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Lipoxygenase-induced autoxidative degradation of terrestrial particulate organic matter in estuaries: A widespread process enhanced at high and low latitude

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ABSTRACT

There exists a substantial amount of research on abiotic (e.g. photochemical) degradation pertaining to organic matter (OM) in the marine realm. While recent research has shown its importance in the degradation of terrestrial particulate OM (TPOM), the mechanisms involved in the induction of autoxidation in estuaries remain unclear. In this study, we propose for the first time the involvement of lipoxygenase (LOX) activity in the induction of autoxidation in mixed waters. The observation of unusual profiles of palmitoleic acid oxidation products and the presence of jasmonic acid in suspended particulate matter (SPM) collected close to the Rhône River, as well as in samples from the Mackenzie and Amazon rivers, is attributed to strong LOX activity. We show the role played by salinity in the induction of this LOX activity and provide an explanation for the differences in estuarine autoxidation level. At high latitude, lower temperatures and irradiance favor photooxidative damage to higher plant debris and, consequently, hydroperoxide production. High hydroperoxide content strongly contributes to LOX activation in mixed waters. The high resulting LOX activity enhances alkoxyl radical production and thus autoxidation. On the contrary, at low latitude, photooxidative effects are limited, and riverine autoxidation is favored. The higher hydroperoxide content of TPOM may, as a consequence, thereby also contribute to a high level of LOX activity and autoxidation in estuaries. In temperate zones, land and riverine photooxidative and autoxidative damage is limited, unlike estuaries where we observed significant LOX-induced and autoxidative damage.

1. Introduction

Riverine particulate organic matter (POM), which consists in part of highly degraded residues from terrestrial higher plants, has long been considered to be refractory compared with

marine-derived POM (de Leeuw and Largeau, 1993; Wakeham and Canuel, 2006). The assumption is not supported by the unexpected relatively low proportion of land-derived OM detected in marine sediments (Hedges and Keil, 1995), suggesting extensive remineralization of this material at sea (Hedges et al., 1997). In fact, the notion of OM being inherently refractory and/or labile in terrestrial and aquatic systems has been recently challenged, with the suggestion that the all this material is utilized by microbes under the “right” environmental conditions (Bianchi, 2011; Schmidt et al., 2011). Moreover, several studies have demonstrated

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that POM delivered by rivers is sensitive to microbial remineralization in some shelf areas (Aller et al., 1996; Aller, 1998; Mayer et al., 2008; van Dongen et al., 2008; Sampere et al., 2008; Vonk et al., 2010; Bourgeois et al., 2011; Karlsson et al., 2011). More recently, it has been shown that processes such as biodegradation and autoxidation (free radical reaction of organic compounds with O₂) play a key role in the degradation of vascular plant-derived lipids discharged by the Mackenzie River to the Beaufort Sea (Rontani et al., 2014a).

Autoxidation is not spontaneous but autocatalytic; once started, it is self-propagating and self-accelerating (Schaich, 2005). The mechanisms of initiation have been debated for many years, but likely involve the homolytic cleavage of photochemically produced hydroperoxides in phytodetritus (Girotti, 1998; Rontani et al., 2003). The cleavage may be induced by heat, light, metal ions and lipoxygenases (Schaich, 2005). Autoxidation, largely ignored for the coastal zone, proceeds by a radical chain reaction and acts mainly on organic compounds possessing C=C bonds or C-H bonds whose bond energy is relatively low (e.g. allylic, tertiary, α to oxygen, etc.; Fossey et al., 1995). It can act not only on unsaturated lipids [e.g. sterols, unsaturated fatty acids (FAs), chlorophyll phytyl side chain, alkenes, tocopherols and alkenones; Rontani, 2012], but also on amino acids (Seko et al., 2010), sugars (Lawrence et al., 2008) and polyphenols (Hathway and Seakins, 1957). It can also affect biopolymers (Schmid et al., 2007) and kerogen, inducing ring opening and chain cleavage.

To explain this induction, a mechanism involving homolytic cleavage of photochemically produced hydroperoxides, resulting from the senescence of higher plants on land, was proposed (Galeron et al., 2016c). Cleavage was attributed to some redox-active metal ions released from suspended particulate matter (SPM) in the mixing zone of riverine water and marine water. Using new lipid tracers specific to the degradation of terrestrial higher plants at the mouth of the Rhône River (Galeron et al., 2016a,b; Rontani et al., 2015), we confirmed the role played by autoxidation in the degradation of terrestrial POM (TPOM) in estuaries (Galeron et al., 2017). As in the Beaufort Sea (Rontani et al., 2014a), autoxidation rate and salinity level appeared to correlate well within the river plume in the Mediterranean Sea (Galeron et al., 2017). However, it was not possible to detect a significant release of metal ions (able to catalyze hydroperoxide cleavage) during that study. Four important questions thus remained to be elucidated: (i) Which processes promote induction of autoxidation of TPOM in estuarine waters, (ii) why is autoxidation induced in TPOM in mixed waters (i.e. during an increase in salinity), (iii) why is autoxidation of TPOM enhanced in Arctic vs. temperate estuaries and (iv) what is the role of autoxidation in tropical zones? In order to answer these questions, the degradation of TPOM in several SPM and surface sediment samples collected from three contrasting key areas was monitored: (1) Arctic Ocean (Mackenzie River and Shelf), (2) tropical Atlantic Ocean (Amazon River and Shelf) and (3) Mediterranean Sea (Rhône River and Shelf).

The Arctic Ocean receives the largest riverine input of all the oceans, relative to its size. More specifically, its upper layer, which represents only 0.1% of the global ocean volume, receives 11% of the global riverine discharge (Fichot et al., 2013). Moreover, since global warming has accelerated twice as rapidly in the northern Hemisphere (Kug et al., 2015) than other regions of the globe, the Arctic remains a key focal point for climate change research. With a huge watershed (1.8×10^6 km²) and annual water discharge of 330 km³/yr delivered mainly in the short summer period, the Mackenzie is the fourth largest Arctic river and the primary source of particulate matter (PM) in the Arctic Ocean (e.g. Hilton et al., 2015). Its total suspended matter load was estimated at 40–160 Mt/yr and its particulate organic carbon (POC) flux at 0.2–2.2 Mt/yr (Macdonald et al., 1998; Doxaran et al., 2015).

The Mediterranean is a semi-enclosed sea subject to both anthropogenic pressure and climate change. Since the damming of the Nile, the Rhône is the main supplier of fresh water (55 km³/yr), suspended matter (2–8 Mt/yr) and POC (0.2 Mt/yr) to the Mediterranean (Sempéré et al., 2000; Sadaoui et al., 2016). It is 816 km long with a drainage area of 97,800 km².

The Amazon is the largest river in the world, with a drainage basin area of 6.1×10^6 km² (Goulding et al., 2003) covered by diverse vegetation, including tropical rainforest, inundated floodplain ('várzea') forest, floating grass and extensive grassland/savannah (Hedges et al., 1986). Its discharge has a strong seasonal variation, with a maximum of 7500 km³/yr in May–June and a minimum of 2500 km³/yr in October–November (Lentz, 1995). The total export of POC from the historic gauging station, Óbidos, to the ocean was estimated at 12.8–14.4 Mt/yr (Moreira-Turcq et al., 2013). However the POC load has been reported to decrease by ca 50% between Óbidos and the river mouth, 850 km further downstream (Ward et al., 2015).

Here we evaluate the mechanisms that induce autoxidation across a diverse suite of high to low latitude river-to-ocean gradients. Since it is well known that peroxidation of membrane lipids by lipoxygenases (LOXs) may play an important role in promoting oxidative damage during environmental stress (Thompson et al., 1987), we hypothesize that LOX activity is directly related to level of estuarine TPOM autoxidation and that the balance between photo-, bio-, and auto-oxidative TPOM breakdown depends on salinity, temperature, and light penetration.

2. Methods

2.1. Sampling of SPM and sediments from the Rhône, Mackenzie and Amazon rivers and shelves

SPM samples were collected in February 2012 along a transect following the salinity gradient in the Rhône plume (Fig. 1). Sampling and hydrographic conditions have been described by Galerón et al. (2017). High frequency monitoring of the nutrients and of the PM input from the Rhône to the Mediterranean has been carried out since 2010 in the framework of the national program MOOSE (Mediterranean Ocean Observing System for the Environment). Monitoring is undertaken at the Arles station (43°40'44"N, 4°37'16"E), 40 km upstream from the river mouth (Fig. 1). In-river sampling included particle collection using a Teflon-coated high speed centrifuge (CEPA Z61). Sediment material (0–1 cm) was collected in August 2006 at stations R28 and R30 in the Rhône pro-delta (Fig. 1) using a box multitube corer. Sediments were immediately frozen at –20 °C on board, then freeze-dried and stored in the dark in the laboratory until analysis.

SPM samples from the Mackenzie River were collected in Tsiigehtchic (67°27'7"N, 133°45'32"W) in June 2011 (Fig. 1), using the same protocol as that used for the Rhône plume SPM collection (Galerón et al., 2017). Sediment material was collected at station M434 near to the river mouth (70°10'12"N, 133°35'24"W) as part of the ArcticNet and IPY-CFL system studies on board the CCGS Amundsen in 2008. A surface sample (ca. 0–1 cm) was collected from a box core, freeze dried and stored (< 4 °C) prior to analysis.

