Autoxidation as a major player in the fate of terrestrial particulate organic matter in seawater

Marie-Aimée Galeron, Olivier Radakovitch, Bruno Charrière, Frederic Vaultier, Jean-Francois Rontani

RESEARCH ARTICLE

Key Points:
- Long overlooked autoxidation is a major terrestrial POM degradation process in estuaries, strongly increasing in mixed waters
- Terrestrial POM autoxidation levels are well correlated with salinity in mixed waters
- There is no evidence of an estuarine metal desorption from terrestrial POM, a factor previously thought to induce this strong autoxidation

Supporting Information:
- Supporting Information S1

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Autoxidation as a major player in the fate of terrestrial particulate organic matter in seawater

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Abstract The Rhône River plays a major role in the Mediterranean Sea, being both its main freshwater source and its major particulate matter provider. This survey of the fate of terrestrial particulate organic matter (POM) was conducted along the salinity gradient of the Rhône River plume, between 2012 and 2014. It revealed that autoxidation acts rapidly and intensely upon the POM's arrival at sea, with α-amyrin and β-amyrin autoxidation rates going from 12.9 ± 2.9% to 45.0 ± 6.4% and 10.7 ± 4.0% to 50.3 ± 4.4%, respectively, between fresh water (salinity 0) and seawater (salinity 38). These compounds, being unambiguous markers of the terrestrial origin of POM, allow us to unequivocally characterize the POM as terrestrial. While it was originally believed that a desorption of redox-active trace metal ions was the favoring factor that kick-started this intense autoxidation, this study evidences no trace metal desorption in the Rhône River mixing zone and hence no correlation between high autoxidation rates and the presence of trace metal ions. Autoxidation rates however were very well correlated with salinity levels within the river plume, with r^2 reaching 0.801, 0.962, and 0.943 for sitosterol, α-amyrin, and β-amyrin, respectively, in November 2014.

1. Introduction

The fate of terrestrial organic matter in the ocean has intrigued scientists for decades. Riverine organic matter of terrestrial origin was long believed to be refractory to marine degradation since it had already been highly degraded during riverine transport [*De Leeuw and Largeau*, 1993; *Wakeham and Canuel*, 2006]. However, it appeared that coastal sediments showed very little trace of terrestrial organic matter [*Hedges and Keil*, 1995]. These findings raised a question that has been at the center of our research efforts: are the reservoir estimates wrong, or is the terrestrial organic matter undergoing an intense oxidation upon its arrival at sea, limiting the amount of particulate organic matter (POM) delivered to coastal sediments?

Previous studies undertaken in the Arctic [*Rontani et al.*, 2012a, 2012b, 2014] have evidenced the existence of an intense degradation of terrestrial particulate organic matter (TPOM). Specific lipidic tracers allowed researchers to pinpoint the degradative process at play in the Mackenzie estuary: if photooxidation appears to be a major driver of TPOM degradation during river transport, autoxidation acts intensely on TPOM once it reaches seawater. Are these results a local anomaly? For a better understanding of carbon fluxes, it is crucial to gain better knowledge of the fate of TPOM in the ocean [*Hedges et al.*, 1997] and to find out if this intense coastal degradation phenomenon is global.

Preliminary studies on the Rhône River [*Galeron et al.*, 2015] highlighted the involvement of autoxidation in terrestrial organic matter during riverine transport but also evidenced the lack of unambiguous tracers allowing us to determine the origin of TPOM as well as to pinpoint the degradative processes it has undergone. With this challenge in mind, we developed a set of terrestrial tracers specific enough to distinguish between different types of higher-plant organic matter sources (angiosperms versus gymnosperms) but also allowing us to identify the type of oxidative damages endured by POM [*Galeron et al.*, 2016b, 2016c; *Rontani et al.*, 2015].

This work follows another preliminary study [*Galeron et al.*, 2016a] undertaken in order to corroborate the original hypothesis formulated by *Rontani et al.* [2014] on the Mackenzie, stating that the intense autoxidation observed in the river plume could be catalyzed by the release of redox-active metal ions in the estuarine
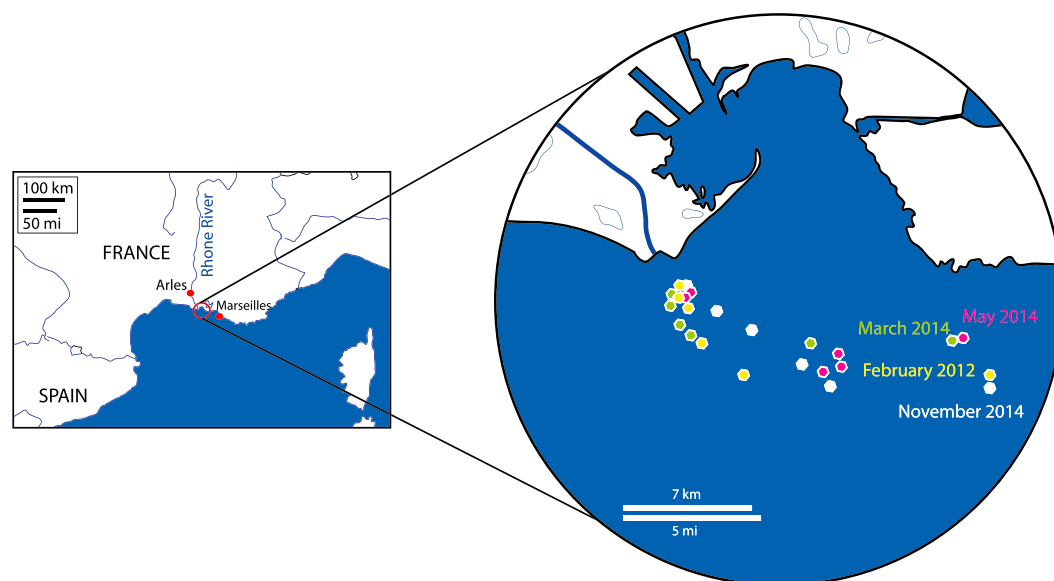
Figure 1. Sampling area: the Arles riverine station as well as the Rhône River plume area showing all campaign stations sampled.

mixing zone. The aforementioned study confirmed the effects of the presence of metal ions on autoxidation rates but also highlighted hydroperoxide content as a major driver of autoxidative processes in organic matter [*Galeron et al.*, 2016a].

