

Reply to the referee 1

We thank Dr Volkman for his review that helps us to improve the quality of the manuscript. We have taken into account his remarks and modified deeply our manuscript according to his comments. We hope these modifications will correspond to his attempts.

We also realised that most of his remarks arise from the limited description we made on the functioning and specificities of the Rhône deltaic system. Since this paper was intended for a special issue on a program (CHACCRA) dealing with the fate of the Rhône inputs in the pelagic and benthic ecosystems, we thought that it was not necessary to repeat what was already in the other publications of that issue. But this information appears necessary for the understanding of our results. Accordingly, we have added to the description of the study area a more thorough description on the specificities of the system. Here are the main points of this description:

In the Gulf of Lions, oceanographic circulation is dominated by the Liguro-Provençal Current (LPC), which generally flows southwest along the coast. Movement of the LPC is determined by a geostrophic equilibrium established between two opposite forces: (1) the force due to the pressure gradient existing between the lighter (less salty) shelf waters and the denser water masses over the continental slope, and (2) the Coriolis force induced by Earth's rotation. This situation results in a corridor through which the LPC flows, separating the shallower waters from the offshore ones. Most often, river plumes issued from the Rhône mouth are rapidly deviated toward the southwest and onshore, contributing to the formation of a coastal cloudy layer (Arnau et al., 2004). No significant tidal signal can be recorded in the Gulf of Lion. The structure of the Rhône dilution zone results therefore from a balance between the stratifying influence of buoyancy and the net stirring effect induced by wind and waves. Surface velocities are high and carry away the freshwater plume. For example, Naudin et al. (2001) recorded at station A, surface velocities between 69 and 79 $\text{cm}\cdot\text{s}^{-1}$. If we make a gross calculation using an average value of 70 $\text{cm}\cdot\text{s}^{-1}$, it would take ~ 1 hour for the desalted water to spread from the river outlet to station A, and 30 more minutes to reach station B. Consequently, as highlighted by Naudin et al. (2001) "In the vicinity of the river mouth little production occurred and the sharp salinity gradient prevents marine species to develop. As the interface friction forces are enhanced, the halocline is eroded. In that case, salt exchanges occurred and benefit first to the marine phytoplankton with the higher salinity tolerance. Then the mixing regime is enhanced yielding to an equilibrium between nutrient availability and osmotic tolerance of microbial communities." The enhanced production zone establish thus further offshore and could be quite far from the river mouth in case of high velocity (Conan and Pujo-Pay, 1995). The formation of a salt-wedge that prevents early mixing and euryhaline species development is a particularity of microtidal estuaries. Moreover, one needs to keep in mind (1) the relatively limited size of the Rhône in comparison to the Amazon or the Mississippi (as regards to river discharge) and (2) the bathymetry of the continental shelf south of the Rhône mouth (with a steep slope). The area of prodelta (defined as the immersed portion of the delta) is therefore limited and only extends for about 5 kilometres. As a consequence, most of the processes such as enhanced primary production occurred on the shelf which begins after the slope.

The answers to the Referee #1's comments are given below (abbreviations are RC: referee comment; AC: author comment). Note that the order of the different comments has been reorganized for more coherence.

RC: *“No samples of the proposed end-members were obtained.”*

AC: We agree with the reviewer that marine and riverine end-members are important to discuss our results. We actually have performed analyses on the riverine end-member (referred in the text as Rhône suspended organic matter) and these results are discussed in the manuscript (pages 3372 & 3375). Page 3372 line 20: “In good agreement with the potential inputs of freshwater microalgae, EPA and DHA were found in suspended organic matter from the Rhône river”. Page 3375 line 20: “The analysis of suspended particulate organic matter from the Rhône river indicates that riverine inputs were more enriched in THAA (234.0 mg gOC⁻¹, data not shown) than the superficial deposits off the Rhône river (148.3 mg gOC⁻¹, station A, data not shown)”. These results have been added to our revised manuscript and discussed more thoroughly. As regards to the marine end-member, it has not been possible during this oceanographic field campaign to get a “pure marine end-member” because in the whole studied area suspended matter was composed of terrestrial and marine particles as shown by the uniform $\delta^{13}\text{C}$ signature (-23 to -25 ‰, Kerhervé unpublished). There is however a wide literature on that subject, especially on fatty acid composition of marine phytoplankton (Volkman et al., 1989; Zhukova and Aizdaicher, 1995; Mansour et al., 1999). The question of the proportion of continental and marine organic matters will be discussed in detail with the comment on bulk carbon isotopic measurements.

