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Title: The allochthonous material input in the trophodynamic system of the shelf sediments of the Gulf of Tigullio (Ligurian Sea, NW Mediterranean)

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Abstract: The organic allochthonous material input in the benthic system of a NW Mediterranean shelf area was studied using a three-pronged approach, focusing firstly on the evaluation of the sedimentary stable isotope ratios and organic matter (OM) composition, then on the OM recycling processes performed by the microbial organisms, and finally on the potential trophic relationships between the macrobenthic organisms. The highest allochthonous signal, indicating continental input, was observed within the 50-m isobath, while at the 80-m isobath the marine signal was higher, pointing to a rather low continental influence approximately 5 km from the shore. Heavier rainfall, often generating abrupt allochthonous inputs by river outfalls, led to a wider spread of fine sediment particles. Carbohydrates were the compounds that best represented the continental input and these compounds were associated with potential recycling activities by microbiota, pointing to the entry of these C-containing allochthonous materials into the microbial food web. The macrofaunal deposit-feeders used sedimentary OM characterised by a continental signature as a food source, although the isotopic ratios of the organisms also pointed to selective feeding on materials that had a marine signature, especially at our offshore sampling stations. Predators fed on deposit- or suspension-feeders, with a potential selection of the latter during the highest inputs of continental materials occurring in winter.

Detailed Response to Reviewers

The manuscript has been modified following carefully all the comments and suggestions of the Reviewers. In the Title and elsewhere we changed “diffusion” with “allochthonous inputs”. The Introduction has been expanded, in order to explain the meaning and use of the variables we considered. More information was provided in the Material and Methods on: (i) the Entella river features, adding the average monthly discharge and the rainfall of the study period; (ii) sampling and analytical details previously not reported. The polyphenols have been excluded. The two-end-member model for the isotopic ratios was deleted. The Results of the deeper layer of the sediment were briefly considered and reported as Appendix 1, excluding them from the figures. Statistical analyses were changed as suggested. The Discussion was rearranged, highlighting some comparisons with other researches on small and large-river systems. In paragraph 4.3 the two isotopic ratios were coupled. Result-like sentences have been deleted. The text has been corrected by an English native speaker.

The point-by-point responses to the Reviewers are reported below. Black: Reviewer comment, red: our response/action

Reviewer #1:

Here is my review of the ms MERE-D-16-00132 by Misic et al about the use of several markers to assess the diffusion of terrigenous OM to coastal zones. I have few comments about this ms, which can be suited for publication after considering the minor comments I made below.

My main concern about this paper is about the way authors calculated relative contribution of OM to sediment. I do not succeed to understand the rationale behind the equation proposed by the authors. In addition, I would suggest another design for their model, taking into account that sediment OM can come from terrigenous, marine pelagic or benthic primary production. And I would also suggest using a recently developed model, which may allow integrating variabilities in SI values, and also considering C and N isotope to calculate the contribution.

The paragraph related to the calculation of the terrigenous contribution via-mixing model has been deleted

Regarding the low isotopic value measured for marine POM, did you have any information about the composition of marine phytoplankton locally? Rau et al. (1990) observed very low $\delta^{13}\text{C}$ values for phytoplankton sampled in Villefranche sur Mer bay, ie not so far from your sampling site, and pico/nanophytoplankton is classical in oligotrophic waters. Since authors describe their sampling site as oligotrophic, it may be another potential explanation of low isotopic ratios.

Rau et al. 1990 $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. Mar Ecol Prog Ser 59: 33-38

This observation has been added (page 11).

The introduction of the paper is rather short despite there is a large body of work about the understanding of the trophic couplings between marine and terrestrial systems, and about the influence of terrigenous inputs on coastal ecosystems functioning. I would suggest increasing the length of the intro by providing more details about this topic. Information on the meaning of the used variables was added and also, shortly, on the relevance of small river systems in the transfer of allochthonous materials to the sea (pages 3-4, first and third paragraphs of section 1).

P6L49: even if this "simple" equation may be useful, I would suggest the authors to use some more recently developed models, like Bayesian SIAR, since this tool would allow providing information about the variability of contribution of each source. In addition, it would allow increasing the accuracy, by calculating the contribution based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

The paragraph related to the calculation of the terrigenous contribution via-mixing model has been deleted

Regarding the figures, they appear blurred in the ms, and may benefit from some improvements. By example, the sites where river and marine POM were sampled is not really clear in the map, the arrows are hard to find. Using another symbol may be needed.

The arrows have been enlarged and coloured to be easily seen.

Similarly, the legends of y-axes in fig3 are blurred. Please consider improving the quality of these figures.

Figures have been redrawn.

Highlights and graphical abstract are missing and should be provided.

Highlights have been provided.

I'm also quite disappointed that the large, complex and potentially time-consuming work made to identify all individuals at species level was underused. Such large taxonomical analyses are not so common, such a dataset should be better exploited.

The manuscript was rather long and complex to add other detailed observations. The macrofaunal complete data set was provided in order to give a general idea of the kind of organisms we found and chose for the isotopic analyses. Anyway, a more detailed study on the macrofaunal communities has been planned, using also other data collected previously in this area as a temporal comparison.

Detailed minor comments:

P3L58: collecting water from (and not form) **corrected**

P4L54-60: Was the sediment sample dedicated to isotope analyses stored in formalin? This would be a major bias, so I'm pretty sure this was not done, but the way the sentence is written may let it think. Would you mind modifying this sentence to avoid misunderstanding? **corrected**

P5L20: "silt and clay and sand". The way this sentence is written does not allow separating the two groups. I would suggest writing "[...] silt and sand (<0.063 mm), and clay (>0.063 mm)[...]" **corrected**

P5L53: Were the sediment samples freeze-dried? **No, they weren't**

P6L28 (and potentially elsewhere): please amend the writing of polychaetes, using a y and not an i **corrected**

P7L5: Newman-Keuls (and not Kneuls) **corrected**

P10L10: please define what EF is. This is classical in isotopic ecology but it may be useful to define it more clearly for non-isotopist readers. **A definition has been added (page 10, fourth paragraph of section 3.4).**

P12L21 (and in reference section): the correct writing of author's name is Adin (Adin and Riera's reference) **corrected**
But I'm quite concerned that authors do not cite here a Mediterranean study whereas numerous papers used stable isotope ratios to assess the trophic role of *P. oceanica* (see by examples papers by Alcoverro, Lepoint, Deudero, Vizzini, Prado etc.). I would also suggest the paper of Papadimitriou et al, which used SI to track the contribution of *P. oceanica* to sediment OM

Papadimitriou et al. 2005. Sources of organic matter in seagrass-colonized sediments: A stable isotope study of the silt and clay fraction from *Posidonia oceanica* meadows in the western Mediterranean. *Organic Geochemistry* 36: 949-961
Some of these references have been added (page 13, eighth paragraph of section 4.1).

P12L60: Darnaude (final e is missing) **corrected**

P14L28: Gulf of Lions (and not Lyons) **corrected**

Reviewer #2:

This paper presents an original study on the transfer of terrestrial organic matter in multitrophic compartments of the Ligurian Sea shelf sediments. The aim of the study is assessed using a multitracer approach including carbohydrates, lipids, chlorophyll a, organic carbon, total nitrogen and natural stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$). However, some of the analyzed biomarkers such as polyphenols and proteins are not included in the discussion. This comment is also relevant for 2-10cm sediment layer which was included everywhere in the manuscript except in the discussion. Such inclusions burden the manuscript, which also suffer from spelling, vocabulary, grammatical and statistical errors.

See detailed responses below

However, the paper provide interesting information on the impact of terrestrial organic matter on the benthic food web of such small rivers that would need to be compared to other similar small systems to expand the scope of this manuscript.

Some comparisons with other small river systems have been provided (for instance page 3, paragraph 1 of section 1; pages 11 and 12, paragraphs 3 and 4 of section 4.1)

Title

P. 1: Are we really dealing with a diffusion process of terrigenous material? I'm not sure were discussing of such a fine process of material transfer. I will also suggest to reformulate the end of the title as follow: "[...]of the Tigullio Golf shelf sediments (Ligurian Sea, NW Mediterranean)" **corrected, we used the generic term "input" to avoid reference to the diffusion processes.**

Abstract

P. 2, L. 5: please modify "stable isotopes ratios [...]" **corrected**

P. 2, L. 6: replace organic matter "characteristics" by "composition", also avoid excessive use of "the" everywhere in the MS **corrected, a native English speaker corrected the whole text.**

P. 2, L. 7: please define what "trophic features" belongs to
The sentence has been rephrased in order to be more clear.

P. 2, L. 8: "60%" Include a space between a numerical value and its corresponding unit of measurement according to the ISO 31-0 prescription, or at least be consistent throughout the manuscript ex: "30 %" (P. 2, L. 10) **corrected, all the numbers and units have now the same format**

P. 2, L. 12: Delete the sentence "Seasonal variations occurred", there is no construction in this sentence, and the MS is not dealing with seasonal variations has no temporal replicates have been done (see comment below) **corrected**

P. 2, L. 14: Please do not consider carbohydrates as an "organic material". Actually, carbohydrates are compounds of organic matter (OM) and must be considered in this study as a tracer of OM transfer from rivers. Relevant elsewhere in the MS and for other tracers such as lipids **corrected**

P. 2, L. 19: It's not appropriate to said "trophic web", prefer the use of "food web". **corrected**

Also, please define the "connection" between macrofauna and sedimentary material.

P. 2, L. 21: "by a certain marine signature", please be more specific
The sentences have been rephrased in order to be more clear.

p. 2, L. 23-25: please reformulate the sentence as follow: "Predators fed on deposit- or suspension-feeders, with a potential selection of the latter during highest inputs of terrigenous materials." **corrected**

Also please specify in which period "highest inputs of terrigenous materials" occurred.

Unfortunately an up-to-date measure of the daily flow of the Entella R is not available. Technical papers published by the local agencies for environmental management reports monthly-averaged data that are based on observations collected from approximately 1950 to 2000. We provided in Figure 2A these data (and the on-line reference) together with the respective averaged rainfall data. Anyway, data on the monthly-averaged rainfall for 2012 and 2013 have been found and provided in Figure 2B.

Introduction

P. 3, L. 1: replace "coastal sites" by "coastal areas" **corrected**

P. 3, L. 3-5: Does all these references mandatory to describe geo-sedimentological and bio-ecological studies? **Deleted.**

P. 3, L. 10: please rephrase the sentence as follow: "At coastal areas, phytoplankton production [...]" **corrected**

P. 3, L. 17-19: First remove one space before "In oligotrophic conditions", then specify what "foraging the ecosystem" belongs to. I assume you try to say that in such ecosystems, allochthonous inputs play a higher role in nutrient supply. Then please rephrase the sentence, as the use of "foraging" is not appropriate in that context. **corrected**

P. 3, L. 21: Please define how "Allochthonous substances ?enter? in biogeochemical processes"? I would say "Biogeochemical processes drive allochthonous material accumulation into detritus and newly produced biomass"? **corrected**

P. 3, L. 28-30: Please rephrase the sentence, also what "deeper layers of the sea" means? "deep waters"?, what "potential environmental anomalies" are? Please, this is not science fiction. **Corrected with "deep waters"**

P. 3, L. 33-36: Please rewrite the sentence as follow: "Different scales and ecological processes were considered." **corrected**

P. 3, L. 37-39: remove "major input", "seasonal conditions" and reformulate the sentence **corrected**

P. 3, L. 39-43: Delete and rephrase the entire sentence, you're focusing on organic tracers to characterize allochthonous inputs in the microbial food web. **corrected**

Material and methods

P. 3, L. 55: replace "torrents" by "rivers" and elsewhere in the MS, same comment for the "Golfo del Tigullio" into "Tigullio Golf" **corrected**

P. 3, L. 55-59: Correct the sentence as follow: "The Entella R. [...] tributaries, has a total of [...], with a catchment area of 372 km2 [...]" **corrected**

P. 4, L. 1: Start a new sentence with: "However, in recent years, overflow occurred every autumn, [...]" **corrected**

P. 4, L. 3: replace "take place" by "occurs", also replace "adds up" by "account" similar comment in this section **corrected**

P. 4, L. 9: who have reported "hydrocarbons or sewage"? please provide a reference
Local newspapers and personal observations, added.

P. 4, L. 10: replace the sentence as follow "The Boate River is characterized by a smaller catchment area (32.2 km2), [...] cross the Rappalo Town and pours [...]" **corrected**

P. 4, L. 23: Same comment as above, "Several towns along the sea-shore, account for over 65 000 [...]" **corrected**

P. 4, L. 25: I don't catch what you mean by "upcurrent respect to the sampling stations"? **deleted**

P. 4, L. 28-30: move "small patches" before "Posidonia oceanica", and replace "dimension" by "surface area" or synonym **corrected**

P. 4, L. 37-43: what do you mean by "to the area of the Entella R."? Also as indicated above, you're not doing a seasonal comparison, but a temporal comparison; replace therefore "The two seasons" by "The two periods" and elsewhere in the MS. **corrected**

Provide a reference also for the increased continental inputs in November 2012.

Figure 2B provide the data on the heavy rainfalls of that period, and the Regional Agency for the Protection of the Environment (on-line reference provided) reported these anomalous meteorological events. No direct influence on the quantity of continental input may be quantified, but the short river course and its mountainous nature make a sudden input of freshwaters to the sea rather likely.

P. 4, L. 46: Why only one station for shallow depth? I assume that you wanted to focus on the mouth of the Entella R., but why then you include the Boat R. in your study without sampling at its mouth? This raise question on the robustness of the data analysis, as for example, when you make spatial comparison, you only compare one station (A1, with three samples replicates) with 3 stations (A2, B2, C2, with three samples replicates, or similarly for A3, B3, C3).

This comparison has been deleted, due to the limited shallow-depth data. Previous studies (see for instance Albertelli et al., 1999) pointed to the Entella R. as the sole freshwater input to the continental shelf area. The other torrents are so small and irregular that they are considered only for their very local influence on human activities when exceptional rainfall occurs. The sampling for Boate water was thought in order to have a comparison for the terrestrial end-member for the mixing model, but we deleted this part.

My concern on data analysis and sampling strategy is reinforced by the occurrence of the fish farm that may induce variability in OM composition according to water currents in the golf.

Previous studies (see Doglioli et al., 2004) found that a long-distance transport of particulate OM from the fish farm was not likely (page 13, last paragraph of section 4.1).

P. 4, L. 52-53: Correct the sentence as follow: "Four replicates were sieved at 0.5 mm mesh, in order to retrieve macrofaunal organisms." **corrected**

P. 4, L. 58-60: This was not the first occurrence of "organic matter" in the MS, so pleased provide the corresponding abbreviation to its first occurrence. **corrected**

Also following my above comment, I would suggest to remove the 2-10 cm layer from the entire MS
The information on the 2-10 cm layer has been provided as appendix 2, the figures have been changed. Only few sentences were maintained to point to the higher variability of the 0-2 cm layer than the deeper layer (page 9, last paragraph of section 3.3).

P. 5, L. 1: For $\delta^{13}C$, you should use the words "composition" or "signature" instead of "value" corrected

P. 5, L. 3: replace "before" by "preceding" corrected

P. 5, L. 7-9: rephrase the sentence as follow: "[...] during sampling campaigns, at approximately 1.5 km upstream of the Entella R. mouth and 1.2 km upstream for the Boate R.." corrected

P. 5, L. 10-12: remove the line break before this last sentence. Also find an alternative to "treated" e.g. "prepared", "analyzed"... corrected

P. 5, L. 16: as commented above, replace "treatment" by "preparation" corrected

P. 5, L. 19-20: and elsewhere in the manuscript reformulate the two fraction for more clarity: "silt-clay and sand" corrected

P. 5, L. 23: replace "placing" by "adding" corrected

P. 5, L. 42-48: cut out the sentence, indicating first that "Boiled sediments were used as a blank [...]" and then that "Samples and controls (blanks) were incubated [...]" corrected

P. 5, L. 53-57: move the first sentence after the second modified as follow: "The main OM biochemical fractions of sediments were determined following colorimetric methods. These analyses were made on sediments stored at -20°C." corrected

P. 5, L. 58: please provide a reference for the "Lowry method" if proteins have to be kept in the MS It was an error of "cut and paste", proteins are not reported in the manuscript.

P. 6, L. 30-32: Rephrase the sentence as follow: "Glyceridae and Nephtyidae polychaete families were selected as predators." corrected

P. 6, L. 35-40: this is the elemental analyzer that is connected to the mass spectrometer not the opposite otherwise the results are nonsense.

The method has been corrected, adding some specific information that was not present before.

If your sediment samples are ^{13}C enriched compared to your marine end-member, then it means that your were not far enough from the influence of your river. Other possibilities include a contamination through input from the fish farm or reprocessing of carbon through the microbial loop. Also, some recent paper (Schaal et al. 2016) indicates that reaching some depth (80-150m) signature of sediments and bottom waters become very similar.