Particles from the Amazon were collected in the Solimões River (near the city of Manacapuru, 03°19'29"N, 60°32'58"W) in June 2005 and March 2006, and in the Madeira River (near its mouth where it reaches the Amazon; 03°27'24"N, 58°47'57"W) in June 2005 (Fig. 1). Surface sediments were collected along the tidally influenced reaches of the lower Amazon near the city of Almeirim (1°34'428"S, 52°40'79"W) and in the two main north/south channels at the river mouth near the city of Macapá (00°05'240"S, 50°37'212"W and 00°09'249"S, 51°03'120"W, respectively).

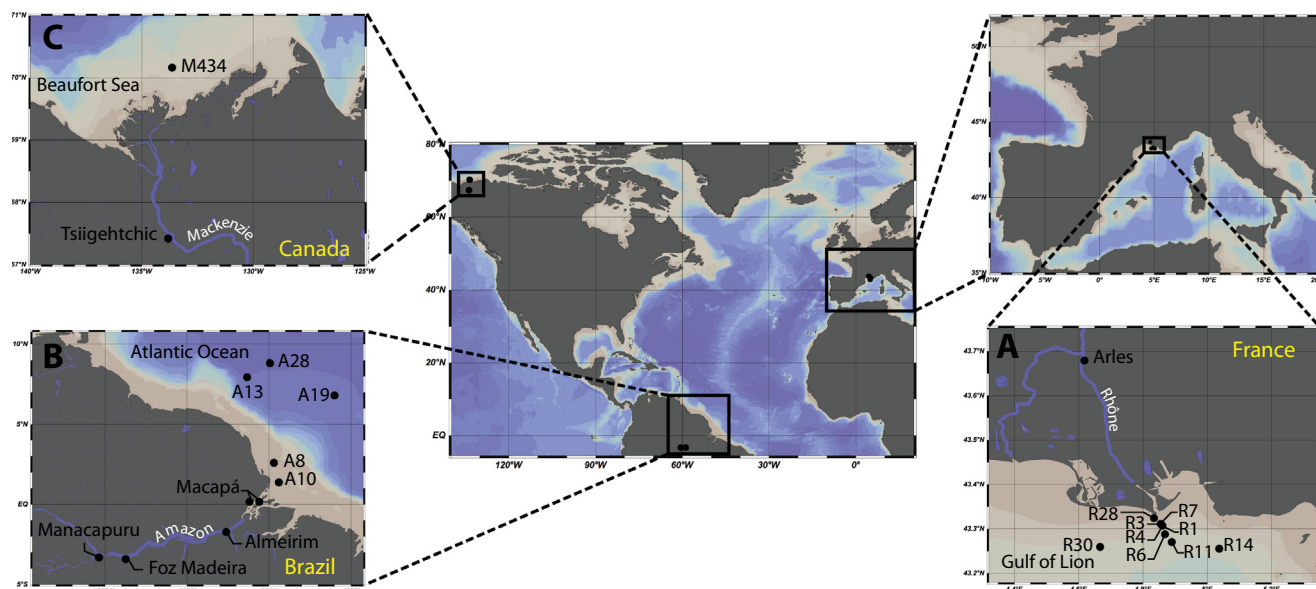


Fig. 1. Summary map showing sampling locations: (A) Rhône River and shelf, (B) Amazon River and shelf and (C) Mackenzie River and shelf.

Sediment samples from the lower Amazon were collected near the river margin using a Van Veen grab. Samples were stored in 50 ml sterilized centrifuge tubes and frozen at -20°C prior to analysis.

In the tropical Atlantic, particle samples were collected in 2011 and 2012 at the surface (ca. 2 m) using a gentle impeller pumping (modified Rule 1800 submersible pump) through 10 m of Tygon tubing to the ship's deck. Immediately after collection, 2–5 l of plume water were filtered through $0.7\ \mu\text{m}$ Whatman GF/F filters (pre-combusted at 450°C for 5 h), carefully wrapped using pre-combusted Al foil, and kept frozen at -20°C until analysis. Sediments were collected using a multi-corer with 9.8 cm (i.d.) core tubes from depths of 4000–4600 m (Table 3). Immediately after recovery, cores were transferred to a temperature-controlled cold van (ca. 2°C), where sediments were sectioned in 1 cm intervals, and the top layer was transferred to pre-combusted Al foil, carefully wrapped and kept frozen at -20°C until analysis.

2.2. Incubation of SPM at different salinity values

In order to determine the degradation kinetics of the Rhône SPM upon reaching seawater, we incubated SPM from the river at different salinity values. Rhône water was collected on November 18, 2014, during a flood (water flow $3700\ \text{m}^3/\text{s}$, SPM $217.1\ \text{mg/l}$, POC $119.01\ \mu\text{M}$) and seawater (salinity 38 g/kg) during the MORTIMER sampling campaign of November 12, 2014 (Galeron et al., 2017). Dilution was carried out with 1 l glass flasks using Rhône water and filtered $0.7\ \mu\text{m}$ seawater in order to reach salinity of 0 (Rhône water only), 15 and 25 g/kg. No extra SPM was added to the dilutions since the collected Rhône water displayed a sufficiently high level of SPM for incubation. Flasks were kept at room temperature in the dark, and mixed and oxygenated every other day. Filtering for analysis occurred after 102 days, for each salinity level. For each incubation flask, $3 \times 250\ \text{ml}$ incubated water (triplicate) were filtered on pre-combusted GF/F filters (Whatman). Filters were immediately frozen at -20°C until analysis.

2.3. Treatment of SPM and sediment samples

After thawing, filters were reduced with NaBH_4 and saponified. NaBH_4 reduction of hydroperoxides to alcohols is essential for estimating the importance of photooxidative and autoxidative

degradation in natural samples (Marchand and Rontani, 2001). Without this treatment, these labile compounds could be thermally cleaved during alkaline hydrolysis or gas chromatography (GC) analysis and thereby be overlooked during conventional organic geochemical studies. All manipulations were carried out using foil-covered vessels in order to exclude photochemical artifacts. It is well known that metal ions can promote autoxidation during hot saponification (Pokorny, 1987). Prior reduction of hydroperoxides with NaBH_4 allowed us to avoid such autoxidation artifacts during the alkaline hydrolysis.

Filters were placed in MeOH (20 ml) and hydroperoxides were reduced to the alcohols with excess NaBH_4 (70 mg; 30 min at 20°C). During this treatment, ketones are also reduced to alcohols and the possibility of some ester cleavage cannot be totally excluded. Saponification was carried out on reduced samples. After NaBH_4 reduction, 20 ml water and 2.8 g KOH were added and the mixture directly saponified by refluxing for 2 h. After cooling, the flask contents were acidified with HCl (pH 1) and extracted ($3\times$) with dichloromethane (DCM). The combined DCM extracts were concentrated to give the total lipid extract (TLE). After solvent evaporation, the residue was taken up in $300\ \mu\text{l}$ pyridine/*N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco; 2:1, v:v) and silylated for 1 h at 50°C to convert OH-containing compounds to trimethylsilyl ether or ester derivatives. After solvent removal under a stream of N_2 , the derivatized residue was taken up in $100\ \mu\text{l}$ BSTFA (to avoid desilylation of FAs) and an amount of solvent (EtOAc) depending on the mass of the TLE.

2.4. GC-EI tandem mass spectrometry (GC-EIMS-MS)

GC-EIMS-MS was performed in multiple reaction monitoring (MRM) mode using an Agilent 7890A/7000A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France). Operating conditions are described by Rontani et al. (2016).

2.5. GC-quadrupole time of flight mass spectrometry (GC-QTOF)

Accurate mass measurements were carried out in full scan mode with an Agilent 7890B/7200 GC-QTOF System (Agilent

Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). Operating conditions are described by Rontani et al. (2017).

2.6. Standards

Quantification of lipids and their oxidation products was carried out using GC-EIMS-MS or GC-QTOF with the following external standards. FAs, sterols, amyryns, betulin and jasmonic acid were from Sigma-Aldrich. NaBH₄ reduction of commercial jasmonic acid (containing a small proportion of *iso*-jasmonic acid) afforded isomeric cucurbitic acids. Oxidation products of monounsaturated FAs, sitosterol, amyryns and betulin were produced according to previously described procedures (Marchand and Rontani, 2001; Rontani et al., 2014a; Galeron et al., 2016a,b). A standard of *threo* 7,10-dihydroxyoctadec-8(*E*)-enoic acid containing 10% of *threo* 7,10-dihydroxyhexadec-8(*E*)-enoic acid produced by *Pseudomonas aeruginosa* PR3 (Suh et al., 2011) was obtained from H.R. Kim (School of Food Science and Biotechnology, Kyungpook National University, Daegu, South Korea).

2.7. Estimation of autoxidative, photooxidative and LOX degradation

Sitosterol and more specific triterpenes (betulin, α - and β -amyryns) were used to estimate the oxidation state of higher plant material. The proportions of photooxidation and autoxidation of sitosterol were estimated from 24-ethylcholest-4-en-3 β ,6 α/β -diol and 24-ethylcholesta-3 β ,5,6 β -triol concentration, respectively,

using equations proposed by Christodoulou et al. (2009) and Rontani et al. (2009). Autoxidation state of α - and β -amyryns was estimated thanks to the proportion of 11-oxo- α -amyryn and 11-oxo- β -amyryn, respectively (Galeron et al., 2016b). In the case of betulin, estimation of autoxidation was carried out with lupan-20-one-3 β ,28-diol (Galeron et al., 2016a). The part played by autoxidation in the degradation of palmitoleic acid was estimated thanks to the proportion of its specific *Z*-oxidation products (Frankel, 1998) and of the water temperature according to the approach described by Marchand and Rontani (2001). After subtraction of the amounts of oxidation products of autoxidative origin, it remained to determine the relative parts played by photooxidative and enzymatic processes in the degradation. Taking into account the production of equal amounts of 9-*E* and 10-*E* oxidation products during the photooxidation of the Δ^9 monounsaturated FA (Frankel, 1998) and their specific allylic rearrangement to 11-*E* and 8-*E* isomers, respectively (Porter et al., 1995), the part played by photooxidative degradation was estimated to be $2 \times (9\text{-}E + 11\text{-}E)$. Concerning 10*S*-DOX degradation, this was obtained from the difference between (10-*E* + 8-*E*) and (9-*E* + 11-*E*) oxidation products, to which was added the amount of *threo* 7,10-dihydroxyhexadec-8(*E*)-enoic acid formed.

