig. 1) and purification by a combination of open column chromatography (SiO$_2$; hexane) and Ag$^+$ HPLC as described previously in detail by Belt et al. (2012).

2.3. Production of IP$_{25}$ oxidation products

All procedures were carried out on a ca. 10–50 µg scale. Oxidation of IP$_{25}$ using RuCl$_3$ and tert-butyl hydroperoxide in cyclohexane at room temperature for 16 h (Seki et al., 2008) produced 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradec-1-en-3-ol (1) and 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradec-2-en-1-ol (2) with yields of 5% and 2%, respectively.

OsO$_4$ oxidation of IP$_{25}$ in anhydrous dioxane/pyridine (McCloskey and McClelland, 1965) afforded 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradecan-1,2-diol (6) as the major product (ca. 45%) together with smaller amounts of 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanoic acid (7) (ca. 6%) resulting from diol cleavage and subsequent oxidation of the aldehyde thus formed.

The structures of all IP$_{25}$ oxidation products are shown in the Appendix. Due to the very low amounts of IP$_{25}$ available, compounds 1, 2, 6 and 7 could not be produced in sufficient amounts to permit quantification, although comparison of their mass spectra and retention times with compounds detected in sediments confirmed their identification.

2.4. Induction of autoxidation in solvent
Autoxidation experiments were performed under an atmosphere of air in 15 ml screw-cap flasks containing IP$_{25}$ (10 µg), tert-butyl hydroperoxide (200 µl of a 6.0 M solution in decane), di-tert-butyl nitroxide (1.2 mg) and hexane (2 ml). After stirring, the flask was incubated in the dark at 65 °C. A relatively high temperature was selected in order to accelerate the autoxidation reactions. Aliquots (200 µl) were withdrawn from the reaction mixture after incubation for different times. Each sub-sample was evaporated to dryness under a stream of nitrogen and analyzed by gas chromatography–electron ionization quadrupole time of flight mass spectrometry (GC–QTOFMS) after NaBH$_4$ reduction (see Section 2.5) and derivatization (see Section 2.8) for identification of hydroxylated oxidation products.

2.5. Reduction of oxidation products

Hydroperoxides resulting from IP$_{25}$ oxidation were reduced to the corresponding alcohols by reaction with excess NaBH$_4$ in diethyl ether:methanol (4:1, v:v, 10 mg/mg of residue) at room temperature (1 h). After reduction, a saturated solution of NH$_4$Cl (10 mL) was added cautiously to remove any unreacted NaBH$_4$. The pH was then adjusted to 1 with dilute HCl (2 N) and the mixture shaken and extracted with hexane:chloroform (5 ml, 4:1, v:v; ×3). The combined extracts were dried over anhydrous Na$_2$SO$_4$, filtered and evaporated to dryness under a stream of nitrogen.

2.6. Aerobic biodegradation of phytoplankton sterols

Aerobic biodegradation of phytoplankton cells was performed using the upper layer (0–1 cm) of Arctic sediments collected in July 2016 from Davis Strait (70°29′55.56″ N, 59°31′30.24″ W) during the GreenEdge cruise on board the CCGS Amundsen as bacterial inoculum. Enrichment cultures were incubated in the dark in 250 ml Erlenmeyer flasks containing 50 ml portions of an enrichment medium consisting of LB medium (20 ml) and...
phytoplankton suspension (10 ml) (10 mg dry weight) as carbon source. Samples were
maintained at 2 °C (a temperature close to that of Arctic waters) and agitated using a
reciprocal shaker for different times. The amounts of 24-methylenecholesterol and epi-
brassicasterol in the sediment inoculum were negligible relative to those in the phytoplankton
suspension. These phytoplankton cells (mainly composed of diatoms) were collected in
Commonwealth Bay (East Antarctica, 66°56'S; 142°27'E) during the IPEV-COCA2012 cruise
in January 2012 as described previously (Rontani et al., 2014). After incubation,
phytoplankton material was recovered by filtration on GF/F filters and saponified as described
in Section 2.7.

2.7. Sediment and sea ice algal treatment

Sediments from box cores (i.e., STN 4, 308, 408) or sea ice algae (19.3 mg dry
weight) were placed in MeOH (15 ml) and hydroperoxides were reduced to the corresponding
alcohols with excess NaBH₄ (70 mg, 30 min at 20 °C). Following the reduction step, water
(15 ml) and KOH (1.7 g) were added and the mixture saponified by refluxing (2 h). After
cooling, the contents of the flask were acidified with HCl to pH 1 and extracted three times
with dichloromethane (DCM) (30 ml). The combined DCM extracts were dried over
anhydrous Na₂SO₄, filtered and concentrated to give a total lipid extract (TLE). Since IP₂₅
oxidation product content was quite low relative to other lipids, accurate quantification
required further separation of the TLE using column chromatography (silica; Kieselgel 60, 8
cm × 0.5 cm i.d.). IP₂₅ was obtained by elution with hexane (10 ml) and its oxidation products
by subsequent elution with DCM (10 ml). Additional elution with MeOH (10 ml) was carried
out to recover the more polar lipid compounds. Relative IP₂₅ content was determined using
the method of Vare et al. (2009) and Belt et al. (2010) and some uncalibrated data (STN 308)
were presented previously by Brown (2011). Here, all previous GC–MS data were re-
analysed and converted to absolute concentrations using instrumental response factors derived
from solutions of known IP$_{25}$ concentration (Belt et al., 2012). Biomarker data were further
normalised to total organic carbon (TOC) to accommodate possible changes in burial
efficiency. TOC data were obtained following removal of inorganic carbonate from sediment
material according to the method of Berben et al. (2017).