The hypothesis of the influence of the river has been proposed. As previous reported, Doglioli et al. (2004) modelised the potential influence of the fish farm on the environment, joining hydrodynamic model application and in-situ observations. The authors concluded that the dissolved materials could be spread in the entire Gulf, depending on wind dominance and current direction. The particulate fraction, instead, settled next to the farm. A transfer of particulate materials to the sediment of our stations is, therefore, not likely. The sampling for marine POM was performed at ca. 15 m above the bottom, therefore this was certainly a "deep" water but not exactly a "bottom" water.

The last point is that particulate OM is used to estimate the contribution of terrestrial organic matter to marine sediments. However, POM will undergo degradation process while settling, which suggest the occurrence of an enrichment factor between POM and sediment that is not included in your model. For all these reasons, I will suggest extreme caution while using such model for terrestrial OM contribution to sediment.

The paragraphs on the calculation of the terrigenous contribution via-mixing model have been deleted

P. 7, L. 3: "One-way Anova", where are the assumptions for normality and homoscedasticity? I assume that given your sampling effort, there will be no normality in sample distribution which avoid the use of One-Way Anova without transformation

Data have been normalised.

P. 7, L. 10-16: SIMPER analysis is definitely not appropriate to analyze such variables that are not consistent. SIMPER, ANOSIM, Cluster, nMDS are analyze realized with PRIMER software that need a table where the sum of all variables will be 100%. For example the macrofaunal composition of your sediment, with species as variable can be considered with such statistical tools. You can't realize a SIMPER on a same table that include, bacterial abundances, chlorophyll, carbohydrates... At least you may eventually be able to realize a PCA to see whether these variables are correlated and how they explain your variability.

The SIMPER analysis has been deleted, as suggested we propose a PCA for the sedimentary OM variables (macrofauna excluded, it has a cluster on its own) and the significant correlations between variables.

P. 7, L. 25: "values (close to 0), little or no separation." This is true but more appropriate to say that below R of 0.3, intragroup variability is high, meaning high heterogeneity within your groups.

corrected

Results

In all this section, you can remove the word "significantly" as you provide the corresponding ($p < 0.05$) corrected

P. 7, L. 44-50: Here comes my concern on spatial comparison, and elsewhere in the MS. I assume that you can't compare the shallower station A1 to other depths, as you only have one station for the shallower depth with pseudo-replicates in it.

We deleted the statistical values when the shallow-depth station was concerned, leaving only a generic description of the fact that the coarser grain size, as generally observed in marine systems, often leads to lower OM contents.

P. 7, L. 44-48: Also prefer the use of "concentrations" instead of "values" and the word "composition" instead of "content" corrected

P. 7, L. 60: "Some variations [...], except for some variables [...]" please be more specific. However, this provides information on the 2-10 cm sediment layer so just delete it.

The 2-10 cm data were removed from the text and presented in Appendix 1. Few lines allowed us to point to the fact that the main variations were confined within the surface sediment layer and justified the focus on this layer.

P. 8, L. 3: why some time the use of "OC/TN ratio" and some time the "TN/OC ratio"? this provide confusion in the MS. Also what is a "trophic quality"? Please define that concept I'm not familiar with although I'm a trophic ecologist.

Corrected, now the used ratio is only OC/TN. The use of the other ratio depended only on the comparison we made with other studies in order to highlight possible sources of OM.

P. 8, L. 14: please rephrase this sentence for a better clarity. Also you present higher "values" of what?

corrected

P. 8, L. 14-25: In this section, you says both that there are higher Chl a in shallow depth stations without differences in the deeper ones, and that chl a is higher in deeper station without difference in the shallow one... do you mean in the different sediment layers? Are you doing a seasonal comparison? I'm lost.

The paragraph was shortened and simplified.

P. 8, L. 33: replace "ant" by "and" corrected

P. 8, L. 35-40: how do you characterize such "strong" differences? "with the season" what this mean? Also, you mention a three-fold and six-fold increase between depth, reaching similar "values" in the entire study area? Again I'm lost

Strong was changed with significant. The paragraph was shortened and simplified.

P. 8, L. 42: "BG and LA activity" please specify the acronym correspondence at their first occurrence (in the material and method) **corrected**

P. 8, L. 50-53: delete this section according to my previous comment on SIMPER utilization
deleted

P. 8, L. 58: do not start a sentence by "Fig. 5 [...]" or an acronym. Also what are isotopic "features" or "features" of 4 possible OM? Please be more specific or define them.
The paragraph has been rephrased.

P. 9, L. 1: from where come the "possible OM sources"? From "Tesi et al"?
Yes

P. 9, L. 5: "particular position", again please be more specific **deleted**

P. 9, L. 7: not necessarily needed to say that "marine algae" are "not derived from continental inputs" **deleted**

P. 9, L. 10: "Station A1 showed variable results" useless **deleted**

P. 9, L. 17-34: not sure if appropriate, see my previous comment on mixing model
The paragraphs on the calculation of the terrigenous contribution via-mixing model have been deleted

P. 9, L. 53-55: "different composition of the assemblages." Please be more specific
Rephrased

P. 9, L. 39 to P. 10, L. 5: are we dealing with proportion, density, taxa? I'm lost again...
We started describing the total density of organisms for the different depths, then we gave information on the composition. The cluster analysis showed that the species/taxa that composed the assemblages divided the stations depending on depth and period.

P. 10, L. 12-18: although I often use ANOSIM and knows that such results are hard to present, I'm confused and can't follow the sentence, please consider reformulating this last sentence.
Reformulated

P. 10, L. 19: "From the trophic point of view" again a meaningless concept **deleted**

P. 10, L. 21: We went from "suspension-deposit-feeder" in the methods section to "mixed-strategy deposit-suspension feeder" in the result section. Please, be more consistent throughout the MS. **corrected**

P. 10, L. 21-25: Does these contribution results come from a SIMPER analysis? Then it may be appropriate in this case but need to be specified. **No, it wasn't. These were only the contribution of each taxa to the total density (100%)**

P. 10, L. 33: $\delta^{13}C$ is commonly used to distinguish food sources within a food web instead of trophic strategy.
corrected

Discussion

P. 10, L. 44: please provide a reference for the "climatic reports" **the reference was added, for this specific area it belongs to public documents provided by the local government (Provincia of the city of Genova, the link was provided)**

P. 10, L. 57-58: "could have the high-outflow features" please rephrase it **deleted**

P. 11, L. 1: please provide a reference for this proxy **provided**

P. 11, L. 2: replace "-30%" by "-30‰" **corrected**

P. 11, L. 5-9: What about the impact of the fish farm? Do you have any information on fish feed $\delta^{13}C$ signature or POM composition outflowing from the farm?

Unfortunately, no information on the isotopic ratios of the POM coming from the farm is now available.

P. 11, L. 12-29: this is the most interesting part of the MS, I would suggest going further on residence time and diagenesis of terrigenous detritus.

This is certainly interesting, but it would increase the length of the manuscript and need more specific sampling and analytical approaches.

P. 11, L. 26-26: I don't see whether these references to a Japanese estuary or the Arenas Bay, are mandatory for the MS and whether they are useful for a comparison with the present study.

deleted

P. 11, L. 43: what do you mean by "Carbonaceous OM"? High carbon concentration? Also, the justification provided between brackets was previously provided in the result section

deleted

P. 11, L. 30-51: given the oligotrophic regime of the Ligurian sea, are there any references on phytoplankton blooms at the vicinity of estuaries that will very quickly use these allochthonous nutrient inputs from rivers?

We added a reference

P. 11, L. 60: "Several environmental factors [...], starting from the higher winter hydrodynamism [...]" what are the other environmental factors? I don't catch them later in the MS.

The paragraph has been changed to be clearer, the hydrodynamic forcing and the surrounding seagrasses, urban pipes have been suggested to contribute to the peculiar isotopic ratios of the winter A1 station.

P. 12, L. 33: Actually, carbohydrates are not a type of OM, but a compound of OM. Same comment later in the MS on lipids, which are not used as a distinct food source. corrected

P. 12, L. 38-42: "The carbohydrates we detected, in fact, contain a large part of polymers [...]" this is a general feature of carbohydrates and you didn't go deeply in details to characterize the polymers variability of your samples. Also please consider reformulating the end of this sentence that is not understandable.

The paragraph has been shortened and sentences made clearer.

P. 12, L. 48: "photoautotrophic" why such specification at this point? Are you discussing chemoautotrophic biomass elsewhere?

deleted

P. 12, L. 48-49: "stronger pelagic-benthic coupling" this is speculation; you don't have enough data to conclude on this point.

deleted

P. 12, L. 53-55: Similar comment for carbohydrates. These compounds are highly refractory, and not responsible for OM accumulation in the sediment.

deleted

P. 13, L. 2: Avoid the use of the word "reduce" except to discuss of oxydo-reduction processes. Also how lipids can be diminished to only indicating anthropogenic influence due to pollutants such as hydrocarbons? At least alkane or hydrocarbons themselves can provide such information, but lipids are common constituent of all dead and living organisms.

deleted

P. 13, L. 9-10: Again, Lipids are not a trophic resource, also caution on repetition: "resource less influenced by anthropogenic influence."

deleted

P. 13, L. 16: replace "types" by "tracers" or "compounds" corrected

P. 13, L. 23: please define what are the "trophic conditions"

Changed with "lower quality of OM"

P. 13, L. 23: "Anyway, it didn't mean" please avoid the use of such colloquial language **corrected**

P. 13, L. 26: please provide a reference on "hydrolytic enzymes"
provided

P. 13, L. 34: "a certain trophic value" please be more specific and define this concept if not previously asked
Changed with "semi-labile"

P. 13, L. 39: "position" do you mean "depth"? **corrected**

P. 13, L. 48-49: what do you mean by "build up metabolic structures devoted to carbohydrates degradation"? I thought it was hydrolytic enzymes? Also I don't understand what you mean by "forage with energy"?
deleted

P. 13, L. 50-51: "The energy [...] would help." Meaningless **deleted**

P. 13, L. 51-57: this is full speculation. **deleted**

P. 14, L. 1-7: First we lost shallow depth stations and then C2 macrofauna(Methods section). Now we lost winter sampling. What are we going to lose next?

P. 14, L. 9-14: taxa tolerant to "high" organic load are found in stations with high OM content... Is this a discussion? I don't even see any references. Please rewrite this section.

Deleted, one sentence was linked to another consideration.

P. 14, L. 23: how far is the difference between Darnaude et al. and Martinetto et al. enrichment factors? Also enrichment factors acronym EF was previously provided in the result section. **corrected**

P. 14, L. 30: remove the line break before "However" **corrected**

P. 14, L. 30-37: this belongs to a result section, not to a discussion
deleted, the observation was briefly discussed.

P. 14, L. 48: "anomalously enriched d15N" may also be associated to anthropogenic (terrestrial derived material) nitrogen rich effluents (see Riera et al. 2000).
deleted

P. 15, L. 1-35: I don't understand why d13c and d15N stable isotopes are not considered jointly in this study. Indeed, d15N provide information on the trophic position and d13C on the carbon source. The discussion provided here appears relatively limited in the end of the MS due to such decoupling between both stable isotopes.

We simplified the discussion and couple the information coming from the two isotopic ratios.

Tables

Table 2: delete this table that is not appropriate **deleted**

Figures

Fig. 3 and 4: please remove the 2-10cm layer and increase the font size **corrected**

Reviewer #3:

General overview

The ms of Misic et al deals with the diffusion of terrigenous material in the sediment and macrofauna in a small coastal area of the Ligurian Sea in the North-Western Italy. The ms is very clear and easy to read. The approach that was used (combining stable isotopes, markers and measures of activity) is sound and interesting. Consequently, the overall data set is of interest.

However, there are some issues that authors should solve before the ms can be acceptable for publication. Especially, 1) there are many lacks of information in Section Material and Method and I wonder if the methods were correct enough for getting correct data sets for some of the parameters, 2) description of the results are not always in full agreement with figures, 3) interpretation of the sediment data sets is not deep enough. I have other comments that are detailed below, in addition to the above.

Thus, I recommend major revision.

See detailed responses below

Detailed comments

C/N versus N/C ratio

Throughout the manuscript, authors present the elemental ratio either as C/N or N/C ratio. This is a bit confusing for the reader. Even if these ratios have not exactly the same meaning (C/N ratio is a proxy of TN quality whereas N/C ratio is a proxy of OC quality: see the great article of Perdue and Koprivnjak, 2007), authors should prefer to present and discuss only one of the ratios. Since values of C/N ratio are more well-known by a broad scientific community and since the use of the one or the other one ratio does not have deep implication on the discussion and interpretation of the data sets, I would encourage authors to use the C/N ratio.

We used the C/N ratio as suggested.

Description/interpretation of the results

In many occurrences, the description or interpretation of the results is erroneous and many sentences should be reworded.

- Section 3.1, first sentence: reword in order to indicate that the sand fraction decrease from to the deepest stations and then to the mid-depth stations (Fig. 2).

reworded

- Section 3.2, last sentence of the first paragraph ("no variation was instead found for the shallow-depth station"): this is false since there was a huge difference in chlorophyll a content between summer and winter in the 0-2cm layer.

The reviewer is right, it was a mistake, corrected

- Section 3.2, first sentence of the second paragraph ("the bacterial abundance did not vary with season"): there are huge seasonal variations depending on station and layers!

It was referred to the shallow-depth station, we corrected the sentence

- Page 12, second sentence ("The shallow depth allowed the autochthonous primary production, for instance benthic diatoms with high $\delta^{13}\text{C}$ as shown by the significant accumulation of photoautotrophic biomass (chlorophyll-a)"): this is correct for summer but does not stand for winter season.

The sentences have been reworked, a change of the phyto-benthic community due to the allochthonous inputs, that are known to sustain irregular blooms in Liguria, was suggested as an event that potentially may increase the $\delta^{13}\text{C}$ values.

- Page 14, last sentence of the fifth paragraph ("The deposit feeders of the shallow-depth station, in particular, changed notably their seasonal isotopic values depending on the previously reported variations of the sediment"): this is valid for $\delta^{15}\text{N}$ but does not stand for $\delta^{13}\text{C}$.

It was reported in the part related to $\delta^{15}\text{N}$, indicator of trophic level, while the $\delta^{13}\text{C}$ indicates the C source

- Page 14, sixth paragraph ("This may explain the anomalously enriched $\delta^{15}\text{N}$ values for sediment and deposit feeders during winter at the shallow-depth station, when the bacterial abundance was the highest"): why not, but in contrast, bacterial abundance was similarly high in summer whereas $\delta^{15}\text{N}$ of sediments and deposit feeders was low. Consequently the explanation does not stand.

Deleted

Introduction: description of the tools is missing

Author should add a paragraph dedicated to the tools that are used in the study (C and N elemental and isotopic ratios, lipids, carbohydrates, polyphenols, LA, BG, etc.): why these tools are used? How can they be interpreted? What are the messages that they can deliver in the context of this kind of study? etc. Such a brief state-of-the-art would help the reader to understand the results and their discussion. Indeed, future readers are probably not all very well-used with all these tools. In the present version of the manuscript, some information appears in the results (page 8, lines 3-7). This sentence should be removed from section Results and placed in the introduction.

We added a paragraph summarising the meaning of the tools we used (pages 3-4 , third paragraph of section 1).

Material and methods: additional figure needed

A figure showing the mean daily flow of the two main torrents with the indication of the sampling dates is needed in order to contextualize the sampling periods in the seasonal variation of terrestrial inputs. It is also needed for the discussion (section 4.1, page 10, lines 57-59).

Unfortunately an up-to-date measure of the daily flow of these two torrents is not available. Technical papers published by the local agencies for environmental management reports monthly-averaged data only for the Entella river, based on observations collected from approximately 1950 to 2000. We provided these data (and the on-line reference) together with the respective averaged rainfall data. Anyway, data on the monthly-averaged rainfall for 2012 and 2013 have been found and provided in Fig. 2. The increase of rainfall of November 2012 is rather clear.

Material and methods: lack of information The required information has been added. We highlighted that the chlorophyll-a data have been not corrected for the water content of the sediment, therefore the reader is alerted of the qualitative nature of such results.