2.8. $\delta^{13}\text{C}$ analysis

Compound specific carbon isotope ($\delta^{13}\text{C}$) analysis was performed on the TLE using a HP7890B gas chromatograph coupled

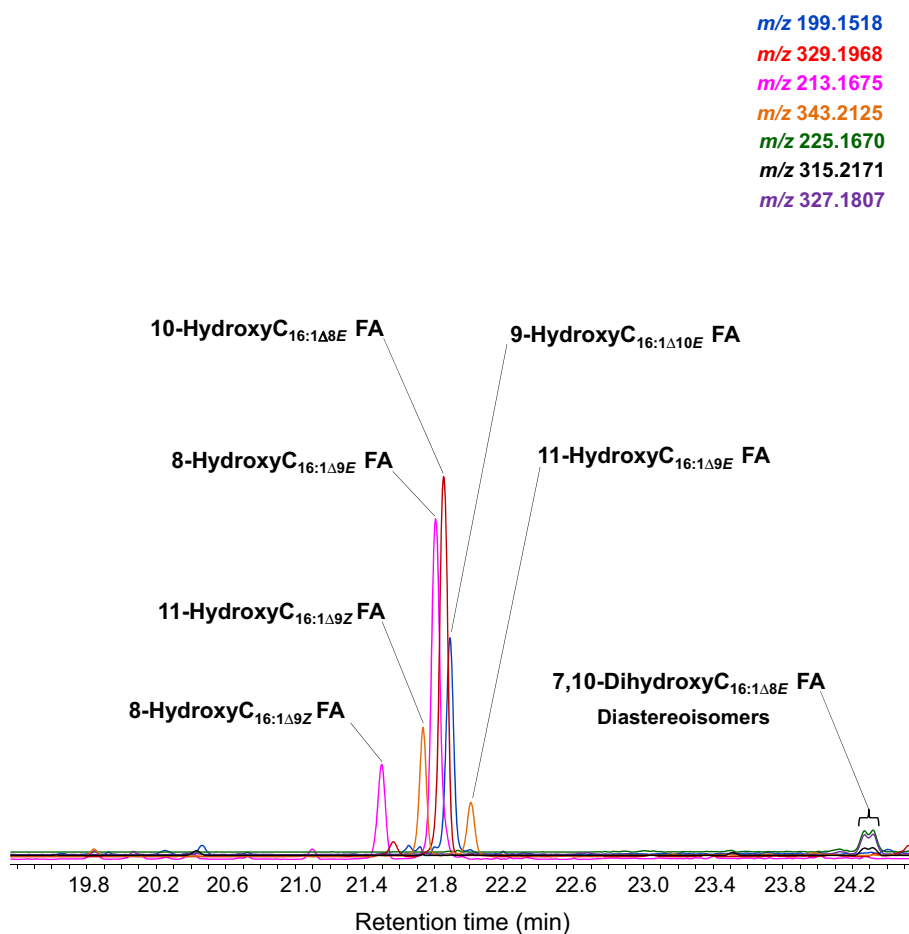


Fig. 2. Partial *m/z* 199.1518, 213.1635, 329.1968, 343.2125, 225.1670, 315.2171, 327.1807 chromatograms showing presence of oxidation products of palmitoleic acid in SPM sample collected in February 2012 at station R7 (43°15'55"N, 4°58'6"E) from the Rhône River plume. (Abbreviated names: e.g. 10-hydroxyC_{16:1Δ8E} FA = 10-hydroxyhexadec-8(*E*)-enoic acid).

Table 1
 $\delta^{13}\text{C}$ signature (‰) of FAs and sterols at stations R1 (43°18'57"N, 5°1'49"E) and R7 (43°15'55"N, 4°58'6"E) in the Rhône River plume.

Compounds	Station R1	Station R7
C _{14:0} FA	-28.6 ± 1.0 ^a	-
Branched C _{15:0} FAs	- ^b	-
C _{16:1Δ9} FA	-30.9 ± 0.4	-27.8 ± 1.1
C _{16:0} FA	-28.0 ± 0.2	-30.4 ± 0.2
C _{18:1Δ9} FA	-29.5 ± 0.7	-28.6 ± 1.1
C _{18:1Δ11} FA	-	-
C _{18:0} FA	-25.6 ± 0.4	-29.2 ± 1.6
Cholesterol	-20.6 ± 0.5	-23.6 ± 0.6
24-Methylenecholesterol	-	-21.6 ± 1.4
Sitosterol	-	-27.1 ± 2.2

^a Analytical error.

^b Not measured (below detection limit).

to an Isoprime Vision stable isotope ratio mass spectrometer via a GC-5 combustion interface at 870 °C. The GC instrument was equipped with a BPX5 column (30 m × 0.25 mm × 0.10 μm film thickness) and a cool on-column injector, with He as carrier gas (1 ml/min). Samples were injected at 60 °C and the oven temperature was ramped to 130 °C at 20 °C/min, then to 300 °C (held 30 min) at 4 °C/min. Samples were analyzed in duplicate and the $\delta^{13}\text{C}$ values were corrected for instrument deviation using the Indiana University B4 mixture and for the BSTFA derivatizing agent (Jones et al., 1991).

3. Results

3.1. Re-examination of SPM samples from Rhône estuary

In order to identify the processes causing the induction of autoxidation in coastal waters, TLEs from different SPM samples previously collected (Galeron et al., 2017) from the Rhône and along a transect in its estuary (Fig. 1) were re-examined. These samples were subjected to NaBH₄ reduction (allowing analysis of labile hydroperoxides in the form of the corresponding alcohols) and subsequent alkaline hydrolysis (allowing hydrolysis of esterified lipids). Unusual profiles of C_{16:1Δ9} (palmitoleic) acid oxidation products dominated by 10-hydroxyhexadec-8(*E*)-enoic and 8-hydroxyhexadec-9(*E*)-enoic acid were observed at stations R1 and R7 close to the river mouth (Fig. 2). Indeed, singlet oxygen-mediated photooxidation of Δ^9 monounsaturated FAs such as palmitoleic acid produces (after NaBH₄ reduction) equal proportions of isomeric 9- and 10-hydroxyacids with an allylic *E* double bond (Frankel, 1980), which can subsequently undergo highly stereoselective radical allylic rearrangement to 11-*E* and 8-*E* hydroxyacids, respectively (Porter et al., 1995). In contrast, autoxidation (free radical induced oxidation) produces a mixture of 9-*E*, 10-*E*, 11-*E*, 11-*Z*, 8-*E* and 8-*Z* hydroxyacids also exhibiting equal proportions of the major 9-*E* and 10-*E* isomers (Frankel, 1998). The strong predominance of the 10-*E* isomer (and its allylic rearrangement product 8-*E*) relative to the 9-*E* isomer (and its allylic rearrangement product 11-*E*) observed at the stations closer to the river mouth (Fig. 2) is thus particularly interesting. It should be noted that diastereoisomers of 7,10-dihydroxyhexadec-8(*E*)-enoic acid could be also detected at these stations (Fig. 2).

To obtain information about the origin of palmitoleic acid and its oxidation products, the stable carbon isotopic ($\delta^{13}\text{C}$) signatures of the main lipids were measured in SPM samples from R1 and R7 (Table 1). This monounsaturated FA is usually a minor component of higher plants (Bridson, 1985), in these samples it was therefore more likely derived from bacteria and/or phytoplankton. Based on its depleted $\delta^{13}\text{C}$ signatures (-30.9‰ and -27.8‰ at R1 and R7, respectively), we can likely assume that the contribution from

marine phytoplankton ($\delta^{13}\text{C}$ values generally -19‰ to -24‰; Tolosa et al., 2013) to this acid was negligible. Moreover, the relatively enriched $\delta^{13}\text{C}$ signature of 24-methylenecholesterol (a well-known diatom marker; Volkman, 2003) at R7 (-21.6‰) (Table 1) attests to the lack of freshwater phytoplankton [which typically exhibits more depleted $\delta^{13}\text{C}$ values (-34‰ to -36‰; Tolosa et al., 2013)] in these samples. Thus, the main source of palmitoleic acid was likely riverine bacteria growing on C₃ terrestrial plant material.

The preferred substrates for LOXs in plants are C_{18:2ω6} (linoleic) and C_{18:3ω3} (α -linolenic) FAs (Siedow, 1991). α -Linolenic acid is converted to 7-*iso*-jasmonic and jasmonic acids by a series of enzymatic reactions in the chloroplast (Kazan and Manners, 2008; Fig. 3). To determine if LOX activity increased in higher plant detritus at stations closer to the Rhône mouth, we quantified, using GC-MS-MS in MRM mode, isomeric cucurbitic acids resulting from the reduction of 7-*iso*-jasmonic and jasmonic acids during the treatment (Fig. 3). The transitions employed were based on specific fragmentations of isomeric cucurbitic acid trimethylsilyl ether derivatives recently elucidated by Rontani et al. (2016). Interestingly, the values of the ratio Σ cucurbitic acids/parent α -linolenic acid appeared to be more than two orders of magnitude higher at station R1 than in the Rhône River (Table 2).