2.8. Derivatization of hydroxyl-containing products

In order to analyse for hydroxylated products (i.e. alcohols and carboxylic acids),
DCM- and MeOH-eluted fractions were derivatized by dissolving them in 300 µl
pyridine/bis-(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco; 2:1, v:v) and silylated (50
°C, 1 h). After evaporation to dryness under a stream of N$_2$, the derivatized residue was re-
dissolved in 100 µl BSTFA (to avoid desilylation of fatty acids), together with an amount of
cosolvent (ethyl acetate) dependent on the mass of the TLE, and then analyzed using GC–
QTOFMS.

2.9. GC–QTOFMS analyses

Accurate mass spectra were obtained with an Agilent 7890B/7200 GC–QTOFMS
System (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A
cross-linked 5% phenyl-methylpolysiloxane (Macherey Nagel; Optima 5-MS Accent) column
(30 m $\times$ 0.25 mm, 0.25 µm film thickness) was employed. Analysis was performed with an
injector operating in pulsed splitless mode at 280 °C and the oven temperature programmed
from 70 °C to 130 °C at 20 °C/min, then to 250 °C at 5 °C/min and then to 300 °C at 3
°C/min. The carrier gas (He) was maintained at 0.69 $\times$ 10$^5$ Pa until the end of the temperature
program. Instrument temperatures were 300 °C for transfer line and 230 °C for the ion source.
Accurate mass spectra were recorded across the range m/z 50–700 at 4 GHz with nitrogen as
collision gas (1.5 ml/min). The QTOFMS instrument provided a typical resolution ranging from 8009 to 12252 from \( m/z \) 68.9955 to 501.9706. Perfluorotributylamine (PFTBA) was utilized for daily MS calibration. Structural assignments were based on interpretation of accurate mass spectral fragmentations and confirmed by comparison of retention times and mass spectra of oxidation products with those of authentic compounds, when available.

3. Results

3.1. Autoxidation and biodegradation rates of epi-brassicasterol, 24-methylenecholesterol and IP\(_{25}\)

Autoxidation rates of 24\( \alpha \)-methylcholesta-5,22E-dien-3\( \beta \)-ol (epi-brassicasterol) and 24-methylcholesta-5,24(28)-dien-3\( \beta \)-ol (24-methylenecholesterol) were previously measured in phytoplankton cells (Rontani et al., 2014). In order to compare biodegradation rates of these two sterols, phytoplanktonic cells were incubated in the presence of sediment inoculum under oxic conditions. We observed a strong depletion of both sterols (close to 90% after incubation for 1 month at 2 °C), although their biodegradation rates were quite similar (Table 1). The pseudo-first order rate constant (k) for the biodegradation of each sterol was obtained from the gradient of the regression lines determined according to the relationship \( \ln(C/C_o) = -kt \), where \( C \) is the concentration of an analyte at the time of sampling, \( C_o \) is the initial concentration, and \( t \) corresponds to the duration of the incubation. For these experiments, a microbially mediated change in the sterol content is supported by the near invariance of the concentration of 24-ethylcholesta-3\( \beta \),5\( \alpha \),6\( \beta \)-triol, a well-known autoxidation product of sitosterol (Rontani et al., 2009).

Incubation of hexane solutions of IP\(_{25}\) in the presence of tert-butyl hydroperoxide and di-tert-butyl nitroxide at 65 °C, with subsequent NaBH\(_4\)-reduction and silylation, yielded several HBI alcohol TMS derivatives (resulting from the reduction and the silylation of the
corresponding, hydroperoxides, respectively) that could be identified by GC–QTOFMS.

Specifically, the formation of 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradec-1-en-3-ol (1) and 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradec-2-en-1-ol (2) was supported by comparison of their accurate mass spectra (Fig. 2A) and retention times with those of reference compounds prepared by oxidation of purified IP (see Section 2.3). Furthermore, 2,6,10,14-tetramethyl-9-(3-methylpent-4-enyl)-pentadecan-2-ol (3), 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl)-pentadecan-2-ol (4) and 2,6,10,14-tetramethyl-9-(3-methylpent-4-enyl)-pentadecan-6-ol (5) could be tentatively identified on the basis of their accurate mass fragmentations (Fig. 2B and 2C).

3.2. Degradation of IP, epi-brassicasterol and 24-methylenecholesterol in Arctic sediments

IP concentrations (Supplementary Table S1) and the ratio epi-brassicasterol/24-methylenecholesterol (Bra/24-Me) were monitored in the upper sections (up to ca. 20 cm) of three short sediment cores collected from different regions of the Canadian Arctic, which possessed contrasting near-surface redox properties. Thus, sediments from Viscount Melville Sound (STN 308) and the western Amundsen Gulf (STN 408) exhibited a thick oxic layer (11 cm and 8 cm, respectively), while the redox boundary was much shallower (ca. 2 cm) in the box core from Barrow Strait (STN 4). After an increase in the first 3 cm, IP concentration (expressed relative to TOC) decreased substantially in the 3–11 cm sections of (oxic) sediments from Viscount Melville Sound (STN 308). Similarly, a reduction in IP concentration was identified in the top 3 cm of oxic sediments from the western Amundsen Gulf (STN 408) before a subsequent increase (ca. 3–11 cm) and then decrease (Fig. 3). In contrast, IP concentration remained relatively constant in the case of Barrow Strait (STN 4) sediments (Fig. 3). Concerning the two main sterols, the ratio Bra/24-Me remained relatively constant in anoxic sediments from Barrow Strait (STN 4), although it increased steadily in
oxic sediments from Viscount Melville Sound (STN 308) (Fig. 3). Sediments from the
western Amundsen Gulf (STN 408), Bra/24-Me increased strongly in the first 4 cm, before
decreasing and then stabilizing (Fig. 3).

