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Organic biomarkers in deep-sea regions affected by bottom trawling: pigments, fatty acids, amino acids and carbohydrates in surface sediments from the La Fonera (Palamós) Canyon, NW Mediterranean Sea

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Abstract. Deep-sea ecosystems are in general adapted to a limited variability of physical conditions, resulting in high vulnerability and slow recovery rates from anthropogenic perturbations such as bottom trawling. Commercial trawling is the most recurrent and pervasive of human impacts on the deep-sea floor, but studies on its consequences on the biogeochemistry of deep-sea sediments are still scarce. Pigments, fatty acids, amino acids and carbohydrates were analysed in sediments from the flanks of the La Fonera (Palamós) submarine canyon (NW Mediterranean Sea), where a commercial bottom trawling fishery has been active for more than 70 yr. More specifically, we investigated how trawling-induced sediment reworking affects the quality of sedimentary organic matter which reaches the seafloor and accumulates in the sediment column, which is fundamental for the development of benthic communities. Sediment samples were collected during two oceanographic cruises in spring and autumn 2011. The sampled sites included trawl fishing grounds as well as pristine (control) areas. We report that bottom trawling in the flanks of the La Fonera Canyon has caused an alteration of the quality of the organic matter accumulated in the upper 5 cm of the seafloor. The use of a wide pool of biochemical tracers characterized by different reactivity to degradation allowed for us to discriminate the long-term effects of trawl-induced sediment reworking from the natural variability caused by the seasonal cycle of production and sinking of biogenic particles. Differences between untrawled and trawled areas were evidenced by labile amino acids, while differences between spring and autumn samples were detected only by the more labile indicators chlorophyll *a* and monounsaturated fatty acids. These results suggest that changes in the biochemical composition of the sedimentary organic matter caused by bottom trawling can be more relevant than those associated with natural seasonality and pose serious concerns about the ecological sustainability of deep-sea trawling activities.

1 Introduction

Commercial bottom trawling is a fishing activity that consists in pulling heavy fishing gear over the seafloor, with negative effects on the sedentary macrofauna and on fish stocks (Jones, 1992; McConnaughey et al., 2000; Thrush and Dayton, 2002; Morato et al., 2006; Thrush and Dayton, 2010). Over the last decades, commercial fishing has been extending its operations over larger and deeper areas of the world's oceans (Bensch et al., 2009). It has been recently estimated that the overall oceans' area (including continental shelf regions and seamounts) used as trawling grounds accounts for 20 million km^2 (World Resources Institute, 2000), of which about one-fourth is located on the continental slope regions (Puig et al., 2012). Due to their low resilience, deep-water areas (> 200 m) are more vulnerable to anthropogenic disturbance than shallow, high-energy environments (McConnaughey et al., 2000), and for this reason, the effects of bottom trawling on the deep benthic communities and their habitats need to be studied carefully.

Submarine canyons are morphological incisions traversing continental margins that have the capacity to facilitate the transport of dissolved and particulate matter from continental shelves to deeper waters (Granata et al., 1999; Allen and Durrieu de Madron, 2009; Palanques et al., 2011), and therefore are sites of high productivity and organic matter enrichment (Vetter and Dayton, 1999; García and Thomsen, 2008; De Leo et al., 2010; Vetter et al., 2010) where benthic communities are favoured by food availability. Not surprisingly, submarine canyons harbour valuable stocks of living resources and their surroundings are often targeted by commercial fisheries for purposes such as bottom trawling (Company et al., 2012).

This study focuses on the flanks of the La Fonera Canyon (also known as Palamós Canyon), where a monospecific otter trawl fishery targeting the deep-water shrimp *Aristeus antennatus* has been active for more than 70 years to depths of up to 800 m (Tobar and Sardà, 1987). Previous studies have shown that bottom trawling along the flanks of the La Fonera Canyon can trigger sediment gravity flows (Palanques et al., 2006), which transport sediments downslope from the fishing grounds into the canyon axis (Martín et al., 2006, 2007), affecting sediment accumulation rates in the lower canyon (Martín et al., 2008) and ultimately altering the morphology of the canyon flanks as a result of chronic reworking and removal of sediments (Puig et al., 2012).