2. Methods

2.1. Sampling

The Mediterranean Sea represents only 0.7% of the global ocean surface, but it gathers over 11% of the world's coastal population. Its semi-enclosed nature is often why oceanographers consider it a "miniature" ocean, since physical processes such as thermohaline circulation happen there within a tenth of the global timescale [*Schroeder et al.*, 2016]. The Rhône River is one of the main freshwater sources—and TPOM provider—in the Mediterranean Sea [*Sempéré et al.*, 2000] (Figure 1), making it a river of interest in the area.

Because the Rhône river is prone to seasonal flooding [*Eyrolle et al.*, 2012], sampling campaigns span a wide range of water flow conditions in order to be as representative as possible (Table 1).

In order to quantify particulate organic matter (POM) degradation rates in the estuarine zone outside the Rhône River mouth, we sampled surface waters using a bucket, along a transect following the salinity gradient (Figure 1). Since tides have a very low amplitude in this area (30–40 cm), the river inputs form a hypopycnal turbid plume which can spread over several kilometers on the shelf, with a classical depth of 0.5 to 2 m [*Many et al.*, 2016]. To monitor seasonal changes, campaigns took place in the winter (February 2012 and March 2014), in the spring (May 2014), and in autumn (November 2014; Figure 1). This last sampling (12 November 2014) was done immediately after an annual flood of the Rhône river with discharges of 3000–5000 $m^3 s^{-1}$ on 4 and 5 November, decreasing slowly until 12 November.

Fresh and saltwater layer mixing in the Rhône plume on sampling dates was extremely variable, depending highly on river flow, wind, and weather conditions. Salinity within the first centimeters of the surface layers could rapidly go from 3 to 33, making it difficult to sample sites with similar salinity during each campaign. Table 1 gathers all information on campaigns and stations sampled.

Monitoring of the nutrients and particulate matter input by the Rhône to the Mediterranean Sea has been carried out since 2010 in the framework of the program MOOSE (Mediterranean Ocean Observing System for the Environment; http://data.datacite.org/10.6096/MISTRALS-MOOSE.767). Monitoring is undertaken at the Arles station (30 km upstream of the mouth; Figure 1), and the particulate organic carbon (POC) and suspended particulate matter (SPM) data obtained have been used throughout this work.

Table 1. Summary of Surface Stations, Salinity, and River Conditions on the Dates Sampled[a]

Campaign Date	Rhône River Water Flow ($m^3 s^{-1}$)	SPM ($mg L^{-1}$)	POC (%)	Surface Station Number	Salinity
22 February 2012	980	7.1	9.7	Station 1	33.0
				Station 3	15.0
				Station 4	20.0
				Station 6	26.5
				Station 11	38.0
				Station 14	40.0
18 March 2014	1622	19.0	3.0	Station 1	9.6
				Station 2	3.7
				Station 3	24.0
				Station 4	36.8
				Station 5	20.7
				Station 6	37.0
6 May 2014	1230	7.8	5.0	Station 1	3.9
				Station 2	5.7
				Station 3	4.3
				Station 4	6.4
				Station 5	29.1
				Station 6	37.6
12 November 2014	2833	109.2	1.7	Station 1	8.4
				Station 2	7.1
				Station 3	11.3
				Station 4	17.0
				Station 5	37.5
				Station 6	38.3

[a]During the February 2012 campaign, a large number of stations and depths were sampled. In this work, we will only be looking at the surface stations listed here.

In-river sampling also included filtering between 100 and 400 mL of water on GF/F glass fiber filters (precombusted at 450°C for 4 h to remove all traces of organic matter), as well as particle collection using a Teflon-coated high-speed centrifuge device (CEPA Z61). Samples were kept at −20°C until analysis. For suspended matter determination, water samples were filtered on preweighed GF/F filters. After drying, filters were weighed to determine SPM contents (in $mg L^{-1}$). Nonweighted filters were used to quantify POC contents: the filters were acidified with 50–100 μL 0.5 N sulfuric acid and dried overnight at 60°C [*Raimbault et al.*, 1999]. POC contents were determined using the high combustion (900°C) procedure on a CN Integra mass spectrometer (Sercon). Uncertainty of SPM is estimated to be 0.05 $mg L^{-1}$, while the analytical error for POC determination is 5 μM (both errors were calculated during method validation, using replicates, and taking into account scale and spectrometer precision and detection limits).

2.2. Trace Metals Sampling

Surface seawater (0–50 cm) was collected 3 m out from the side of the boat using a pole, a prewashed (MilliQ + HNO_3) Teflon tube, and a peristaltic pump. The water was filtered immediately through prewashed 0.2 μm syringe filters and acidified with ultrapure HNO_3 and kept in an acid-cleaned LDPE 60 mL bottle. River samples were collected in Arles with a similar protocol.

2.3. Chemical Treatment of the Samples

After thawing, filters collected were reduced with $NaBH_4$ and saponified. The $NaBH_4$ reduction of hydroperoxides to alcohols—in order to make them more amenable to gas chromatography-quadrupole time-of-flight (GC-QTOF)—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 GC analysis and thus be overlooked during conventional organic geochemical studies. Lipids and their degradation products in the resulting total lipid extracts (TLEs) were then quantified by GC-QTOF. 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]. The prior reduction of hydroperoxides with $NaBH_4$ allowed us to avoid such autoxidation artifacts during the alkaline hydrolysis.