Next, we aimed to identify IP$_{25}$ autoxidation products in the DCM fractions of the
TLEs of different sediments by comparison of accurate mass fragmentations and retention
times with the oxidation products characterised during the thermal incubation reactions. Using
this approach, we detected compounds 1, 3, 4 and 5 in sediments from Barrow Strait (STN 4),
which also contained the highest concentrations of IP$_{25}$ (Fig. 4). The combined relative
abundance of these compounds (estimated on the basis of similar TOFMS responses to that of
IP$_{25}$) reached 8.8% of the amount of IP$_{25}$ in the 1–2 cm layer and then decreased rapidly to
1.2% in the 3–4 cm horizon. In addition, 2,6,10,14-tetramethyl-7-(3-methylpenten-4-yl)-
pentadecan-6-ol (8), which was absent in the incubation experiments, was also identified,
albeit tentatively (Fig. 4B). In contrast, since the mass spectrum of the TMS derivative of the
saturated tertiary C$_{25}$ HBI alcohol (C-7) had already been reported (Robson, 1987), we were
able to investigate if the corresponding mono-unsaturated oxidation product was also present;
however, no characteristic fragmentation ions corresponding to oxidation at C-7 of IP$_{25}$ could
be identified. Analysis of extracts by GC–QTOFMS did, however, enable us to detect 3,9,15-
trimethyl-6-(1,5-dimethylhexyl)-tetradecan-1,2-diol (6) in sediments from Barrow Strait (STN 4) and the western Amundsen Gulf (STN 408) (Fig. 5), while traces of 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanoic acid (7) could be identified in Barrow Strait (STN 4) and
Viscount Melville Sound (STN 308) sediments (Fig. 6). These two compounds were formally
identified by comparison of their accurate mass spectra (Fig. 7) and retention times with those
of standards. On the other hand, we failed to detect compounds 1-8 in floating sea ice algal
aggregates from Resolute Passage despite the presence of relatively large amounts of IP$_{25}$
within these samples (Brown et al., 2014).
4. Discussion

4.1. Autoxidation of IP$_{25}$

According to our product identifications, autoxidation of IP$_{25}$ involves hydrogen atom abstraction by peroxyl radicals on the allylic carbon C-22 and the tertiary carbon atoms C-2, C-10 and C-14. Subsequent oxidation of the resulting radicals together with hydrogen abstraction from other substrate molecules leads to the formation of various hydroperoxides (Fig. 8). These labile compounds were reduced to their corresponding alcohols (1-5) during NaBH$_4$-reduction and silylated prior to analysis by GC–QTOFMS. The failure to detect any autoxidation product resulting from reaction with either of the tertiary carbons C-6 or C-7 is likely due to increased steric hindrance during hydrogen abstraction by the bulky tert-butylperoxyl radicals employed during the incubation. Indeed, when comparing our data from laboratory and environmental samples, we note that the relative abundances of IP$_{25}$ oxidation products are very different in Arctic sediments (Fig. 4B) compared to those from incubations in solvent (Fig. 4A), likely reflecting the contrasting nature of the peroxyl radicals involved during autoxidation. For example, the bulky tert-butylperoxyl radical pertinent to the laboratory-based incubations probably favours the attack of the less hindered external carbon atoms of IP$_{25}$ (i.e. C-2 and C-14), while the unknown (structurally) peroxyl radicals acting in sediment seem to be less sensitive to such steric hindrance. This conclusion is further supported by the detection of an additional oxidation product in sediments (Fig. 4B), tentatively attributed to 2,6,10,14-tetramethyl-7-(3-methylpenten-4-yl)-pentadecan-6-ol (8), which was absent in the incubation experiments (Fig. 4A).

Finally, although each of 1-5 could be readily identified during the incubation reactions, they were only ever present in low abundances and none accumulated over time. We attribute this to the likely secondary oxidation of primary hydroperoxides to polar and
oligomeric compounds (Fig. 8), which are not detectable using the GC–QTOFMS method employed here. This kind of secondary oxidation was described previously for other HBIs (Rontani et al., 2014).

