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Seasonal variation in stable C and N isotope ratios of the Rhone River inputs to the Mediterranean Sea (2004–2005)

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Abstract The temporal variation in the stable carbon and nitrogen isotope ratios of particulate organic matter (POM) in the Rhone River was investigated on a monthly basis during a 2-year period (2004–2005). In spite of high month-to-month variation, interannually consistent seasonal trends appeared, with significantly lower $\delta^{13}C$ (<−28.2‰) in spring than in the other seasons. In contrast, $\delta^{15}N$ did not exhibit significant temporal variation. In spring and summer, high chlorophyll *a* and *b* concentrations were associated with low C/N values (<8) and a high percentage of organic carbon (%C) and organic nitrogen (%N), testifying to high development of autochthonous riverine phytoplankton (mainly diatoms and chlorophytes). In fall and winter, higher $\delta^{13}C$ (>−27.2‰) and C/N (>8) values, and lower %C, %N, and chlorophylls concentrations indicated the predominance of allochthonous terrestrial detritus material in the river POM. The lower $\delta^{13}C$ values recorded in spring–summer, when the phytoplankton biomass was high, were related to the lower carbon isotopic signatures of freshwater diatoms and chlorophytes compared to those of terrestrial plants. Overall, Rhone River POM was mainly composed of terrestrially derived material (90%), with autochthonous phytoplankton representing only 10% as a mean, in spite of a higher mean contribution of phytoplankton (27%) to river POM in summer.

Keywords Chlorophyll · C/N · Freshwater organic matter · Gulf of Lions · Stable isotopes

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Introduction

Rivers discharge large quantities of organic matter and nutrients into marine coastal areas, which significantly affect the productivity and trophodynamics of near-shore systems (Caddy 2000). The role of river inputs is particularly prominent in deltaic coastal areas, like in the Gulf of Lions (NW Mediterranean) where fisheries catches fluctuate with the Rhone River discharge (Lloret et al. 2001; Salen-Picard et al. 2002). In rivers, particulate organic carbon derives from two major sources, autochthonous material like river phytoplankton and aquatic macrophytes, and allochthonous terrestrial material stemming from soil and litter of the river drainage basin and from industrial and domestic waste waters (Mook and Tan 1991; Finlay and Kendall 2007). Carbon and nitrogen isotopic signatures are widely used as natural tracers to elucidate the origin and fate of continental particulate organic matter (POM) in estuarine and marine coastal ecosystems, and determine its influence on food webs dynamics (Riera and Richard 1997; Rolff and Elmgren 2000; Darnaude et al. 2004; Wissel and Fry 2005). The chemical and isotopic compositions of organic matter sources have been studied in different rivers, from the St Lawrence (Hélie and Hillaire-Marcel 2006) and different rivers in the United States (Kendall et al. 2001), to the Amazon (Cai et al. 1988) and others, as well as in numerous estuarine environments (Matson and Brinson 1990; Martinotti et al. 1997; Hellings et al. 1999; Cloern 2002; De Brabandere et al. 2002; Goñi et al. 2003).

The Rhone River, with a mean annual discharge of 1700 m^3 s^{-1}, is the most important inflow to the Mediterranean Sea since the damming of the Nile. It contributes to the high productivity of the Gulf of Lions located in the north-western Mediterranean Sea (Lochet and Leveau 1990; Sempéré et al. 2000). Its river basin, remarkable due to its high climatic and geologic diversity, extends over 97,800 km^2 (Pont et al. 2002). Dissolved inorganic carbon (DIC) dominates the total river carbon fluxes (82%), while dissolved organic carbon (DOC) and particulate organic carbon (POC) represent respectively 6 and 8% only of the total carbon exported by the Rhone River (Sempéré et al. 2000).

Salen-Picard et al. (2002) demonstrated that Rhone River POM plays a major role in the functioning of coastal ecosystem functioning and benthic fisheries in the Gulf of Lions. River POM is incorporated into coastal food webs via depositivorous polychaetes and influences landings of the commercially important food fish common sole, which are correlated with river discharge with a 5-year time lag. This delay results from a two-step biological process (increase in juvenile survival and female fecundity within 2–3 years) combined with the mean age of individuals in catches (2–3 years). The finest particles of terrestrial origin are transported westwards hundreds of kilometres away from the river mouth all over the Gulf of Lions (Tesi et al. 2007), and influence the isotopic signatures of surface water POM (Harmelin-Vivien et al. 2008) and deep settling POM (Kerhervé et al. 2001). It is thus important to know the variations in isotopic signatures of river derived POM to determine its fate and role in coastal ecosystem functioning. A difference in isotopic signatures of the Rhone River POM was evidenced between spring and autumn 2001 (Darnaude et al. 2004), but the seasonal variation over the year has not been analysed. The present study was thus performed to (1) assess the seasonal temporal variability of the C and N isotopic composition of Rhone River POM on a monthly basis over a 2-year period, (2) identify the mechanisms controlling this temporal variability and (3) quantify the relative importance of autochthonous and allochthonous sources of organic carbon in the river POM.