Filters were placed in methanol (MeOH; 20 mL), and hydroperoxides were reduced to the corresponding alcohols with excess NaBH$_4$ (70 mg; 30 min at 20°C). During this treatment, ketones are also reduced, 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 content of the flask was acidified with HCl (pH 1) and extracted (3 × 15 mL) with dichloromethane (DCM). The combined DCM extracts were concentrated to give the TLE. After solvent evaporation, residues were taken up in 300 μL of pyridine/N,O-bis(trimethysilyl)trifluoroacetamide (BSTFA; Supelco; 2:1, vol/vol) and silylated for 1 h at 50°C to convert OH-containing compounds to TMSi-ether or ester derivatives. After evaporation to dryness under a stream of N$_2$, the derivatized residues were taken up in 100 μL BSTFA (to avoid desilylation of fatty acids) and an appropriate amount of hexane, depending on the concentration in lipids in each sample, in order to get the best possible GC-QTOF reading. It should be noted that under these conditions, steran-3β,5α,6β-triols were silylated only at C3 and C6 and thus need to be analyzed with great care [*Rontani et al.*, 2014].

2.4. GC-QTOF Analyses

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). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert; 30 m × 0.25 mm, 0.25 μm film thickness) capillary column was employed. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C and the oven temperature programmed from 70°C to 130°C at 20°C min^{-1} and then to 300°C at 5°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 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. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12,252 from m/z 68.9955 to 501.9706. Perfluorotributylamine was utilized for daily MS calibration.

2.5. Tracers Used

Previous studies have evidenced that sitosterol (24-ethylcholest-5-en-3β-ol) alone cannot be unambiguously attributed to POM of terrestrial origin [*Galeron et al.*, 2015]. These results highlighted the need for a set of tracers specific enough to pinpoint the origin of organic matter and with identified oxidation products specific to degradation processes we are looking at here. For this purpose, we previously studied and identified a number of tracers which, coupled with their degradation products, formed a specific set allowing us to unambiguously attribute a higher-plant terrestrial origin to the suspended particulate matter we are studying [*Galeron et al.*, 2016b, 2016c; *Rontani et al.*, 2015].

These tracers include betulin (lup-20(29)-en-3β,28-diol) and its oxidation products [*Galeron et al.*, 2016c], α-amyrin and β-amyrin (urs-12-en-3β-ol and olean-12-en-3β-ol), with their oxidation products [*Galeron et al.*, 2016b], and dehydroabietic acid (8,11,13-abietatrien-18-oic acid) (DHAA) and its oxidation products [*Rontani et al.*, 2015] (Figure 2).

Betulin is abundant in birch wood [*Schnell et al.*, 2014] and has been used as a tracer of peat-derived organic matter in coastal sediments [*Volkman et al.*, 2000], or even as a tracer of mangrove-derived organic matter in sediments in tropical areas [*Koch et al.*, 2003, 2005]. While a number of oxidation products have been identified, we will only be looking at lupan-20-one-3β,28-diol here, since it is specific to autoxidation [*Galeron et al.*, 2016c].

α-Amyrin and β-amyrin are commonly found in higher-plant material such as leaves, bark, roots, or wood [*Vázquez et al.*, 2012] and are useful markers of organic matter of a terrestrial angiosperm origin. Of all the oxidation products identified, we selected 3β-hydroxy-urs-12-en-11-one (11-oxo-α-amyrin) and 3β-hydroxy-olean-12-en-11-one (11-oxo-β-amyrin) as specific tracers of autoxidative degradation [*Galeron et al.*, 2016b].

Dehydroabietic acid is a gymnosperm biomarker found in sediments [*Brassell et al.*, 1983] and aerosols often used as tracer of biomass burning [*Oros and Simoneit*, 2001; *Simoneit*, 2002]. Although only a minor component in the fresh resin of conifers, its abundance increases on aging at the expense of the corresponding abietadienic acids [*Schao et al.*, 1995]. Further, if oxygen is available, DHAA can be oxidized abiotically to
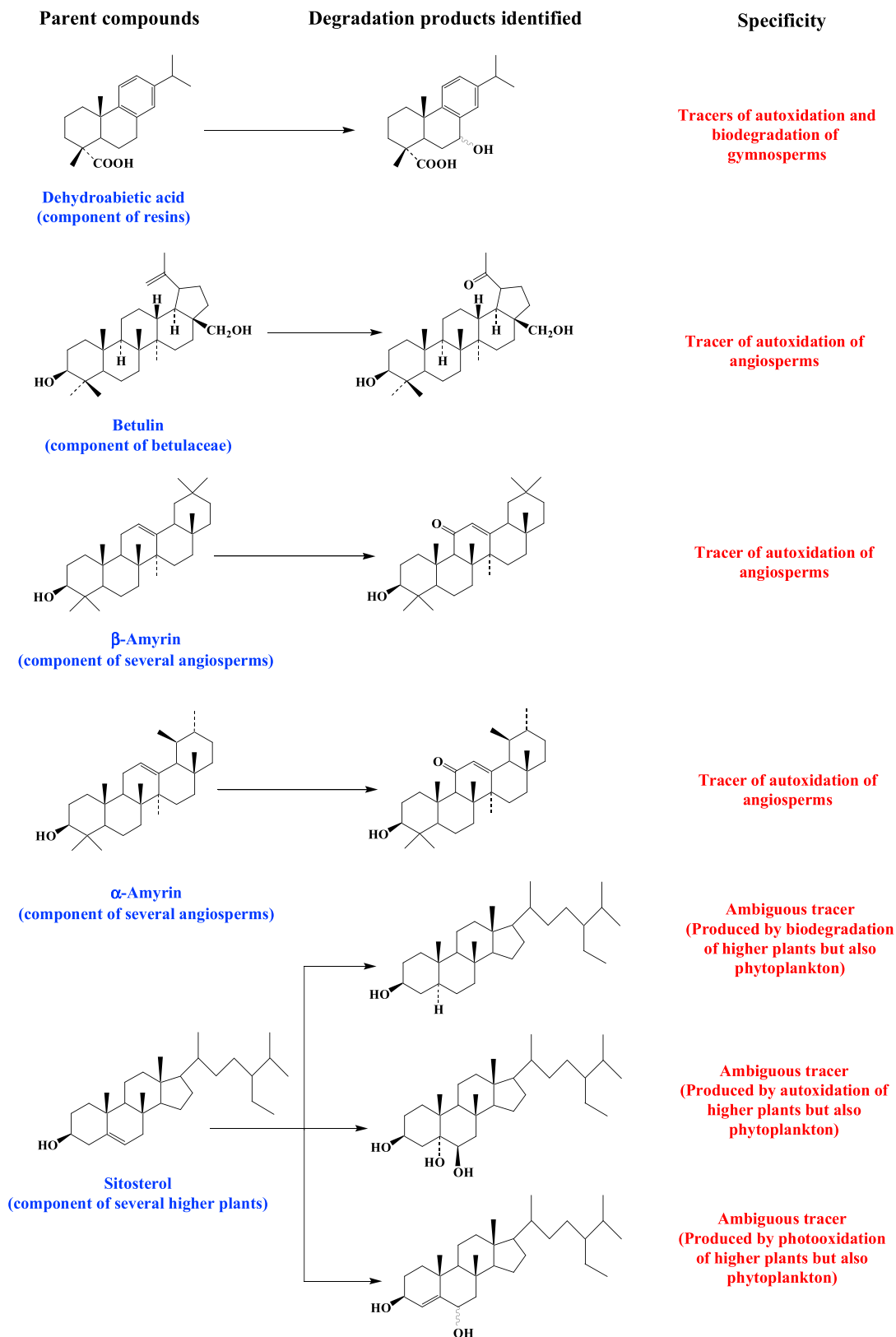
Figure 2. Lipid tracers used throughout this work, along with their oxidation products.