3.2. Incubation of SPM from Rhône River at different salinity values

Past work indicates that LOX activity increases significantly in higher plants cultivated in a NaCl-supplemented medium (Rodríguez-Rosales et al., 1999; Mittova et al., 2002; Zhang et al., 2012). The effect of this higher LOX activity can be either deleterious (increasing formation of FA hydroperoxides and free radicals inducing membrane deterioration) or beneficial (increasing formation of jasmonic acid, which participates in the onset of defense reactions to biotic and abiotic stress) to the cells (Rodríguez-Rosales et al., 1999). To determine if LOXs were activated in higher plant debris carried by rivers during the mixing of riverine and marine waters, SPM from the Rhône was incubated at room temperature under darkness at different salinity values. Concentrations of isomeric cucurbitic acids (NaBH₄-reduced 7-*iso*-jasmonic and jasmonic acids) after incubation for 102 days increased significantly with increasing post incubation salinity (2.8 ± 0.4 ng/g dry wt at S = 0 g/kg, 4.3 ± 0.7 ng/g dry wt at S = 15 g/kg and 14.5 ± 5.0 ng/g dry wt at S = 25 g/kg) (Table 3).

3.3. Examination of SPM from the Amazon

In SPM samples from the Solimões and Madeira rivers, in the central Amazon River basin, we observed relatively low sitosterol photooxidation, averaging 11.8 ± 2.2%, with photooxidation products of phytol, oleic and palmitoleic acids below limits of detection. Autoxidation was, on the other hand, much higher than for the Rhône River (Galeron et al., 2017), with conversion averaging 55.0 ± 1.6% for sitosterol, 73.8 ± 7.0% for α -amyrin and 56.8 ± 15.5% for β -amyrin. A high proportion of sitostanol (ranging from 40 to 78% of the parent sterol for the different stations) was also detected in these samples.

3.4. Comparison of efficiency of autoxidative degradation in vascular plant debris of Arctic, temperate and tropical coastal zones

To compare the efficiency of autoxidation in vascular plant debris discharged by temperate and tropical rivers with that previously measured in Arctic sediments (Rontani et al., 2017), oxidation products of specific tracers of vascular plants (α - and β -amyrins and betulin) were quantified in surface sediment (0–1 cm) and SPM samples from the Amazon River, fan and shelf and

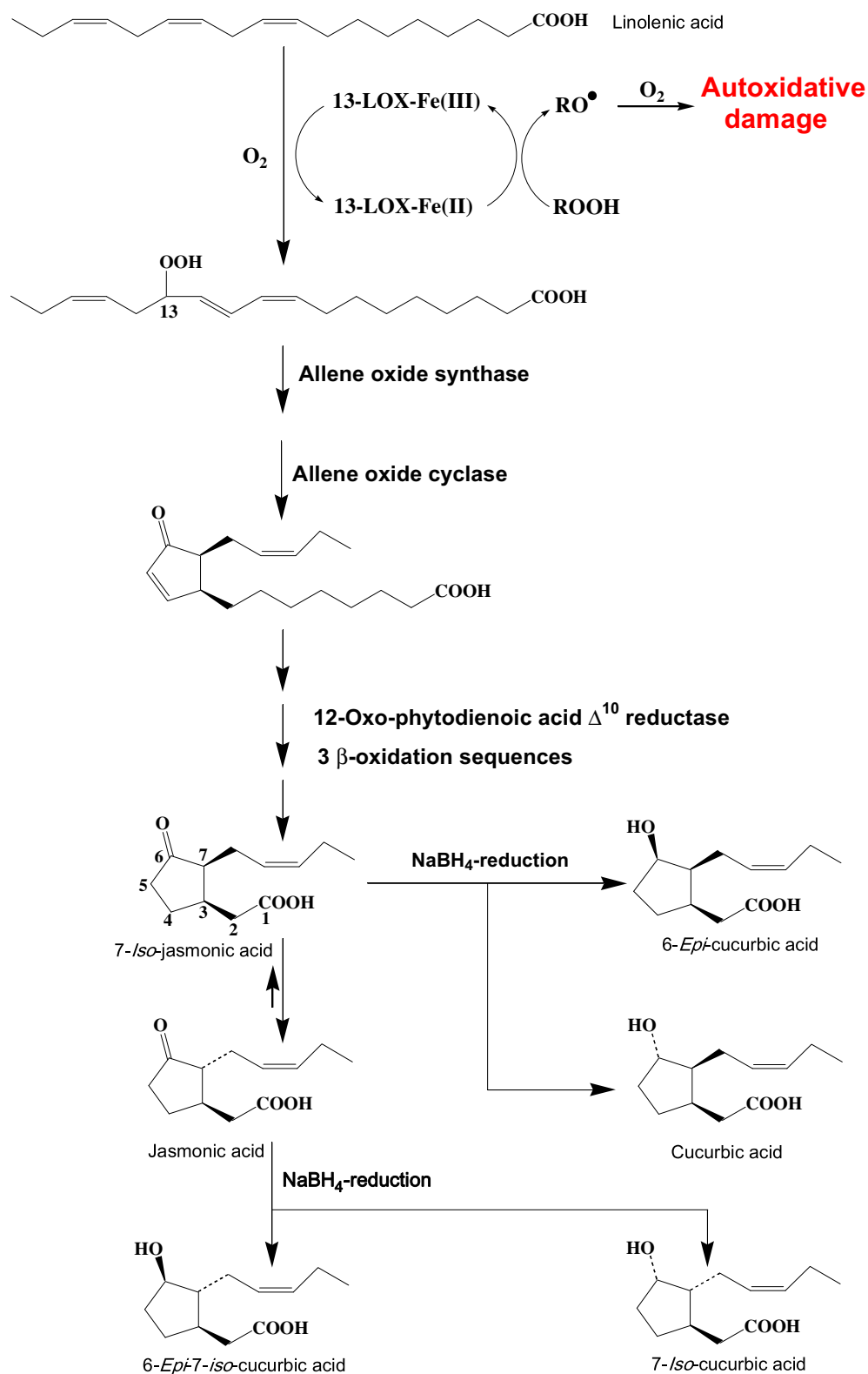


Fig. 3. Degradation of linolenic acid by 13-lipoxygenase.

surface sediment (0–1 cm) from the Rhône Shelf. The extent of autoxidation of vascular plant debris appeared to be considerably higher in the Amazon Shelf than in the Rhône Shelf (Table 4). Interestingly, diastereoisomeric 7,10-dihydroxyhexadec-8(*E*)-enoic acids could be detected in sediments from M434 and in SPM from A8 (Fig. 4).

3.5. Quantification of 11- α -hydroperoxyamyrins in SPM from Rhône, Mackenzie and Amazon rivers

It was previously demonstrated that autoxidation of α - and β -amyrins produces stable 11- α -hydroperoxides (Galeron et al., 2016b). These compounds, which are not affected by NaBH₄ reduc-

Table 2

Proportions of isomeric cucurbitic acids and their parent linolenic acid in SPM samples from Rhône River and shelf and Amazon and Mackenzie rivers.

Sample	Location	Σ cucurbitic acids/parent α -linolenic acid ^a
SPM Rhône Shelf (station R1)	(43°18'57"N, 5°1'49"E)	$3.1 \pm 2.2 \times 10^{-3}$
SPM Rhône River	(43°40'44"N, 4°37'16"E)	$3.8 \pm 1.7 \times 10^{-5}$
SPM Mackenzie River	(67°27'7"N, 133°45'32"W)	0.14 ± 0.13
SPM Amazon River	(03°27'24"N, 58°47'57"W)	0.20 ± 0.17

^a Average of triplicates.

Table 3

Production of isomeric cucurbitic acids (from NaBH₄ reduction of jasmonic acids) after incubation of SPM from Rhône River at different salinity values for 102 days.

Post incubation salinity (g/kg)	7- <i>iso</i> -cucurbitic acid (ng/g dry wt)	6- <i>epi</i> -7- <i>iso</i> -cucurbitic acid (ng/g dry wt) ^a
0	0.12 ± 0.07^a	2.64 ± 0.30
15	0.30 ± 0.10^a	4.00 ± 0.70
25	0.79 ± 0.13^a	13.8 ± 4.9

^a Average of triplicates.

tion, are thermally cleaved to the corresponding ketones during GC injection (Galeron et al., 2016b). Thus, they were quantified in the form of 11-oxoamyrins in SPM samples from the Rhône, Mackenzie and Amazon rivers and their hydroperoxide nature was confirmed in subsamples after reduction with LiAlD₄. The results clearly showed the presence of a higher proportion of 11-hydroperoxyamyrins in SPM from the Mackenzie and Amazon rivers than in SPM from the Rhône River (Table 5).

3.6. Quantification of isomeric cucurbitic acids in SPM from Amazon and Mackenzie rivers

Isomeric cucurbitic acids resulting from NaBH₄ reduction of 7-*iso*-jasmonic and jasmonic acids (products of linolenic acid degradation by lipoxygenases; Kazan and Manners, 2008), were also quantified in SPM from the Amazon and Mackenzie rivers (Fig. 5). The results showed considerably higher values of this ratio in the Amazon and Mackenzie rivers than in the Rhône estuary and shelf (Table 2).