Much information is lacking in this section. Details regarding sampling, processing and storage procedures are needed for getting a critical overview of the following results. Here is a list of the lacks:

- Page 4, lines 52-59: were these processings performed on board? **added**
- What was the delay between sampling and lab-processing and how the samples (water and sediment) were stored from the field to the lab? **added**
- Water samples: how particles were recovered? Using centrifugation (not optimal)? Using filtration? If the latter, what filters were used and how the filters were processed before the filtration? How the filters were stored before processing for analysis? **added**
- Sediment texture: how many time after sampling were samples processed? **added**
- Chlorophyll: how many time after sampling were samples processed? Was the extraction performed on dry or wet sediment (I guess wet sediment)? If dry sediments, how were they dried (centrifugation versus oven-drying versus freeze-drying)? If wet sediment, how the water content has been taking into account for 1) the dilution of the acetone/water solution (note that the efficiency of the extraction varies depending on the acetone:water ratio), and 2) reporting the value in $\mu\text{g/g}$ units (i.e. is it reported against dry or wet weight of sediment)? I am afraid that the chlorophyll was not correctly analyzed. **Added and specified**
- Bacterial counts and hydrolytic enzymatic activities: centrifuged, dry or wet sediment was used? What quantity of sediment was used? Are the values reported against wet or dry weight of sediment? **added**
- Sediment stored at -20°C : were sediment dried? If yes, how? Are the values reported against wet or dry weight of sediment? **added**
- Water samples for elemental and isotopic analysis: were samples decarbonated before $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ analyses? If yes, how? **added**
- Macrofaunal samples: was the stomach content removed before analysis? If yes, how? Were samples decarbonated before $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ analyses? If yes, how? **added**
- Why the mass spec was calibrated against gas of high $\delta^{13}\text{C}$ and not against solid standard of values similar to sample values? How the mass spec was calibrated for $\delta^{15}\text{N}$? **added**
- Page 6, line 44: PDB is the reference for $\delta^{13}\text{C}$ only (and not $\delta^{15}\text{N}$. Add information regarding $\delta^{15}\text{N}$). **added**
- Why station C2 was not analyzed for sediment isotopes? **added**
- Have a look at Coplen (2011) for reporting delta values in figures and equations.

Results: additional table needed

Numerous correlations are reported in this section. A table reporting significant and non significant correlations between the tested parameters is needed. **We reported the significant correlations in Table 2.**

Results- section 3.3: choice of the values for running the mixing model

The $\delta^{13}\text{C}$ value used as the marine end-member comes from Darnaude et al (2004). This value comes from samples performed in the gulf of Lion, in the vicinity of the Frioul Island. I wonder if this value is suitable as marine

end-member for the study area of the present ms (Gulf of Tigullio, Ligurian Sea). Indeed, when comparing $\delta^{13}\text{C}$ values performed since some years near the Frioul Island and at the outer end of the Bay of Villefranche (eastern Mediterranean French coast; western boundary of the Ligurian Sea), there is 1‰ difference: -22.4‰ near Frioul versus -23.4‰ in the outer Bay of Villefranche (data sets can be seen at and even retrieved from the SOMLIT web site: <http://somlit.epoc.u-bordeaux1.fr/fr/>). Thus, I think that the value of -22.4‰ is not useful for the present study. I encourage authors to find a better value from the literature (I would be very surprised if no data from the literature would be useful) or to use the Somlit data.

The part on the calculation of the terrigenous contribution via-mixing model has been deleted

Enrichment factor (EF)

Since it is not demonstrated in the ms what consumers uses what resource, the term "EF" should be replaced by "potential EF" or "apparent EF" throughout the ms. Added and corrected (page 10, paragraph 4 of section 3.4).

Use of the word "depleted"

In the discussion, the term "depleted" is used in many occurrences. This term alone does not stand. The terms " ^{13}C -depleted" and " ^{15}N -depleted" should be preferred. See Coplen (2011) for the correct use of the terminology associated to stable isotopes. The terms "lower $\delta^{13}\text{C}/\delta^{15}\text{N}$ " may also be used since they are easier to understand for readers that are not well-used with these proxies.

Corrected

Section 4.1 - interpretation of sediment results

The interpretation of the data sets regarding sediment origin and the diffusion of the terrigenous material is not always convincing, especially regarding station A1. First, it should be noted that station A1 exhibited the highest sand fraction and the lowest %OC and %TN. Thus, organic matter deposition is the lowest at this station.

As it is stated by the authors, one may a priori consider that station A1 is the station the more influenced by terrestrial material since it is located the closest to the Entella mouth. However, this is not the case because %OC and %TN are the lowest and because N/C ratios are almost the highest. Also, it should be noted that river N/C ratio of the river POM are high, indicated that river POM may not be dominated by terrestrial POM but may also have large fractions of river phytoplankton and heterotrophic bacteria. Consequently, the terrestrial origin of sediment at mid-depth stations may come from other river than the local torrents. What about a large river located in the south of the study area (e.g. the Arno River?)?

Specific answers below

Authors should deeply re-interpret the sediment data sets considering that

- Station A1 exhibited the lowest %OC and %TN and thus may receive the lowest OM load (is there a local current that may flush the OM from this station? Is there man-managed processes like sand deposition on the shore line and beaches that may increase the sand fraction at this station?)

Actually our summer values pointed to a significant allochthonous input at station A1, while winter was the anomaly. We suggested some reasons for this. The text has been reorganised. The presence of local currents is possible, but the sediment texture points to a continuous influence and doesn't explain the differences between summer and winter. The shore line management, instead, was not reported by the local agencies.

- Terrigenous material may come from a southern large river (is there along shore currents from the South to the North in the Ligurian Sea?)

Larger rivers may be found southward (Arno, for instance) but they are rather far to be more relevant than Entella river. Previous papers (Vignudelli et al., 2004 for instance) showed that the Arno influence is limited to the coastal waters of Tuscany.

- There is a fish farm in the study area. Could part of the sediment organic matter come from this farm?

A previous study (Doglioli et al., 2004) modelised the potential influence of the fish farm on the environment, joining hydrodynamic model application and in situ observations. The authors concluded that the dissolved materials could be spread in the entire Gulf, depending on wind dominance and current direction. The particulate fraction, instead, settled next to the farm. A transfer of particulate materials to the sediment of our stations is, therefore, not likely.

Another comment regarding the interpretation of A1 data:

- Page 12, the seagrass hypothesis: this may stand for $\delta^{13}\text{C}$ data but not for N/C ratio since seagrass N/C ratio is low.

Probably all the suggested processes were involved, not only the seagrass. Anyway, the main variation was due to the $\delta^{13}\text{C}$, while OC/TN ratio showed no significant changes

Section 4.3 - discrepancies between apparent EF and usual EF values

Authors interpret the isotopic data and the comparison between consumers and resources in a static point of view. However, isotopic data in a consumer gives indication on what they have assimilated at the time scale of their tissue turnover. This turnover varies between days or few weeks for small animals (meiofauna, small macrofauna) to months (e.g. bivalves, large macrofauna). Consequently, isotope ratios of a consumer reflect isotope ratios of their preys that were consumed from days to months ago. Isotope ratios of preys that were consumed months ago are different to isotope ratio of the same species sampled at the time of the consumer sampling. Consequently, estimating EF from consumers and preys that were sampled at the same time may not be valid.

Author should include these considerations for revising section 4.3

Included (page 14, paragraph 3 of section 4.3).

Minor points

- Section 3.2, first two sentences: refer to Fig. 4A at the end of the first, and not the second, sentence. **corrected**

- Section 3.3: the title of the section should be "Elemental and isotopic..." **corrected**

- Page 9, line 28 ("Fig. 6A"): I guess you mean Fig. 5A. **Yes, corrected**

- Page 10, line 7 ("The contribution of the taxa tolerant to the organic load"): indicate which taxa are tolerant to the organic matter load. For instance, it could be indicated as an '*' in the appendix. **Asterisks added as suggested**

- Page 11, first line "...proxy of terrigenous influence": add a ref. **added**

The way of using $\delta^{13}\text{C}$ as a proxy of terrigenous influence should also be stated in the introduction (see my detailed comment "Introduction").

added

- Page 11, lines 57-59 ("The $\delta^{15}\text{N}$ values of the winter sampling..."): this is also valid for $\delta^{13}\text{C}$ and N/C ratio. **corrected**

- Page 11, last line: replace "were" with "may be" since it is not demonstrated in the ms. **corrected**

- Page 12, last line: add a "e" after "Darnaud". **corrected**

- Page 14, line 23: replace "and" with "or". **corrected**

- Figure captions (page 31): Fig. 2: indicate what sediment layer is of concern. Fig. 3, 4 and 8: indicate what error bars correspond to. Fig. 5: remove one of the two "Fig. 5"; replace "Isotopic" with "Elemental and isotopic". **Corrected**

Fig. 8: indicate to how many samples, individuals and/or species the dots correspond to. **In the figure legend we indicated that the averages have been performed on 1, 2 or 3 stations for shallow-depth, mid-depth and high-depth, respectively. The average (min-max) numbers of individuals for each analysis were, instead, provided in the Material and methods section (page 7, last paragraph of section 2.3).**

- Table 1: replace "EF" with "potential EF" or "apparent EF". **corrected**

Additional references

Coplen, 2011. Guidelines and recommended terms for expression of stable isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25, 2538-2560.

Perdue, E.M., Koprivnjak, J.-F., 2007. Using the C/N ratio to estimate terrigenous inputs of organic matter to aquatic environments. *Estuarine, Coastal and Shelf Science* 73, 65-72.

Reviewer #4:

This paper presents interesting results on the diffusion and role of terrigenous materials in the benthic food webs of shelf sediment in the north of the Ligurian Sea. The authors combined different methods to improve our knowledge on the role of terrigenous input in the trophodynamics of this system (stable isotopes of the different sources and of some macrobenthic organisms, organic carbon and nitrogen contents, enzymatic activity). This paper provides new data on the trophic functioning of these systems according to depth and season, particularly concerning the microbial activity in sediments. The bibliography is appropriate and up to date. However, it needs major corrections before being published.

General comments

The English should absolutely be corrected by a native English speaking person.

Corrected by a native English speaking

The paper is rather long, particularly the Discussion which needs to be clarified and shortened, particularly the § 4.1 and 4.3, which are too long and confusing.

The discussion has been re-arranged following the suggestions of all the reviewers. Discussion has been shortened.

Specific comments

- p. 4, lines 1-2: what is the maximum flux of the Entella River during flooding events?

This information was provided in the discussion (page 11, paragraph 4 of section 4.1).

- p. 4, line 10: the direction of the main coastal current seems to be from south-east to north-west (rather than east-west) **corrected**

- p. 6, lines 17 to 20: correct polychaetes (not polichaetes); Nephtyidae (not Nephtydae); precise Apseudes elisae (Tanaidacea); Ampelisca typica (Amphipoda); Metaphoxus simplex (Amphipoda). **corrected**

- p. 6, line 28: why the sediment of station C2 was excluded? **Problems in the sample conservation, we added this information**

- p. 7, line 3: Newman-Keuls (not Newman-Kneuls) **corrected**

- p. 8, § 3.2: change the 2nd sentence 'The depth of the A1 station...' by 'The shallower depth of station A1 explained the highest ...' (as all stations are located in the euphotic zone). **corrected**

Change the next sentence by 'Significantly higher values were found in shallow water than at the two other depths, which did not differ significantly.' **corrected**

- p. 9, line 4: complete the sentence in giving indication on d13C as follows: "..., while the mid-depth stations showed a clear terrigenous signature with low d13C values." **corrected**

- p. 9, line 8: precise "In the 0-2 cm layer, d13C values were significantly negatively correlated to ..." **corrected**

- p. 9, lines 15 and 16: correct the number of the figure. Fig. 5A (instead of 6A). **corrected**

- p. 10, lines 5 to 11: indicate p values as usual $p = 0.025$ and not $p = 2.5\%$. 4 corrections to make in this paragraph.

- Discussion and throughout the text: correct "Gulf of Lions" (like the animal, and not Lyon like the town). **corrected**

- References: Walling et al.: change 'fluvial sospende' by "fluvial suspended sediment" **corrected**

Table 1: complete the table with $S > W$ or $S < W$ when the difference are statistically significant. The direction of the difference should be indicated clearly in the table. **The table was completed**

Fig. 1: circle the arrows of stations where OM was sampled as they were difficult to locate.

The arrows have been enlarged and coloured to be easily seen.

Highlights for:

“The allochthonous material input in the trophodynamic system of the shelf sediments of the Gulf of Tigullio (Ligurian Sea, NW Mediterranean)”

by Cristina Mistic, Luigi Gaozza, Mario Petrillo, Anabella Covazzi Harriague.

- The input of continental organic matter (OM) was studied in the trophodynamic system of a shelf area (NW Mediterranean).
- Stable isotopic composition and biochemical components of sedimentary OM were used as input proxies.
- The highest continental influence was observed within the 50-m isobaths (3-4 km from the coast).
- Allochthonous carbohydrates were recycled by microbiota, allowing terrigenous OM entrance in the lower food web.
- Macrofaunal deposit-feeders consumed allochthonous OM, but also selected autochthonous OM.
- Macrofaunal predators potentially changed their prey during high- or low-input periods.

**The allochthonous material input in the trophodynamic system of the shelf sediments of the Gulf of
Tigullio (Ligurian Sea, NW Mediterranean)**

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Abstract

1 The organic allochthonous material input in the benthic system of a NW Mediterranean shelf area was
2 studied using a three-pronged approach, focusing firstly on the evaluation of the sedimentary stable
3 isotope ratios and organic matter (OM) composition, then on the OM recycling processes performed by the
4 microbial organisms, and finally on the potential trophic relationships between the macrobenthic
5 organisms. The highest allochthonous signal, indicating continental input, was observed within the 50-m
6 isobath, while at the 80-m isobath the marine signal was higher, pointing to a rather low continental
7 influence approximately 5 km from the shore. Heavier rainfall, often generating abrupt allochthonous
8 inputs by river outfalls, led to a wider spread of fine sediment particles. Carbohydrates were the
9 compounds that best represented the continental input and these compounds were associated with
10 potential recycling activities by microbiota, pointing to the entry of these C-containing allochthonous
11 materials into the microbial food web. The macrofaunal deposit-feeders used sedimentary OM
12 characterised by a continental signature as a food source, although the isotopic ratios of the organisms also
13 pointed to selective feeding on materials that had a marine signature, especially at our offshore sampling
14 stations. Predators fed on deposit- or suspension-feeders, with a potential selection of the latter during the
15 highest inputs of continental materials occurring in winter.
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Key-words

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30 Continental input, organic matter, enzymatic activity, macrofauna, sediments, Ligurian Sea, NW
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1. Introduction

Continental inputs in coastal areas are largely carried by rivers, and they are a heterogeneous mixture of organic matter (OM) with different chemical features and origins (soil, vegetal debris, freshwater production etc.) (Tesi et al., 2007). These inputs enter the coastal area at different rates, depending on the morphology of the coastline, the catchment area and marine hydrodynamic conditions. In small river systems a higher temporal coherence between river discharge and sea conditions has been recorded. A huge input of continental materials at sea is often contemporaneous with rainfall events, leading to differences in OM quality and fate between small rivers and large river systems (Weathcroft et al., 2010).

In coastal areas phytoplankton production is generally the main source of OM, together with benthic production. Autochthonous primary production strongly sustains secondary production (Chanton and Lewis, 2002). The presence of allochthonous inputs, such as those due to river outflow, may further increase the general productivity (Darnaude et al., 2004; Cresson et al., 2012) and allow the presence of ecoclines (Attrill and Rundle, 2002). In oligotrophic conditions the allochthonous inputs may play a higher role in sustaining the ecosystem with organic nutrients, due to limited autochthonous production. Nowadays, such processes may have a heavy anthropogenic signature (Halpern et al., 2007; Muniz et al., 2010; Venturini et al., 2012), especially in small river systems (Weathcroft et al., 2010). The release of huge quantities of human-derived materials into the environment points to the need for a better understanding of the fate and pathways of allochthonous inputs in the coastal system and, subsequently, in the deep waters of the sea (Weathcroft et al., 2010).