4.2. Degradation of epi-brassicasterol and 24-methylenecholesterol

Due to the different positions of the double bonds in their alkyl chains (see Appendix), an enhanced autooxidative and photooxidative reactivity of epi-brassicasterol compared to 24-methylenecholesterol would be expected. Indeed, the C-H bond energy for allylic hydrogens is lower for internal double bonds than it is for terminal double bonds (77 kcal/mole vs 85 kcal/mole) (Schaich, 2005), thus making allylic hydrogen abstraction more favourable in epi-brassicasterol. Moreover, on the basis of degradation rates of singlet oxygen ($^1\text{O}_2$) with terminal and internal double bonds ($4.0 \times 10^3$ and $7.7 \times 10^3$ M$^{-1}$ s$^{-1}$, respectively; Hurst et al., 1985), Type II photosensitized oxidation of epi-brassicasterol should also be favoured compared to 24-methylenecholesterol. However, in natural settings, it was previously reported that autoxidation (Rontani et al., 2014) and photooxygenation (Rontani et al., 2012; 2016) processes act more intensively on 24-methylenecholesterol than on epi-brassicasterol, at least in mixed phytoplanktonic assemblages. These differences in reactivity can be attributed to the involvement of intra-cellular compartmentalization effects, which may significantly modify the reactivity of lipids towards autooxidative and photooxidative processes according to their location in phytoplanktonic cells (Rontani, 2012). This enhanced reactivity of 24-methylenecholesterol towards autooxidation in phytoplanktonic cells suggests that an increase in the Bra/24-Me ratio may be a good indicator of autoxidation processes in sediments, especially as the main autooxidative products of these two sterols are unspecific and labile $7\alpha/\beta$-hydroperoxy steroids (Christodoulou et al., 2009; Rontani et al., 2009).
In contrast to autoxidation reactions, aerobic microbial degradation of \( \Delta^5 \)-sterols involves two processes: side-chain elimination and ring opening (Rostoniec et al., 2009). The degradation is initiated by oxidation of the 3\( \beta \)-hydroxyl moiety and isomerization of the \( \Delta^5 \) double bond to the \( \Delta^4 \) position (Sojo et al., 1997). Further degradation of the resulting 4-steren-3-one proceeds via hydroxylation at C\(_{26}\) to initiate side-chain degradation, or oxidation of rings A and B resulting in the cleavage of the ring structure (9,10-seco-pathway; Philipp, 2011). In the case of cholesterol, the degradation of the 26-hydroxylated alkyl chain may be carried out after oxidation to the corresponding acid by classical sequences of \( \beta \)-oxidation (Rostoniec et al., 2009). In contrast, in the case of epi-brassicasterol and 24-methylenecolesterol, due to the presence of a methyl or methylene group at C-24, the involvement of alternating \( \beta \)-decarboxymethylation (Cantwell et al., 1978) and \( \beta \)-oxidation sequences is needed (Fig. 9). The very close degradation rates of these two sterols observed after incubation of phytoplanktonic cells in the presence of sediment inoculum under oxic conditions (Table 1) may be attributed to the involvement of a 2,3-enoyl-CoA isomerase (Ratledge, 1994). Indeed, these widely distributed enzymes may catalyze the isomerisation of the methylidene double bond to the C24-25 position in the case of 24-methylenecolesterol (Fig. 9), thus permitting the involvement of a similar degradation process of the alkyl side-chain in the case of the two sterols.

Under anoxic conditions, ring cleavage of \( \Delta^5 \)-sterols may be mediated by oxygen-independent enzymatic processes (Chiang et al., 2007). In the case of cholesterol, only hydroxylation of the side chain at C-25 has been shown to occur, with the resulting tertiary alcohol not oxidized further (Chiang et al., 2007). For sterols with more substituted or unsaturated side chains, such as sitosterol, fucosterol and isofucosterol, similar degradation rates were observed following incubation of cells of the microalga \textit{Nannochloropsis salina} in anoxic sediment slurries (Grossi et al., 2001). This suggests that changes to the sterol side
chain have little impact on the overall degradation rates under anoxic conditions. As such, in the absence of any reported experimental data, it is reasonable to propose similar anaerobic degradation rates for epi-brassicasterol and 24-methylenecolesterol, especially given their common ring structure. Overall, therefore, aerobic and anaerobic bacterial degradation processes should not induce significant changes to the Bra/24-Me ratio in sediments.

4.3. Degradation of IP$_{25}$, epi-brassicasterol and 24-methylenecolesterol in Arctic sediments

Due to the extremely low rate of autoxidation of IP$_{25}$ in solution, even at higher temperature (e.g., 65 °C), it was suggested previously that it should be largely unaffected by autoxidative degradation processes in the Arctic, at least in comparison with lipids of similar structure such as other HBIs with greater unsaturation (Rontani et al., 2014). However, here we show that autoxidative degradation of IP$_{25}$ may occur under more ‘forced’ conditions and such processes may also take place in Arctic surface sediments. Indeed, due to recent evidence of strong lipoxygenase activity (a well-known source of radicals; Fuchs and Spiteller, 2014) in bacteria associated with ice algae (Amiraux et al., 2017) and in terrestrial particulate organic matter discharged from Arctic rivers (Galeron et al., 2017), autoxidative degradation reactions can even be dominant in Arctic sediments (Rontani et al., 2012; 2017), despite the low temperatures. The autoxidation of IP$_{25}$ in sediments possessing a thick oxic layer, where the contact of ice algal detritus with oxygen may be relatively long, therefore represents a viable degradation pathway of this biomarker in near-surface sediments.