Materials and methods

Studied site and sampling procedure

The Rhone River basin is characterized by a largely mountainous catchment area with a high mechanical erosion rate due to a steep altitudinal gradient (2300 m from the source to the sea) for a rather short river length (812 km) (Pont et al. 2002). The watershed touches three large mountain areas, (1) the Alpine mountains to the east consisting mainly of calcareous sedimentary rocks and siliceous crystalline and metamorphic rocks, (2) the Jura consisting mainly of calcareous sedimentary terrains and drained by the largest tributary of the Rhone, the Saone, and (3) the Massif Central, located on the western bank of the Rhone valley, consisting mainly of crystalline siliceous rocks. The Rhone catchment ends in the Camargue delta, which is the largest river delta in Western Europe (Pont et al. 2002). Three main rainfall regimes responsible for different types of floods can be characterized over different regions of the Rhone basin. Specifically, so-called oceanic floods originate from rainfall over the Saone basin in the north, Cevenol floods from the Massif Central, and Mediterranean floods from the eastern bank including the Alps (Pont et al. 2002; Antonelli et al. 2008). The soil of the Rhone basin is characterized by a rather homogeneous clay repartition, with a generally higher sand fraction in the mountainous parts (Ottle et al. 2001).

Vegetation is dominated by forests, with conifers spreading mainly in the mountains, and deciduous forest in the Cevennes and the northern Jura. In the valley, agriculture is highly diversified, with three dominant crops, wheat, corn, and rice, the latter being limited to the river delta (Ottle et al. 2001). The vegetation of the Rhone watershed generally follows the C3 photosynthetic pathway, except for corn (C4 pathway). However, as corn covers less than 10% of agricultural areas in the basin, its influence on the carbon isotopic signature of the Rhone River organic matter is negligible (Aucour et al. 1999).

Monthly sampling of subsurface water (0.5 m depth) was performed in the Rhone River 6 km upstream from the mouth, far from the influence of any large city, from January 2004 to December 2005. Sampling river water close to the river mouth allowed obtaining representative values of the isotopic signatures of the Rhone River POM discharged to the sea. Suspended particulate matter (SPM) samples were obtained by filtering surface water with preweighed Whatman GF/F filters (0.7 µm pore size) precombusted for 4 h at 500°C. Prior to this step, samples were prefiltered through a 250-µm-mesh sieve to remove large detritus particles. The size of analysed particles thus ranged from 0.7 to 250 µm. At least six filters were done at each sampling date. SPM was quantified by reweighing filters that had been dried at 60°C for 48 h. Filters were then stored at −20°C until stable isotope analysis. From January to December 2005, additional samples for pigments were filtered using Whatman GF/F filters, which were immediately frozen at −20°C wrapped in an aluminium foil and kept frozen in the dark until analyses. River discharge data measured at Beaucaire, 65 km upstream from the river mouth, were obtained from the Compagnie Nationale du Rhône daily for the period 2004–2005. No other tributaries occur from this point to the river mouth. The mean monthly discharge ($m^3\ s^{-1}$) was then computed to correlate POM parameters to the river discharge.

Stable isotope analysis

Stable carbon and nitrogen isotope analyses were conducted on two sub-samples of SPM surface water for each sampling date, with at least 3 replicates per treatment. One sub-sample was analysed without any prior treatment for $\delta^{15}N$ analysis. The other was acidified with 1% HCl solution to remove carbonates, rinsed with distilled water and oven-dried at 40°C for 24 h for $\delta^{13}C$ analysis, as carbonates present a higher $\delta^{13}C$ than organic carbon (Jacob et al. 2005). The quick acidification with 1% HCl solution is sufficient to remove carbonates but does not change the isotopic composition of the SPM organic content (Darnaude et al. 2004). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the samples were determined using continuous flow isotope mass spectrometry at the Mylnefield Lipid Analysis-SCRI, using a Europa Scientific ANCAT-NT 20-20 Stable Isotope Analyser with ANCA-NT solid–liquid preparation module (Europa Scientific Ltd., Crewe, UK), operated in dual mode. The analytical precision was 0.2‰ for both N and C, estimated from internal standards analysed along with the samples (1 mg leucine prepared by freeze drying 50 µl of a 20 mg ml^{-1} stock solution and calibrated against 'Europa flour' and IAEA standards N1 and N2). Isotope ratios were expressed as parts per thousands (‰) differences from a standard reference material:

$$\delta X = \left[\left(R_{\text{sample}}/R_{\text{reference}}\right) - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N, and R the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard reference materials were Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen.

Percentages of organic carbon (%C) and organic nitrogen (%N) in the river POM were given in addition to the stable isotopic signatures.

HPLC pigment analysis

Pigment analysis was performed using High Performance Liquid Chromatography (HPLC) according to Mantoura and Llewellyn (1983). Frozen filters were cut into slices and placed in 6 ml of 90% acetone, crushed with a glass stick and left in the dark at 4°C for 24 h for pigments extraction. Extracts were then filtered on GF/F filters with a SWEENEX system. The HPLC system used (Waters) consisted of a quaternary low pressure mixing dual pump, a photodiode-arraydetector, and a fluorimeter set at a detection wavelength of 430 nm for excitation and 670 nm for emission. The separating column was a bio-sil C-18 HL 90-5S (150 * 4.6 mm, 5 µm particle sizes), and the two solvents consisted of 100% acetone and methanol (80% methanol + 20% distilled water). The injected sample volume was 500 µl and the flow rate 1.2 ml min^{-1} for 30 min. Pigments quantified in the HPLC analyses were the chlorophylls *a* and *b*, by comparison with pigment standards (Sigma).