The chemical characterisation and the stable isotopic ratios of OM have been used to identify its origin and potential lability. Carbon sources (allochthonous as well as autochthonous) display peculiar C and N isotopic ratios that are widely used as a proxy for discriminating between the multiple sources of OM (from riverine inputs to benthic and pelagic marine producers for instance, Bănaru et al., 2007; Cresson et al., 2012). Terrestrial plant debris carried by riverine inputs generally shows lower $\delta^{13}\text{C}$ values (from -28 to -25‰ and down to -30‰ if C3 plants such as deciduous and coniferous trees are dominant, Sanchez-Vidal et al., 2013) than marine phytoplankton (from -22 to -19‰) (Hedges et al., 1997). Within the different components of the OM, the OC/TN ratio has been used as an indicator of trophic quality but also of terrigenous influence (the higher the ratio, the lower the trophic quality and the higher the terrigenous influence, Goñi et al. 2003, Burone et al., 2013 Cresson et al., 2012). The transfer of OM through the food web may be followed starting from the consumption and degradation of detrital OM by microbes, carried out especially (but not exclusively) by bacteria. Due to their small size, bacteria have firstly to cut the polymeric compounds into smaller units by means of extracellular enzymatic activities, whose features have been considered as proxies of OM turnover and bioavailability (Manini et al., 2003; Caruso et al., 2005). Once the OM components are channelled along the microbial food web and are reworked in the sediment, metazoans may benefit from this food source. This supply may be studied by focusing on its

1 lability or refractivity to consumption, measuring the TN or lipid content to assess the lability and
2 carbohydrate and OC content as an indicator of the semi-labile/refractory fraction (Cresson et al., 2012;
3 Venturini et al., 2012; Arndt et al., 2013). The complexity of the food web may be studied by determining
4 the isotopic ratios of the organisms. The $\delta^{15}\text{N}$ values of the organisms have been used to define the trophic
5 level. Lower values are characteristic of low trophic levels (deposit-feeders, for instance), while predators
6 show the higher values (Darnaude et al., 2004; Martinetto et al., 2006). The $\delta^{13}\text{C}$ values are often
7 considered less useful for the determination of the trophic position (Egger and Jones, 2000), but they can
8 provide information on the origin of the C supply for consumers.
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10 In the present study we aimed to define the extent of the continental input in the shelf area of the NE
11 Ligurian sea (NW Mediterranean), characterised by oligotrophic conditions. Different scales and ecological
12 processes were considered. Firstly we aimed to assess the spatial extent of the input of continental OM in
13 the sediments during the lowest and the highest freshwater input periods. We focused on sedimentary
14 organic tracers to characterise the allochthonous inputs in the microbial food web. Finally we tested the
15 trophic relationships between the small metazoans of the sediment (macrofauna), determining whether
16 direct relationships could be established between sedimentary OM, deposit-feeders, suspension-feeders
17 and predators.
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30 **2. Material and Methods**

31 *2.1 Study site*

32 The Entella River is one of the main natural rivers of Liguria, flowing into the Gulf of Tigullio (Fig. 1). The
33 Entella R. itself is 8 km long and, together with its three main tributaries, has a total length of ca. 38 km,
34 with a catchment area of 372 km² (Tomaselli et al 2009). The Entella R. discharge is rather low, on average
35 20 m³ s⁻¹ during autumn and winter and less than 10 m³ s⁻¹ during summer (Tomaselli et al. 2009). However,
36 in recent years, overflow occurred every autumn, spreading continental-derived material in the entire Gulf
37 of Tigullio area. The Entella R. flows through rural and highly urbanised areas, where agriculture and small
38 manufacturing activities occur. The population is more than 71 000 inhabitants, increasing to 90 000 during
39 the summer (Regione Liguria, www.ambienteinliguria.it). Sometimes pollution events (such as the
40 appearance of hydrocarbons or sewage on the water surface) have been reported by local newspapers and
41 personal observations.
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51 In the Gulf of Tigullio (Fig. 1) the main coastal current flows from SE to NW, carrying the continental waters
52 towards the Portofino Promontory, although a nearshore eastward current sometimes occurs (Doglioli et
53 al., 2004). Wind forcing, which is known to deeply influence the surface-water movement (Astraldi and
54 Manzella 1983), may spread the continental water in the entire area or rapidly carry it offshore (Doglioli et
55 al. 2004). Several towns along the sea-shore (Fig. 1) have more than 65 000 inhabitants (up to 180 000 with
56 the tourist contribution). An offshore fish-farm, on the 30-40 m isobath, lies to the east of our sampling
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1 stations. At the Entella R. mouth occasional and irregular meadows of *Cymodocea nodosa* have been
2 observed, at between 10 and 20 m depths, while to the west small patches of *Posidonia oceanica* have
3 been recorded, increasing in surface area approaching the Portofino Marine Protected Area (Diviacco and
4 Coppo 2006).
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7 8 2.2 Sampling 9

10 Samplings were carried out in summer (July 2012) and winter (February 2013) on the continental shelf from
11 the Portofino Promontory to the area of the Entella R. (Fig. 1). The two periods were those characterised by
12 the minimum and maximum river discharge, which have variable delays with respect to the rainfall in the
13 area (Tomaselli et al., 2009, Fig. 2A). The rainfall of the sampling years is reported in Fig. 2B. Irregular
14 flooding may occur during autumn with increased continental inputs in the sea, as observed by the
15 Regional Agency for the Protection of the Environment (ARPA-Liguria, www.arpal.gov.it) for late 2012.
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18 Seven stations along three transects (A, B and C) were sampled (Fig. 1), covering the main part of the
19 continental shelf in the Gulf of Tigullio, with special focus on the Entella R. outflow. Station A1 was, in fact,
20 placed in front of the river mouth at a 20 m depth (shallow station), stations A2, B2 and C2 were located at
21 53±2 m (mid-depth stations), stations A3, B3 and C3 were located at 84±5 m (deep stations).
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24 At each station sediment samples were collected using a Van Veen grab. Four replicates were sieved with a
25 0.5 mm mesh, on board of our research vessel, in order to retrieve the macrofaunal organisms. The
26 replicates for organism classification were then treated with formalin (10% final concentration), while the
27 sub-samples for isotopic measurements were kept frozen until sorting and analysis.
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30 One replicate was sampled for the sediment texture analysis and three replicates were sampled for the OM
31 and stable isotope analyses using PVC corers. The cores were cut into two sections: 0-2 cm and 2-10 cm, on
32 board.
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35 Seawater and freshwater were sampled for the determination of the $\delta^{13}\text{C}$ signal of the particulate OM. In
36 particular, seawater was collected at approximately an 80-m depth during the sampling and during the
37 month preceding each sampling (June and July 2012, January and February 2013) at an offshore station (95
38 m depth) placed next to station C3 (Fig. 1). Freshwater was collected at approximately 1.5 km upstream
39 from the Entella R. mouth during the sampling campaigns. Immediately after collection the samples were
40 transported to the laboratory (within 3 h), where they were filtered on pre-combusted (450°C for 4 h)
41 Whatman GFF filters (glass fiber). The filters were stored at -20°C until analysis.
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53 54 2.3 Sample preparation and analysis 55

56 The sediment texture analysis was performed within 2 months of the sampling, following Buchanan and
57 Kain (1971). The sediments were sieved after H₂O₂ treatment and drying (60°C, 48 h). We considered two
58 dimensional fractions: silt-clay (<0.063 mm), and sand (>0.063 mm).
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1 The chlorophyll-a samples were immediately prepared on board, adding approximately 1 g of wet sediment
2 to a solution of acetone diluted with deionised water (9:1 v:v acetone and water, respectively) and buffered
3 with sodium carbonate. The samples were stored at -20°C until analysis the day after. The pigment was
4 measured following the method of Holm-Hansen et al. (1965), using a Jasco FP50 spectrofluorometer
5 calibrated with chlorophyll-a from spinach. No correction was made for the water content of the sediment.
6 After analysis each sample was dried at 60°C for 24 h to calculate the dry weight (DW). The results were
7 expressed as ng g^{-1} DW.
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11 The samples for the bacterial counts (approximately 1 g of wet sediment) were immediately stored in 0.2-
12 μm -prefiltered and autoclaved seawater with the addition of formalin (2% final concentration), on board,
13 then stored at 4°C until analysis, which was carried out within 1 month of sampling. The bacteria counts
14 were performed with epi-fluorescence microscopy (Zeiss Universal Microscope) using 0.2- μm -black
15 Nuclepore filters after acridine orange staining (Hobbie et al. 1977). After analysis each sample was dried at
16 60°C for 24 h to calculate the dry weight (DW). The results were expressed as $\text{cells} \cdot 10^8 \text{ g}^{-1}$ DW.
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19 The samples for the hydrolytic enzymatic activities (β -glucosidase- BG- and leucine aminopeptidase - LA)
20 (approximately 0.5 g of wet sediment) were immediately incubated on board following Hoppe (1983), using
21 artificial substrates: 4-methylumbelliferyl glucoside (excitation at 365 nm and emission at 460 nm) for β -
22 glucosidase and L-leucine 7-amido-4-methylcoumarin hydrochloride (excitation at 380 nm and emission at
23 440 nm) for leucine aminopeptidase. Boiled sediments were used as a blank to counteract accidental
24 contamination due to handling and abiotic cleavage of the artificial substrates. Samples and controls
25 (blanks) were incubated in duplicate with 0.5 ml of substrate solution (saturating concentrations) for 3 h at
26 in-situ temperatures in the dark. Fluorescence was then measured with a Jasco FP50 spectrofluorometer,
27 previously calibrated with 4-methylumbelliferone and 7-amino-4-methylcoumarin solutions. After analysis
28 each sample was dried at 60°C for 24 h to calculate the dry weight (DW). The results were expressed as
29 $\text{nmol g}^{-1} \text{ h}^{-1}$ DW.
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42 The main OM biochemical fractions of the sediments were determined following colorimetric methods.
43 These analyses were made on sediments stored at -20°C within two months of sampling. Total
44 carbohydrates, mainly structural polymers such as cellulose, were evaluated following Dubois et al. (1956),
45 a method based on the reaction of the carbohydrates to phenol (5% in water solution) in an acid medium
46 (sulphuric acid). Total lipids (including all the non-polar substances soluble in organic solvents) were
47 extracted from the sediment with a solution of chloroform and water (Bligh and Dyer 1959) and measured
48 following carbonisation with sulphuric acid (Marsh and Weinstein 1966). A Jasco V-500 spectrophotometer
49 was calibrated with D-glucose (carbohydrates, absorbance 490 nm) and tripalmitin (lipids, absorbance 350
50 nm) solutions.
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58 The organic carbon (OC) and total nitrogen (TN) contents, and the stable isotope compositions were
59 measured by a Finnigan DeltaPlus XP mass spectrometer directly coupled to a FISIONS NA2000 Element
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1 Analyzer via a ConFlo III interface for continuous flow measurements (Tesi et al., 2007). The samples for the
2 OC analyses were first decarbonated with acid treatment (HCl, 1.5 M). Samples for the TN contents and
3 $\delta^{15}\text{N}$ were weighed in tin capsules and directly inserted in an EA autosampler. The average standard
4 deviation of each measurement, determined by replicate analyses of the same sample, was $\pm 0.07\%$ for OC
5 and $\pm 0.009\%$ for TN. All isotopic compositions are presented in the conventional δ notation and reported as
6 parts per thousand (‰). For $\delta^{13}\text{C}$ determinations, the IAEA reference sample IAEA-CH7 (polyethylene,
7 -32.15% vs. VPDB) was used for the mass spectrometer calibration. Uncertainties were lower than
8 $\pm 0.05\%$, as determined from routine replicate measurements. The internal standard for ^{15}N isotopic
9 measurements was IAEA-N-1 (ammonium sulphate, $+0.4\%$ vs. air). Errors for replicate analyses of the
10 standards were $\pm 0.2\%$.

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17 The macrofaunal specimens were recognised, when possible, down to species level within 2 months of
18 sampling. Some of the more common species or families were isolated at different stations and periods to
19 be processed for the stable isotope analyses. The polychaetes *Sternaspis scutata* and the Paraonidae family
20 were selected to represent the deposit-feeders. The Glyceridae and Nephtyidae polychaete families were
21 selected as predators. The crustaceans *Apseudes elisae* (Tanaidacea) and *Ampelisca typica* (Amphipoda)
22 were selected as suspension-deposit-feeder and *Metaphoxus simplex* (Amphipoda) as suspension-feeder.
23 The total body of the specimens was utilised and the samples were not decarbonated before analysis. For
24 each analysis a variable number of organisms was isolated, on average they were: 6 (from 2 to 15) for
25 deposit-feeders, 2 (2-6) for predators, 2 (2-6) for suspension-deposit-feeders, 1 (1-3) for suspension-
26 feeders.
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37 2.4 Statistics

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39 The one-way ANOVA (Statistica software) was performed on the normalised data to highlight significant
40 differences in the single variables between periods. The one-way ANOVA was coupled with a Newman-
41 Keuls post-hoc test. A Spearman-rank correlation analysis was performed to test the relationships between
42 the various variables.
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46 Multivariate analyses were performed using the PRIMER program package. A PCA was carried out on the
47 normalised OM, microbial and isotopic ratios of the sediment. A cluster analysis was carried out on the
48 macrofaunal data, after fourth-root transformation and resemblance with the Bray-Curtis similarity index.
49 We tested for differences in community composition at different depths with the analysis of similarities
50 (ANOSIM), a resampling technique that uses permutation/randomization methods on Bray-Curtis similarity
51 matrices to identify differences among groups of samples. Large values of the test statistic (R) indicate
52 complete separation of groups, and small values, below 0.3, point to high intra-group variability, meaning a
53 high heterogeneity within them.
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3. Results

3.1 Elemental and isotopic signature of particulate OM and sedimentary OM

The isotopic signature of the OM in the surface 0-2 cm sediment layer (except for station C2 due to problems in sample conservation) and of the particulate OM collected in the freshwaters and in the offshore marine area are reported in Fig. 3. Following the observations of Tesi et al. (2007) for the nearby Gulf of Lions, the compositions of four possible OM sources (heterotrophic bacteria, marine algae, soil-OM and vascular plant detritus) were superimposed to the $\delta^{13}\text{C}$ values and the OC/TN ratios of our samples in Fig. 3A. The data showed a complex mixture of different sources for our stations, with all the deep ones tending to autochthonous and marine-derived OM, while the mid-depth stations showed a clear continental signature with low $\delta^{13}\text{C}$ signal. The shallow station showed signals of marine algae and heterotrophic bacteria accumulation. The $\delta^{15}\text{N}$ values (Fig. 3B) also highlighted differences between the mid-depth stations, showing lower values than the deep ones.

The $\delta^{13}\text{C}$ values recorded for the Entella R. particulate OM were $-26.7\pm 0.5\text{‰}$ (Fig. 3A). The values obtained for the particulate OM of the seawater were $-25.1\pm 0.1\text{‰}$ and $-25.1\pm 0.5\text{‰}$ for summer and winter, respectively (Fig. 3A).

3.2 Sediment texture, sedimentary OM distribution, microbial variables

In the Gulf of Tigullio shelf area the surface layer of the sediment showed a generally rather high sand fraction, with the highest contribution at the shallow station A1 ($91\pm 4\%$), followed by the deep stations ($69\pm 7\%$) and the mid-depth stations ($54\pm 11\%$). On average, the mid-depth stations showed a higher silt-clay fraction than the deep ones. Different contributions were recorded for the two periods, with a higher silt-clay contribution in winter at the mid-depth stations (A2, B2 and C2, from $37\pm 1\%$ to $55\pm 7\%$) and the deep stations (A3, B3 and C3, from $26\pm 7\%$ to $36\pm 5\%$).

The sedimentary OM distribution (Fig. 4 for the 0-2 cm layer and Appendix 1 for 0-2 cm and 2-10 cm layers) was influenced by the sediment texture. The coarsest station A1 generally had lower values than the other stations. The stations with the highest sedimentary OM content were the mid-depth ones, also characterised by the finest sediment texture. The OC/TN ratio showed the lowest values at the shallow station (for the 0-2 cm layer 8.1 ± 0.7 for summer and 5.6 ± 0.5 for winter) and the highest at the mid-depth ones (14.4 ± 1.8 for summer and 13.3 ± 1.7 for winter). Intermediate values were found for the deep stations (8.9 ± 1.8 for summer and 9.8 ± 0.3 for winter).

In many cases, the 0-2 cm layer of the stations showed differences between the summer and winter samplings, especially for carbohydrates and enzymatic activities (Table 1). At the mid-depth stations, C2 showed a lower OM accumulation than A2 and B2, due to its position, further to the west than the other stations, which limited the direct influence of the Entella R. outflow and lowered the differences between the two periods. The deeper layer (2-10 cm), instead, was more conservative (the entire data set is

1 presented in Appendix 1). Some variations in the contents were observed but were lower than in the
2 surface layer, except for carbohydrates at station A1, which increased during the winter sampling ($p < 0.01$).