Consistent with this suggestion, the decrease in IP$_{25}$ concentration observed in the oxic layer of sediments from Viscount Melville Sound (STN 308) (between 3 and 10 cm) and the western Amundsen Gulf (STN 408) (between 0 and 3 cm) (Fig. 3) may potentially be attributed to the involvement of oxic degradation processes such as aerobic biodegradation (Robson and Rowland, 1988) or autoxidation, and this last suggestion is supported further by
the increase of the Bra/24-Me ratio within the same sediments (Fig. 3). In contrast, the strong
decrease in Bra/24-Me observed in the bottom of the oxic layer of sediments from the western
Amundsen Gulf (STN 408) is potentially due to an input of fresh algal material (with a low
Bra/24-Me ratio) during this period. This suggestion is supported by the observation of a 10-
fold increase in phytoplanktonic sterol concentration in the 6–7 cm horizon compared to the
4–5 cm layer. Further, Brown (2011) proposed that rapid decreases in sedimentary IP25
congestion in some other cores from the Canadian Arctic could potentially reflect
degradation processes, more generally. In contrast, the more consistent concentration of IP25
in anoxic sediments from Barrow Strait (STN 4) and the western Amundsen Gulf (STN 408)
(Fig. 3) is likely indicative of enhanced resistance to oxidation under such conditions.
Unfortunately, we were not able to detect the primary autoxidation products of IP25 in
sediments other than from Barrow Strait (STN 4), likely due to: (i) their further oxidation (as
suggested from the incubation reactions), especially in the oxic layers of cores from Viscount
Melville Sound (STN 308) and the western Amundsen Gulf (STN 408) and (ii) the detection
limits of GC–QTOFMS analyses. However, despite the general resistance of IP25 towards free
radical oxidation, as reported previously (Rontani et al., 2011, 2014), the detection of
compounds 1, 3, 4 and 5 (Fig. 4) shows that this HBI alkene can be susceptible to
autoxidation in Arctic sediments, an environment where such processes have previously been
shown to be enhanced for some other lipids (Rontani et al., 2012; 2017). Further, this
vulnerability towards autoxidation may be especially prevalent in cases where sequestered ice
algal material experiences long residence times in the oxic layer.

Interestingly, compounds 6 and 7 could be detected in anoxic sediments from Barrow
Strait (STN 4) and oxic sediments from Viscount Melville Sound (STN 308) and the western
Amundsen Gulf (STN 408) (Figs. 5 and 6). We attribute the formation of such compounds to
aerobic or anaerobic bacterial metabolism of IP25. In contrast, a mechanism involving
autoxidative production (via epoxidation and subsequent hydrolysis; Schaich, 2005) is discarded on the basis of: (i) the detection of only one pair of enantiomers of compound 6 in sediments (Fig. 5) and (ii) the lack of compounds 6 and 7 observed during our in vitro autoxidation experiments. Aerobic bacterial degradation of IP25 may be initiated either via attack on the double bond or by the same mechanisms associated with n-alkane metabolism (i.e., attack of terminal methyl groups; Morgan and Watkinson, 1994). Oxidation across the double bond in IP25 can produce diol 6 via the corresponding epoxide 9 (Soltani et al., 2004) (Fig. 10). Previously, it was demonstrated that various pristenes and phytenes (also isoprenoid alkenes) can be rapidly biodegraded by sedimentary bacteria under anaerobic conditions, mainly by hydration reactions (Rontani et al., 2013). Enzymes that catalyze the addition of water to isolated and electron-rich carbon-carbon double bonds are termed hydratases and display a high degree of enantioselectivity (Resch and Hanefeld, 2015). In the case of IP25, addition of water to the C23-24 double bond results in the formation of 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradecan-2-ol (10) (Fig. 10), which subsequently oxidises to the corresponding ketone (11). Mechanisms involving hydration of the enol forms of the keto group have been proposed for the anaerobic metabolism of isoprenoid ketones by denitrifiers (Rontani et al., 1999; 2013). Hydration of the enol form under kinetic control of the ketone 11 affords the diol 6 (Fig. 10). This diol may be subsequently cleaved to form 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanal (12), which may then be fully metabolized via 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanoic acid (7) by alternating β-oxidation and β-decarboxymethylation sequences (Cantwell et al., 1978; Rontani and Volkman, 2003). These interesting results suggest that IP25 may be also affected by bacterial degradation processes in Arctic sediments, although the extent to which this occurs remains to be determined.

4.4. Implications for palaeo sea ice reconstruction
The identification of some degradation pathways of IP$_{25}$ in some Arctic marine sediments raises potentially important questions regarding the use of this biomarker as a reliable proxy measure of past sea ice. However, the failure to investigate the occurrence of any of the degradation products described herein in previous studies, prevents a comprehensive evaluation of the importance of IP$_{25}$ degradation from being made at this stage. In the meantime, analysis of an extensive set of surface sediments from different Arctic regions has revealed excellent agreement between IP$_{25}$ content and known sea ice cover (e.g., Müller et al., 2011; Stoynova et al., 2013; Navarro-Rodriguez et al., 2013; Xiao et al., 2013, 2015; Belt et al., 2015; Köseoğlu et al., 2017; Ribeiro et al., 2017), while IP$_{25}$ data obtained from several short core records (typically covering recent decades to centuries) show generally good agreement with known sea ice conditions derived either from historical records or satellite data (Alonso-García et al., 2013; Weckström et al., 2013; Cormier et al., 2016), including examples where IP$_{25}$ even increases with depth (e.g., Massé et al., 2008; Andrews et al., 2009; Vare et al., 2010; Cabedo-Sanz and Belt, 2016). However, in a recent study from the Chukchi-Alaskan margin, a decline in IP$_{25}$ abundance in near-surface sediments was suggested to indicate a combined influence of diagenesis and long-range sediment transport (Polyak et al., 2016). Further, the previously reported surface sediment datasets (and their relationship to known sea ice cover) might need re-examination in light of the evidence described herein for at least partial IP$_{25}$ degradation in some near-surface sediments.

Interestingly, although there is a clear decline in IP$_{25}$ concentration with depth in the box core from Viscount Melville Sound (STN 308) (Fig. 3), a similarly continuous negative trend was not apparent in the cores from either Barrow Strait (STN 4) or the western Amundsen Gulf (STN 408) (Fig. 3), despite the detection of IP$_{25}$ oxidation products in both cases (Fig. 5D). This suggests that climatic influences likely exceeded those from...
degradation, although the possible impact of bioturbation, a feature in some near-surface sediments, cannot be totally ruled out at this stage. However, preliminary $^{210}\text{Pb}$ data suggest that bioturbation is negligible in cores from Barrow Strait (STN4) and Viscount Melville Sound (STN308), and confined to the (at most) upper 2 cm in the core from the western Amundsen Gulf (STN408) (S. Schmidt, personal communication).