RC: *“Furthermore discussions about degradation are confounded because it is not clear whether this occurs mainly in the water column (so that transit time from the river would be important) or in the sediments...”*

AC: We agree with the reviewer that degradation might occur in the water column and in the sediments, and that as distance to the Rhône mouth increases degradation before sedimentation will increase (see page 3375 line 23). We doubt it is possible to answer the question of how much degradation occurs before sedimentation from our dataset. For this reason, we discussed in the text of the possible mechanisms of OM degradation without trying to estimate their relative importance. Anyway, our purpose was to provide a picture of the quality and degradation state of the OM in the sediments at a given moment (April 2007), not to study the mechanisms of OM degradation. This will be the subject of other papers dealing with degradation kinetics in the sediments. Nonetheless, our results and others from the literature (Pastor et al., 2011; Durrieu de Madron et al., 2000) suggest that close to the river mouth (A and B) particles settle rapidly and are essentially degraded in the sediments, while more offshore sediments contain older OM that has undergone many cycles of deposition/resuspension/degradation.

RC: *“The lack of data for bulk delta13C and delta15N values is rather a surprise, especially since delta13C data are provided for individual fatty acids... The C/N ratios all fall within a narrow range consistent with mixed marine and terrestrial organic matter sources rather than showing a trend to proportionally greater contributions of marine-derived organic matter with distance from the river mouth.”*

AC: Bulk $\delta^{13}\text{C}$ analyses have been performed by a colleague from another institute. These results have not been included in our manuscript because they are part of a paper by Cathalot and collaborators that is intended for *Geochimica and Cosmochimica Acta*. Our colleague, Dr P. Kerhervé, will be an author of our revised manuscript and a discussion on bulk isotopic measurements has been included. A two end-member model of $^{13}\text{C}_{\text{OC}}$ signatures has been used to estimate the fraction of terrigenous organic carbon (Gordon and Goni, 2003) with the following assumption: OC is composed exclusively of terrestrial matter delivered by the Rhône ($^{13}\text{C}_{\text{OC}}$

of Rhodanian suspended OM = -27.4 ‰, mean of a monthly survey realised from 2006 to 2008) and marine inputs ($^{13}\text{C}_{\text{OC}} = -20.1$ ‰, Harmelin-Vivien et al., 2008). The terrestrial contribution is comprised between 97% (station A) to 89% (station B) in the prodelta area, whereas terrestrial and marine contributions are equivalent in the shelf area. These results are in good agreement with previous estimates based on organic tracers, bulk isotopic signatures and plutonium isotope distribution (Lansard et al., 2009; Bouloubassi et al., 1997; Lansard et al., 2007). In addition, Tesi and collaborators (2007) have shown that the Rhône inputs are composed of fine soil organic matter and plant debris. The wood fragments characterised by high OC and lignin contents are retained within the prodelta area, while the soil-derived OM adsorbed onto fine fraction (with a low lignin content), is selectively transported out of the prodelta along the main sediment dispersal pathway.

It is true that C:N ratios do not exhibit much variability (11.2 ± 0.4 to 15.6 ± 1.4), but they are correlated with distance to the coast when the whole dataset is considered ($p < 0.0001$, 16 stations, $n = 48$). According to Lansard et al. (2009), the fate of terrestrial POC in the Rhône delta is governed by the hydrodynamic sorting of riverine particles of different compositional character rather than by simple dilution with marine OM, which might explain the behaviour of this proxy.