7-oxo-dehydroabietic acid, 7α/β-hydroxy-dehydroabietic acids, and a number of other products not quantified here.

Even though it is nonspecific of higher plants (also present in phytoplankton [*Volkman*, 1986, 2003]), we still compared autoxidation rates of the aforementioned compounds with those of sitosterol, in order to get an exhaustive picture of the degradation state of POM. Free radical autoxidation of this sterol yields mainly Δ^5-3β,7α/β-hydroperoxides and in smaller quantities 24-ethyl-5,6-epoxycholestan-3β-ols and 24-ethylcholestan-3β,5α,6β-triol. 24-Ethylcholestan-3β,5α,6β-triol was previously proposed as a tracer of autoxidation processes [*Christodoulou et al.*, 2009; *Rontani et al.*, 2009]. $^1O_2$-mediated photooxidation (type II photoprocesses) of sitosterol yields mainly Δ^6-5α-hydroperoxides and to a lower extent Δ^4-6α/β-hydroperoxides, but due to the instability of the former, the latter were selected as tracers of photooxidation processes [*Christodoulou et al.*, 2009; *Rontani et al.*, 2009]. Finally, 24-ethyl-5α-cholestan-3β-ol derived from aerobic bacterial hydrogenation of sitosterol was selected as tracer biodegradation. The choice of sitosterol oxidation products as tracers for degradation processes was extensively described in our previous work [*Galeron et al.*, 2015].

Oxidation rates were calculated relatively to the amount of the parent compound remaining, using equation (1):

$$\text{Oxidation product}/(\text{parent compound} + \text{oxidation product})^* \, 100 \qquad (1)$$

For sitosterol, degradation rates were calculated using previously described equations [*Galeron et al.*, 2015]. Sitosterol autoxidation percentages were estimated using equation (2) based on autoxidation rate constants calculated by *Morrissey and Kiely* [2006].

$$\text{Sitosterol autoxidation\%} = ([\text{24-ethylcholestan-3}\beta, 5\alpha, 6\beta\text{-triol}] \times 2.4)/$$
$$([\text{sitosterol}]. + ([\text{24-ethylcholestan-3}\beta, 5\alpha, 6\beta\text{-triol}] \times 2.4)) \times 100 \qquad (2)$$

The percentage of sitosterol photooxidation was estimated using equation (3) [*Christodoulou et al.*, 2009], based on the ratio Δ^4-3β,6α/β-hydroperoxides/Δ^6-5α-hydroperoxides found in biological membranes (0.30) [*Korytowski et al.*, 1992].

$$\text{Sitosterol photooxidation\%} = ([\text{24-ethylcholest-4-en-3}\beta, 6\alpha/\beta\text{-diol}] \times (1 + 0.3)/0.3)$$
$$/([\text{sitosterol}] + ([\text{24-ethylcholest-4-en-3}\beta, 6\alpha/\beta\text{-diol}] \times (1 + 0.3)/0.3)) \times 100 \qquad (3)$$

Sitosterol biodegradation rates were calculated using equation (1).

2.6. Trace Metal Analyses

Samples were analyzed for Fe, Mn, and Cu using a 7700 series ICP-MS (Agilent) equipped with an HMI kit. We focused on these elements because they are suspected to play a role in the induction of autoxidation processes due to their high concentrations and redox activity. ICP-MS accuracy was checked using a coastal seawater reference CASS-5 and estuarine water reference SLEW-3 (NRCC). Measured concentrations were in line with recommended values ±10% for Fe and Cu and ±15% for Mn.

3. Results

3.1. SPM Sources and Degradation

Selected tracers have been identified and quantified in all Rhône River plume samples. Their distribution, however, varied greatly along the salinity gradient within the plume (Figure 3). It is worth noting that river conditions were normal on the first three campaigns while the 12 November cruise occurred immediately after a major flood (Table 1). The extra amount of suspended organic matter transported made it easier for us to detect and quantify compounds that were not otherwise detected during previous campaigns.