For longer records (i.e. those beyond recent centuries), a common feature in many IP$_{25}$-based sea ice reconstructions has been a reduction in IP$_{25}$ concentration over time, especially during the Holocene (e.g., Vare et al., 2009; Belt et al., 2010, Fahl and Stein, 2012; Müller et al., 2012, Hörner et al., 2016; Kölling et al., 2017; Stein et al., 2017). Such changes have generally been interpreted as reflecting an increase in sea ice extent or duration from the warm early Holocene through neoglacial conditions towards present, an interpretation generally supported with other paleoclimatic proxy data. The often higher IP$_{25}$ concentrations observed in older sections of the same (or related) records, covering the Younger Dryas stadial (ca. 12.9–11.5 kyr BP) (C Abedo-Sanz et al., 2013, Müller and Stein, 2014; Belt et al., 2015; Méheust et al., 2015; Jennings et al., 2017; Xiao et al., 2017) and the Last Glacial Maximum (LGM; e.g., Müller and Stein, 2014; Hoff et al., 2016) provide further evidence of substantial climatic overprinting within biomarker profiles.

Resolving the relative contributions of climatic influence and diagenetic alteration on downcore IP$_{25}$ (and other biomarker) distributions is likely to remain a challenge from an analytical perspective, however, not least because, on the basis of our new results described here, the oxidation products of IP$_{25}$ are unlikely to accumulate in sufficient amounts to enable their quantification (or even detection), especially since IP$_{25}$ content itself is often quite low in Arctic marine sediments. On the other hand, the measurement of certain biomarker ratios such as Bra/24-Me may prove useful for assessing such degradation processes, especially when used alongside IP$_{25}$ concentration profiles; however, the potential for changes in
environmental conditions to also influence such ratios should also be considered. Further, the measurement of redox boundary layers in upper sections of sediment cores might also provide additional insights into the nature of different degradation processes.

Finally, it is interesting to note that we were not able to detect any IP$_{25}$ oxidation products in our sample of sea ice algae, which supports conclusions from previous studies that it is largely resistant to abiotic alteration in the host matrix (Rontani et al., 2014) and also in the water column soon after ice melt (Brown et al., 2016; Rontani et al., 2016).

5. Conclusions

This study represents the first attempt to evaluate, via oxidative product identification, the possible fate of IP$_{25}$ in Arctic sediments. Laboratory-based autoxidation of the Arctic sea ice diatom biomarker IP$_{25}$ results in the formation of a series of oxidation products that could be characterised using high resolution GC–MS methods. Some of the same oxidation products could also be identified in sediment material from the Canadian Arctic although their accumulation was very low, likely due to further oxidation. The detection of bacterial metabolites of IP$_{25}$ showed that this HBI alkene may also be affected by aerobic and/or anaerobic degradation processes in sediments. We suggest that complementary evidence for autoxidation and biodegradation processes may potentially be obtained from measurement of certain phytoplankton sterol ratios, although these may also be influenced by changes to the overlying climatic conditions.

Although degradation of IP$_{25}$ has, to date, not been considered in detail within IP$_{25}$-based sea ice reconstructions, our initial overview of previous studies suggests that climatic contributions to sedimentary IP$_{25}$ distributions likely exceed the impact of sedimentary degradation, at least in the albeit still limited number of case studies thus far reported. On the other hand, oxidative degradation may have a significant impact on IP$_{25}$ concentration in
some near-surface material, especially in cases where the oxic layer represents relatively long
time intervals. In any case, we suggest that such degradation processes should be considered
more carefully in future sea ice reconstructions based on IP$_{25}$.

Acknowledgments

Financial support from the Centre National de la Recherche Scientifique (CNRS) and
the Aix-Marseille University is gratefully acknowledged. Thanks are due to the FEDER
OCEANOMED (N° 1166-39417) for the funding of the apparatus employed. We are grateful
to Lindsay Vare for providing us with the redox boundary information used in this study and
for carrying out some of the sediment extractions for IP$_{25}$ analysis. Patricia Cabedo-Sanz is
acknowledged for conducting calibration experiments that enabled us to subsequently
quantify IP$_{25}$ from archived GC–MS data. We also thank Thomas Brown for fruitful
discussions regarding the potential degradation of IP$_{25}$ in Arctic sediments. We are grateful to
Lindsay Vare, Guillaume Massé, André Rochon and the officers and crew of the CCGS
Amundsen for help with obtaining box core sediment material. Finally, we thank the
Associate Editor, Kirsten Fahl and an anonymous reviewer for their useful and constructive
comments.

Associate Editor—Marcus Elvert
APPENDIX

Epi-brassicasterol

24-Methylenecholesterol
References


Andrews, J.T., Belt, S.T., Olafsdottir, S., Massé, G., Vare, L.L., 2009. Sea ice and climate variability for NW Iceland/Denmark Strait over the last 2000 cal. yr BP. The Holocene 19, 775–784.