The contribution of river phytoplankton (C_{Phyto}) to total organic carbon was determined from chlorophyll *a* concentration according to the mass ratio, R = C_{Phyto}/Chl *a*, with R = 35 for freshwater phytoplankton (Canuel et al. 1995; Müller-Solger et al. 2002). R can vary widely (20–160) with phytoplanktonic origin and composition (Taylor et al. 1997). It was estimated to be 60 on average for temperate marine phytoplankton (Aminot and Kerouel 2004) and 35 for temperate freshwater phytoplankton (Canuel et al. 1995). The percentage of phytoplankton (% Phyto) in the particulate organic carbon (POC) was determined from the relation, % Phyto = (C_{Phyto} * 100)/POC, with POC = (%C * SPM)/100. Finally, the mean seasonal quantity of river phytoplanktonic carbon (Q_{phyto}) brought to the sea by the Rhone was computed according to the formula, $Q_{Phyto} = C_{Phyto}$ * mean monthly discharge.

Data analysis

After testing for the homogeneity of variance (Levene test), two-way ANOVA was used to analyse the effect of year and season on the main parameters determined for the Rhone River water. Appropriate means were compared using Student–Newman–Keuls test. Relationships between all parameters were tested by Pearson linear regressions for the 2-year period.

Results

Seasonal variations

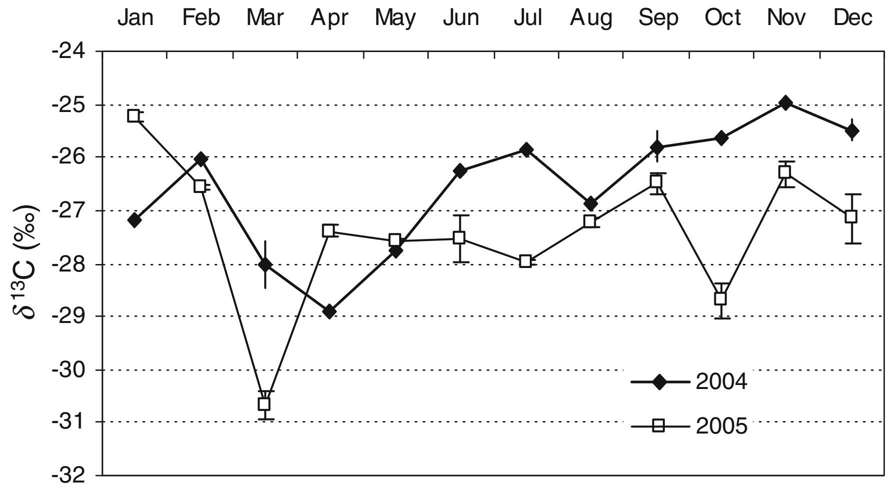
Seasonal variation of δ^{13}C, δ^{15}N, C/N and other parameters of the Rhone River water were evidenced both in 2004 and 2005 (Table 1). Month-to-month variation in all parameters was high, as illustrated for δ^{13}C, δ^{15}N (Figs. 1, 2) and mean monthly discharge (Fig. 3). At the same time, seasonal trends that were consistent between years could be noticed for some parameters. Seasonal means therefore appeared more appropriate to study temporal variation of Rhone River water characteristics. Two-way ANOVAs indicated that year was not a significant factor in explaining temporal variations for all the parameters analysed, whereas season was a significant factor for most of them (Table 2). The absence of interactions between year and season indicated that seasonal patterns were consistent between years. The δ^{13}C of POM was significantly lower in spring than in the other seasons, whereas δ^{15}N did not show any significant seasonal differences, due to the high variability observed for this element (Table 1, Fig. 2). The C/N ratio was significantly lower in spring and summer (<8) compared to fall and winter (>8), whereas %C and %N showed the opposite

Table 1 Seasonal means (± SD) of the particulate organic carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signatures, C/N mass ratio, percentages of organic carbon (%C) and organic nitrogen (%N), and quantity of particulate organic carbon (POC) in the suspended particulate matter (SPM) of the Rhone River water collected in 2004 and 2005, with the mean seasonal monthly discharge of the river (Month. discharge)