Table 4

Autoxidation of vascular plant lipid tracers (betulin and amyrins) in surface sediment (0–1 cm) and SPM samples from Amazon River, fan and shelf and in surface sediment (0–1 cm) from Rhône shelf (nd, not detected).

Sample	Location	Betulin autoxidation (%)	α -Amyrin autoxidation (%)	β -Amyrin autoxidation (%)
<i>Amazon</i>				
Surface sediment (Almeirim)	(01°34'428"S, 52°40'79"W)	26.9	16.9	23.2
Surface sediment (Macapá south channel)	(00°09'249"S, 51°03'120"W)	nd	20.0	18.0
Surface sediment (Macapá north channel)	(00°05'240"S, 50°37'212"W)	nd	15.9	17.9
SPM (station A10)	(01°20'26"S, 49°22'20"W)	85.6	38.0	29.4
SPM (station A8)	(02°35'30"S, 49°38'55"W)	94.5	72.2	53.5
Surface sediment (station A13)	(07°57'7"S, 51°19'40"W)	94.2	93.5	81.6
Surface sediment (station A19)	(06°48'52"S, 46°02'02"W)	91.0	93.3	81.9
Surface sediment (station A28)	(09°00'5"S, 49°59'59"W)	93.5	90.7	72.8
<i>Rhône</i>				
Surface sediment (station R28)	(43°15'22"S, 04°50'12"W)	39.9	28.1	40.6
Surface sediment (station R30)	(43°15'30"S, 04°40'03"W)	20.8	13.8	10.9

4. Discussion

4.1. Rhône River and Mediterranean shelf water

4.1.1. Origin of induction of autoxidation processes in POM in coastal water

$\delta^{13}\text{C}$ analyses revealed that palmitoleic acid at R1 and R7 likely derived from riverine bacteria that appeared to grow on C₃ terrestrial plant material (Table 1). The similar $\delta^{13}\text{C}$ values of C_{18:1 ω 7} (vaccenic) and branched pentadecanoic acids (FAs specific to bacteria; Sicre et al., 1988) measured by Bourgeois et al. (2011) in sediments from the same deltaic region of the Rhône River provide further evidence for the presence of FAs derived from bacteria utilizing vascular plant sources.

The predominance of 10-*E* and 8-*E* hydroxyacids among the oxidation products of palmitoleic acid was thus attributed to the involvement of a specific bacterial enzymatic process – and more precisely a LOX-like activity. LOXs are non-heme iron dioxygenases responsible for a wide range of functions (e.g. regulation of host defense, stress response, inflammation and development) in eukaryotes (Garreta et al., 2016). They are also found in some prokaryotes, where their possible biological role remains unclear. A 10S-DOX-like lipoxygenase was notably isolated from *Pseudomonas aeruginosa* 42A2 (Guerrero et al., 1997; Busquets et al., 2004). Interestingly, the activity of this enzyme is highest in the case of FAs containing a double bond at Δ^9 . In fact, this 10S-DOX-like lipoxygenase converts palmitoleic acid to 10(S)-hydroperoxyhexadec-8(*E*)-enoic acid, which may then undergo stereoselective allylic rearrangement (Porter et al., 1995) to 8-hydroperoxyhexadec-9(*E*)-enoic acid (Fig. 6). The involvement of such enzymes could thus explain the unusual dominance of isomers 10-*E* and 8-*E* among palmitoleic acid oxidation products at stations closer to the Rhône mouth (Fig. 2). This assumption is supported by the detection of *threo* 7,10-dihydroxyhexadec-8(*E*)-enoic acid in these samples (Fig. 2). Indeed, the 10(S)-hydroperoxyhexadec-8(*E*)-enoic acid produced by *P. aeruginosa* 42A2 is then converted to 7(S),10(S)-dihydroxyhexadec-8(*E*)-enoic acid by way of a diol synthase (Fig. 6; Gardner and Hou, 1999). The presence of *erythro* isomers (Fig. 2) likely resulted from the involvement of hydroperoxide isomerase, well known to produce these enantiomers (Jernerén et al., 2010). The combined bacterial (10S)-DOX and diol synthase activities, which may be expressed extracellularly (Kim et al., 2000), may be associated with the modification of the membrane lipids or could contribute to detoxification of FAs in the bacterial environment, promoting colonization and growth (Martínez et al., 2010).

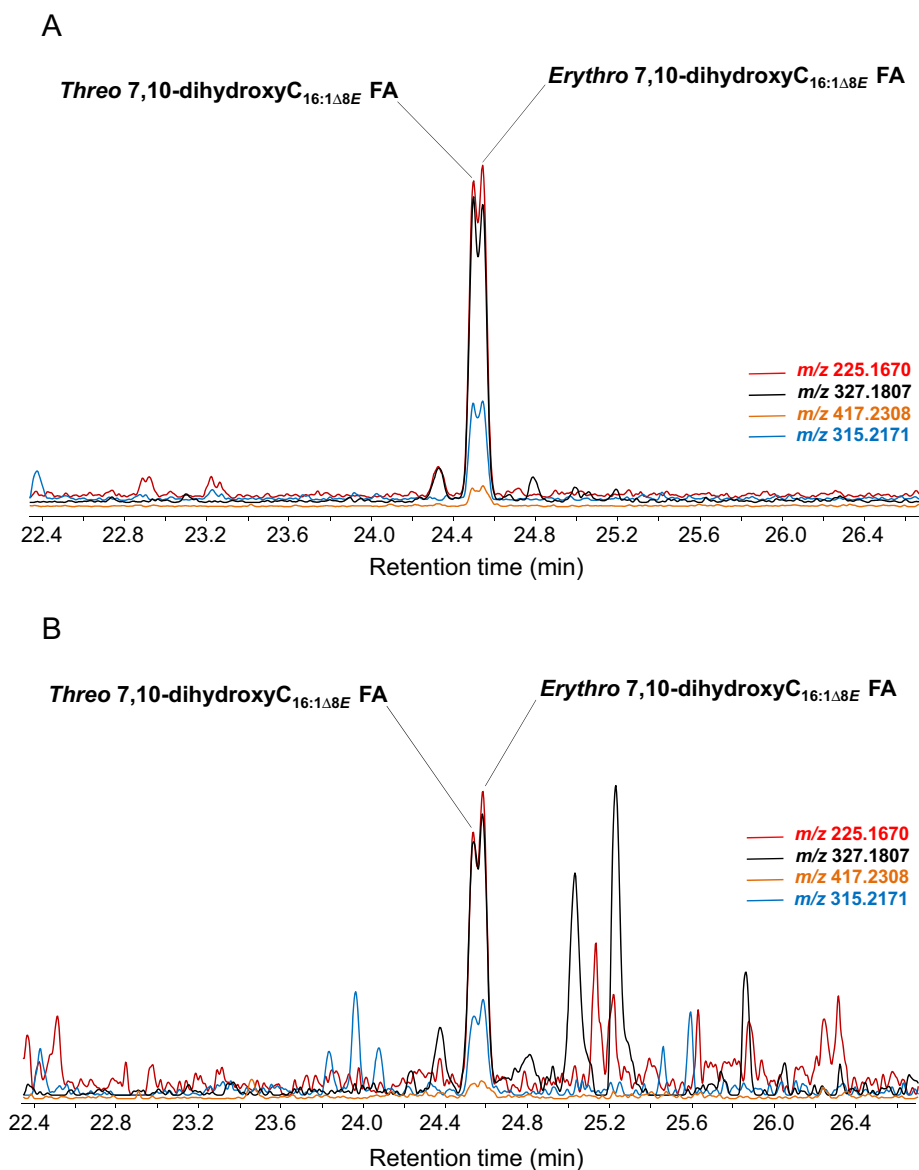


Fig. 4. Partial *m/z* 225.1670, 315.2171, 327.1807, 487.3090 chromatograms showing presence of 7,10-dihydroxyhexadec-8(*E*)-enoic acid in: (A) surface sediment from the Mackenzie River plume (Station M434) (70°10'12"N, 133°35'24"W) and (B) in SPM from station A8 (02°35'30"S, 49°38'55"W) in the Amazon Estuary. (Abbreviated name: 7,10-dihydroxyC_{16:1Δ8E} FA = 7,10-dihydroxyhexadec-8(*E*)-enoic acid).

The relative parts played by autooxidation, photooxidation and LOX activities in the degradation of this acid could be estimated for the Rhône River and along the coastal transect investigated. The results showed a lack of LOX reactions in the Rhône River itself, with a greater contribution of LOX reactions to degradation at stations closer to the river mouth and a dominance of autooxidation at the more distant marine stations (Fig. 7). The increasing proportions of reduction products of 7-*iso*-jasmonic and jasmonic acids (isomeric cucurbitic acids) observed between the Rhône River and the station R1 (Table 2) showed that LOX activity increased at stations closest to the Rhône Mouth, not only in bacteria, but also in higher plant material.

4.1.2. Induction of autooxidation in POM in estuarine waters

The concentrations of isomeric cucurbitic acids (resulting from NaBH₄ reduction of 7-*iso*-jasmonic and jasmonic acids), after incubation for 102 days, increased significantly with increasing post incubation salinity (Table 3), attesting to the key role played by salinity in the induction of LOXs in riverine SPM. Indeed, 7-*iso*-

jasmonic and jasmonic acids are specifically produced during LOX oxidation of α -linolenic acid in higher plants (Kazan and Manners, 2008). Thus, the increase in LOX reactions observed at R1 and R7 (Fig. 7) can be attributed to the strong change in salinity between the Rhône (S 0 g/kg) and these off-shore stations (S 33 g/kg and 38 g/kg at R1 and R7, respectively).

