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Interactive comment on "Organic biomarkers in deep-sea regions affected by bottom trawling: pigments, fatty acids, amino acids and carbohydrates in surface sediments from the La Fonera (Palamós) Canyon, NW Mediterranean Sea" *by* E. Sañé et al.

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Interactive comment on "Organic biomarkers in deep-sea regions affected by bottom trawling: pigments, fatty acids, amino acids and carbohydrates in surface sediments from the La Fonera (Palamós) Canyon, NW Mediterranean Sea" by E. Sañé et al.

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Interactive Discussion

Discussion Paper

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I enjoyed reading this paper; I think it adds to the literature in terms of the effects of habitat disturbance (by fishing) in deep water. I definitely recommend publication and find little of a substantive nature to criticise in the manuscript in terms of the analysis presented.

1. However, I think the paper could be stronger by being more focused. To me it is not clear if the focus of the paper is on fishing impacts on biomarkers or on the utility of biomarkers. I would recommend the former as the latter has been covered in the literature cited in this paper.

REPLY: The main objective of the paper is to study the effects of chronic trawling disturbance, and to reach this objective we had first to analyse the information associated to each biomarker and this is why the referee may find too verbose the description and the discussion of the results for each marker. Furthermore, we believe that the analysis biomarker by biomarker which let us conclude that amino acids is the biomarker group that best identifies differences between trawled and untrawled areas, may be useful in the future for other researchers studying trawling impacts at temperate latitudes. Nonetheless, we agree with the referee that the paper had to be more focused; therefore, we have introduced some changes in the manuscript to highlight the main objective of the work. Also, the conclusions have been updated to make clearer the main point of the paper (organic matter in sediments from trawled areas is degraded in comparison with control sites) (see response to point #14 in this document).

2. I think it is important to be clear right from the start why only measurements in the upper 5 cm of the sediment are presented. Is this simply a sampling issue or related to the expected effects of the disturbance? I suspect this is only a sampling issue and so the focus of this study is really on surface sediment and changes in biomarkers.

REPLY: Since there is no definition of how many centimetres can be considered "surface sediments", we had set that range in the topmost 5 cm. In previous works in which

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Comment

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Interactive Discussion

Discussion Paper

the organic matter present in sediment cores has been characterized, that range has been set in the upper 0.5 cm (Ingalls et al., 2003; García and Thomsen, 2008), 1 cm (Cowie et al., 1992; Grémare et al., 2002; Grémare et al., 2005), 2 cm (Henrichs and Farrington, 1987; Haugen and Lichtentaler, 1991), 3 cm (Henrichs et al., 1984) and, like on our study, 5 cm (Keil et al., 1998; Dalto et al., 2006).

3. Also in the regard, I would add in a line or two indicating what the animals do in the sediment (bioturbation) with respect to mobilising and transforming biomarkers. Given the study focuses on a shrimp fishery, I assume these animals are doing some work on the seafloor.

REPLY: We assume that Aristeus antennatus (the commercial target species) does not impact significantly the seafloor. This deep-sea shrimp is a demersal predator and scavenger, it does not need to make burrows in the sediment to feed. We therefore can safely assume that its capacity to mix or alter surface sediments is very limited compared to burrowing infauna or other decapods that dig tunnels in the seafloor.

4. I am a little disappointed with the referencing in the paper, it would be better to include some of the early work on fishing impacts in soft sediments. I also think citing Collie et al 2000 is inappropriate – this is a case of meta-analysis with no reality check. Simply put how can intertidal cockle populations possibly be the most impacted benthic community by fishing? There are plenty of other reviews that make your point and in fact relate to your study more closely (I have cited the original work in Thrush, S. F. and P. K. Dayton. 2002. Disturbance to marine benthic habitats by trawling and dredging - Implications for marine biodiversity. Annual Review of Ecology and Systematics 33:449-473. And Thrush, S. F. and P. K. Dayton. 2010. What can ecology contribute to ecosystem-based management? Annual Review of Marine Science 2:419-441.) If you really want to cite Collie and colleagues then this group did update that review – I think Kaiser was the senior author, but I do not have the reference.

REPLY: We accept the criticism and have substituted Collie et al. (2000) with the

references proposed by the reviewer. Also, we are now quoting the comprehensive review by Jones (1992).