	δ^{13}C (‰)	δ^{15}N (‰)	C/N	%C	%N	SPM (mg l^{-1})	POC (mg l^{-1})	Month. discharge (m^3 s^{-1})
2004								
Winter	−26.24 ± 0.87	5.42 ± 2.63	8.99 ± 2.86	2.70 ± 0.89	0.31 ± 0.13	26.32 ± 35.40	0.746 ± 0.988	1875 ± 581
Spring	−28.23 ± 0.61	5.55 ± 5.55	5.82 ± 0.93	4.17 ± 0.58	0.73 ± 0.15	12.71 ± 5.76	0.546 ± 0.307	1348 ± 262
Summer	−26.33 ± 0.51	5.57 ± 0.54	5.94 ± 0.37	2.92 ± 0.86	0.50 ± 0.16	13.11 ± 10.82	0.326 ± 0.168	995 ± 210
Fall	−25.46 ± 0.45	6.10 ± 2.62	8.24 ± 0.79	2.08 ± 0.12	0.26 ± 0.04	4.89 ± 1.11	0.103 ± 0.028	1322 ± 466
2005								
Winter	−26.32 ± 0.99	4.60 ± 1.34	8.18 ± 0.31	2.10 ± 0.51	0.26 ± 0.06	5.56 ± 1.50	0.121 ± 0.054	1299 ± 313
Spring	−28.55 ± 1.84	5.52 ± 1.36	7.70 ± 2.47	4.03 ± 1.17	0.61 ± 0.43	18.99 ± 9.85	0.739 ± 0.328	1744 ± 664
Summer	−27.57 ± 0.37	4.92 ± 0.52	7.45 ± 1.68	4.02 ± 0.70	0.57 ± 0.20	6.10 ± 1.65	0.239 ± 0.042	797 ± 132
Fall	−27.17 ± 1.33	4.27 ± 0.50	9.13 ± 1.94	2.19 ± 0.31	0.24 ± 0.04	6.45 ± 2.61	0.145 ± 0.076	881 ± 45

The size of the particles analysed ranged from >0.7 μm to <250 μm. Means corresponded to 3 values for river flow and to 9 values for the other parameters

Fig. 1 Monthly means (±SD) of $\delta^{13}C$ of the Rhone River particulate organic matter (POM) in 2004 and 2005



pattern, with higher values in spring–summer than in fall–winter. Mean monthly river discharge was significantly higher in winter and spring than in summer and fall, but the quantity of SPM and POC in water did not differ significantly between seasons due to large month-to-month variation. Similarly, a one-way ANOVA performed on Chl *a* and Chl *b* concentrations failed to indicate any seasonal difference due to the high standard deviations of the data (Table 3). However, after data were grouped (spring–summer and fall–winter, resulting in a reduction of the variance relative to the ungrouped datasets) a significant difference between the two periods was observed for some parameters, with higher values in spring–summer than in fall–winter for Chl *a* and C_{Phyto} (t-test $= -3.006$, $P < 0.05$ for both parameters). Overall, the resulting quantity of freshwater phytoplankton carbon (Q_{Phyto}) carried down to the sea by the Rhone River was significantly higher in spring than in the other seasons ($F = 4.352$, $P < 0.05$).

Quality of the organic matter

The only parameter significantly correlated with Rhone river discharge was the quantity of SPM, which increased with discharge (Table 4). The stable carbon isotope signature of the river POM was significantly negatively correlated with both %C and %N, i.e., $\delta^{13}C$ decreased when %C and %N increased. The C/N ratio followed the same pattern, i.e., it significantly decreased when %C and %N increased. In contrast, C/N increased with the quantity of SPM. Chl *a* and Chl *b* concentrations were both significantly positively correlated with %C and %N, but Chl *a* was negatively correlated with $\delta^{13}C$ and C/N (Table 4). Taken all together, these correlations show that the C/N ratio and the $\delta^{13}C$ of the organic matter decreased when chlorophylls, carbon, and nitrogen concentrations were important in the river water, reflecting an increase in phytoplankton carbon in the river water (Table 3). The quantity of Chl *a* and Chl *b* in river water presented two peaks, the first in March and the second in June (Fig. 4), with the second peak in Chl *b* lasting longer (June to September) than the first. The % Phyto in Rhone water peaked in summer and reached a minimum in spring. Low values in spring despite the high concentrations of phytoplanktonic carbon during that season were related to the dilution by high SPM quantity (Table 3).

Discussion

Mean $\delta^{13}C$ values ranging from −31‰ (De Brabandere et al. 2002) to −26‰ (Martineau et al. 2004) have been reported for river POM, with most values varying between −29 and −27‰ (Cai et al. 1988; Hellings et al. 1999; Kendall et al. 2001; Bănaru et al. 2007). In the present study, the mean stable isotope signatures of the suspended particulate matter of the Rhone River were -26.56 ± 1.19‰ for $\delta^{13}C$ and 5.66 ± 1.81‰ for $\delta^{15}N$ in 2004, and -27.40 ± 1.36‰ for $\delta^{13}C$ and 4.83 ± 0.99‰ for $\delta^{15}N$ in 2005.