This work aims to assess the effects of bottom trawling on the quality of the organic matter in surface sediments by comparing regions affected and unaffected by this fishing technique. The quantity and the quality of the organic matter settling on the seabed represent important factors regulating benthic biomass (Grebmeier et al., 1988) and are important for the development of benthic communities (Thompson and Nichols, 1988; Graf, 1989). Organic matter indicators have been already used to address the biochemical composition of sediments in submarine canyons (García and Thomsen, 2008; Pusceddu et al., 2010; Pasqual et al., 2011). Here, we utilized biomarkers characterized by different susceptibilities to environmental conditions. Pigments and unsaturated fatty acids, the most labile compounds (Haddad et al., 1992; Wakeham et al., 1997), are good indicators of fresh organic matter, whereas carbohydrates are considered the most refractory macromolecules (Wakeham et al., 1997). Protein and neutral amino acids are less labile than pigments and unsaturated fatty acids, but like them, they are utilized as indicators of labile material (Mayer et al., 1995; Lee et al., 2004). The use of this combination of biomarkers will allow for us to study the effects of sediment reworking by trawling on the quality of the organic matter as well as compare them with those related to seasonal variability, being that sediment samples were collected both in spring and in autumn.

2 Materials and methods

2.1 Sampling

Coring stations were chosen based on the distribution of fishing grounds in the La Fonera Canyon, which is well known thanks to the satellite-based navigation tracks of bottom trawlers (i.e. vessel monitoring system (VMS) data) (Fig. 1). Trawling activities are conducted on the flanks of the canyon between 350 and 800 m depth along two main fishing grounds: Sant Sebastià on the northern flank and Rostoll on the southern flank (Fig. 1). The offshore sector of the southern flank remains unexploited by the trawling fleet and therefore will be used as a control site.

Samples were taken on board RV *García del Cid* during two oceanographic cruises: HERMIONE I in spring 2011 (SPR), and HERMIONE II in autumn 2011 (AUTM). Station names, depths, coordinates and sampling dates are listed in Table 1 (see also Fig. 1). Two regions were targeted in the study area: an untrawled region in the offshore southern canyon flank (UTR: stations SF-3, SF-4, SF-5 and SF-6) and a trawled region comprising both the northern canyon flank and the inshore southern flank (TR: stations NF-1, NF-2, NF-3, NF-4, NF-5, NF-6, SF-1 and SF-2). All sampling stations were located within a water depth range of approximately 450–600 m. Sediment samples were taken using a KC multi-corer equipped with six polycarbonate tubes with an inner diameter (i.d.) of 9.4 cm. From each station, a tube with an undisturbed sediment–water interface was selected for analysis. During the SPR cruise, the multi-corer tubes were sub-sampled down to 5 cm with smaller tubes (i.d. 3.5 cm) that were immediately frozen at $-20\,^{\circ}$C. During the AUTM cruise, the multi-corer tubes were directly sub-sampled on board in 1 cm slices from the top to 5 cm depth and subsamples were stored in plastic bags at $-20\,^{\circ}$C.

Except for the offshore part of the northern canyon flank (NF-5 and NF-6), the top 5 cm of the sediment column at sampled sites was basically composed of silty mud with variable amounts of sand (1–14 %). We noticed that along the northern canyon flank the coarseness of the topmost sediments increased seawards. Given the wide differences in the textural properties compared with all the other stations, the station NF-5 was thereafter discarded for statistical analysis. Regarding NF-6, it could not be sampled due to the stiffness of bottom sediments at that station. In previous works, no substantial differences between the northern and southern canyon flanks were observed in terms of the composition of downward particulate matter fluxes collected by sediment traps (Martín et al., 2006); hence, for the purpose of this study, we assume that there are no major natural compositional differences between the trawled and untrawled pool of samples.

Table 1. Names, depths, coordinates and sampling dates of the coring stations. Trawled (TR) and untrawled (UTR) regions as well as spring (SPR) and autumn (AUTM) sampling seasons corresponding to each station are also indicated.