RC: *“The term “preferential” implies that the microbial populations prefer to mineralize terrestrial organic matter rather than marine organic matter, but the evidence for this seems quite weak.”*

AC: The term “preferential” was indeed excessive in this context (page 3354 line 20, page 3377 line 15). We corrected the 2 sentences as followed: “In the proximal prodelta, bacteria-specific fatty acids were abundant ($1.65 \text{ mg.g}^{-1}\text{OC}$ at the mouth site) and were relatively depleted in ^{13}C confirming that bacteria predominantly utilize terrestrial OM in this area”. “Relatively depleted ^{13}C of these bacteria-specific fatty acids in the nearshore sites indicates the predominant utilization of terrestrial OM by bacteria while in shelf sites, marine-derived OM is the main source for microbial synthesis of these compounds.”

RC: *“the pigments are almost certainly derived from planktonic sources (despite some speculation about the source of chlorophyll b) and the source of the amino acids is not stated, but is also likely to be from planktonic sources. Thus neither of these data sets provides evidence for degradation of terrestrial-derived organic matter.”*

AC: We agree that in another context, data such as DI or pigment composition would not be sufficient by themselves to demonstrate that continental OM is efficiently degraded. However, as explained earlier continental OM is largely predominant in nearshore sediments off the Rhône river during this field campaign ($\delta^{13}\text{C} = -27.18$ ‰ at 2.8 km off the river mouth) and others (Tesi et al., 2007; Lansard et al., 2009). A simple calculation may clarify the origin of the amino acids. Close to the river mouth (station A), 9% of the organic carbon is contained in amino acids, but there is only 3% of marine organic carbon. Therefore, a large proportion of the amino acids must derive from continental sources. This affirmation is also supported by the close similarity of the composition of river SOM and surface sediments (see Table 1): the proportions of C and N contained in the amino acid pool as well as the ratio EHAA/THAA are comparable. Obviously, the proportion of continental amino acids decreases offshore, but we cannot estimate the amounts of marine and terrestrial amino acids.

Table 1: Proportion of carbon and nitrogen contained in the amino acid fraction, as well as values of the digestibility index of Mayer et al. (1995) for river suspended organic matter and surface sediments (station A).

	River SOM	Surface sediments (st A)
THAA-C%	10.02	8.94±0.49
THAA-N%	30.97	34.46±3.63
EHAA/THAA	28.19	28.21±1.07

As regards to the origin of pigments in the Rhône prodelta, phytoplankton biomass and primary production are extremely limited in the close vicinity of the Rhône river mouth (Conan and Pujo-Pay, 1995; Pujo-Pay et al., 2006). Thus, the input of marine organic matter to the sediment is not significant. Alliot and collaborators (2003) realised a five year bi-monthly survey to study the impact of the dilution plume on coastal sediments. They showed that changes in the composition of organic matter were linked with the inputs of the Rhône River and associated with variations of chlorophyll-*b*. Chlorophyll-*b* content in the surface sediments decreased linearly with distance for 2 miles off the river mouth and showed statistically significant negative correlation with the five year bi-monthly mean surface salinity values. Similar observations were made for 9 European estuaries, where Lemaire and collaborators (2002) found that chlorophyll-*b* and lutein only occurred in the upstream part of the estuaries. The concentrations of these pigments decreased along the salinity gradient showing once again that chlorophyll-*b* mainly derived from terrestrial higher plants and freshwater chlorophytes in estuaries. Chlorophyll-*b* may also be found in marine phytoplankton species (Chlorophytes). However several studies on phytoplankton community structure indicate that Bacillariophyceae (diatoms) dominate phytoplankton blooms in the Mediterranean (Sarhou et al., 2005; Marty et al., 2002; Latasa et al., 2010), whereas chlorophyll-*b* was attributed to Prasinophytes and was restricted to summer months (Marty et al., 2002).

Considering the low inputs of marine OM to the seafloor in the vicinity of the Rhône river mouth and the high loads of OM deriving from continental sources (debris of higher plants, freshwater phytoplankton and soil-derived OM), one may legitimately consider that part of the organic matter that is degraded in the sediments comes from continental sources. Pastor and collaborators (2011) recently estimated using the OMEXDIA model that the most labile fraction ranged from 50 % to 94 % in the prodelta area, confirming that part of the continental OM is reactive. Note that in the revised version, we use the term “continental” instead of the more ambiguous term “terrestrial” that does not include freshwater sources.