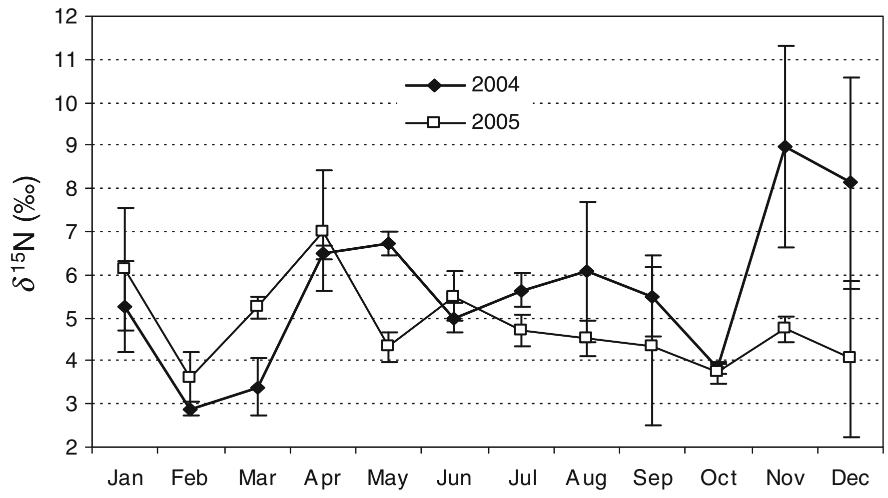


Fig. 2 Monthly means (±SD) of δ^{15}N of the Rhone River particulate organic matter (POM) in 2004 and 2005

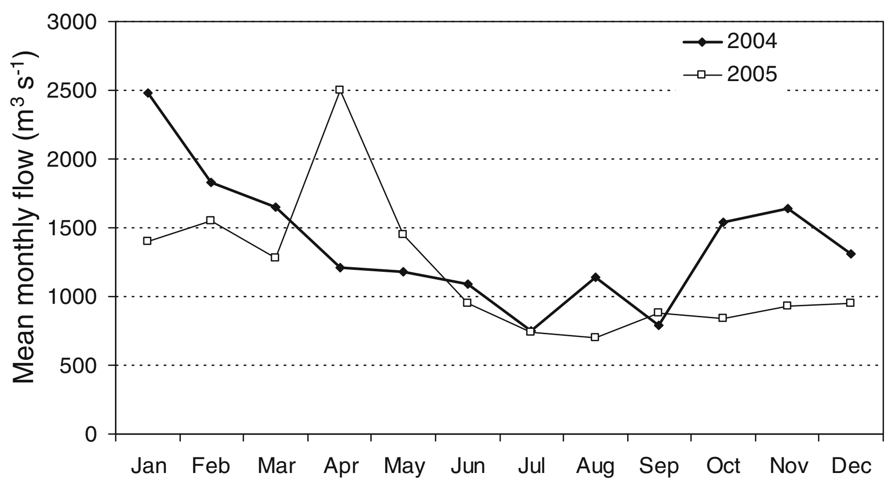


Fig. 3 Mean monthly discharge of the Rhone River in 2004 and 2005

These values were within the range of those found for Rhone POM in 1996 (mean $\delta^{13}C = -27.4$‰ (Aucour et al. 2003)), 2001 (mean $\delta^{13}C = -26.1$‰ (Darnaude et al. 2004)), and in 2006 ($\delta^{13}C = -27.55 \pm 0.70$‰; $\delta^{15}N = 5.29 \pm 0.79$‰ (Kerhervé and Sanchez-Vidal 2007)), and in the range of those observed for smaller rivers flowing into the Gulf of Lions (Kerhervé and Sanchez-Vidal 2007). However, despite the abundance of previous information on annual mean values, no information on seasonal variability of Rhone POM signatures has been published to date

Seasonal patterns

Isotopic composition of river POM can be highly seasonally variable because it is composed of a mixture of organic matter derived from autochthonous aquatic production and allochthonous terrestrial detritus of various origins (Maksymowska et al. 2000; Finlay and Kendall 2007). In the Rhone River, δ^{13}C was significantly lower in spring compared to the other seasons and in particular the fall–winter period. This confirms previous findings of lower POM δ^{13}C values in spring in other rivers (Mook and Tan 1991; Hellings et al. 1999; Maksymowska et al. 2000; Kendall et al. 2001; De Brabandere et al. 2002), which are generally related to an increase in phytoplankton biomass. In contrast, no significant seasonal trend in POM δ^{15}N appeared in the Rhone River, due to both high month-to-month variation and high variance of data. These variations are more difficult to interpret, as a variety of

Table 2 Annual and seasonal variations in some parameters of the Rhone River water analysed by two-way ANOVAs (year and season)

Parameter	Factor	F	*p*	Post-hoc
$\delta^{13}C$ (‰)	Year	4.269	0.060 n.s.	SP < WI = FA = SU
	Season	5.919	0.006 **	
	Year × season	0.904	0.461 n.s.	
$\delta^{15}N$ (‰)	Year	1.527	0.234 n.s.	
	Season	0.105	0.956 n.s.	
	Year × season	0.309	0.818 n.s.	
C/N	Year	1.457	0.245 n.s.	
	Season	3.040	0.050 *	SP = SU < FA = WI
	Year × season	1.076	0.387 n.s.	
%C	Year	0.157	0.697 n.s.	
	Season	9.856	0.001 **	
	Year × season	1.483	0.257 n.s.	SP = SU > WI = FA
%N	Year	0.118	0.735 n.s.	
	Season	6.645	0.004 **	SP = SU > WI = FA
	Year × season	0.258	0.855 n.s.	
SPM (mg l^{-1})	Year	0.787	0.388 n.s.	
	Season	0.803	0.510 n.s.	
	Year × season	1.118	0.371 n.s.	
POC (mg l^{-1})	Year	0.563	0.464 n.s.	
	Season	1.926	0.166 n.s.	
	Year × season	1.250	0.325 n.s.	
Monthly river discharge (m^3 s^{-1})	Year	1.639	0.219 n.s.	
	Season	4.492	0.018 *	WI = SP > FA = SU
	Year × season	1.808	0.186 n.s.	