The organic matter sampled within the plume, regardless of date, station, or river conditions, showed amounts of phytol and sitosterol overshadowing the other compounds quantified (Figure 3), both nonspecific markers of higher plant-derived material and major constituents of our samples. Amyrins (undifferentiated in Figure 3) and DHAA concentrations were much more variable, with amyrins more important in samples from November 2014 than in samples from the other campaigns, probably due to the extra terrestrial material carried by rain and flood waters on that date.
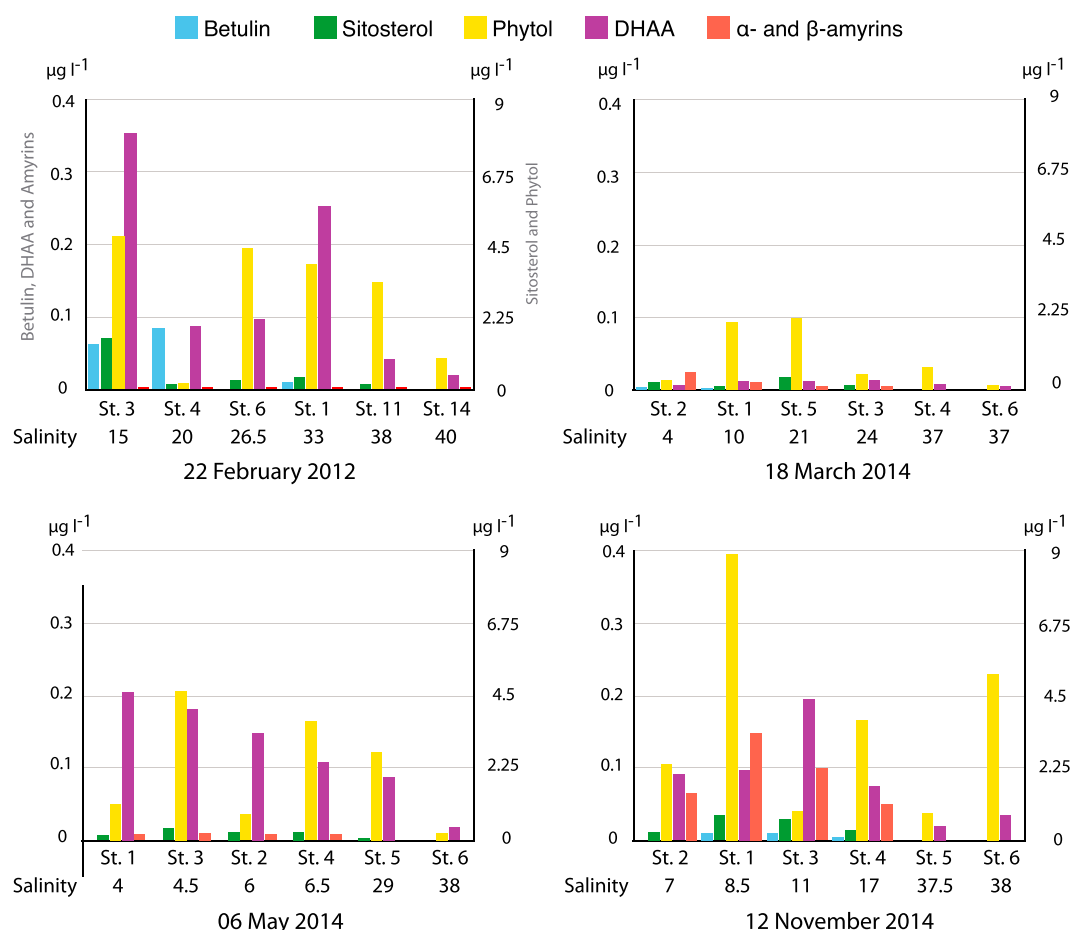
Figure 3. Quantification (in $\mu g\,L^{-1}$) of lipid tracers within the Rhône River plume, along the salinity gradient (on all graphs, betulin, dehydroabietic acid (DHAA), and amyrins are plotted against y axis 1, while phytol and sitosterol are plotted against y axis 2).

In the Rhône River, there is an evident variability in OM degradation rates across the year (Table 2, Figure 4, and Data Set S1). For comparison purposes, we will be comparing the plume oxidation rates with this of riverine (averaged values) for each compound across all four dates sampled. On average, the Rhône River displays variable autoxidation rates, as low as $3.9 \pm 1.6\%$ for sitosterol [*Galeron et al.*, 2015] and as high as $27.2 \pm 9.0\%$ for DHAA. Betulin and α-amyrin and β-amyrin were scarcely present in river samples, apart from the February 2012 and November 2014 samples (Tables 2 and supporting information S1). α-Amyrin and β-amyrin average autoxidation rates reached $13.0 \pm 2.9\%$ and $10.7 \pm 4.0\%$, respectively. These degradation rates contrasted starkly with the rates observed within the plume (Table 2, Figure 4, and Data Set S1). Photooxidation remained limited on the dates sampled, averaging $4.3 \pm 1.6\%$ for sitosterol. The autoxidation rates observed in the samples studied here appear lower than those presented in *Galeron et al.* [2016b, 2016c]. In these previous studies, autoxidation rates for different compounds were compared in order to validate their use as a tracer, but it appears autoxidation rates were overestimated due to our use of an external standard having suffered autoxidation. Even with this overestimation, the overall trend as well as our previous conclusions remains unchanged. However, corrected values are much lower and in line with the results presented here.

In February 2012, autoxidation rates of betulin, α-amyrin, and β-amyrin peaked upon reaching seawater, with $76.2 \pm 1.4\%$, $81.5 \pm 0.9\%$, and $74.0 \pm 1.0\%$, respectively (Table 2, Figure 4, and Data Set S1).

In March 2014, within the plume, α-amyrin and β-amyrin autoxidation rates first peaked at $51.0 \pm 1.5\%$ and $39.0 \pm 1.9\%$, respectively, and were attained at the sampling station #5, where salinity reached 20.7 (Tables 2 and S1). The same was observed in May 2014, where α-amyrin autoxidation rates did not peak

Table 2. Summary Table of Autoxidation Rates (%) for All the Compounds Studied, for All Stations Across All Four Sampling Dates