3 The highest chlorophyll-a values were found at the shallow station A1 (Fig. 4E). At this station the summer
4 values were higher than the winter ones, while at the mid-depth and deep stations higher chlorophyll-a
5 contents were observed in winter than in summer (Table 1).
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7 Fig. 4F showed that the bacterial abundance at the shallow station was higher than at the others, and the
8 bacterial abundance did not vary with the period. At the other stations, instead, a rise in bacterial
9 abundance was observed for the winter sampling (Table 1).
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11 The enzymatic activities (Figs. 4G and 4H) showed different distributions: the BG activity was, on average,
12 the lowest at the shallow station and the highest at the mid-depth stations; the LA activity showed no
13 spatial differences. Significant differences between the two periods were observed for the two enzymatic
14 activities (Table 1), very sharp for the LA activity (up to a six-fold increase at the deep stations, which in
15 winter reached activities similar to the shallow and mid-depth stations, Fig. 4G). The BG activity, although it
16 did not increase at the same rate as the LA activity, was strongly stimulated in winter in the 0-2 cm layer
17 (Table 1) and also in the deeper layer ($p < 0.05$) (Appendix 1).
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28 The PCA (Fig. 5) was applied to the sedimentary isotopic ratios, OM and microbial variables of the 0-2 cm
29 layer. This layer showed variations between the two periods, while the deeper layer was more stable
30 showing only a few differences and only at the shallow station. These differences were likely due to
31 higher sediment mechanical mixing and the coarser texture, which allowed a higher pore water exchange
32 between the layers. PC1 explained 48.12% of the variance, PC2 24.2%. The plot confirms the similarities
33 previously observed for the stations based on their depth (especially for the mid-depth and deep ones,
34 showing respectively higher and lower OM and isotopic ratio values) and the period (winter generally richer
35 than summer), but also the differences at station A1 in summer and winter. Moreover, the variables
36 showed significant correlations (Table 2), in particular carbohydrates with β -glucosidase activity, OC and
37 $\delta^{13}\text{C}$ and also with OC/TN ratios .
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48 *3.3 Macrofaunal community and isotopic ratios*

49 The complete data set for the macrofaunal density is reported in Appendix 2. Macrofaunal total density
50 showed a clearly decreasing pattern from the coast to the deep stations in the summer samplings (on
51 average, 1152.4 ± 167.1 and 443.8 ± 66.7 ind m^{-2} for the shallow station and the deep stations, respectively)
52 (Fig. 6). In winter, a similar pattern was maintained for transects B and C. The mid-depth station A2,
53 instead, showed the maximum density (1129.5 ± 448.0 ind m^{-2}). Density values were higher in summer than
54 in winter ($p < 0.05$). The major contributor to the total density were the polychaetes (82%), followed by
55 crustaceans (8%), molluscs (5%), echinoderms (1%) and other taxa (4%). The assemblages were composed
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1 principally by deposit feeders, which dominated most stations (Fig. 6). Predators and suspension-deposit
2 feeders showed proportionally higher contributions in winter (on average 30 ± 2 and $35\pm 4\%$, respectively)
3 than in summer (19 ± 1 and $22\pm 4\%$). The suspension feeder contribution rarely exceeded 2%.

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5 The cluster analysis applied on the macrofaunal density (Fig. 7) showed that the assemblages at the
6 different stations depended on depth and on sampling period. The first cut-off separated the shallow
7 station (SuA1 and WiA1), due to the peculiar composition of these assemblages. In fact, 38% of the taxa
8 found at the shallow station were exclusive to this area. In accordance with the sediment texture, we found
9 species characteristic of sandy bottoms, such as: *Tellina nitida*, *Owenia fusiformis*, *Lucinella divaricata*,
10 *Spisula subtruncata*, *Gastrosaccus sanctus*, *Perioculodes longimanus longimanus* and *Tellina compressa*.
11 The second cut-off isolated the deep stations (WiA3, WiB3 and WiC3) in winter, characterised by a lower
12 number of taxa. The other stations, especially the mid-depth ones in summer, showed a higher number of
13 taxa. The anomalous winter A2 station clustered with the summer mid-depth ones, displaying the highest
14 number of taxa in this group.

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16 The contribution of the taxa tolerant of the organic load (indicated by asterisks in Appendix 2) ranged, on
17 average, from 22 ± 1 (deep stations in summer) to $50\pm 5\%$ of the total density (mid-depth stations in winter).
18 For the entire winter data set the contribution of the organic-load tolerant taxa was higher than for
19 summer (on average $37\pm 6\%$ vs $29\pm 5\%$, respectively), although the communities showed a high
20 heterogeneity within the groups (ANOSIM: $R=0.2$, $p<0.5$). The highest contribution was found at the mid-
21 depth stations (on average $41\pm 7\%$) and the lowest at the deep ones ($24\pm 3\%$). The shallow station showed a
22 contribution of $36\pm 4\%$. The multivariate ANOSIM pointed to different assemblages for the three depths
23 (mid-depth vs. shallow stations: $R=0.99$, $p<0.5$; mid-depth vs. deep stations: $R=0.65$, $p<0.1$; deep stations
24 vs. shallow stations $R=0.90$, $p<0.5$).

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26 The results of the isotopic ratio analyses performed on the selected organisms have been averaged by
27 period, station depth and trophic strategy (Fig. 8). Given that consumers become enriched relative to their
28 food by 3 to 4‰ for $\delta^{15}\text{N}$ and 1 to 2‰ for $\delta^{13}\text{C}$ (Darnaude et al., 2004; Martinetto et al., 2006), we
29 calculated the enrichment factors (EF) for our samples in order to highlight potential trophic relationships
30 between them. Since it was not possible to directly demonstrate which consumers uses which resource, the
31 EFs have to be considered potential EF. A clear division based on the $\delta^{15}\text{N}$ values between the level of the
32 predators and their potential prey (deposit-feeders and suspension-deposit feeders) was found at all the
33 stations, confirmed by potential EF values for $\delta^{15}\text{N}$ ranging from 1.4 and 5.2‰ (Table 3). The $\delta^{13}\text{C}$ signal,
34 instead, did not allow us to clearly separate the organisms by food sources (potential EFs between -2.0 and
35 1.9‰). The results for the deposit-feeders were different from the sediment ones, showing notable
36 potential EFs especially for the $\delta^{13}\text{C}$ signal (4.4-6.3‰).

37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 **4. Discussion**

4.1 Spatial and temporal input of continental OM in the Gulf of Tigullio

1 Similarly to other Mediterranean areas (Sanchez-Vidal et al., 2013), the climatic reports for the natural
2 rivers of the Gulf of Tigullio, principally represented by the Entella R., indicated the lowest outflow during
3 summer and the highest during winter (Tomaselli et al., 2009), although the heaviest rainfall was generally
4 observed during autumn. The time-lag between rainfall maxima and outflow maxima was not constant,
5 depending on the degree of saturation of the catchment soil (Tomaselli et al., 2009). However, the
6 mountainous-nature of the area pointed to the occurrence of exceptional events that may carry huge
7 amounts of allochthonous material to the sea and quickly spread it on the continental shelf (Kao and
8 Milliman, 2008; Weathcroft et al., 2010).

9 The sediment texture of our winter samples indicated a notable sedimentation of fine particles, which were
10 spread especially at the mid-depth and deep stations. Given that the peak in suspended-sediment load is
11 associated with finer suspended sediment (Walling et al., 2000), our winter sampling showed a quantitative
12 rise in continental input in the area where the least turbulent water conditions allowed sedimentation (Tesi
13 et al., 2007).

14 There are numerous methods for studying the entrance of continental OM into marine systems. In this
15 case we firstly considered the $\delta^{13}\text{C}$ composition for OM, which is widely used as a proxy to discriminate
16 between the multiple sources of OM (Bănaru et al., 2007; Cresson et al., 2012). Focusing firstly on the
17 marine particulate OM in the open-sea waters next to the Portofino Promontory, we found rather low and
18 stable $\delta^{13}\text{C}$ values. This result may depend on the autochthonous OM, namely small phytoplankton that is
19 known to have lower $\delta^{13}\text{C}$ values (Rau et al., 1990) and that is typical of oligotrophic waters especially in
20 summer (Vidussi et al., 2000 for the Ligurian Sea). Anyway, our values were similar to those found by
21 Darnaude et al. (2004) for the plume particulate OM of the nearby Rhone river and to the values of Cresson
22 et al. (2012) for the Huveaune River, a small river flowing into the Bay of Marseilles. This pointed to a rather
23 continuous influence of continental water in our sampling area even when the river outflow was at its
24 minimum. In our study area the presence of the Portofino Promontory could slow the main current and
25 generate counter-currents (Doglioli et al., 2004), that increase the residence time of the freshwater in the
26 gulf and allow diagenesis of continental detritus.

27 Although the outflow of the Entella R. is small (with outflow maxima up to $450 \text{ m}^3 \text{ s}^{-1}$, www.arpal.gov.it, but
28 representing a water flow 30 times higher than the average), due to its mountainous bed (Kao and
29 Milliman, 2008) it may bring a contribution of allochthonous OM proportionally similar to large rivers
30 (Banaru et al., 2007) to the Gulf sediment. In fact, the $\delta^{13}\text{C}$ values of our sedimentary OM fall within the
31 ranges or are slightly lower than those observed for the nearby coastal area of the Gulf of Lions,
32 characterised by the large outfall of the Rhone River (Darnaude et al., 2004, Tesi et al. 2007). On the other
33 hand, our results indicate a tighter coupling between continental inputs and sedimentary OM than that

1 observed by Cresson et al. (2012) for the Bay of Marseilles, where the contribution of autochthonous OM
2 was higher.

3 All these observations are in agreement with the oligotrophic regime of the Ligurian Sea (Raick et al., 2005;
4 Misic and Fabiano, 2006) and, in particular, of the Gulf of Tigullio (Albertelli et al., 1999), where
5 accumulation of autochthonous OM is generally limited, increasing the relevance of allochthonous inputs.
6

7 The stations that showed the highest continental OM signal were those placed on the 50-m-depth isobath.
8 This was in accordance with the significant accumulation of nearly all the types of sedimentary OM at the
9 mid-depth stations. A higher content of OM in the sediment of the mid-depth stations has already been
10 observed in a previous study carried out in the same area (Albertelli et al., 1999). This indicated that such
11 accumulation was a distinctive and permanent feature of the Gulf of Tigullio. The small outflow of the
12 Entella R. did not allow the creation of a consistent frontal area (Albertelli et al., 1999), therefore the
13 reasons of such distribution remains unknown. On the other hand, such a distribution was also typical of
14 the nearby Gulf of Lions (Darnaude et al., 2004), where the influence of the Rhone river was clear.
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16 The significant correlations of the carbohydrates with $\delta^{13}\text{C}$ values in both periods of our study and also with
17 the OC/TN ratio values, pointed to their continental origin. Carbohydrates such as cellulose, for instance,
18 are mainly produced by higher plants. Phytoplankton, instead, shows a proportionally lower cellulose
19 content (Yamamuro and Kamija, 2014). On the other hand, the rather low and constant values of
20 sedimentary lipids, consistent with the values previously reported for the same area (Albertelli et al., 1999),
21 indicated that they are not dependent on continental inputs as carbohydrates are, but have a prevalent
22 autochthonous nature, such as plankton detritus deposited through the water column after
23 phytoplanktonic blooms (Gómez-Erache et al., 2001) and/or depending on faecal-pellets of zooplankton
24 (Baldi et al., 2010).
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26 We expected that the shallow station A1 would show the highest continental signature, being in front of
27 the Entella R. mouth. Instead, at this station the isotopic values and the OC/TN atomic ratios indicated
28 multiple origins for the sedimentary OM and also changes in the source depending on the period, with the
29 winter sampling showing values similar to those of the marine-influenced stations. This station showed
30 higher hydrodynamic forcing than the others, as shown by the coarser and more stable sediment texture,
31 also during the period with the highest continental loads. Therefore, some processes had masked or limited
32 the continental input. On the other hand, the higher winter hydrodynamism may have decreased the
33 supply of allochthonous OM to the station. A higher mechanical forcing modifies the sedimentation rates of
34 coarse and fine debris (Muniz et al., 2010), increasing the transfer of the finer particles (richer in OM) to the
35 offshore areas (Tesi et al., 2007). In other areas, characterised by small mountainous river systems, solids
36 delivered during high discharges are rapidly removed from the surface plume in shallow water, where they
37 cannot deposit because wave stresses are high (Wheatcroft et al., 2010). In addition, patchy *Cymodocea*
38 *nodosa* meadows lay next to the sampling site (Diviacco and Coppo 2006). The seagrass detritus may have
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been occasionally transported to the surface sediment of station A1 by the winter hydrodynamic forcing, modifying the isotopic features. It is well known, in fact, that seagrasses show high $\delta^{13}\text{C}$ values and that sometimes the presence of meadows may mask the actual terrigenous origin of sediments (Lepoint et al., 2000; Adin and Riera 2003; Papadimitriou et al., 2005; Prado et al., 2010; Dubois et al. 2012). On the other hand a change in autochthonous primary production, for instance benthic diatoms with high $\delta^{13}\text{C}$ ($> -18\text{‰}$, Moens et al., 2002), may have contributed to these results. Allochthonous nutrients carried by the small rivers of Liguria may sustain pulses of autotrophic biomass development at coastal sites (Misisic et al., 2011), and the irregular regime of such supply may lead to variations in the autotrophic community.

A fish farm lying to the east on the 50 m isobaths, instead, would have had a negligible influence. Previous studies (Doglioli et al., 2004) highlighted a very limited spatial extension for the spread of particulate debris coming from the farm, and also the $\delta^{15}\text{N}$ signal of the winter A1 station was rather low compared to those reported for other fish farms (Vizzini and Mazzola, 2004).

4.2 Potential input of the continental OM in the benthic microbial food web

Accumulation at the mid-depth stations was a common feature of nearly all the OM compounds we analysed, indicating a higher trophic availability for consumers in these areas, irrespective of the lower trophic quality. The bacterial abundance showed a different trend, with significantly higher values at the shallow station, maybe also due to input from urban sites. However, this pointed to a decoupling of the bacterial proliferation and OM supply, thus somehow responding to the lower quality of the OM.

The functional tools of microbes, especially of bacteria, to consume the OM are the hydrolytic enzymes (Hoppe, 1983). The determination of enzymatic activity, although potential, may give clues to the main trophic pathways of the microbial compartment. The significant correlation between the carbohydrates and the BG activity pointed to a good adaptability of the microbial consumers to exploiting the available resources, especially those coming from the continental sources, which are semi-labile (cellulose for instance, Arndt et al., 2013), and points to a potential entrance of continental OM into the microbial food web.

As with the BG activity, the LA activity was higher for the winter sampling, but it increased irrespective of the distance from the coast and of the specific substrate (TN) availability, indicating that the microorganisms were also exploiting N-OM pools other than the sedimentary one. Maybe a rise in continental inputs had carried dissolved organic N to the sea (Dagg et al., 2004), or autochthonous dissolved-N sources were available. This trophic behaviour would partially separate the N-metabolism of microorganisms from the particulate allochthonous fraction of the OM.