SPM suspended particulate matter, *POC* particulate organic carbon, *SP* = spring, *SU* summer, *FA* fall, *WI* winter

n.s. not significant, * significant at $P < 0.05$, ** significant at $P < 0.01$, *Post-hoc* post-hoc comparisons of means

Table 3 Seasonal variation (mean ± SD) of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and phytoplanktonic carbon (C_{Phyto}) concentrations in Rhone River water in 2005, with the mean percentage of freshwater phytoplankton (% Phyto) and the quantity of phytoplanktonic carbon (Q_{Phyto}) inputs to the sea

	Chl *a* (μg l^{-1})	Chl *b* (μg l^{-1})	C_{Phyto} (μg l^{-1})	% Phyto	Q_{Phyto} (g m^{-3})
2005					
Winter	0.376 ± 0.253	0.010 ± 0.008	13.145 ± 8.866	9.9 ± 5.0	18.807 ± 13.840
Spring	1.606 ± 1.072	0.023 ± 0.033	56.213 ± 37.521	8.5 ± 5.2	87.177 ± 37.251
Summer	1.715 ± 0.835	0.043 ± 0.010	60.031 ± 29.240	27.2 ± 18.4	50.435 ± 33.235
Fall	0.622 ± 0.523	0.015 ± 0.021	21.768 ± 18.319	16.0 ± 17.1	18.650 ± 15.553

different processes may contribute to these patterns. Specifically denitrification processes, bacterial degradation of organic matter, and the large range of $\delta^{15}N$ of anthropogenic derived inputs (e.g., high for manure and low for fertilisers) (De Brabandere et al. 2002; Finlay and Kendall 2007) may play a role. Several millions of people inhabit the Rhone River bassin, with diverse agricultural practices and waste water treatments (Bravard and Clémens 2008), which prevents any reliable explanation of POM $\delta^{15}N$ variations at the river mouth. The absence of seasonal variation in POM $\delta^{15}N$ has also been noticed in other rivers (Kendall et al. 2001). However, in the Vistule River, $\delta^{15}N$ is inversely correlated to ammonium concentration and the lowest values are recorded in winter (Maksymowska et al. 2000). In the Danube River, $\delta^{15}N$ is positively related with river discharge with higher values in spring than in autumn (Bănaru et al. 2007),

Table 4 Significant Pearson linear correlations between physical, biochemical and isotopic parameters of the Rhone River water during the period 2004–2005

	r	*p*
SPM vs monthly discharge	0.667	<0.001
δ^{13}C vs %C	−0.656	<0.001
δ^{13}C vs %N	−0.641	<0.001
C/N vs SPM	0.411	<0.05
C/N vs %C	−0.476	<0.05
C/N vs %N	−0.742	<0.001
%C vs %N	0.914	<0.001
Chl *a* vs δ^{13}C	−0.705	<0.05
Chl *a* vs C/N	−0.627	<0.05
Chl *a* vs %C	0.868	<0.001
Chl *a* vs %N	0.898	<0.001
Chl *a* vs Chl *b*	0.826	<0.001
Chl *b* vs %C	0.764	<0.05
Chl *b* vs %N	0.771	<0.05

SPM = suspended particulate matter

due to both phytoplankton blooms and an increase in animal waste inputs during spring flooding. Low δ^{15}N in winter and high δ^{15}N in spring–summer is also observed in the Scheldt estuary, where it is related to the development of ^{15}N-enriched phytoplankton and bacteria (De Brabandere et al. 2002).

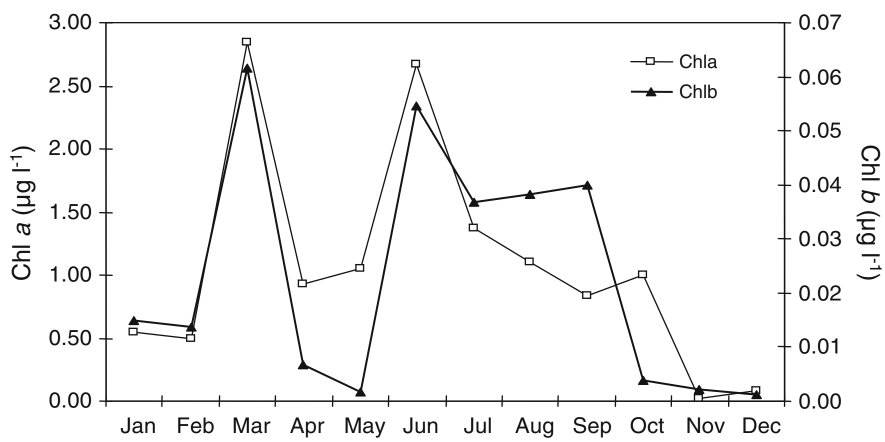
The C/N ratio is a useful indicator of the quality of organic matter, with low values indicating fresh "high quality" (i.e. labile) POM. C/N of freshwater phytoplankton is generally lower than 8, whereas C/N is often between 10 and 12 for soil, and reaches 20 for leaf detritus (Kendall et al. 2001; Finlay and Kendall 2007). A decrease in δ^{13}C linked with low C/N ratios indicates a higher contribution of phytoplankton to total river POM. Thus, the lower δ^{13}C values corresponding to low C/N of river POM recorded in the Rhone River in spring and summer compared to fall and winter, testified of increased development of phytoplankton during these seasons. This was also evidenced by high concentrations in organic carbon, organic nitrogen and chlorophyll *a*. Our findings are in accordance with high development of phytoplankton in spring–summer in many other rivers, like the Schelde (Hellings et al. 1999; Lionard et al. 2008), the Vistule (Maksymowska et al. 2000), and other temperate rivers (Kendall et al. 2001).