Interestingly, it is generally considered that LOXs play a central role in promoting oxidative injury in plants during environmental stress (Bhattacharjee, 2014). Indeed, because the Fe present in LOXs is usually in the inactive ferrous Fe²⁺ state, an activation step is required for these enzymes to enter the catalytic cycle. This activation involves the reaction of the ferrous enzyme with FA hydroperoxides, producing an active ferric (Fe³⁺) enzyme and an alkoxy radical (Ivanov et al., 2005; Fig. 7). This generation of radicals in the course of the LOX catalytic cycle may also act like a catalyst in autooxidation (Fuchs and Spiteller, 2014). Moreover, when LOX activity becomes very high, increasing amounts of free radicals may damage the active site of LOXs and release Fe²⁺ ions (Sato et al., 1992; Fuchs and Spiteller, 2014), which may very effi-

Table 5

Proportion of hydroperoxyl amyryns (relative to residual parent compound) in SPM from Rhône, Mackenzie and Amazon rivers.

	11 α -Hydroperoxy- α -amyryn	11 α -Hydroperoxy- β -amyryn
Rhône (43°40'44"N, 4°37'16"W; Feb. 2012)	20.7 \pm 6.2 ^{a,b,c}	6.3 \pm 1.8 ^{a,b,c}
Rhône (43°40'44"N, 4°37'16"W; March 2014)	7.5 \pm 0.3 ^{b,c}	6.8 \pm 0.4 ^{b,c}
Rhône (43°40'44"N, 4°37'16"W; Nov. 2014)	20.4 \pm 0.2 ^{b,c}	9.4 \pm 1.3 ^{b,c}
Mackenzie (67°27'7"N, 133°45'32"W; June 2011)	70.9 \pm 21.6 ^{a,c}	25.2 \pm 6.8 ^{a,c}
Amazon (03°19'29"N, 60°32'58"W; June 2005)	75.9 \pm 1.7 ^{a,d}	70.8 \pm 6.8 ^{a,d}
Amazon (03°19'29"N, 60°32'58"W; June 2006)	72.0 \pm 1.2 ^{a,d}	71.4 \pm 0.4 ^{a,d}
Amazon (03°27'24"N, 58°47'57"W; June 2005)	74.8 \pm 1.0 ^{a,d}	67.7 \pm 9.9 ^{a,d}

^a This work.^b Galeron et al. (2017).^c Average of triplicates.^d Average of duplicates.

ciently catalyze the reduction of hydroperoxides to alkoxy radicals (Schaich, 2005) and thereby induce free radical oxidation chains.

To explain the induction of autoxidation of TPOM observed in estuaries, we therefore propose the scenario in Fig. 8. Higher plant debris carried by rivers contains various amounts of hydroperoxides produced by photo- or autoxidation during the senescence of higher plants on land as well as inactive LOXs. When this material is delivered from rivers to coastal waters, the increase in salinity induces LOX activation, allowing additional production of FA hydroperoxides and alkoxy radicals. The increase in the concentration of these free radicals can result to the following processes: (i) autoxidation of the components of higher plant debris and (ii) degradation of LOXs, resulting in the release of Fe²⁺ ions, which strongly enhance alkoxy radical production and thus autoxidation. Accordingly, while it is well known that estuaries are reactive zones for the processing of OM (e.g. Bianchi, 2007), we contend that the role of autoxidation in these systems needs to be further examined as a potentially important process affecting the fate of TPOM.

4.2. Arctic estuarine water

4.2.1. Enhancement of TPOM autoxidation

The important role played by autoxidation in the degradation of TPOM in coastal waters, initially observed in the Mackenzie Estuary (Canadian Arctic) (Rontani et al., 2014a), was subsequently confirmed in the temperate zone of the Rhône Estuary (Galeron et al., 2017). The results demonstrated that the level of autoxidation in Arctic coastal waters was extraordinarily high. For example, autoxidation of 24-ethylcholest-5-en-3 β -ol (sitosterol) can reach

98% in SPM from surface waters of the Beaufort Sea shelf (Rontani et al., 2014a), compared with only 10% of similar material in the Mediterranean Sea (Galeron et al., 2017). Moreover, autoxidation of specific biomarkers of vascular plants (α - and β -amyryns and betulin) reached 99.8, 99.7 and 93.5%, respectively in surface sediments from different regions of the Canadian Arctic (Rontani et al., 2017), while they appeared to be considerably lower in similar material collected from the Rhône Shelf (Table 4). In order to explain the high level of autoxidation in the Arctic, we compared several parameters (efficiency of photooxidation in terrestrial higher plant debris, hydroperoxide concentration and LOX activity in river SPM) in the Mackenzie and Rhône rivers.

4.2.2. Synergy between TPOM photo- and autoxidation

Work has shown exceptional efficiency of type II (i.e. involving singlet oxygen) photosensitized oxidation in Arctic phytoplankton in summer (Rontani et al., 2012). In order to explain this unexpected efficiency, we recently carried out in vitro incubation of the diatom *Chaetoceros neogracilis* RCC2022 at different temperature and irradiance values. Interestingly, the results allowed us to show that type II photosensitized oxidation in senescent phytoplanktonic cells is strongly favored at low temperature and low irradiance (Amiriaux et al., 2016). This apparent paradox has been attributed to: (i) the relative preservation of the sensitizer (chlorophyll) at low irradiance, which permits a longer production time for singlet oxygen, and (ii) the slower diffusion rate of singlet oxygen through the cell membranes at low temperature (Ehrenberg et al., 1998), thereby favoring the intracellular involvement of type II photosensitized reactions. Although never measured, enhanced

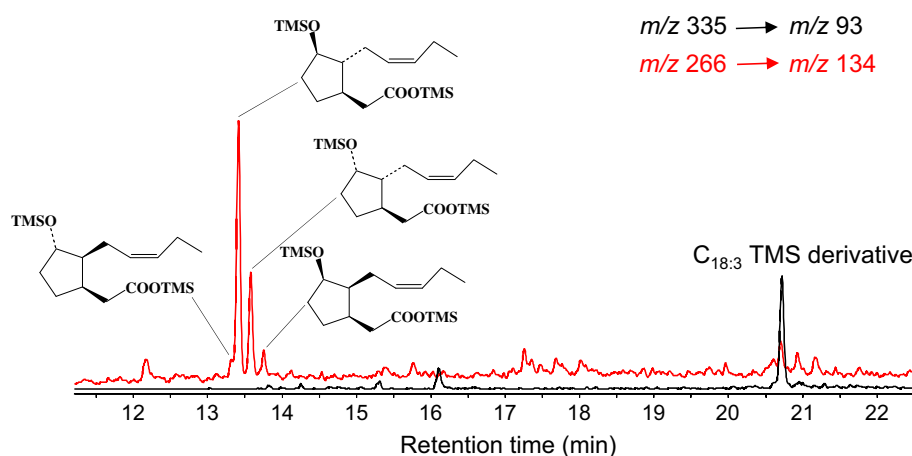


Fig. 5. MRM chromatograms (m/z 266 \rightarrow 134 and m/z 335 \rightarrow 93) showing proportions of isomeric cucurbitic acids and their parent linolenic acid in SPM samples collected from station Tsiigehtchic (67°27'7"N, 133°45'33"W) in the Mackenzie River.

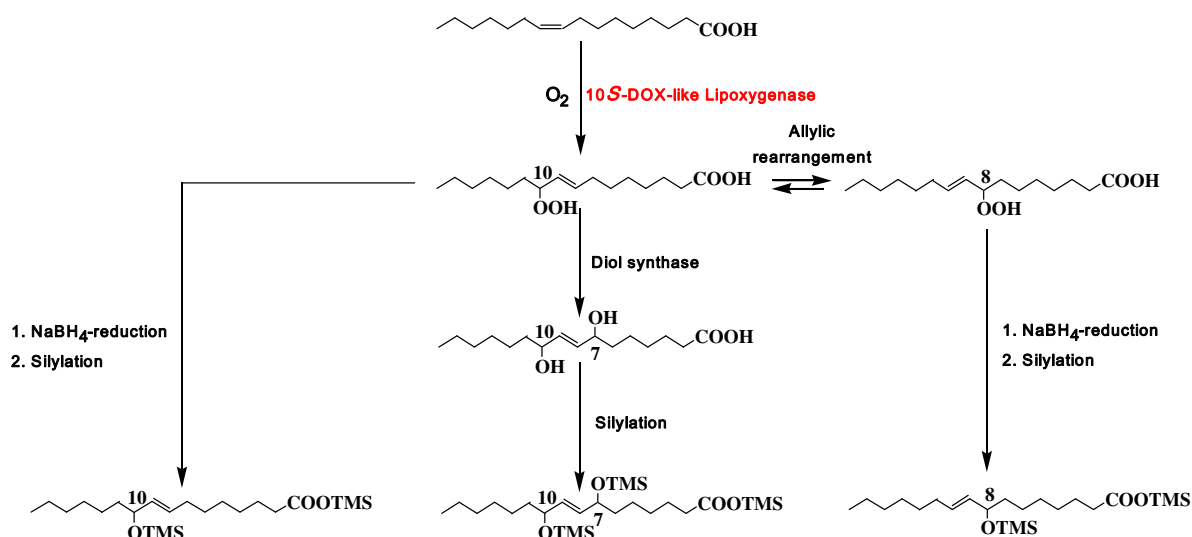


Fig. 6. Main products resulting from oxidation of palmitoleic acid by 10S-DOX-like lipoxygenase.