	Station	Salinity	Betulin	Sitosterol	β-Amyrin	α-Amyrin	DHAA
22 February 2012	Rhône	0	—	0.45	4.50	14.21	18.12
	3	15.0	76.25 ± 1.39	10.87 ± 1.16	74.00 ± 0.97	81.55 ± 0.89	29.25 ± 2.66
	4	20.0	10.53 ± 2.00	11.91 ± 3.40	67.62 ± 21.31	72.31 ± 22.73	31.41 ± 4.09
	6	26.5	13.79	13.31 ± 0.51	13.89 ± 1.22	32.19 ± 0.60	30.61 ± 6.87
	1	33.0	55.92 ± 13.84	13.30 ± 7.89	47.49 ± 21.11	71.06 ± 21.97	33.97 ± 9.78
	11	38.0	23.31 ± 1.77	15.39 ± 0.79	21.08 ± 3.40	30.91 ± 2.88	40.45 ± 0.49
	14	40.0	25.23	22.30 ± 11.23	57.72 ± 21.16	60.79 ± 19.68	29.65 ± 6.77
18 March 2014	Rhône	0	—	4.92 ± 4.19	6.78 ± 0.38	7.53 ± 0.34	1.15 ± 0.03
	2	3.7	—	10.67 ± 0.62	8.45 ± 1.21	20.96 ± 0.61	76.68 ± 5.02
	1	9.6	21.92	12.75 ± 1.13	20.02 ± 5.54	29.55 ± 2.13	51.49 ± 8.16
	5	20.7	—	8.24 ± 0.71	38.97 ± 1.86	50.98 ± 1.46	57.64 ± 3.79
	3	24.0	—	8.62 ± 0.43	22.05 ± 2.74	25.17 ± 1.81	28.05 ± 2.20
	4	36.8	—	19.01 ± 1.16	45.25 ± 2.28	37.09 ± 2.15	58.08 ± 2.38
	6	37.0	—	13.44 ± 4.20	56.57 ± 8.10	47.70 ± 6.58	42.92 ± 6.53
6 May 2014	Rhône	0	41.20	2.37 ± 1.68	22.21 ± 10.73	9.59 ± 0.93	2.49 ± 0.86
	1	3.9	—	8.00 ± 3.34	16.88 ± 1.07	22.59 ± 3.29	11.41 ± 8.98
	3	4.3	—	7.51 ± 1.06	14.08 ± 2.25	24.45 ± 1.50	25.42 ± 1.36
	2	5.7	—	4.21 ± 0.61	16.82 ± 3.58	18.98 ± 2.02	18.82 ± 7.21
	4	6.4	—	9.01 ± 2.03	13.38 ± 1.11	27.58 ± 1.75	27.13 ± 3.55
	5	29.1	—	3.14 ± 1.94	25.35 ± 5.13	35.37 ± 3.10	27.19 ± 6.17
	6	37.6	—	13.76 ± 8.77	38.82 ± 2.62	30.31 ± 1.56	45.90 ± 6.01
12 November 2014	Rhône	0	7.31	7.75 ± 0.01	9.42 ± 1.31	20.43 ± 0.14	36.21 ± 12.96
	2	7.1	—	3.99 ± 2.23	3.71 ± 0.43	11.05 ± 1.77	31.34 ± 8.14
	1	8.4	—	2.40 ± 1.72	8.29 ± 0.68	14.43 ± 0.86	39.30 ± 15.01
	3	11.3	—	7.36 ± 1.18	9.07 ± 1.76	16.98 ± 1.57	20.24 ± 11.79
	4	17.0	—	10.46 ± 0.53	7.33 ± 1.98	16.74 ± 1.57	64.80 ± 5.61
	5	37.5	—	11.81 ± 3.74	40.98 ± 11.16	35.65 ± 8.93	50.75 ± 5.85
	6	38.3	—	14.26 ± 2.09	48.15 ± 1.95	41.23 ± 0.53	49.13 ± 5.94

until the very edge of the plume, with an autoxidation rate of 35.4 ± 3.1% at the surface station where salinity reached 29.1, while none of the stations within a few hundred meters displayed a salinity over 6 (Tables 2 and S1)—calmer conditions brought the freshwater plume farther out on the shelf. In November 2014, α-amyrin and β-amyrin autoxidation rates peaked only when the salinity reached 37.5. It is worth noting that for both amyrins, autoxidation rates reached their highest levels at the marine station, displaying a salinity of 38 or more, located outside the river plume (Figure 4).

3.2. Metal Desorption

Fe, Cu, and Mn all show a rather conservative behavior (Figure 5) in the Rhône plume, with no significant desorption observed. Their concentrations follow a mixing line between river and seawater end-members, except for Fe in November. The November flood induced higher concentrations of dissolved Fe, Cu, and Mn compared to the other campaigns due to higher river concentrations at this time. The trends in the vicinity of the mouth were also more complex during that campaign, with an increase of dissolved Cu between 0 and 5 and a clear decrease of Fe that may be due to an important aggregation or flocculation phenomenon [*L'her Roux et al.*, 1998]. Such a conservative behavior has already been shown in the Rhône mixing zone for Cu and other trace metals by *Elbaz-Poulichet et al.* [1996].