5. Study design, please specify why the control site was selected, is it not fished as there are no shrimps there? Or simply too far from port (an economic refuge)?

REPLY: To answer why fishermen do not operate in that area selected as control would take some lines of lengthy explanations, however the crucial point is that we know that they are not actively fishing there. Thanks to the Vessel Monitoring System (VMS) datasets (see Puig et al., 2012 for details) now we have accurate information on where trawling is being conducted and where not, and hence, we can safely identify impacted and control sites. To provide the reader more trustworthy information of the coring site selection and its adscription to a trawled or untrawled region, the VMS data are now illustrated in Figure 1.

6. Pull in ancillary information on how similar the habitats are and lack of disturbance indicated in the control area from acoustic imagery if you have it.

REPLY: The evidence of contrasting degrees of disturbance related to the absence or presence of trawling grounds was given in Puig et al. 2012 (quoted in the manuscript), where both acoustic imagery (i.e. multibeam bathymetry) and fisheries data (i.e. VMS) are combined to show that the untrawled areas of the canyon flanks show no signs of disturbance. We have quoted this paper in the manuscript. This information is given in the first paragraph of the methods section.

7. Statistics, I do not understand the choice of procedure here. It seems to me you have a two way ANOVA design (location and time and interaction term) not two one-way ANOVAs. But actually you would be better to use a generalised linear model to tease out site and time differences in a regression based analysis. The uneven sample size is a problem with the ANOVA approach. When the difference in sample size between sites and times is large the only solution if you analyse this way is via randomly dropping replicates from the data set. Looking at the tables I am not sure

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Interactive Discussion

Discussion Paper

this is a major problem but a more gradient based approach would be far better both in terms of the design you have and dealing with normality etc (just use the right error structure).

REPLY: The one-way ANOVA was used hypothesizing no interaction between the effects of trawling and seasonality. Following the suggestions of the two referees, we replaced the two one-way ANOVAs with the two-way ANOVA design. The two-way ANOVA confirmed the absence of interaction between the effects of trawling and seasonality (See Table 2c in the updated manuscript). Also, as suggested by the referee, due to the uneven sample size and also to avoid problems related to normality, the generalized linear model (GLM) has been used, and the results obtained (Table 2e in the updated manuscript) have been compared with those of the two-way ANOVA (Table 2c in the updated manuscript). The GLM confirmed the existence of significant differences between the percentage of Chl-a in spring and autumn samples found both with the one-way ANOVA and with the two-way ANOVA. GLM results are now shown in Table 2e of the updated manuscript and explained in the results section. The one-way ANOVA and the two-way ANOVA found significant differences also between the Chl-a to Pheo-a ratio in spring and autumn samples. Nevertheless, neither spring nor autumn samples showed a normal distribution (Table 2a in the updated manuscript), which is one of the assumptions for performing ANOVA. Therefore, an ANOVA p-value lower than the observed 0.004 would have been preferable to consider the two groups significantly different. As a conclusion, even if the results obtained with the one way ANOVA agree with those obtained with the two-way ANOVA, it is preferable to use the two-way ANOVA instead of the one-way ANOVA to verify the absence of interaction between trawling and seasonality effects. Furthermore, the use of the GLM is preferable than the use of the ANOVAs in general to avoid problems related to the uneven sample size and to normality.

8. I also suggest discussing the multivariate analysis first – this is your strongest result and I think most interesting analysis.

REPLY: Since results are discussed macromolecule by macromolecule (first pigments, then fatty acids, amino acids and finally carbohydrates) and each macromolecule includes univariate and multivariate analyses, we think that it would be confusing to discuss first all the results of the multivariate analysis and then all the results of the univariate analysis.

9. Not all the multivariate analysis discussed in the methods seems to be presented in the results – there is some double up here in the analysis you are doing and its purpose is sometimes lost on me, so I suggest you stick to the important and major findings. This will obviously change the results and discussion.

REPLY: We eliminated the old Fig. 3 that doubled the information presented in the old Fig. 2.

10. Whatever you do in this regard when you do not see a significant difference you can ONLY say you did not detect one and if you want to go further than that (as you currently imply in the Discussion) you need to perform some power analysis.