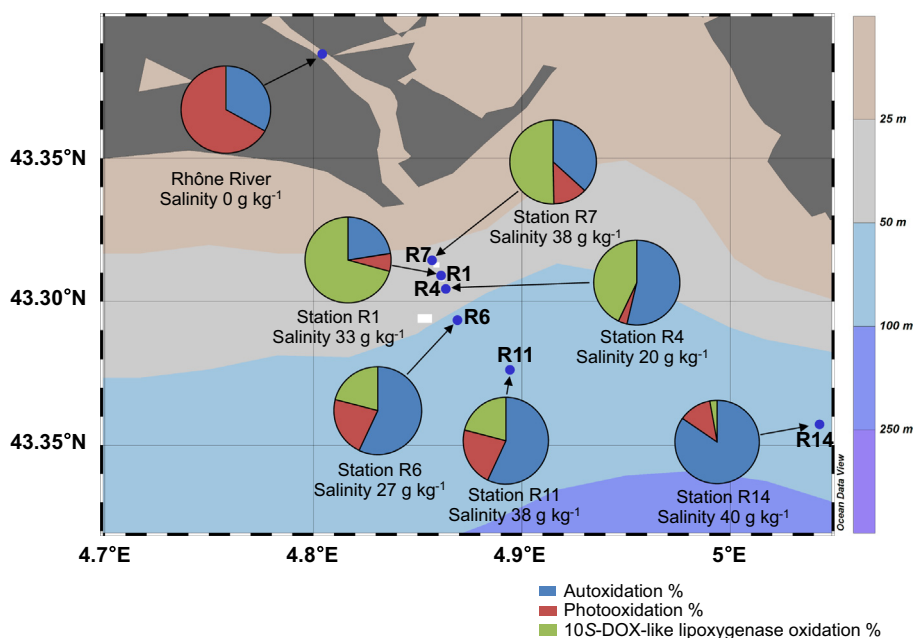


Fig. 7. Estimation of part played by autoxidation, photooxidation and lipoxygenase in the degradation of palmitoleic acid in SPM samples collected in February 2012 along a transect in the Rhône River plume (average of triplicates).

photooxidation of the components of senescent higher plants in the Arctic thus seems likely. This is supported by a comparison between the photooxidation state of sitosterol (arising mainly from terrestrial vascular plants in the Mackenzie and Rhône rivers; Tolosa et al., 2013; Galeron et al., 2015), which showed a greater extent of photooxidation in the Mackenzie River ($48 \pm 2\%$, $n = 3$; Rontani et al., 2014a) compared with the Rhône River ($10 \pm 1\%$, $n = 30$; Galeron et al., 2015). It should be noted that in senescent cells photochemically produced hydroperoxides may undergo one electron reduction, which exacerbates peroxidative damage (Girotti, 1992). These processes result in the formation of alkoxy radicals, which either directly or indirectly (Gardner, 1989) may initiate rounds of free radical peroxidation (autoxidation) by H abstraction. Intense autoxidation may thus be associated in some cases with strong photooxidation in senescent leaves of higher plants (Galeron et al., 2016a).

4.2.3. Hydroperoxide induced LOX activation

Due to the strongest photooxidation of higher plants in the Arctic, we predicted that SPM of the Mackenzie River should contain a higher proportion of hydroperoxides than the Rhône River. In order to confirm this hypothesis, some hydroperoxides resulting from the oxidation of components of higher plants (α - and β -amyriins; Galeron et al., 2016b) in SPM samples from the Rhône and Mackenzie rivers were quantified (Table 5). As predicted, the proportion of hydroperoxides (relative to the residual parent compound) was highest in SPM from the Mackenzie River. These results are particularly interesting due to the high specificity of these compounds (unambiguous tracers of higher plants; Otto et al., 2005; Volkman, 2006). They demonstrate that higher plant debris in the Mackenzie River contain a high proportion of hydroperoxides.

The molar ratio Σ cucurbitic acids (resulting from the reduction of 7-iso-jasmonic and jasmonic acids during the treatment; Fig. 3)/

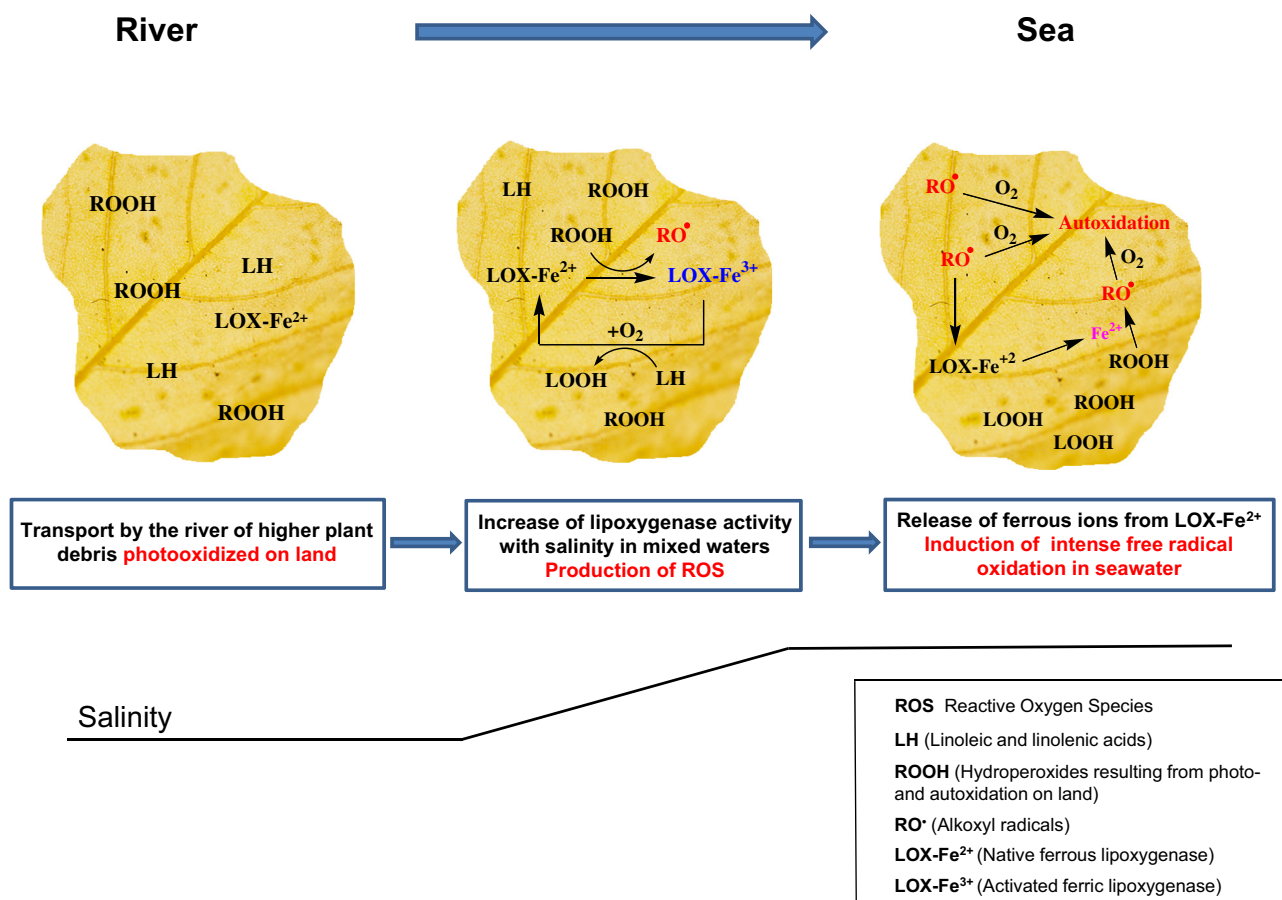


Fig. 8. Proposed pathways for lipoxygenase-induced autoxidative degradation of terrestrial higher plant material discharged by rivers to seawater.

parent α -linolenic acid was more than three orders of magnitude higher in the Mackenzie River (0.14 ± 0.13) than in the Rhône River ($3.8 \pm 1.7 \times 10^{-5}$; Table 2), which confirms greater activity of LOXs in the Mackenzie River particles containing a higher concentration of hydroperoxides (Table 5). This high LOX activation in the Arctic zone is also supported by the detection of *threo* 7,10-dihydroxyhexadec-8(*E*)-enoic acids (arising from 10S-DOX oxidation of palmitoleic acid and subsequent diol synthase activity, Fig. 6) in sediments from station M434 (Fig. 4a).

In Arctic zones (Fig. 9), due to enhanced photooxidation occurring during the senescence of terrestrial higher plants, the detritus from these organisms which is transferred to the rivers, contains a high proportion of hydroperoxides. These compounds strongly contribute to the activation of LOXs (particularly during the increase in salinity in estuarine waters). The resulting high LOX activity strongly enhances alkoxy radical production (Fig. 8) and thereby autoxidation. In contrast, in temperate zones such as the Rhône River (Fig. 9) area, photooxidation in terrestrial higher plant detritus is more limited (Rontani et al., 2014b), probably due to the relatively high temperatures favoring migration of singlet oxygen outside the membranes (Ehrenberg et al., 1998). Consequently, higher plant debris in rivers contains only a moderate proportion of hydroperoxides. Under these conditions, activation of LOXs in estuarine waters is more limited and the induction of autoxidation less significant than in the Arctic. This hypothesis is supported by the strong difference in autoxidative reactivity previously observed during the incubation of SPM from the Rhône and Mackenzie rivers in seawater at room temperature. Indeed, the proportion of sitosterol autoxidation products increased from $2.6 \pm 0.4\%$ to $10.6 \pm 0.7\%$ after incubation of Rhône SPM for 49 days (Galeron et al.,

2016c) and from $16.1 \pm 3.2\%$ to $51.4 \pm 1.6\%$ after incubation of Mackenzie SPM for 15 days (Rontani et al., 2014a).