4.3 Input of continental OM into the macrofaunal community

1 The abundances of the macrofaunal community were comparable with those previously reported for the
2 same area in summer by Albertelli et al. (1999). A higher predator contribution to the total abundance and
3 a lower deposit feeder contribution were observed in our survey, although the comparison was possible
4 only for the summer sampling and, therefore, no consistent evidence of a trophic-guild modification over
5 time could be established.
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8 The different compositions of the assemblages at the three depths and in the two periods highlighted by
9 the cluster analysis was a response of the communities to the variable sedimentary features, intended as
10 texture and OM accumulation and quality. The peculiar communities of the shallow-depth area, adapted to
11 coarser texture and higher hydrodynamic conditions, and the significantly higher contribution of taxa
12 tolerant of the OM load at the mid-depth stations are examples of the adaptation of these assemblages to
13 different environmental conditions.
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15 The stable isotopic ratios were used to gather further insights into the community features, although
16 isotopic data on a consumer give an indication on what it has assimilated at the time scale of its tissue
17 turnover, reflecting isotope ratios of the food that was consumed from days (small organisms) to months
18 (large organisms) before. The $\delta^{15}\text{N}$ values of the organisms were used to define the trophic level, with
19 deposit-feeders showing the lowest values and predators the highest, with EFs between 2‰ and 4.5‰
20 (Darnaude et al., 2004; Martinetto et al., 2006). The $\delta^{13}\text{C}$ values can provide information on the origin of the
21 C supply for consumers (EF of approximately 1‰ - 2‰, Egger and Jones, 2000, Darnaude et al., 2004).
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23 Our $\delta^{15}\text{N}$ values were generally similar to those measured by Sampaio et al. (2010) on the continental shelf
24 off Lisbon (Portugal), a temperate area influenced by multiple OM sources, and also similar to those found
25 by Darnaude et al. (2004) in the Gulf of Lions. The potential EF values based on the $\delta^{15}\text{N}$ values showed
26 that, at each depth, the organisms we selected as predators generally belonged to a higher trophic level
27 than their potential prey, namely deposit-feeders and suspension-deposit feeders. These relationships
28 were particularly true in summer, when the benthic system was quantitatively less influenced by
29 allochthonous inputs and organised its energy fluxes without heavy external influence. During winter,
30 instead, the higher allochthonous input led to changes in the previous trophic relationships. For instance, at
31 the mid-depth stations the potential EF between predators and deposit-feeders was above the threshold
32 proposed by Darnaude et al. (2004). This value depended on the lower deposit-feeder isotopic ratio, while
33 predators maintained the same value observed during summer. This indicated a shift in the feeding activity
34 of predators to partially-new trophic targets. Organisms showing high in $\delta^{15}\text{N}$, other than the selected
35 deposit-feeders, could have entered the predator diet. Fauchald and Jumars (1979) observed that
36 Glyceridae, for instance, have a preference for moving prey, which may have temporarily colonised the
37 mid-depth area in winter but were not abundant during summer. The occurrence, in winter, of $\delta^{15}\text{N}$
38 enriched suspension-feeders may have contributed to the higher $\delta^{15}\text{N}$ values of the predators.
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1 Classically, deposit-feeders have more enriched $\delta^{15}\text{N}$ values than the sediment because they utilize
2 sedimentary OM after bacterial processing and subsequent fractionation (Doi et al., 2005), and probably
3 also incorporate small heterotrophic meiofaunal organisms (Dubois et al., 2007). Our suspension-deposit
4 feeders were trophically related to the sediment only in summer, when the availability of sinking particles
5 was lower due to limited vertical fluxes (Vidussi et al., 2000). The high potential EF for the $\delta^{13}\text{C}$ signal of the
6 deposit-feeders and suspension-deposit-feeders and the sediment indicated that some of these organisms
7 could select marine-autochthonous C, thus reducing their dependence on continental material. Such ability
8 was previously supposed by Dittel et al. (2000) and subsequently confirmed by Darnaude et al. (2004). Our
9 data indicated that lipid-containing particles, influenced to a lesser extent by direct variations in continental
10 inputs, could actually be a food-source for macrofaunal organisms.
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19 **5. Conclusions**

21 The input of continental-origin OM in the sediments of the Gulf of Tigullio showed variable patterns. From a
22 spatial point of view the highest continental influence was observed within, approximately, the 50-m
23 isobath, i.e. at a maximum distance of 3-4 km from the coast. At the 80-m isobath the continental
24 contribution decreased, indicating that the continental influence was rather low starting from
25 approximately 5 km from the shore. Temporal variations may occur, due to the climatic regime of the area.
26 Heavier rainfall, often generating abrupt inputs by river outfalls, was observed in autumn-winter, leading to
27 a widespread diffusion of fine sediment particles. Carbohydrates were the compounds that best
28 represented the continental input. The carbohydrate sedimentary content and its increases in winter were
29 associated with some recycling activities by microbiota, pointing to a diffusion of these C-containing
30 allochthonous materials in the lower food web. Lower matching was, instead, observed for the N fraction,
31 indicating variable trophic strategies depending on the organic nutrient. The macrofaunal deposit-feeders
32 seemed to be strictly connected to the sedimentary materials, although the $\delta^{13}\text{C}$ values indicated the
33 possibility of selective feeding on more attractive materials such as lipids, which showed a higher marine
34 signature. Predators differently fed on deposit-feeders or suspension-feeders, potentially selecting the
35 latter category during the highest continental-input periods. Thus, various pathways of trophic activity have
36 been observed, leading to only a partial exploitation of the continental fraction of the OM. These
37 mechanisms, developed to use the most attractive and labile fraction of the trophic supply, have also an
38 environmental value, potentially reducing the biomagnifications of OM-associated pollutants coming from
39 the anthropogenic activities of the coastal and the hinterland areas.
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Captions to Figures

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2 Fig. 1. Study area: location of the sampling stations and main anthropogenic features of the coastal area
3 such as pipelines (dotted lines), towns (black squares), naturalistic zones (very light grey area), and
4 commercial site (black star). The large grey arrow indicates the dominant current, the small arrows
5 (1 black at sea and 1 white along the Entella R.) the places where particulate matter was sampled
6 for the $\delta^{13}\text{C}$ evaluation.
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10 Fig. 2. A: monthly values for the Entella R. discharge ($\text{m}^3 \text{s}^{-1}$) and for the rainfall (mm) as calculated by
11 Tomaselli et al. (2009) on the basis of multi-decadal observations. The discharge delay with respect
12 to rainfall is clearer in winter. B: monthly values for rainfall (mm) for 2012 and 2013. Samplings are
13 indicated by the arrows.
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17 Fig. 3. Elemental and isotopic features of sediments. A: plot of the $\delta^{13}\text{C}$ composition (‰) and OC/TN ratio
18 for the sedimentary OM in summer (white squares) and winter (black squares). The grey triangles
19 indicate the data for particulate OM in the freshwater (Entella) and in the seawater (average of all
20 the observations), italics indicate the main potential sources of the sedimentary OM. B: plot of the
21 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition (‰) for the sedimentary OM.
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26 Fig. 4. Sedimentary OM and microbial features. A: total nitrogen (TN). B: organic Carbon (OC). C: lipids. D:
27 carbohydrates. E: photoautotrophic biomass as indicated by the chlorophyll-a content of the
28 sediment. F: bacterial abundance. G: leucine aminopeptidase activity (LA). H: β -glucosidase activity
29 (BG). The error bars correspond to standard deviations. The grey dotted lines indicate the
30 approximate average of the entire data set for the shallow A1 station, the mid-depth stations (A2,
31 B2 and C2) and the deep stations (A3, B3 and C3).
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37 Fig. 5. PCA for the OM, microbial and isotopic ratio data for summer (Su) and winter (Wi) (TBN: total
38 bacterial number; chl-a: chlorophyll-a, carbo: carbohydrates; OC: organic carbon, TN: total
39 nitrogen; lip: lipids, ^{13}C and ^{15}N : isotopic ratios for C and N, respectively).
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42 Fig. 6. Total density (ind m^{-2}) of the macrofaunal organisms in the different stations. The bars also report
43 the contribution of the organisms divided by their dominant trophic strategy to the total density.
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46 Fig. 7. Cluster analysis of the macrofaunal density values of each station in summer (Su) and winter (Wi).
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48 Fig. 8. Isotopic ratios (‰, A: $\delta^{15}\text{N}$, B: $\delta^{13}\text{C}$) for the macrofaunal organisms at the shallow stations, mid-
49 depth stations (average for 2 stations) and deep stations (average for 3 stations). The sediment
50 values are reported for comparison (empty circles). The data are divided for season and for trophic
51 strategy (filled circles: predators; triangles: suspension-deposit feeders and suspension feeders;
52 squares: deposit feeders). The error bars correspond to standard deviations, where not visible they
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Table 1. Results of the one-way ANOVA for the 0-2 cm surface layer of the sediment. Normalised data for the different variables in the shallow station (A1), mid-depth (A2, B2 and C2) and deep (A3, B3, C3) for the two periods (summer: S and winter: W) have been compared.

	shallow		mid-depth		deep	
OC	ns		<0.05	S<W	ns	
TN	ns		<0.001	S<W	ns	
OC/TN	ns		ns		ns	
lipids	ns		ns		ns	
carbohydrates	<0.01	S>W	<0.05	S<W	<0.05	S<W
chlorophyll-a	<0.001	S>W	<0.01	S<W	<0.05	S<W
TBN	ns		<0.01	S<W	<0.01	S<W
LA	<0.01	S<W	<0.001	S<W	<0.001	S<W
BG	<0.01	S>W	<0.001	S<W	<0.001	S<W

OC: Organic Carbon; TN: Total Nitrogen, OC/TN: Organic Carbon/Total Nitrogen ratio; TBN: total bacterial number; LA: leucine aminopeptidase activity; BG: β -glucosidase activity.

Table 2. Correlations between the variables for the 0-2 cm layer. ⁺: p<0.05, *: p<0.01, **: p<0.001. Number of observations =14, except stable isotope ratios n=12.

	OC	TN	OC/TN	lipids	carbo	chl-a	TBN	LA	BG	$\delta^{13}\text{C}$
TN	0.87**									
OC/TN	0.89**	0.58 ⁺								
lipids	0.81**	0.64 ⁺	0.88**							
carbo	0.81**	ns	0.79**	0.56 ⁺						
chl-a	ns	ns	ns	ns	ns					
TBN	ns	ns	ns	ns	ns	0.63 ⁺				
LA	ns	ns	ns	ns	ns	ns	0.73*			
BG	0.73*	0.64 ⁺	0.57 ⁺	ns	0.78**	ns	ns	0.66*		
$\delta^{13}\text{C}$	ns	ns	-0.64 ⁺	ns	-0.80*	-0.77*	ns	ns	ns	
$\delta^{15}\text{N}$	ns	ns	-0.62 ⁺	ns	-0.80*	-0.71*	ns	ns	ns	0.98**

OC: Organic Carbon; TN: Total Nitrogen, OC/TN: Organic Carbon/Total Nitrogen ratio; carbo: carbohydrates, chl-a: chlorophyll-a, TBN: total bacterial number; LA: leucine aminopeptidase activity; BG: β -glucosidase activity.

Table 3. Mean potential enrichment factors (pEF, ‰) for N and C in the two periods for the station clustered by depth. Bold numbers fall within the ranges proposed by Darnaude et al. (2004) and Martinetto et al. (2006) for real trophic relationship between organisms and food sources ($\delta^{15}\text{N}$: 2-4.5‰, $\delta^{13}\text{C}$: 1-2‰). For the winter mid-depth stations we used the strict suspension-feeder data (underlined numbers) instead of mixed deposit-suspension feeder ones due to the absence of the latter.

stations	trophic relationship	summer		winter	
		pEF N	pEF C	pEF N	pEF C
shallow	pred vs. dep susp	4.2	0.4	3.9	-2.0
	pred vs. dep	3.1	1.7	1.8	1.9
	dep susp vs. sed	1.5	6.9	1.0	6.5
	dep vs. sed	2.6	5.7	3.1	2.9
mid-depth	pred vs. dep susp	3.3	-1.6	<u>1.4</u>	1.4
	pred vs. dep	4.4	-0.3	5.2	-0.1
	dep susp vs. sed	4.4	6.5	<u>6.6</u>	4.6
	dep vs. sed	3.2	5.2	2.8	6.1
deep	pred vs. dep susp	3.8	-0.8	5.0	0.7
	pred vs. dep	4.9	0.8	3.6	-0.3
	dep susp vs. sed	3.4	5.3	1.3	3.6
	dep vs. sed	2.3	3.7	2.7	4.6

pred: predators

dep-susp: deposit- suspension feeders

dep: deposit feeders

sed: sedimentary organic matter

Figure 1
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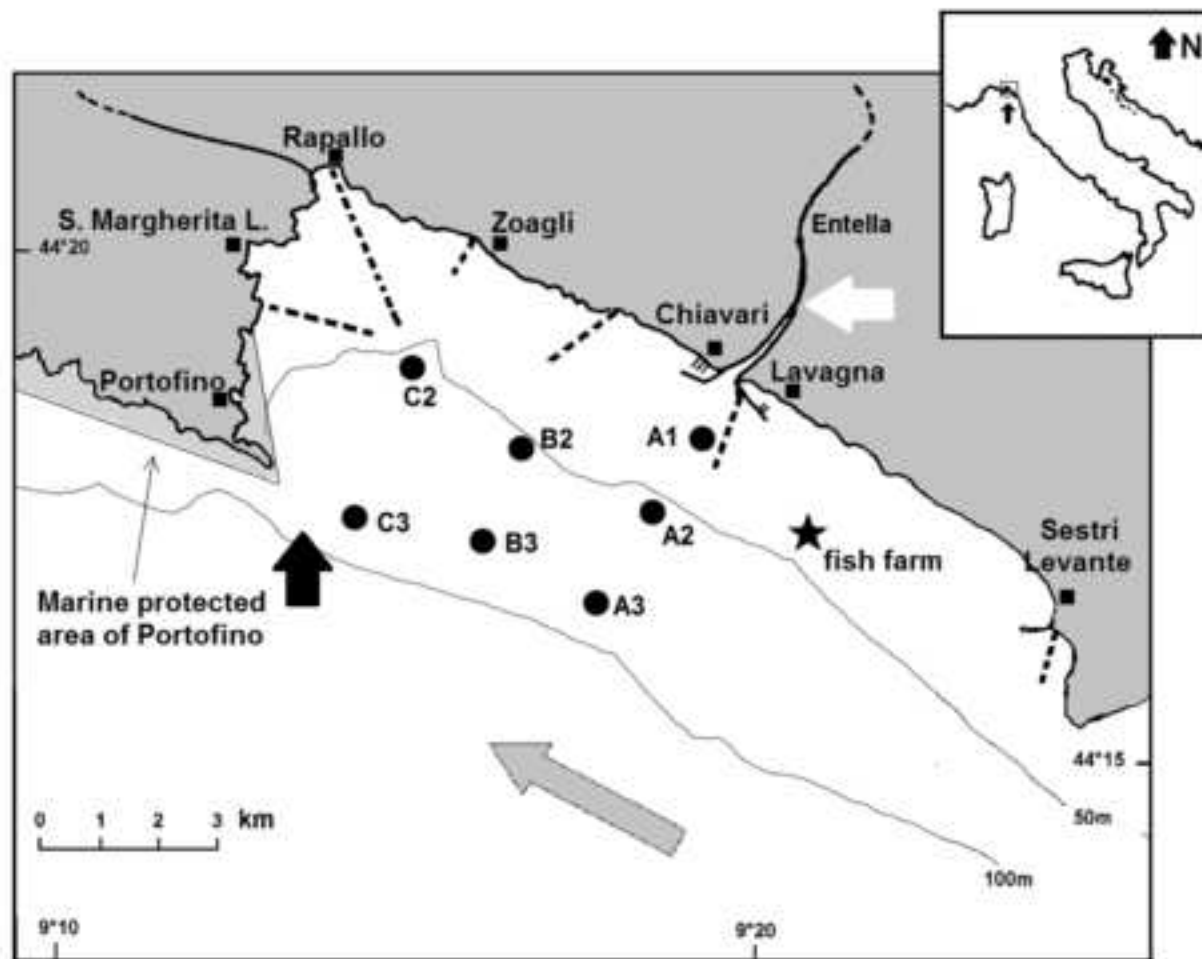


Figure 2
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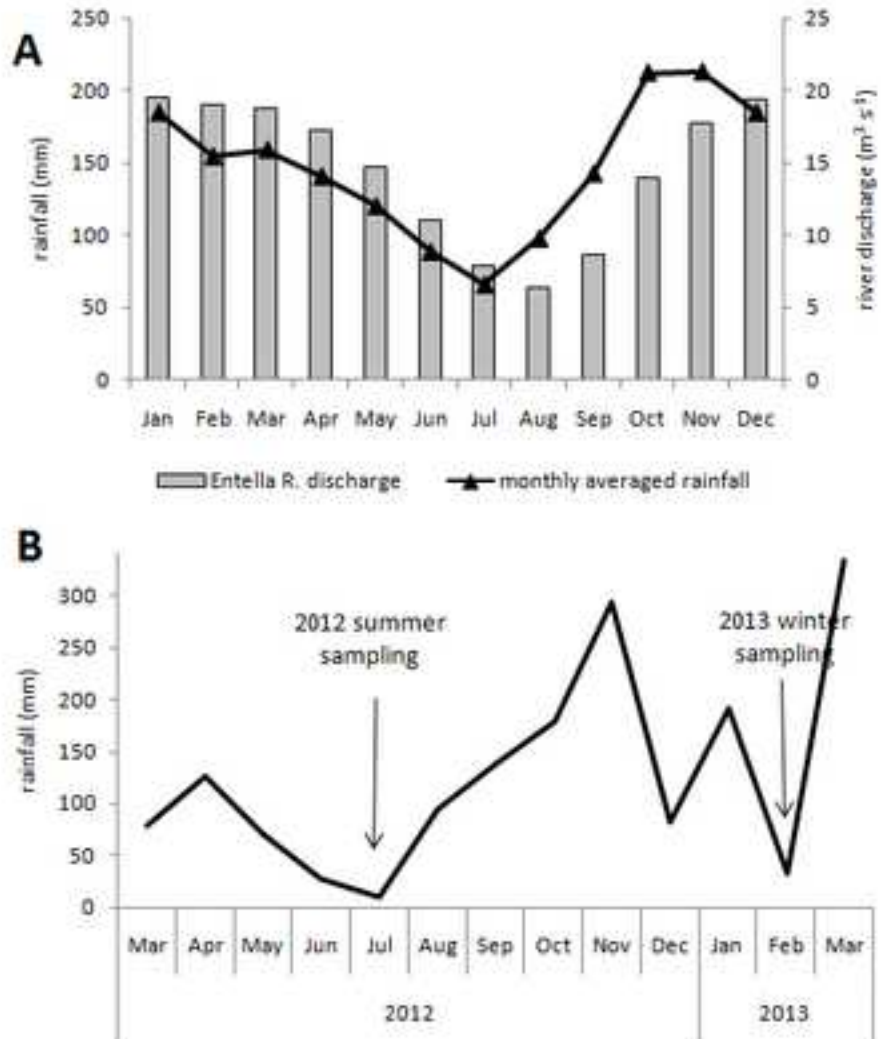