REPLY: We think we performed the necessary statistical analyses to establish if differences in biomarkers exist between the trawled and the untrawled regions or between spring and autumn seasons. Then, we just interpret the results obtained and made some hypothesis to explain them (for example, we think that the absence of significant differences in PUFA and MC-FA should not be considered because PUFA are too labile and MC-FA too refractory to be used as biomarkers in our study, whereas the lack of significant differences in THAA between seasons can be considered as "real"). As said before, this long analysis biomarker by biomarker, was necessary to establish if bottom trawling has a negative impact on the quality of the organic matter present in the sediment.

11. There are some very long tables in this paper that I would suggest are better placed in appendices.

REPLY: We agree with the referee that part of these tables could be available as appendices to the main paper. The old tables #2, #3, #4 and #5 have been placed in appendices (Appendix A, B, C and D, respectively).

12. Discussion, change the first paragraph to focus on what you found rather than what you did. Assuming this paper is more about results that a methods paper.

REPLY: The first paragraph of the Discussion section has been modified accordingly: "The main objective of this study is to characterize the organic matter (OM) present in sediments from two regions of a submarine canyon affected and unaffected by bottom trawling. We found substantial differences in the quality of the organic matter between these two regions. In particular, the organic matter was more degraded in the trawled than in the untrawled region."

13. In the second paragraph there is a lot of detail on what happens to these macromolecules in the water column, unless the water column is different over impacted vs control sites this is not that relevant. If the oceanography is different then you have a problem defining your controls in a non-confounded way. I would focus this more on what is happening on the seabed and expand on the potential role of different organisms in affecting rates of change in macromolecules. Note also the text in this paragraph gets a bit repetitive.

REPLY: The second paragraph of the Discussion section has been modified to focus on what happens in the sediment column and also to avoid repetitions, and some sentences have been deleted.

14. Finally, the conclusion is a bit weak, this will obviously depend on the focus of the paper but some more general statements about either changes in sediment biogeochemistry induced by fishing disturbance or something on the broader ecosystem effects and potential impacts on sustainability.

REPLY: The Conclusions section has been modified considering the focus of the pa-

Interactive
Comment

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Interactive Discussion

Discussion Paper

per on fishing impacts on biomarkers. The conclusions now read like this: "Bottom trawling in the flanks of La Fonera Canyon has caused an alteration of the quality of the organic matter in surface sediments (upper 5 cm), as it has been evidenced by the distribution of amino acids in the study area, which can be interpreted as the result of sediment reworking caused by repeated bottom trawling in the northern flank and in the inshore southern flank of the canyon. The fact that differences between spring and autumn sediments were detected by the most labile biomarkers, Chl-a and MUFA (percentage contribution to the Average Square Distance between SPR and AUTM: > 4.5%), together with the high percentage contribution to the Average Square Distance between SPR and AUTM for Chl-a and MUFA, and the high percentage contribution to the Average Square Distance between UTR and TR for the majority of amino acids (> 4.7%), suggest that alterations in the quality of the organic matter caused by trawling can be considered relatively high if compared with the effects of seasonality. Nutritional value of sedimentary organic matter is crucial for benthic communities; therefore, the changes produced by repeated trawling might imply profound changes on the ecosystem as a whole."

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Minor points (mostly suggestions on English) As there are no line number sin the manuscript I will copy in enough text that the authors' can search and find the text I am referring to.

1. Pulling heavy fishing gears – change to gear

REPLY: "Gears" has been changed with "gear".

2. Over the last decades, this commercial activity – change to "Commercial fishing"

REPLY: "This commercial activity" has been changed with "commercial fishing".

3. And their surroundings are often targeted – omit and their surroundings can trigger sediment gravity flows – later in the paragraph you say - altering the morphology of the

canyon flanks as a result of chronic reworking – This seems incongruous how can a landslide be a chronic effect (at least on ecological time scales).

REPLY: Trawling has been conducted over the same fishing grounds on a daily basis for decades: this is what we mean by "chronic disturbance". Consider also that the type of sediment gravity flows (not exactly 'landslides') triggered by trawl gears are nor the only, nor probably the most relevant, mechanism affecting the long-term shape and structure of the seafloor. The direct physical reworking of sediment by the action of trawl gears is, we believe, the main mechanism changing the shape of the seascape (see also Puig et al., 2012).