4.3. Tropical coastal waters

4.3.1. Role of autoxidation and photooxidation

While the effect of both photo- and autoxidation seems less important in the Rhône than in the Mackenzie estuarine regions, it is reasonable to speculate that tropical areas should have even lower photooxidation and autoxidation rates, assuming a decreasing impact trend is linear. Examination of SPM samples from the Solimões and Madeira rivers, located in the central Amazon River basin, confirmed the low efficiency of photooxidative processes in this zone. Photooxidation is generally low in Amazon waters due to the high sediment load and low light penetration, accounting for $\leq 1\%$ of CO₂ emission from the basin (Amon and Benner, 1996; Remington et al., 2011). As sediments begin to settle along the lower Amazon River and nearshore plume, the potential for photooxidation then increases (Medeiros et al., 2015; Seidel et al., 2015). The low photooxidation state of sitosterol in SPM from the river shows that this process is also limited on land in senescent terrestrial higher plants. As discussed above, such results might seem paradoxical in these highly irradiated regions (solar irradiance ranging from 100 to 250 W/m² in the Amazon Basin; Pinker and Laszlo, 1992), but recent findings have shown that both high temperature and high irradiance increase the diffusion rate of singlet oxygen outside cell membranes and quickly consume the photosensitizer (Amiraux et al., 2016). The conjunction of the short lifespan of the photosensitizer, coupled with the high diffusion rate of singlet oxygen, results in the involvement of a weakly damaging

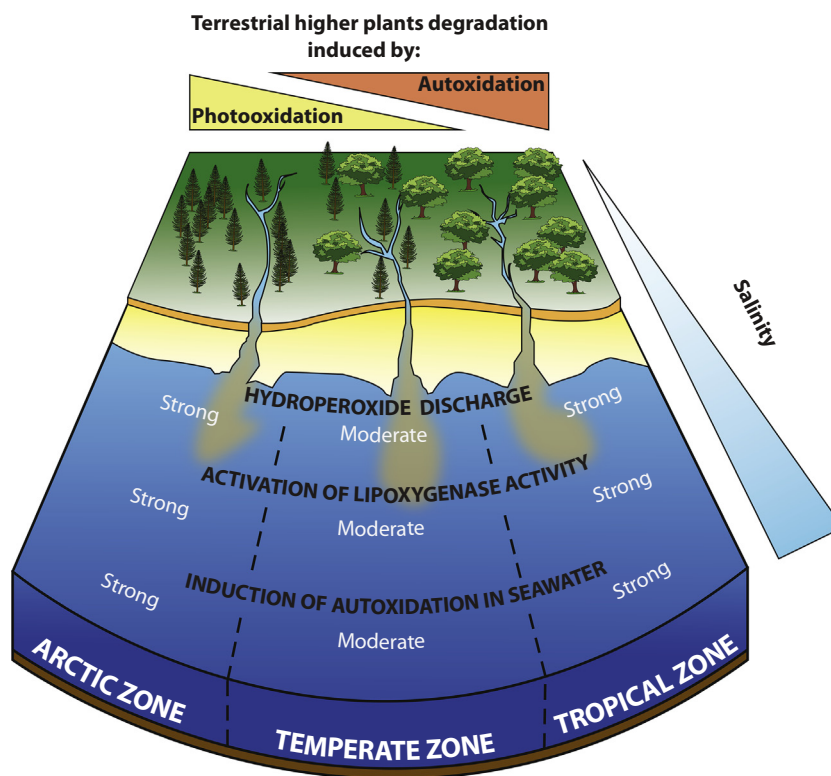


Fig. 9. Degradation of terrestrial higher plant material discharged by rivers in Arctic, temperate and tropical zones.

photooxidative process. In contrast, autoxidation of vascular plant material appears to be strongly enhanced in the Amazon River (Section 3.3). This may be attributed to the high tropical temperature (avg. in the Amazon Basin, 26 °C) well known to favor homolytic cleavage of hydroperoxides (Chaiyasit et al., 2007) and thus initiation of free radical-induced oxidation (Schaich, 2005).

The high proportion (40–70%) of sitosterol (relative to its parent sitosterol) contrasts with values generally considered typical of healthy phytoplanktonic cells (5–10%; Wakeham et al., 1997). Such high values are generally attributed to the involvement of intense bacterial degradation (Wakeham et al., 1997), as previously observed in this river (Amon and Benner, 1996; Ward et al., 2013, 2016). Whereas photooxidation clearly appears to be the driving degradative force in riverine SPM from the Mackenzie River (Rontani et al., 2014a), autoxidation and biodegradation are the major processes at play in the tropical Amazon region.

In order to estimate the involvement of LOX reactions, we also measured the molar ratio Σ cucurbitic acids/parent α -linolenic acid in this material. The values (0.20 ± 0.17) are relatively close (Table 2) to those for the Mackenzie (0.14 ± 0.13), but still more than three orders of magnitude higher than in the Rhône ($3.8 \pm 1.7 \times 10^{-5}$), confirming the highest LOX activation in Amazonian waters. These results allow us to draw a more precise degradation scenario for estuarine areas, whether tropical, temperate, or polar (Fig. 9). In tropical zones, photooxidation does not play a major role in the degradation of riverine SPM, for reasons discussed by Amiraux et al. (2016). However, the higher temperature seems to favor autoxidation, which is clearly the main driver in SPM degradation within the Amazon River, along with biodegradation (Amon and Benner, 1996; Ward et al., 2013). This intense autoxidation produces a large amount of hydroperoxides, favoring in turn important LOX activation in this river, which is able to induce intense autoxidation of TPOM in seawater (Fig. 8).

In order to test this assumption, we examined: (i) different surface sediment samples from the mouth of Amazon and in its north-

west shelf (under the influence of the North Brazil Current; Sun et al., 2016) and (ii) SPM samples from its plume (Fig. 1). The α - and β -Amyrins and betulin (unambiguous tracers of angiosperms; Otto et al., 2005) showed a clear increase in autoxidation state from the Amazon River to the sea (Table 4). It is interesting to note that the high extent of autoxidation observed in the Amazon shelf sediment samples (Table 4) was comparable with that recently observed in Arctic sediments (Rontani et al., 2017). The net increase in autoxidation state (Table 4) in SPM samples from A10 (S 0 g/kg) to 8 (S 29.6 g/kg) confirmed the key role played by the increase in salinity in the induction of autoxidation. The participation of LOXs in this induction is supported by the presence of *threo* 7,10-dihydroxyhexadec-8(*E*)-enoic acids derived from initial attack on palmitoleic acid by 10S-DOX-like lipoxygenase (Guerrero et al., 1997; Busquets et al., 2004) at station A8 (Fig. 4b).

5. Conclusions

Although autoxidation of TPOM has long been overlooked in coastal studies, recent findings demonstrating its importance have rekindled scientific interest in abiotic degradation across salinity gradients. Through the analysis of SPM samples from different rivers and estuaries at high and low latitude, we were able to confirm that autoxidation is favored in estuarine waters, and that its induction seems linked to LOX activation, which increases with salinity – a finding that supports previous lab-based studies confirming an increase in LOX activity in a NaCl-supplemented medium. The LOX catalytic cycle itself, through the generation of radicals, may also induce autoxidative damage. The release of Fe^{2+} , when the LOX activity is high enough that the radicals generated cause damage at the active site of LOX itself, could be another mechanism that induces autoxidation in estuarine waters.

The differences between estuaries, whether they are at high or low latitude, can be explained by the relative importance of each transformation process within the wider OM decay cycle across

steep estuarine gradients. The low temperature and irradiance at high latitude favor photooxidative damage and in turn, hydroperoxide production. This high hydroperoxide content drives LOX activity and autoxidative damage in estuarine waters. At low latitude, photooxidation is less effective, but higher temperature and irradiance favor riverine autoxidation, which, through the production of hydroperoxides, also induces high LOX activation in estuarine waters. In temperate zones, riverine photooxidative and autoxidative damage, and hence hydroperoxide production, are limited, causing the moderate increase observed in autoxidation and LOX activation in mixed waters.

The interactions between biotic and abiotic degradation processes appear to be critical in the overall carbon cycle in coastal systems, but further work is needed. It is interesting to note that autoxidation is not limited to lipids, carbohydrates and amino acids; it can also affect biopolymers (Schmid et al., 2007), lignin (Waggoner et al., 2015) and kerogen (Fookes and Walters, 1990), inducing ring opening and chain cleavage, which may then enhance mineralization of these generally considered recalcitrant substrates via priming (Bianchi, 2011). Thus, the ramifications of autoxidation/photooxidation processes appear to be quite important across a broader spectrum of important compounds found in the coastal zone and thus clearly warrant more attention.

Although the involvement of other radical production mechanisms cannot be totally excluded at this time, LOXs seem to play a key role in the induction of autoxidation in estuaries. Further studies will be needed to better understand the origins and induction mechanisms of these widespread enzymes across the aquatic continuum (Ward et al., 2017), from vascular plants to the streams, rivers, estuaries, and into the marine realm. Investigations of more systems across different latitudes, with different plant communities, light regimes and over multiple seasons are needed to better constrain the important biotic and abiotic drivers of these processes.

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