Station	Depth (m)	Latitude ° N (decimal)	Longitude ° E (decimal)	Sampling date	Trawling region	Sampling season
NF-1	475	41.8853	3.3070	12 October 2011	TR	AUTM
NF-2	500	41.8860	3.3321	13 May 2011	TR	SPR
NF-3	591	41.8903	3.3495	12 October 2011	TR	AUTM
NF-4	486	41.8892	3.3785	12 October 2011	TR	AUTM
NF-5	556	41.8890	3.4093	12 May 2011	TR	SPR
NF-6	470	41.8978	3.4257	12 October 2011	TR	AUTM
SF-1	463	41.8512	3.2912	12 October 2011	TR	AUTM
SF-2	503	41.8340	3.3178	13 May 2011	TR	SPR
SF-3	457	41.8150	3.3433	12 October 2011	UTR	AUTM
SF-4	453	41.8102	3.3652	11 October 2011	UTR	AUTM
SF-5	472	41.8075	3.3872	13 May 2011	UTR	SPR
SF-6	498	41.7942	3.4075	11 October 2011	UTR	AUTM

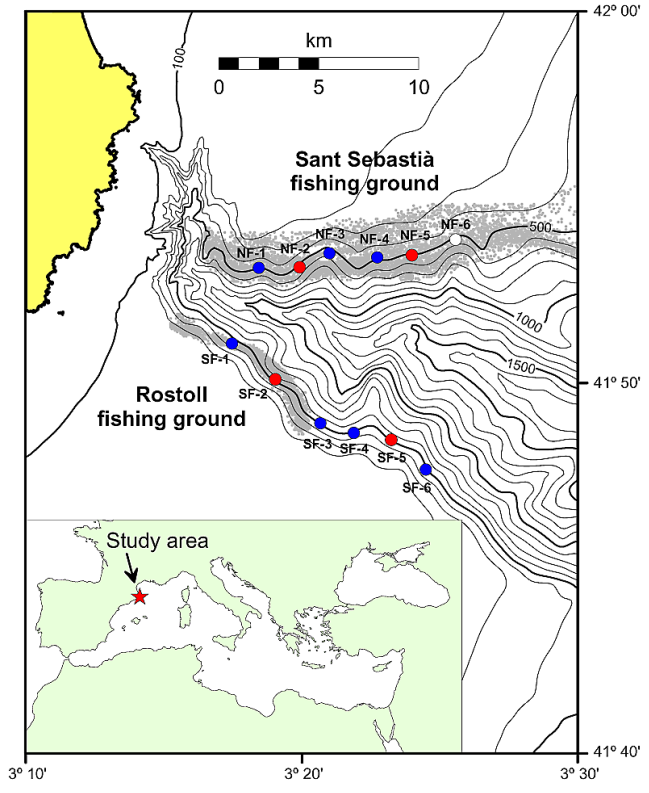

Fig. 1. Bathymetric chart of the La Fonera (Palamós) Canyon showing the locations of sediment sampling stations. The main fishing grounds active on the canyon flanks are defined by the clouds of grey points corresponding to vessel monitoring system (VMS) positions from Palamós harbour trawlers (see Puig et al., 2012 for details). Red dots correspond to coring stations visited in spring 2011 and blue dots to stations visited in autumn 2011. No sediment samples could be retrieved from station NF-6 (white dot) due to the extreme stiffness of bottom sediments at that site (see the text for details).

2.2 Laboratory analysis

Frozen sediment samples were freeze-dried ($P = 0.1$ mbar and $T = -80$ °C) for 24 h and prepared for analysis by high-performance liquid chromatography (HPLC) (pigments, amino acids and carbohydrates) or gas chromatography (GC) (fatty acids). The SPR surface sediment samples kept in the small tubes were subsampled at 1 cm intervals down to 5 cm to have the same vertical resolution as the AUTM sediment samples. Laboratory analyses were carried out on all single samples from 0 to 5 cm depth, although for some analyses there was not enough material left from specific centimetres (see Supplement A, B, C and D).

The percentage of total organic carbon in dried and ground samples was obtained by means of a LECO CN-2000 auto-analyser after treatment with HCl to remove inorganic carbon. Blanks and standards (analytical grade EDTA, Sigma) were intercalated within sets of samples to calibrate the measurements.