4. Discussion

As previously explained, the Rhône River has been selected for this study for its major influence on the northwestern Mediterranean Sea (main SPM contributor) [*Le Fouest et al.*, 2015]. Building on previous results obtained in the Arctic on the Mackenzie River, where it was evidenced that riverine organic matter of terrestrial origin undergoes an intense degradation upon its arrival at sea, it is crucial, if we want to include these findings in global biogeochemical cycles, to show that this phenomenon also takes place in temperate zones.
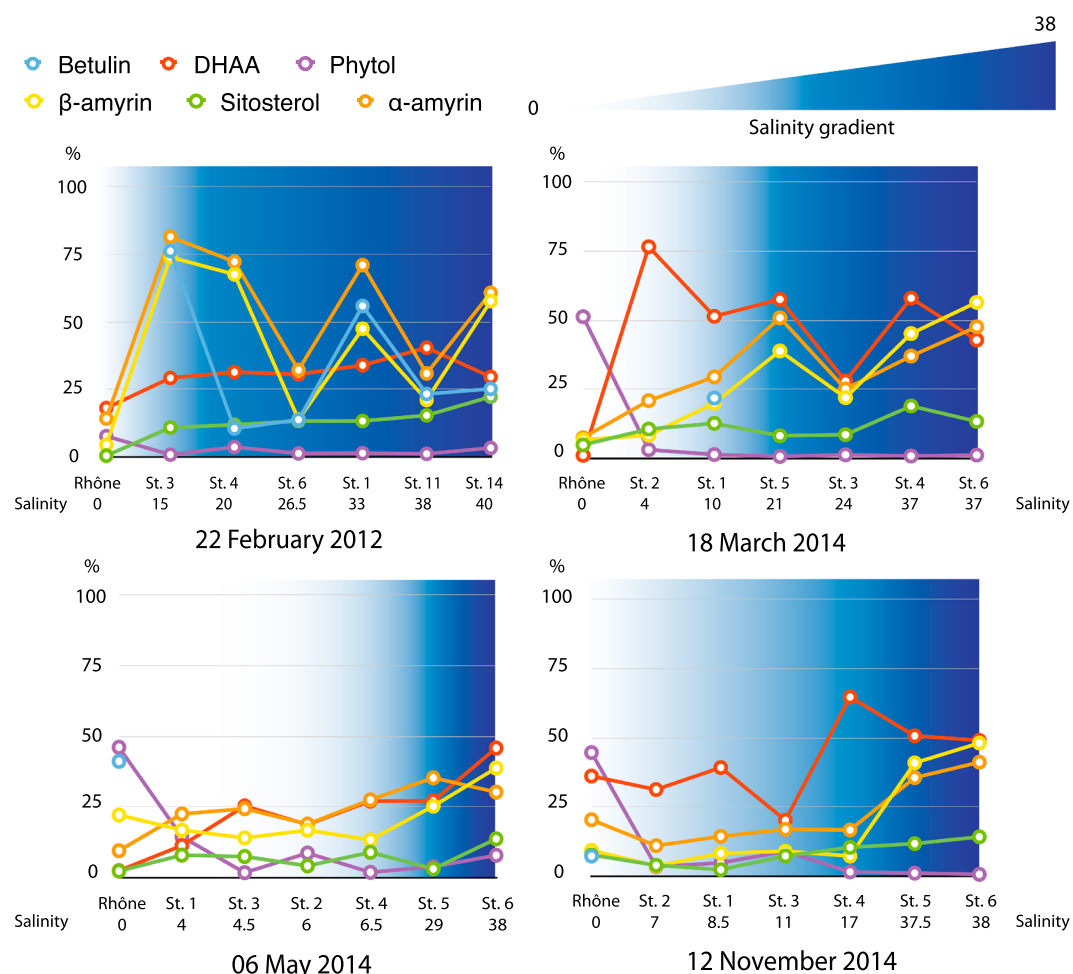
Figure 4. Lipid tracers and their autoxidation rates (%) along the salinity gradient within the Rhône River plume.

The aforementioned study on the Mackenzie River [*Rontani et al.*, 2014] as well as studies on the Rhône River [*Galeron et al.*, 2015] showed the varying degrees of involvement of photooxidation and autoxidation in organic matter degradation. It was evidenced that the role of photooxidation, averaging 10.3 ± 1.1% for sitosterol in the Rhône River between 2011 and 2013 [*Galeron et al.*, 2015], was much more important in the Arctic, where it can reach 48.0 ± 2% [*Rontani et al.*, 2014], than in temperate zones. This paradoxical enhancement of type II photooxidation processes in high-latitude colder regions, receiving less solar irradiance, has recently been explained by a better conservation of photosensitizers and lower diffusion rates of singlet oxygen in biological membranes [*Amiraux et al.*, 2016]. Together, these conditions favor a longer action time for photooxidation, causing extensive degradation and creating hydroperoxide-rich suspended particulate matter. Photooxidation appears to be the driving degradation process acting on riverine SPM in the Mackenzie River, while it only has a limited role in the temperate Rhône River [*Galeron et al.*, 2015].

In higher plant debris, homolytic cleavage (catalyzed by some metal ions, temperature, or UV radiations) [*Schaich*, 2005] of hydroperoxides resulting from type II photosensitized oxidation seems to be at the origin of the initiation of free radical oxidation (autoxidation) processes [*Girotti*, 1998; *Rontani et al.*, 2003; *Galeron et al.*, 2016a]. Thanks to new specific lipid tracers recently identified [*Galeron et al.*, 2016b, 2016c; *Rontani et al.*, 2015], the monitoring of the autoxidative state of terrestrial higher plant-derived organic matter in aquatic environments is now possible.

It is worth noting that the Rhône plume extended further in November after the flood than in February or May. The geographically closest sampling station to the river mouth already reached a salinity of 15 in February because of the very low river discharge, whereas on other sampling dates, surface sampling in
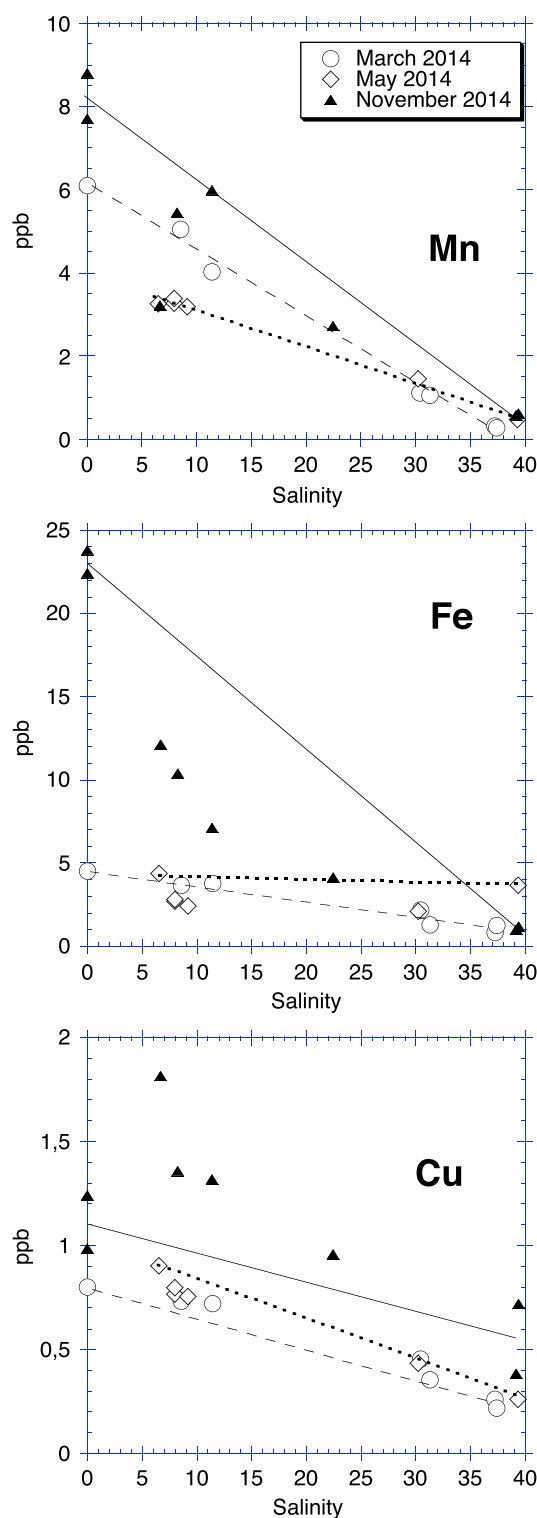
Figure 5. Trace metal concentrations in ppb along the salinity gradient: (a) Mn, (b) Fe, and (c) Cu. The lines represent the theoretical mixing line between fresh and seawater concentrations for the various campaigns.