Figure 3
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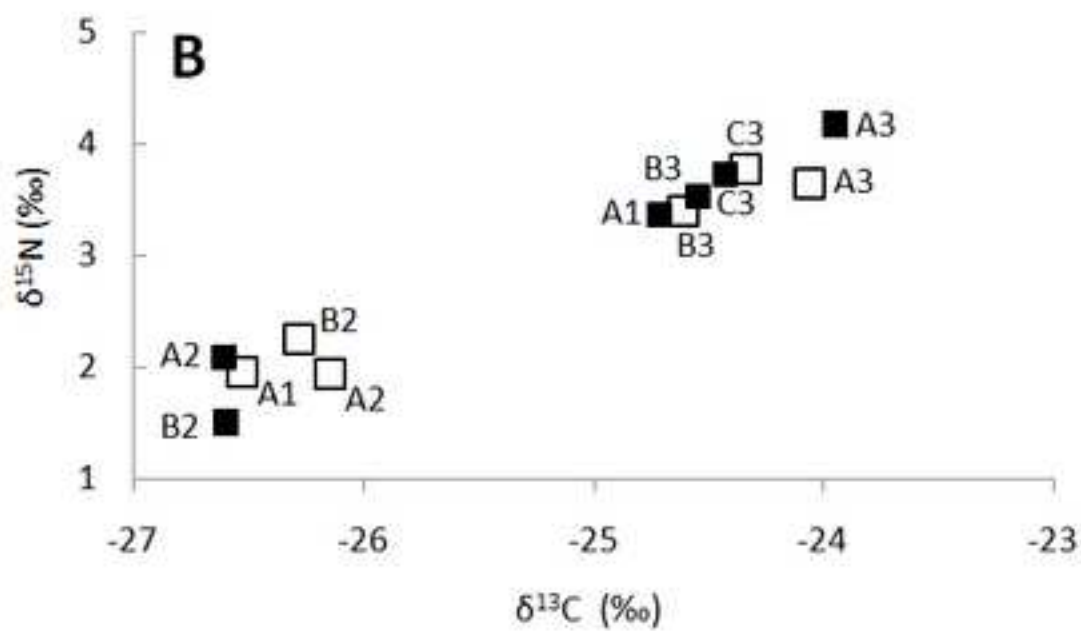
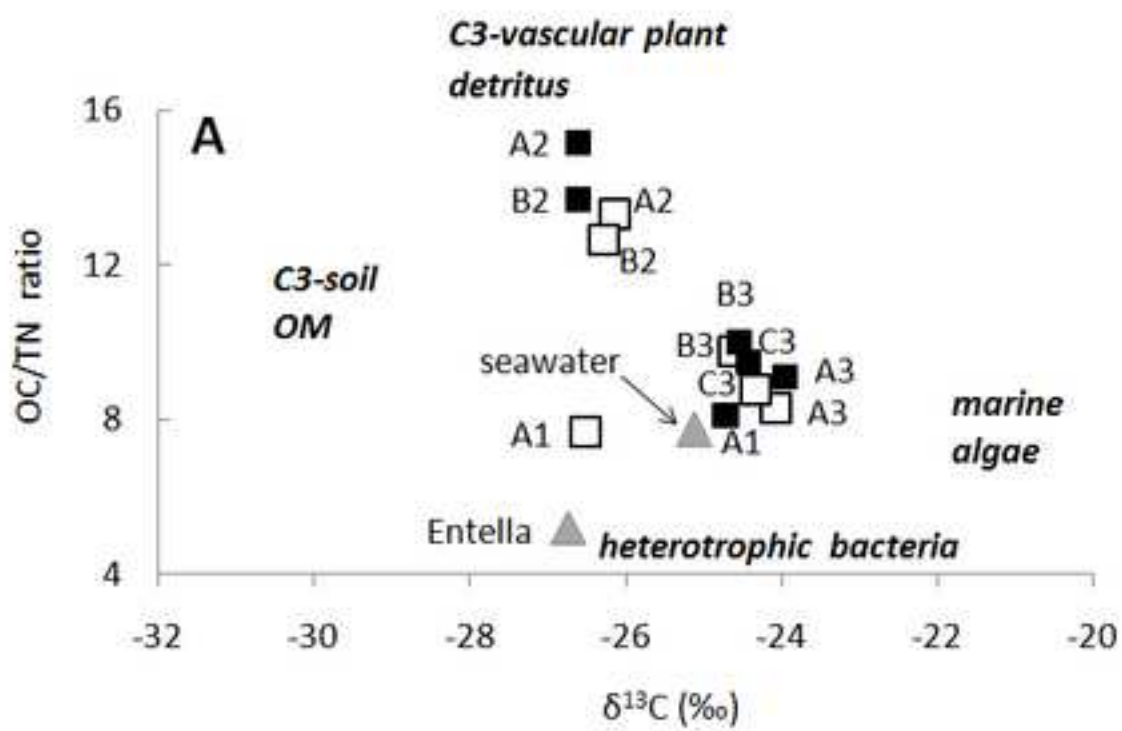


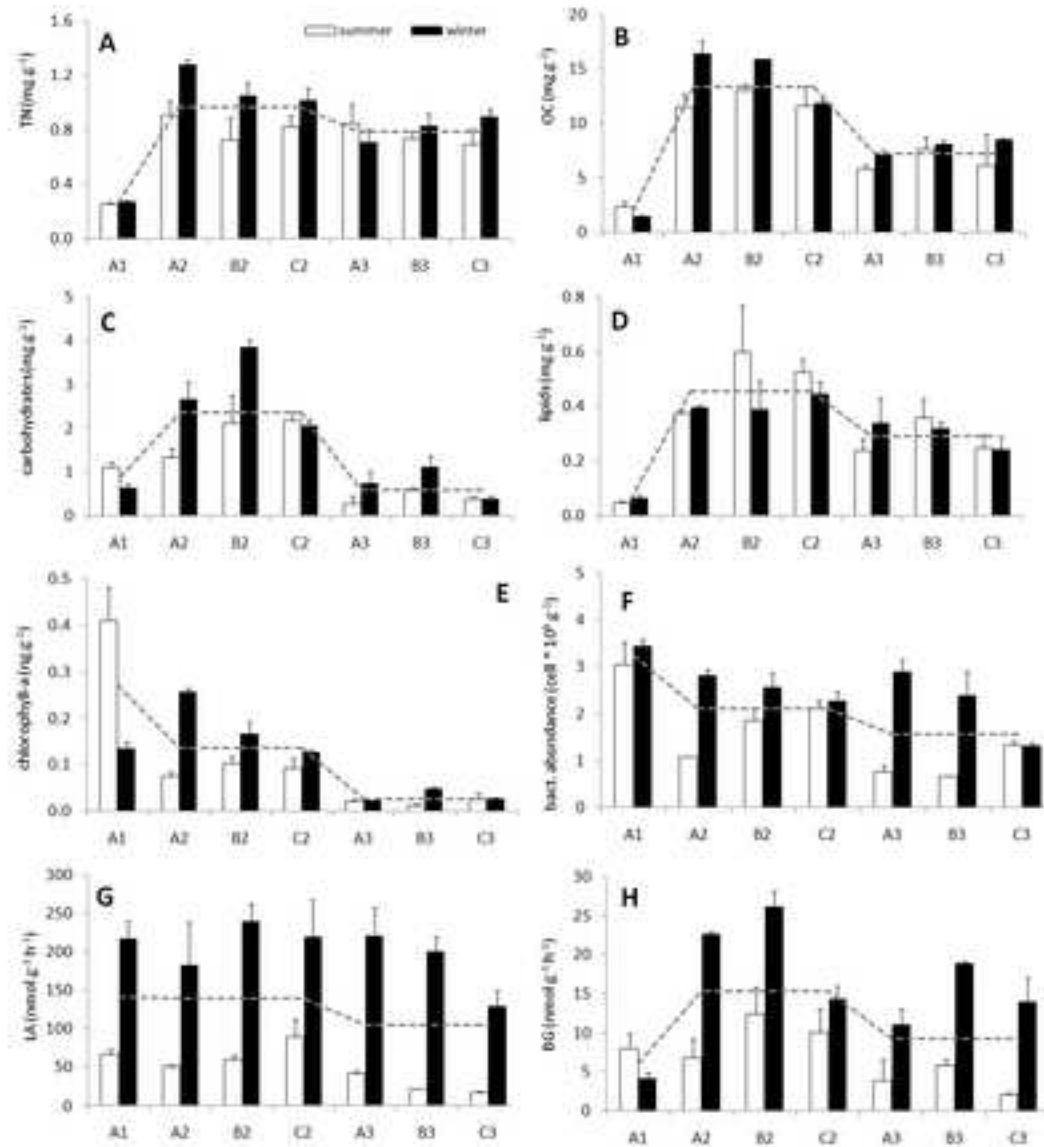
Figure 4[Click here to download high resolution image](#)

Figure 5
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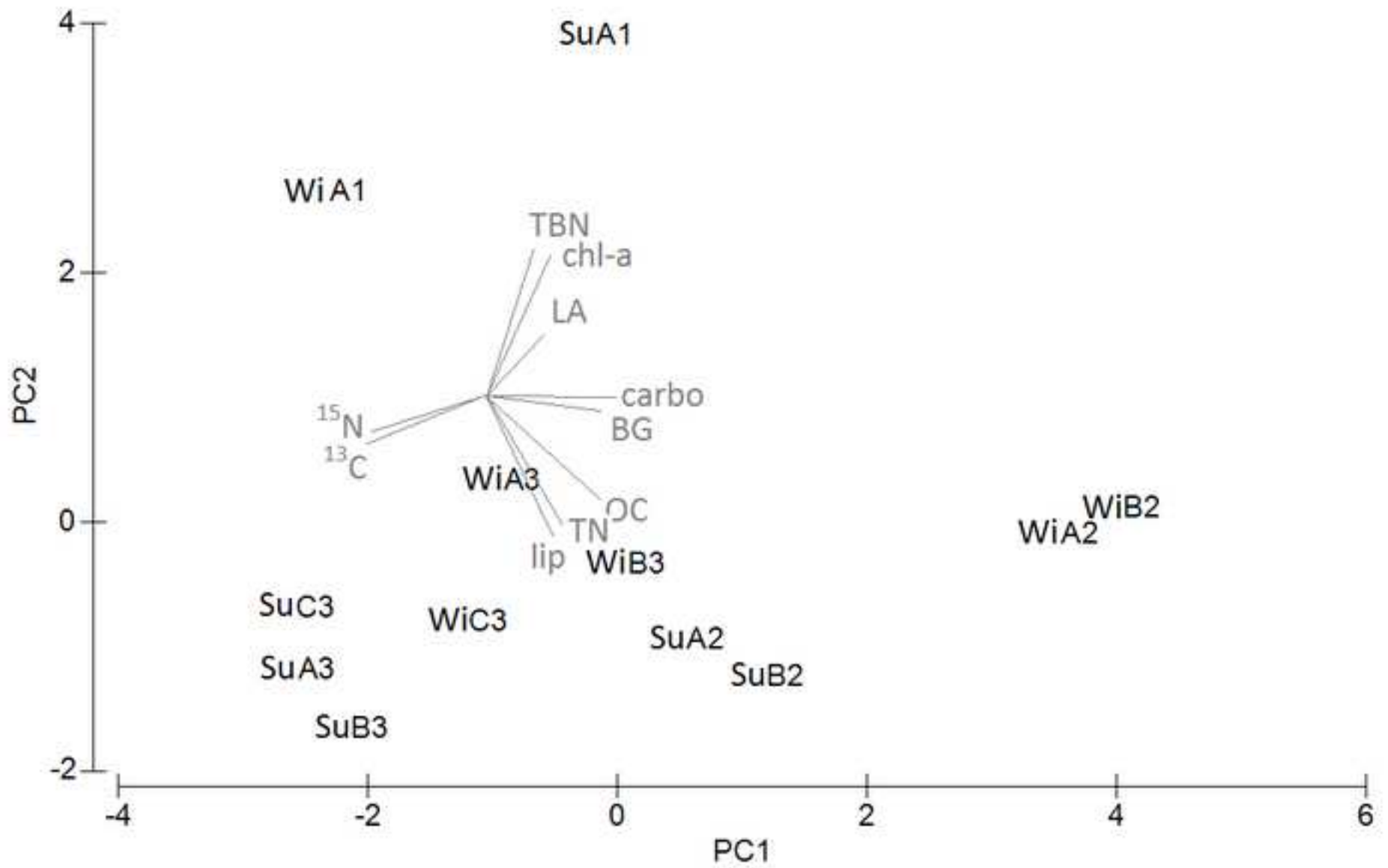


Figure 6
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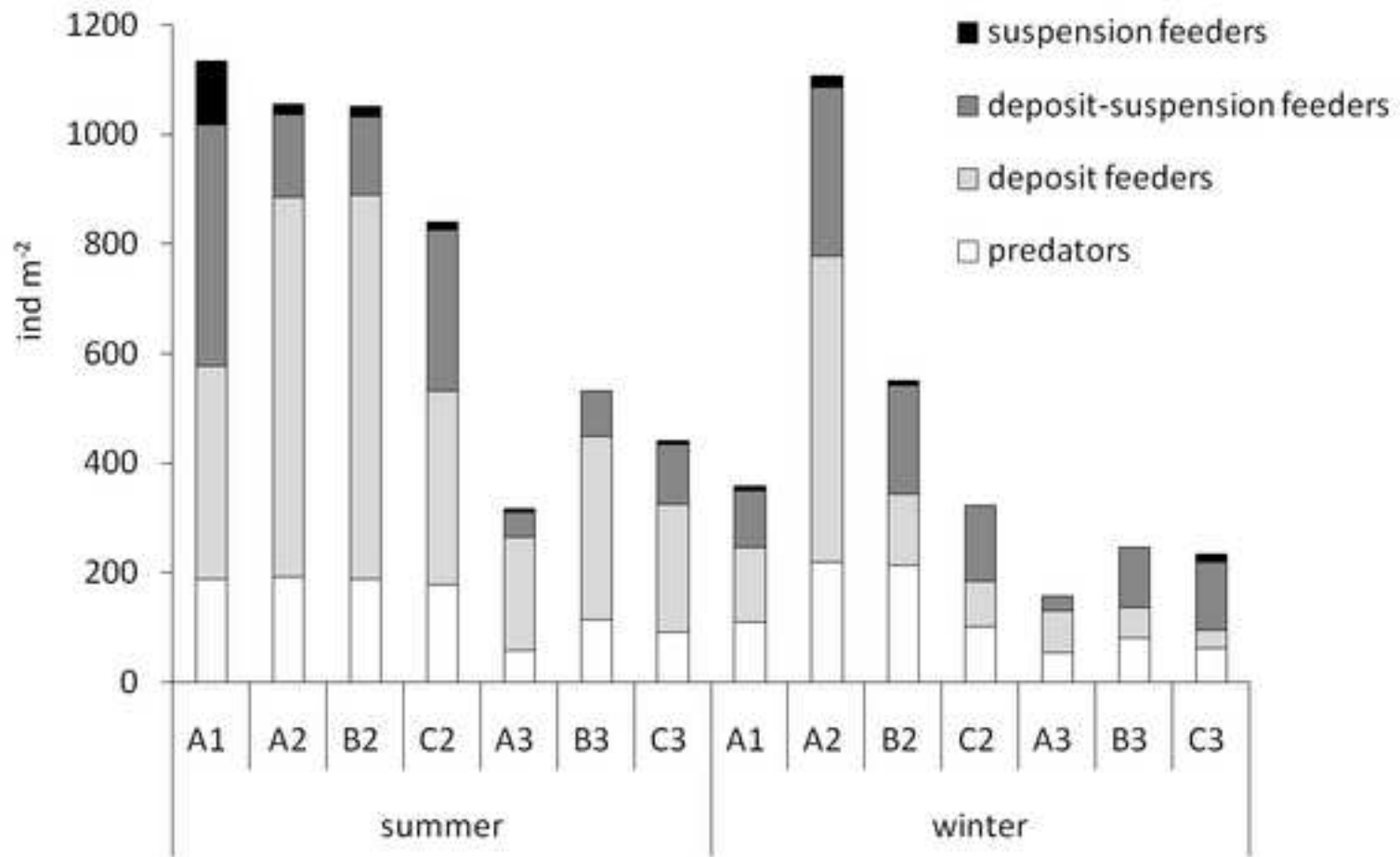