Belt, S.T., Vare, L.L., Massé, G., Manners, H.R., Price, J.C., MacLachlan, S.E., Andrews, J.T. and Schmidt, S., 2010. Striking similarities in temporal changes to seasonal sea ice conditions across the central Canadian Arctic Archipelago over the last 7,000 years. Quaternary Science Reviews 29, 3489–3504.


in Arctic sea ice and underlying sediments: Further considerations for palaeo sea ice

Belt, S.T., Cabedo-Sanz, P., Smik, L., Navarro-Rodriguez, A., Berben, S.M.P., Knies, J.,
Husum, K., 2015. Identification of paleo Arctic winter sea ice limits and the marginal
ice zone: optimised biomarker-based reconstructions of late Quaternary Arctic sea ice.

evolution of Atlantic Water inflow and sea ice distribution in the western Barents Sea.
Climate of the Past 10, 181–198.

Semi-quantitative reconstruction of early to late Holocene spring and summer sea ice


sea ice proxy IP_{25}. Nature Communications 5, 4197.

Quantitative estimates of sinking sea ice particulate organic carbon based on the

Cabedo-Sanz, P., Belt, S.T., 2016. Seasonal sea ice variability in eastern Fram Strait over the
last 2,000 years. Arktos 2, 22.

seasonal sea ice conditions during the Younger Dryas. Quaternary Science Reviews
79, 74–86.


Hörner, T., Stein, R., Fahl, K., Birgel, D., 2016. Post-glacial variability of sea ice cover, river run-off and biological production in the western Laptev Sea (Arctic Ocean) - A high resolution biomarker study. Quaternary Science Reviews 143, 133–149.


degradation of terrestrial vascular plant material in the Canadian Arctic. Organic Geochemistry 108, 43–50.


Figure captions

Fig. 1. Map showing the sampling locations.

Fig. 2. TOFMS mass spectra of HBI alcohol trimethylsilyl derivatives of: (A) 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradec-1-en-3-ol (1), (B) 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl)-pentadecan-2-ol (4) and (C) 2,6,10,14-tetramethyl-9-(3-methylpent-4-enyl)-pentadecan-6-ol (5).

Fig. 3. Downcore plots of IP$_{25}$ concentration and the epi-brassicasterol/24-methylenecholesterol (Bra/24-Me) ratio for the three stations investigated. (The dashed lines represent the redox boundaries).

Fig. 4. Partial ion chromatograms ($m/z$ 131.0885, 143.0883, 201.1670 and 423.4015 (M – CH$_3$)) showing the distribution of IP$_{25}$ oxidation products obtained after incubation in the presence of tert-butyl hydroperoxide and di-tert-butyl nitroxide at 65 °C under darkness (A) and present in the 2–3 cm layer of the core sediment from the station 4 (B). The peak labelled * represents a compound tentatively identified as 2,6,10,14-tetramethyl-7-(3-methylpenten-4-yl)-pentadecan-6-ol (8) trimethylsilyl derivative.

Fig. 5. Partial ion chromatograms ($m/z$ 425.4174 and 513.4520 (M – CH$_3$)) showing the diastereoisomers of 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradecan-1,2-diol trimethylsilyl ether obtained after oxidation of IP$_{25}$ with OsO$_4$ (A) and present in the 2–3 cm (B) and 4–5 cm layers of the core from Barrow Strait (STN 4) (C) and the 2–3 cm layer of the core from the western Amundsen Gulf (STN 408) (D).
Fig. 6. Partial ion chromatograms (m/z 146.0755 and 425.3802 (M – CH₃)) showing 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanoic acid trimethylsilyl derivative obtained after OsO₄ oxidation of IP₂₅ (A) and present in the 2–3 cm layer of the core sediment from Barrow Strait (STN 4) (B) and Viscount Melville Sound (STN 308) (C).

Fig. 7. TOFMS mass spectra of HBI trimethylsilyl derivatives of: (A) 3,9,15-trimethyl-6-(1,5-dimethylhexyl)-tetradecan-1,2-diol (6) and (B) 2,8,12-trimethyl-5-(1,5-dimethylhexyl)-tridecanoic acid (7).

Fig. 8. Proposed mechanisms for the autoxidative degradation of IP₂₅.

Fig. 9. Proposed mechanisms for the aerobic bacterial degradation of the alkyl side-chain of epi-brassicasterol and 24-methylenecholesterol.

Fig. 10. Proposed mechanisms for the aerobic and anaerobic bacterial degradation of IP₂₅.
Figure 2

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Figure 3

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Figure 4

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Figure 5

* Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.*
Figure 6

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Figure 7

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Abstraction of a tertiary hydrogen

Abstraction of the allylic hydrogen

Further oxidation

Figure 8

"Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation."
2,3-enoyl-CoA isomerase
+ CO₂
+ H₂O
- C₂ unit
- C₃ unit
β-oxidation sequences

Figure 9
Figure 10

Aerobic metabolism

Attack of terminal methyl groups

Assimilation

Anaerobic metabolism

+ H$_2$O

Hydratase

- 2H

H$_2$O

Epoxide hydrolase

+ H$_2$O

- CH$_3$O

C2 and C3 units

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Table 1

Pseudo first order degradation rate constants of epi-brassicasterol and 24-methylenecholesterol during in vitro incubations and in Arctic oxic sediments