RC: *“There seems to be an assumption throughout the text that most of the organic matter in the sediments is derived from the Rhône river and yet clearly there must be marine sources as well. The correlation between chlorophyll a and bacterial fatty acids (page 22) does not need to imply a coupling with Rhône river inputs if the chlorophyll is derived from marine inputs.”*

AC: As seen above, marine inputs are extremely low (3 % at station A) and consequently most pigments must derived from continental sources. We added to the revised manuscript pigment analyses of the suspended OM from the Rhône. These results strongly support the hypothesis that Rhône inputs are the likely source for most pigments found in the surface sediments: the ratio chlorophyll-*b*/chlorophyll-*a* is the same (0.14), whereas PRI is only a little higher for suspended OM (40 and 32 % respectively for riverine SOM and surface sediments) showing the ongoing degradation of POM.

RC: *“Similarly, the suggestion that the organic matter delivered by the Rhône river is “well nutritionally balanced” (page 27) goes well beyond the normal meaning of the term “nutritionally balanced” which implies a good balances of all essential nutrients, not just the presence of polyunsaturated fatty acids (which are more likely to be of marine origin anyway).”*

AC: We agree with Referee#1 that the term “well nutritionally balanced” is excessive. As suggested, the term “well nutritionally balanced” was replaced by “nutritionally adequate”. However, we do not agree that “polyunsaturated fatty acids are more likely to be of marine origin”. At least not in the case of sediments located in front of the Rhône mouth (Station A), where bulk $\delta^{13}\text{C}_{\text{OC}}$ signatures indicate that 97 % of the POM is of terrestrial origin (with ~1 % of the carbon contained in fatty acids). Moreover, the isotopic signature of individual polyunsaturated fatty acids (PUFA) confirms their continental origin. In addition, we performed fatty acid analyses on different sources of continental OM delivered to the Rhône prodelta. We found 6.4% of PUFA in riverine suspended matter, while plants detritus like senescent oak leaves and duckweeds contain 19.1 % and 58.9% of PUFA, respectively. Based on compound-specific isotope data and fatty acid composition of several continental end-members, we can not rule out that PUFA originate from continental inputs in our study.

RC: *“The text contains a number of grammatical and stylistic errors and needs careful rewriting. The English expression needs to be checked by a native English speaker.”*

AC: The whole manuscript has been carefully scanned for improper English and grammatical constructions, and rephrased when necessary. The revised manuscript has also been corrected by a native English speaker (Dr Jenifer Guarini).

RC: *“Note that the estimates for chlorophyll degradation (page 13) are minimum estimates since other degradation products (some of which are colourless) are not included in the calculation.”*

AC: Photosynthetic pigments formed many different intermediate products of degradation, some of them may be difficult to quantify. The PRI has been used as a proxy to compare the degradation state of pigments in the 16 stations of our studies, but we agree that it is a just an estimate. Using global fluorometric or spectrofluorometric methods, the term “pheopigments” includes pheophytin, pyropheophytin, pheophorbide, pyropheophorbide and even steryl chlorin esters since these compounds exhibit similar spectral bands that prevent their discrimination through spectral resolution. A group of compounds that cannot be considered by such global assays are the “colourless” pigments. Anyway, most of the degradation products are taken into account by the method of Neveux and Lantoiné (1993), and our under-estimation must be weak. Taking into account the referee comment, we discussed this methodological limitation in the revised paper.

RC: *“It is not correct to state that biosynthetic pathways of some fatty acids are species-specific (page 5), but one could say that the pathways leading to double bond insertion do vary between some algal classes.”*

AC: We agree that the term “species-specific” was improper and we modified the text as proposed by Referee#1. The sentence now states “the pathways leading to double bond insertion do vary between some algal classes”.

Solveig Bourgeois on behalf of all the authors.

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