Figure 7
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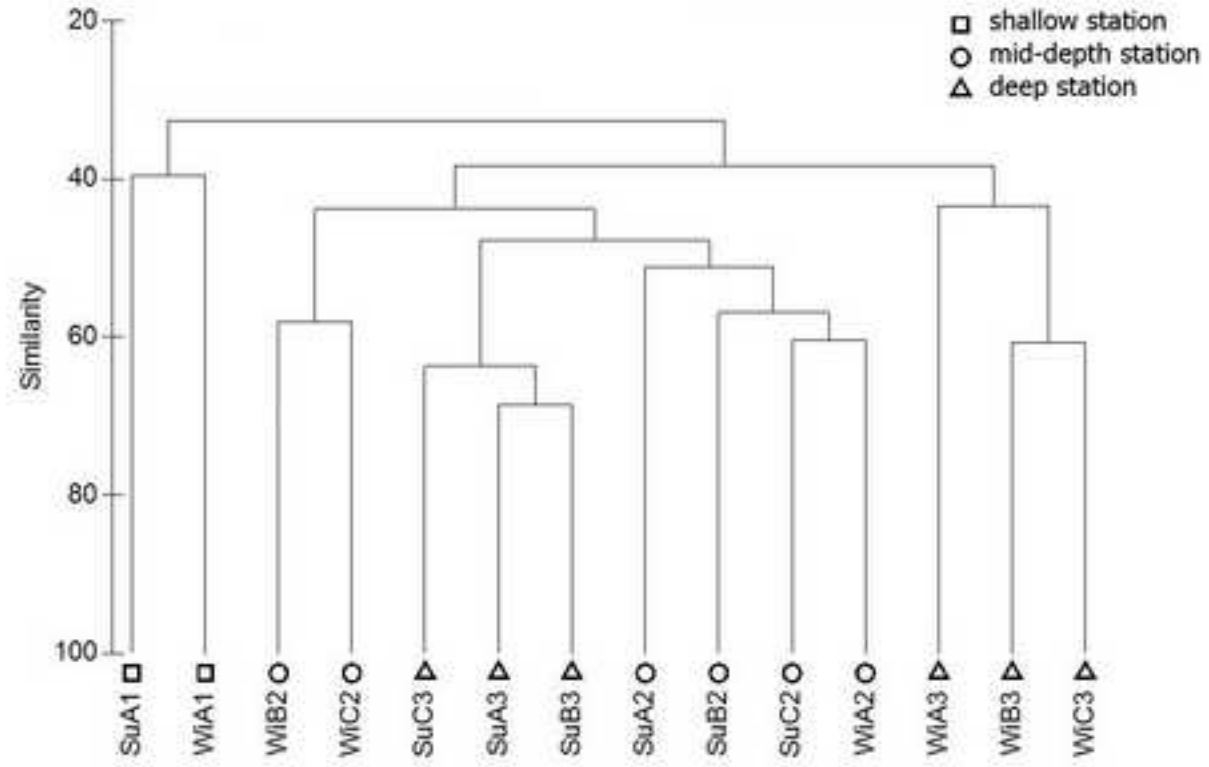
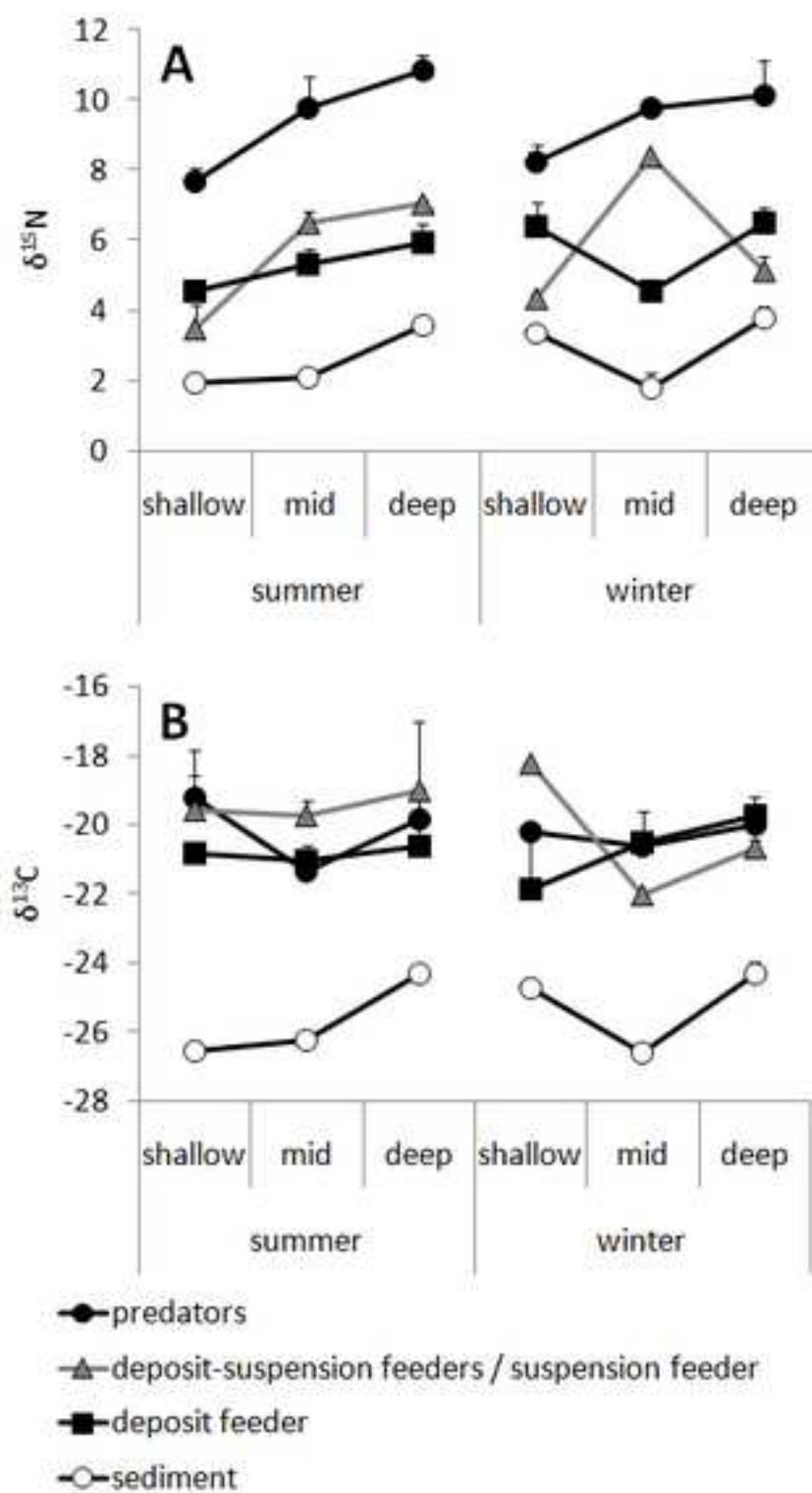


Figure 8

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Appendix

Appendix 1. Complete table for the sedimentary OM and microbial variables for the two layers (0-2 cm and 2-10 cm). Mean values and standard deviations are reported.

period	depth	station	layer (cm)	TN mg g ⁻¹		OC mg g ⁻¹		OC/TN		carbo mg g ⁻¹		lipids mg g ⁻¹		chl-a ng g ⁻¹		TBN cell * 10 ⁸ g ⁻¹		LA nmol g ⁻¹ h ⁻¹		BG nmol g ⁻¹ h ⁻¹	
summer	shallow	A1	0-2	0.26	0.01	2.3	0.5	8.1	0.7	1.12	0.11	0.05	0.01	0.36	0.07	3.0	0.5	66.3	7.4	8.0	1.9
			2-10	0.22	0.02	2.5	0.3	10.9	2.5	0.41	0.03	0.08	0.01	0.17	0.05	2.8	0.4	64.5	12.6	5.0	2.3
	mid	A2	0-2	0.91	0.10	11.5	1.2	12.7	0.7	1.33	0.21	0.37	0.02	0.07	0.01	1.1	0.0	50.8	0.3	6.8	2.4
			2-10	0.91	0.09	11.0	1.5	12.7	2.4	0.97	0.20	0.35	0.07	0.09	0.02	1.6	0.1	37.2	3.9	2.4	1.7
		B2	0-2	0.73	0.16	13.0	0.5	16.3	2.9	2.12	0.63	0.60	0.17	0.10	0.01	1.8	0.3	59.8	5.8	12.4	3.5
			2-10	0.94	0.11	11.5	0.6	12.3	1.1	1.39	0.34	0.37	0.07	0.07	0.01	1.2	0.1	17.4	4.1	5.1	0.4
	C2	0-2	0.82	0.09	11.6	1.8	14.1	1.5	2.17	0.20	0.52	0.05	0.09	0.02	2.1	0.1	89.6	22.6	10.0	3.0	
		2-10	0.72	0.10	9.5	1.9	12.7	2.0	1.17	0.06	0.35	0.06	0.06	0.01	1.7	0.1	22.2	3.31	2.7	1.1	
	deep	A3	0-2	0.85	0.14	5.8	0.4	7.0	1.4	0.28	0.16	0.24	0.04	0.02	0.00	0.8	0.1	41.9	3.4	3.9	2.8
			2-10	0.72	0.07	5.7	0.1	8.1	0.7	0.28	0.19	0.19	0.04	0.02	0.00	1.6	0.1	15.1	7.1	1.0	0.5
		B3	0-2	0.73	0.05	7.6	1.1	10.5	2.2	0.60	0.03	0.36	0.07	0.01	0.00	0.7	0.0	21.5	1.4	5.8	0.8
			2-10	0.68	0.09	7.1	0.5	10.5	1.2	0.60	0.20	0.24	0.05	0.02	0.01	1.5	0.1	9.51	1.6	1.5	0.3
C3		0-2	0.69	0.11	6.2	2.9	9.1	4.7	0.38	0.07	0.25	0.05	0.03	0.01	1.3	0.1	16.4	2.3	2.1	0.1	
		2-10	0.65	0.15	8.4	1.3	11.7	1.3	0.29	0.02	0.30	0.04	0.02	0.00	1.7	0.0	16.5	2.5	1.8	1.4	
winter	shallow	A1	0-2	0.27	0.01	1.5	0.1	5.6	0.5	0.64	0.08	0.07	0.01	0.14	0.01	3.4	0.1	217.5	22.6	4.1	0.8
			2-10	0.28	0.04	2.0	0.3	7.4	1.3	1.45	0.28	0.10	0.04	0.20	0.03	3.6	0.4	34.3	11.0	4.6	0.4
	mid	A2	0-2	1.27	0.04	16.4	1.1	12.9	1.3	2.67	0.40	0.40	0.01	0.26	0.01	2.8	0.1	182.1	57.1	22.6	0.5
			2-10	1.02	0.04	12.7	0.7	12.5	0.5	1.48	0.51	0.25	0.04	0.13	0.02	2.2	0.3	19.7	0.17	13.2	0.4
		B2	0-2	1.05	0.09	15.9	0.1	15.2	1.4	3.86	0.18	0.39	0.10	0.17	0.02	2.6	0.3	239.1	23.2	26.2	2.0
			2-10	1.01	0.06	13.7	1.2	13.9	0.8	1.51	0.26	0.37	0.08	0.10	0.05	1.7	0.3	26.8	4.7	14.7	0.8
	C2	0-2	1.01	0.09	11.9	0.3	11.8	0.8	2.07	0.11	0.45	0.04	0.13	0.00	2.3	0.2	218.9	48.7	14.4	1.7	
		2-10	0.90	0.07	10.8	0.3	12.0	1.3	1.14	0.23	0.24	0.04	0.06	0.01	1.2	0.1	43.6	5.03	8.9	0.9	
	deep	A3	0-2	0.71	0.09	7.1	0.4	10.2	1.7	0.74	0.28	0.34	0.09	0.02	0.00	2.9	0.3	220.3	38.1	11.0	2.0
			2-10	0.70	0.05	6.5	0.6	9.4	0.7	0.53	0.04	0.25	0.04	0.03	0.00	1.2	0.2	17.2	2.0	7.5	1.0
		B3	0-2	0.83	0.08	8.1	0.3	9.8	0.6	1.12	0.25	0.32	0.02	0.05	0.00	2.4	0.5	200.8	18.5	18.8	0.4
			2-10	0.83	0.07	7.2	0.2	8.7	0.4	0.48	0.12	0.16	0.04	0.03	0.00	0.9	0.1	22.8	2.4	7.6	1.4
C3		0-2	0.90	0.06	8.5	0.1	9.5	0.7	0.38	0.07	0.24	0.05	0.03	0.00	1.3	0.1	129.6	20.1	14.0	3.0	
		2-10	0.81	0.07	7.9	0.1	9.8	1.0	0.33	0.07	0.31	0.04	0.02	0.00	1.8	0.1	33.7	2.03	11.5	1.6	

TN: total nitrogen, OC: organic carbon, OC/TN: organic carbon/total nitrogen ratio, carbo: carbohydrates, chl-a: chlorophyll-a, TBN: total bacterial number,

LA: leucine aminopeptidase activity, BG: β-glucosidase activity.

	summer							winter						
	A1	A2	A3	B2	B3	C2	C3	A1	A2	A3	B2	B3	C2	C3
<i>Fustiaria rubescens</i> (Deshayes, 1825)	4.6	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Galeomma turtoni</i> (Turton, 1825)	0	4.6	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda undet.	0	0	0	4.6	0	0	0	0	0	0	0	0	0	0
<i>Gastrosaccus sanctus</i> (Van Beneden, 1861)	0	0	0	0	0	0	0	6.9	0	0	0	0	0	0
Glyceridae *	27.5	59.7	13.8	9.2	23.0	45.9	13.8	41.3	34.4	0	27.5	6.9	34.4	6.9
<i>Goneplax rhomboides</i> (Linnaeus, 1758)	0	4.6	0	0	4.6	9.2	4.6	0	6.9	0	0	0	0	0
Goniadidae	4.6	0	0	0	0	0	4.6	0	0	0	0	0	0	0
<i>Harpiniadellavallei</i> (Chevreux, 1910)	0	0	0	32.1	4.6	9.2	0	34.4	20.7	0	20.7	0	0	0
<i>Harpinia</i> sp.	0	9.2	4.6	0	0	0	4.6	0	0	0	0	0	0	0
Hesionidae	4.6	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hippomedon massiliensis</i> (Bellan-Santini, 1965)	9.2	4.6	0	4.6	0	0	0	0	6.9	0	0	0	0	0
<i>Iphinoe serrata</i> (Norman, 1867)	9.2	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Iphinoe</i> sp.	0	0	0	4.6	0	0	0	0	0	0	0	0	0	0
<i>Kurtiella bidentata</i> (Montagu, 1803) *	4.6	0	0	0	0	0	0	0	0	0	0	0	0	0
Larva decapoda	0	0	0	0	0	0	0	0	6.9	0	0	0	0	0
<i>Leucothoe serraticarpa</i> (Della Valle, 1893)	0	0	0	13.8	0	0	4.6	0	0	0	0	0	0	0
<i>Lucinella divaricata</i> (Linnaeus, 1758) *	13.8	0	0	0	0	0	0	6.9	0	0	0	0	0	0
Lumbrineridae	0	101.0	9.2	133.1	27.5	119.4	27.5	20.7	144.6	20.7	165.3	20.7	48.2	20.7
<i>Maeragrossimana</i> (Montagu, 1808)	0	4.6	0	0	0	0	0	0	0	0	0	0	6.9	0
<i>Magelona</i> sp. *	9.2	13.8	0	4.6	0	27.5	0	0	27.5	0	0	0	6.9	0
Maldanidae	9.2	13.8	4.6	4.6	0	18.4	0	0	41.3	6.9	27.5	0	6.9	0
<i>Metaphoxus simplex</i> (Bate, 1857)	0	0	0	0	0	0	0	0	6.9	0	6.9	0	0	0
<i>Myrtea spinifera</i> (Montagu, 1803) *	0	0	0	0	0	4.6	0	0	0	0	0	0	0	0
<i>Necallia nassatruncata</i> (Giar & Bonnier, 1890) *	0	0	0	0	0	4.6	0	0	0	0	0	0	0	0
Nematoda	18.4	0	0	0	0	4.6	0	0	0	0	0	0	0	6.9
Nemertea	0	4.6	0	9.2	0	0	0	0	6.9	0	6.9	0	0	0
Nephtyidae	9.2	9.2	13.8	4.6	27.5	0	18.4	27.5	0	6.9	6.9	6.9	20.7	13.8
Nereididae	0	13.8	4.6	4.6	0	4.6	0	0	6.9	0	0	0	0	0
<i>Nucula sulcata</i> (Bronn, 1831) *	18.4	0	18.4	4.6	9.2	0	4.6	0	0	6.9	0	0	0	0
Oligochaeta	0	0	0	0	0	0	0	0	27.5	0	0	6.9	0	0
Onuphidae	18.4	0	0	0	0	0	0	20.7	0	13.8	0	6.9	0	0