Total SPM concentration in the Rhone River water in 2004–2005 was related to the water discharge, as already observed by Sempéré et al. (2000). However, Rhone discharge rates were unrelated to the isotopic signatures of the river POM. Hélie and Hillaire-Marcel (2006) obtained similar results for the St Lawrence River. In contrast, strong peaks in discharge can advect large amounts of terrestrial detritus, and thus result in low δ^{15}N and high δ^{13}C values (Hellings et al. 1999).

Phytoplankton importance and composition

Pigment analysis is useful for characterizing river phytoplankton community structure and for tracing the sources of organic matter inputs (Lemaire et al. 2002). In the present study, two main periods were present, as indicated by the observed seasonal variations of stable isotope ratios and of biochemical parameters of Rhone River water: a season of high phytoplankton development (spring–summer) and a season of low phytoplankton development (fall–

Fig. 4 Monthly concentrations of chlorophyll *a* and *b* in the Rhone River water in 2005



winter). During spring and summer, phytoplankton biomass increased, which was attested by the high values of chlorophyll *a* and *b*, high values of %C and %N and the low C/N ratio. The predominance of riverine suspended POM by phytoplankton is effectively correlated to high concentrations of chlorophylls (Hellings et al. 1999; Lemaire et al. 2002; Hélie and Hillaire-Marcel 2006). Descy (1993) and Lemaire et al. (2002) found that in non-stagnant rivers, phytoplankton is dominated by diatoms and chlorophytes. Chlorophyll *a* concentrations mainly reflect the abundance patterns of diatoms and chlorophyll *b* those of chlorophytes (Hellings et al. 1999; Lemaire et al. 2002; Lionard et al. 2008). In Western European rivers, diatoms usually dominate the phytoplankton community (Lemaire et al. 2002; Lionard et al. 2008), as also previously observed in the Rhone River (Fruget et al. 1999). In the present study, two peaks in Chl *a* were observed in the Rhone in 2005, the first in March corresponding to the spring bloom, and the second in June and early summer. Spring blooms of diatoms are observed regularly in other rivers or upper estuaries (Garnier et al. 1995; Lemaire et al. 2002; Lionard et al. 2008), although this group dominates all year round. Chlorophyll *b* concentrations indicate the presence of chlorophytes, which were observed in abundance in upstream portions of the Rhone River by Fruget et al. (1999). Chlorophytes frequently constitute the second-most important component of riverine phytoplankton after diatoms (Fruget et al. 1999; Lemaire et al. 2002). In the Rhone River, a first peak in chlorophyll *b* appeared during the spring bloom (March), and a second peak of longer duration between June and September, indicating the preferential development of chlorophytes during summer. Similar development of chlorophytes in summer has been reported for the Seine (Garnier et al. 1995) and the Shelde River (Lemaire et al. 2002; Lionard et al. 2008).

A consequence of the river phytoplankton bloom, particularly in spring, was a decrease in δ^{13}C values of the organic matter, due to the fact that freshwater phytoplankton is characterised by more negative values than terrestrial detritus matter (Finlay and Kendall 2007). Generally, freshwater phytoplankton has δ^{13}C values ranging from −35 to −5.9‰ and δ^{15}N ranging from −2.1 to 12.8‰, with freshwater diatoms and chlorophytes showing low δ^{13}C values between −32.7 and −26.6‰ (Vuorio et al. 2006). In contrast, terrestrial vegetation has higher δ^{13}C values, ranging from −9 to −14‰ for C4 plants, and from −30 to −23‰ for C3 plants, and is characterized by a larger range in δ^{15}N (3–18‰) (Maksymowska et al. 2000; Finlay and Kendall 2007). Thus, in the spring–summer period phytoplanktonic blooms of diatoms and chlorophytes developed in the Rhone River and induced a decrease in δ^{13}C and C/N, whereas in the fall–winter period terrestrial allochthonous detritus dominated the river POM and resulted in higher values of δ^{13}C and C/N.