4. The principal factors – change to important factors (organic matter is not necessarily THE most important)

REPLY: "The principal factors" has been changed with "important factors".

5. Organic matter indicators have been already used in other works – change to Organic matter indicators have been already used to address

REPLY: "In other works" has been changed with "to address".

6. A pool of four biomarkers – delete a pool of four.

REPLY: "A pool of four" has been deleted.

7. Indicators of labile material (Lee et al., 2004) - this might be useful here too Mayer, L. M., L. L. Schick, T. Sawyer, C. J. Plante, P. A. Jumars, and R. L. Self. 1995. Bioavailable amino acids in sediments : a biomimetic, kinetics-based approach. Limnology and Oceanography 40:511-520.

REPLY: The work of Mayer and co-authors has been cited.

8. Univariate and multivariate statistical tests were carried out on all the analyzed samples. – delete Univariate statistical analyses were performed with the Statistica software v.5.5. – put at the end of the paragraph

REPLY: "Univariate statistical analyses were performed with the Statistica software v.5.5." has been moved to the end of the paragraph.

9. Multivariate statistical analyses were also performed. In this case, the – Omit. Primer software v. 6 was utilized (Clarke, 1993; Clarke and Gorley, 2006). – move to the end of the paragraph.

REPLY: "In this case, the" has been deleted and "Primer software v. 6 was utilized (Clarke, 1993; Clarke and Gorley, 2006) for multivariate statistical analyses." has been moved to the end of the paragraph.

10. Before multivariate analysis, data were pre-treated by a transformation to down-weight contributions from quantitatively dominant macromolecules – How? What is the transformation?

REPLY: Data were Square or Fourth root transformed. The type of transformation was specified in the old Table 3, but as suggested by the referee, it has been specified also in the Methods section.

11. Distances between couples of samples – here and throughout refer to pairs of samples not couples.

REPLY: "Couples" has been replaced by "pairs".

12. Favour, in fact, the preservation – change to favour the preservation

REPLY: "In fact" has been removed.

13. The sinking velocities – change to the sinking velocity.

REPLY: "Velocities" has been replaced by "velocity".

14. Interrelated factors, like oxygen penetration – change to interrelated factors, including oxygen penetration, bioturbation, sediment density and grain size.

REPLY: "Like" has been replaced by "including". "Among others" has been removed.

Interactive
Comment

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Interactive Discussion

Discussion Paper

15. This agrees with a previous work carried - change to This agrees with previous work carried.

REPLY: "A" has been removed.

16. Nevertheless, the distribution of LC-FA in the study area suggested high quantities of refractory material in spring and in the untrawled region, letting us doubt again about the utility of refractory compounds (i.e. carotenoids, MC-FA and LC-FA) as biomarkers in the study area, also due to the considerable lateral component of particle fluxes (Martín et al., 2006). –Sorry but I am not sure what you are trying to say here. Do you mean: Nevertheless, the distribution of LC-FA in the study area suggested high quantities of refractory material in spring in the untrawled region. This further limits the utility of refractory compounds (i.e.carotenoids, MC-FA and LC-FA) as biomarkers (Martín et al., 2006).

REPLY: Yes, this is the meaning of the sentence and it has been changed accordingly.

17. Whereas MC-FA and LC-FA are too refractory to be used as biomarkers – I am a bit confused by the text here, sediment grain size has previously been used as a marker of habitat disturbance by fishing and this is far more conservative than a biomarker. I think this need to be reconciled.

REPLY: Here we are comparing organic compounds and we say that "MC-FA and LC-FA are too refractory to be used as biomarkers". We are not using sediment grain size in this work, and in any case as a "marker". We have only stated, to give a general background for trawling physical impacts, that trawling may alter the grain size distribution of sediments among other impacts. The reason why MC-FA and LC-FA are too refractory for the present study is explained in the text (sixth paragraph of the discussion).

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Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/9/C9015/2013/bgd-9-C9015-2013-supplement.pdf

Interactive comment on Biogeosciences Discuss., 9, 18601, 2012.

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