stations closest to the river mouth evidenced salinities between 3 and 7 (Table 1). Differences in mixing proportions between river and seawater (and then on plume extension) seem to have a major impact on the autoxidative degradation rates observed in March, May, and November 2014. On those dates, the autoxidation rates observed within the river plume have a different distribution than in February 2012 (Figure 4), not peaking until geographically farther stations with higher salinities. This proves the important role that salinity plays in the induction of autoxidation in coastal areas. Across all sampling dates, autoxidation rates and salinity levels were well correlated, especially for sitosterol and α-amyrin and β-amyrin, with r^2 reaching 0.801, 0.962, and 0.943, respectively, in November 2014 (calculated on six stations, p-value < 0.05; Figure 6).

Building upon the results obtained in the Mackenzie estuary and during in vitro experiments [*Galeron et al.*, 2016a], we attributed this induction of autoxidative processes to the desorption of metal ions—adsorbed onto SPM during riverine transport—in the estuarine mixing zone. Upon desorption, these redox-active metal ions could have the potential to kick-start autoxidation, by catalyzing homolytic cleavage of hydroperoxides already present in SPM and resulting from photooxidation and autoxidation processes during its transport.

Desorption of trace metals is a well-researched process in estuaries but is still very difficult to correctly predict. Indeed, previous studies have shown that dissolved Fe or Cu could have a conservative behavior [*Comber et al.*, 1995; *L'her Roux et al.*, 1998; *Millward and Liu*, 2003]. Others have evidenced Cu desorption [*Waeles et al.*, 2004; *Zwolsman et al.*, 1997] or Fe flocculation or adsorption [*Comber et al.*, 1995; *L'her Roux et al.*, 1998] with no clear forcing parameters [*Comber et al.*, 1995; *Hatje et al.*, 2003; *Millward and Liu*, 2003].
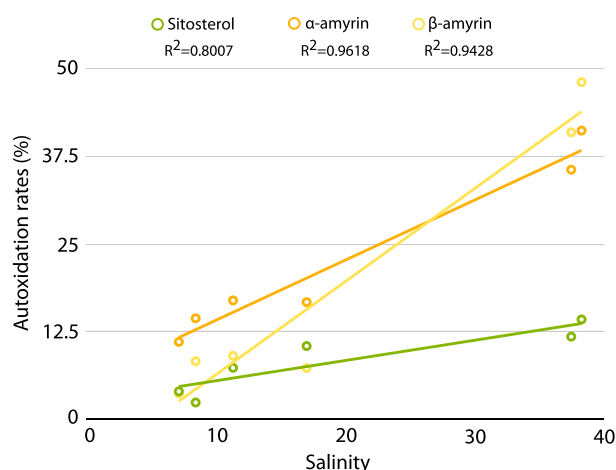
Figure 6. Graph showing the correlation between autoxidation rates and salinity for sitosterol, α-amyrin, and β-amyrin during the November 2014 campaign.

In any case, on all dates sampled, there is no apparent desorption in any particular area of the plume (Figure 5), especially in areas where an intense autoxidation has been evidenced. While metal concentrations do follow a mixing line between fresh and saltwater sources, we have found no evidence linking trace metal concentrations and autoxidation rates, showing that metal ions are clearly not a factor that could have favored autoxidative processes in our area of interest.

5. Conclusion

Previous studies on the Mackenzie River revealed that POM of terrestrial origin undergoes a rapid and intense degradation upon its arrival at sea [*Rontani et al.*, 2014]. The induction of these autoxidative processes was attributed to the presence of redox-active metal ions desorbed from particulate matter in the estuarine mixing zone. This assumption was backed by a recent study on the effect of metals on autoxidation of SPM in vitro [*Galeron et al.*, 2016a].

We show here that there was no apparent metal desorption in the Rhône River plume, while autoxidation rates still showed a rapid increase, well correlated with salinity. This raises once more the question of what is inducing—or favoring—autoxidative processes in the salinity gradient of river plumes? The lesser role played by photooxidation and autoxidation in organic matter degradation during riverine transport in the temperate Mediterranean area, compared to polar areas, has an impact on the amount of hydroperoxides found in SPM, lately found to play a major role in the induction of autoxidation in coastal areas [*Galeron et al.*, 2016a]. Indeed, hydroperoxide levels are much higher at higher latitudes, where photooxidation acts much more intensely, producing hydroperoxide-rich SPM later exported to coastal areas [*Rontani et al.*, 2012a].

The rapid and intense degradation of TPOM can have a major impact on global biogeochemical cycle estimations, with less organic carbon than previously thought making it to deeper layers of the ocean. The Mediterranean Sea is one of the most oligotrophic seawater bodies on the planet [*Karydis and Kitsiou*, 2011], but it is locally increasingly threatened by eutrophication [*Bellan-Santini and Leveau*, 1988]. It is essential to find out if tropical areas—now that we can compare with both polar and temperate areas—are the theater of the same estuarine degradative phenomena, if we want to be able to make global assumptions. Being able to correctly estimate riverine carbon fluxes and organic carbon export in the ocean is crucial if stakeholders and decision-makers are to make the right policy-making choices.

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