It should be noted that somewhat in contrast to these broad patterns, a higher δ^{13}C value was recorded in summer (−26.33‰ in 2004 and −27.57‰ in 2005) than in spring (−28.23‰ in 2004 and −28.55‰ in 2005) in spite of a higher percentage of phytoplankton in summer. A similar seasonal variation of δ^{13}C is observed by Aucour et al. (2003) for the Rhone River POM near the river mouth (−26.0‰ in summer vs −28.3‰ in spring). Importantly, the origin of particles (terrestrial detritus or phytoplankton) in river water, which was responsible for the broad patterns observed in our study, is not the only factor influencing the δ^{13}C of river POM. Specifically, the δ^{13}C of freshwater phytoplankton decreases with high dissolved inorganic carbon (DIC) concentration, low DIC δ^{13}C, and during river floods (Aucour et al. 1999), and increases with temperature and phytoplankton biovolume (Gu et al. 2006). In the Rhone River, Aucour et al. (1999) record a higher DIC concentration and a lower DIC δ^{13}C value in spring than in summer. During the 2 years of our study, the river flow was higher in spring than in summer, while the temperature and the percentage of phytoplankton were higher in summer. Thus, lower DIC concentration, higher DIC δ^{13}C, lower river flow, higher percentage of phytoplankton and higher temperature are occurring in the Rhone River in summer compared to spring, all factors reported to induce an increase in river POM δ^{13}C (Aucour et al. 1999; Gu et al. 2006). It was thus likely the temporal variation of these factors that was responsible for the higher δ^{13}C value of the Rhone POM observed in summer 2004 and 2005 compared to spring.

River POM export to the sea

Our study confirms that the amount of suspended material transported to the sea by the Rhone is related to river discharge, which was previously described by Sempéré et al. (2000). Pont et al. (2002) estimated the mean annual quantity of SPM brought to the Mediterranean by the Rhone River to be 7.4 × 10^6 tonnes yr^{-1}, which are transported mainly during flooding events. In terms of particulate organic carbon, the Rhone River export is estimated to be 0.19 × 10^6 tonnes C yr^{-1} with only 18% of labile POC susceptible to bacterial degradation (Sempéré et al. 2000). Here, we demonstrated that phytoplankton contributed only 10% to the total annual particulate organic carbon load of the Rhone River in 2005, although peak contributions reached 48% at the beginning of summer. Our findings thus show that soil and litter were the major (90%) sources of POC exported to the sea by the Rhone. A similar result (90% of allochthonous POC and 10% of autochthonous phytoplankton) has been recorded for the Garonne River (Etcheber et al. 2007). Low chlorophyll concentrations and phytoplankton biomass are characteristic in fast-flowing rivers (short water residence time) like the Elbe and the Rhine (Lemaire et al. 2002), the Amazon (Cai et al. 1988), or the Rhone (present study). In contrast, in eutrophic rivers or in rivers with high residence time of water, the autochthonous phytoplanktonic contribution increases, particularly during low-water stages (Hein et al. 1999), and may reach 50% of the POC content, as in the Loire River (Etcheber et al. 2007). The low percentage of phytoplankton in the Rhone water observed in spring (8.5%) was due to a dilution phenomenon by the high quantity of SPM, as the total quantity of phytoplanktonic carbon export to the sea (Q_{Phyto}) was maximal in spring (Table 3). Autochthonous aquatically produced POC was important in summer, whereas allochthonous terrestrially derived POC dominated in winter in the Rhone, which has been previously observed in other rivers (Hellings et al. 1999; Hélie and Hillaire-Marcel 2006; Etcheber et al. 2007).

These findings are relevant in the context of climate change, which is predicted to lead to increasing variability in precipitation and river discharge in Europe, with generally increasing freshwater fluxes in Northern Europe, and decreasing fluxes in the South (Bouwer et al. 2008). Considering the patterns that we observed in the Rhone compared to other rivers with longer water residence times, these changes will likely also result in changes in quantity and quality of river POC. In particular, a decreasing discharge projected for South European rivers (Nohara et al. 2006) would be expected to result in decreasing SPM export to marine nearshore ecosystems. At the same time, lower discharge would result in decreased flow velocity and increased water residence times, and consequently, an increase in phytoplankton development and bacterial degradation processes in river waters.

Conclusion

Considering that the Rhone River is the largest river flowing into the Mediterranean, it was important to better characterize the origin of particulate organic matter that it carries to the sea. This information is of particular interest in the context of coastal ecosystem functioning and fisheries, which are strongly influenced by Rhone export both positively (enhanced sole catches, Salen-Picard et al. 2002) but also negatively (export of contaminants to coastal systems, Dierking et al. 2009). The present study demonstrates that both the quantity and the quality of total POC, and the relative role of river phytoplankton and terrestrial material vary significantly between seasons, and may thus result in changes in seasonal availability of POC to coastal systems in the Gulf of Lions. The Rhone phytoplankton production is a potentially significant source of fresh and labile source of carbon for the Gulf of Lions. Freshwater diatoms are observed in the Rhone River plume, particularly in spring (Harmelin-Vivien et al. 2008). The temporal variations of the Rhone river POM isotopic signatures influence those of the surface waters of the Gulf of Lions (Harmelin-Vivien et al. 2008) and can be traced through the coastal food webs of this area (Ferraton et al. 2007), which underlines the importance of the knowledge of river POM isotopic variations in analysing marine ecosystem functioning.

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