<table>
<thead>
<tr>
<th></th>
<th>$k_{BrA}$ (h$^{-1}$)</th>
<th>$r^2$</th>
<th>n</th>
<th>$k_{24-Me}$ (h$^{-1}$)</th>
<th>$r^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoxidation in algal cells (seawater + Fe$^{2+}$)</td>
<td>$2.9 \times 10^{-4}$</td>
<td>0.80</td>
<td>4</td>
<td>$1.1 \times 10^{-3}$</td>
<td>0.85</td>
<td>4</td>
</tr>
<tr>
<td>Aerobic biodegradation of algal cells</td>
<td>$3.5 \times 10^{-3}$</td>
<td>0.95</td>
<td>4</td>
<td>$3.6 \times 10^{-3}$</td>
<td>0.93</td>
<td>4</td>
</tr>
<tr>
<td>Degradation in sediments from station 308</td>
<td>$2.0 \times 10^{-6}$</td>
<td>0.86</td>
<td>6</td>
<td>$3.3 \times 10^{-6}$</td>
<td>0.94</td>
<td>6</td>
</tr>
</tbody>
</table>

$a$ Pseudo first order degradation rate constant of epi-brassicasterol, $b$ Pseudo first order degradation rate constant of 24-methylenecholesterol, $c$ Rontani et al. (2014), $d$ First 10 cm
- **Comment 1**: The answer to the first comment of reviewer #2: "Chronologies of these sediments (which also provide bioturbation depths) are in progress and could not be included in the present paper focusing on degradation processes. It is now indicated that an effect of bioturbation processes in the sediments investigated cannot be totally ruled out (see text p. 21-22 lines 479-481). In Figure 3 concentrations of IP25 are now expressed relative to TOC." does indeed provide a sentence naming the term bioturbation ONCE at the far end of the whole manuscript (line 480) and without reference(s). This needs to expanded because also reviewer #1 was wondering about it and I agree that the answer is not satisfactory. If not (yet) available for the core(s), please provide references that judge to why bioturbation is important (down to which depth?) or can be neglected.

- **Answer**: Bioturbation was neglected on the basis of previous $^{210}$Pb measurements. The following text was added p.22 lines 494-497: “However, preliminary $^{210}$Pb data suggest that bioturbation is negligible in cores from Barrow Strait (STN4) and Viscount Melville Sound (STN308), and confined to the (at most) upper 2 cm in the core from the western Amundsen Gulf (STN408) (S. Schmidt, personal communication).”

- **Comment 2**: The authors now express concentrations relative to TOC (Fig. 3) which is good, but should be actually specified in the Figure text. However, it seems that the concentrations are much too high (now is $\mu$g / mg TOC but should rather be $\mu$g / g TOC from what I am generally aware of). This has to be clarified. Representation of the TOC values (if not visible for all cores in the Berben et al., 2017 reference) should be added to supplementary data, so that concentration calculations are reasonable for everyone.

- **Answer**: There was an error in the TOC unit, which is effectively $\mu$g g$^{-1}$ TOC. The figure 3 was changed accordingly and as suggested by the editor a table showing the calculations was added to supplementary data.

- **Comment 3**: Contrarily to what the authors write in the response, the answer provided to the following comment is not comprehensive: Lines 380-384 (in the original manuscript) and more generally: The lipid profiles observed in the oxic layer of the sediment from station 408 are not discussed. Could authors comment on these profiles showing a decrease in IP25 concentration in the first 2cm of the oxic layer only, and unexpected fluctuations (an increase followed by a decrease) of the Bra/24-Me sterols ratio in the oxic layer? Please directly answer to the question and do not refer to a former response (which was actually a different reviewer comment).

- **Answer**: The following text was added p.19 lines 419-424: “In contrast, the strong decrease in Bra/24-Me observed in the bottom of the oxic layer of sediments from the western
Amundsen Gulf (STN 408) is potentially due to an input of fresh algal material (with a low Bra/24-Me ratio) during this period. This suggestion is supported by the observation of a 10-fold increase in phytoplanktonic sterol concentration in the 6-7 cm horizon compared to the 4-5 cm layer.

- Comment 4: The response given to the following comment is not fully agreed upon by the reviewer: Lines 358-363 (of the first manuscript): This was observed for cholesterol only and not for 24-methyl (or 24-methylidene) structures. The statement of similar anaerobic biodegradation rate for the two sterols is thus very hypothetical and, as such, cannot be taken for granted. The answer that cholesterol, brassicasterol and 24-methylene cholesterol possess the same cyclic structure and differ only by their side-chain is not conclusive. Since under anaerobic conditions the side-chain is not assimilated, it is actually not expected that similar biodegradation rates of these three sterols have to taken for granted. Because we do not clearly know whether the side-chain plays a role (is assimilated) or not during the anaerobic biodegradation of sterols since data are scarce (if not, more references could be given). The presence of an extra methyl group and/or of a double bond may significantly influence the degradation pathways. This should be answered more carefully by the authors. I agree. Please provide further background (with references) on this.

- Answer: The following text was added p. 18 lines 390-397: "For sterols with more substituted or unsaturated side chains, such as sitosterol, fucosterol and isofucosterol, similar degradation rates were observed following incubation of cells of the microalga Nannochloropsis salina in anoxic sediment slurries (Grossi et al., 2001). This suggests that changes to the sterol side chain have little impact on the overall degradation rates under anoxic conditions. As such, in the absence of any reported experimental data, it is reasonable to propose similar anaerobic degradation rates for epi-brassicasterol and 24-methylenecholesterol, especially given their common ring structure." The reference Grossi et al. (2001) was added in the reference list.

- Comment 5: The final paragraph (4.4 Implications for palaeo sea ice reconstruction) is obviously showing a striking difference in English style (much better) in comparison to the rest of the manuscript. I recommend that the rest of the manuscript is re-checked carefully in this direction by all members of the author's team.

- Answer: All the text was carefully checked by Dr. S.T. Belt.