2.2.1 Pigments

Pigments were extracted from sediments using the methodology described by Sun et al. (1991) and analysed by HPLC following the protocol detailed by Wright et al. (1991). For each sample, pigments were extracted from sediments by adding 3 mL of pure acetone to approximately 2 g of freeze-dried sediment. After vortex mixing, samples were placed in an ultrasonic bath for 10 min and then centrifuged at 3000 rpm for 10 min. After recovering 1.5 mL of the supernatant, the entire procedure was repeated a second time in order to obtain for each sample approximately 3 mL of extract. Finally, the pigment extracts were evaporated with nitrogen, re-diluted with 200 µL of pure acetone, filtered with syringe nylon filters (pore diameter 0.45 µm) and transferred to HPLC vials for their later analysis.

Pigments were analysed with a W-600 controller coupled with a W717 autosampler using the technique HPLC-FP-diode array detector (DAD) (Gradient). The column was a C_{18} Prontosil-AA-FMOC 5 µm (250 mm × 4 mm). A Waters DAD 2996 (436 nm) and a Jasco FP-1520 (Ex. 440 nm, Em. 660 nm) were used as detectors. The mobile phase consisted of three solutions, A (methanol and ammonium acetate 0.5 M, 4 : 1 v/v), B (acetonitrile and Milli-Q water, 9 : 1 v/v) and C (ethyl acetate, HPLC grade). The velocity of the flux was 0.9 mL min^{-1} and the pressure 2500 psi. The volume of injection was 100 µL and the run time 34 minutes. The gradient is described in Wright et al. (1991). A mixed pigments standard (DHI) was also analysed. Pigment peaks were identified in the chromatogram using the Empower software, which is specifically for the treatment of chromatographic data. Pigments were identified by knowing their retention times in the column and the wavelength of maximum absorbance characteristic for each pigment.

Among pigments, chlorophyll *a* (Chl *a*) was used as an indicator of labile organic matter, whereas carotenoids (peridin, fucoxanthin, diatoxanthin, lutein, zeaxanthin and canthaxanthin) were used as refractory organic matter indicators. The Chl *a*-to-pheophytin *a* (Pheo *a*) ratio and the sum of the Chl *a* degradation products (pheophytin *a*, pheophorbide *a* and pyropheophorbide *a*) were used as degradation indexes.

Pigment relative abundances are expressed as percentages.

2.2.2 Fatty acids

Fatty acids (FA) were extracted through a one-step transesterification process (Lewis et al., 2000; Christie, 2003; Indarti et al., 2005) modified to be applied on marine matrixes (algae and sediments) (Nahon et al., 2010; Bourgeois et al., 2011).

Approximately 2 g of freeze-dried sediment was extracted in 8 mL of a cold solution of methanol, 98 % sulfuric acid and chloroform in the presence of butyl hydroxytoluene (BHT), an antioxidant at a concentration of 50 mg L^{-1}. The ratio of methanol to chloroform to sulfuric acid in the solvent extraction was 1.7 : 2 : 0.3 $v/v/v$. After adding 20 µL of the internal standard nonadecanoic acid (1 mg mL^{-1}), samples were placed in a preheated oven (at 90 °C for 90 min) for lipid extraction and methylation of the released fatty acids in fatty acid methyl esters (FAME). Ultra-pure water (2 mL) was added to each sample to partition the extract in two phases. Following centrifugation (5 min at 1500 rpm and 4 °C), the inferior chloroform phase was recovered. A second extraction was made with a solution of hexane and chloroform, (4 : 1 v/v) and after centrifugation (5 min at 1500 rpm and 4 °C), the superior phase was recovered and added to the first organic phase. This procedure was repeated twice. The organic phases were pooled and cleaned with a cold solution of potassium carbonate (2 %). After centrifugation (5 minutes at 1500 rpm and 4 °C), the organic phase was recovered, evaporated to dryness at room temperature, and diluted again with 400 µL of pure hexane prior to analysis.

FAME were analysed with a GC coupled to an ion-trap mass spectrometer (MS) GCMS-QP2010. A BPX70 chromatographic column was used. The column had a length of 30 m and an internal diameter of 0.25 mm; film thickness was 0.25 µm. The flow was constant with a velocity of 1 mL min^{-1}. The injector temperature was set to 260 °C and the volume injected was 1 µL. The use of known standards as reference (Supelco 37-component FAME mix) allowed for FA quantification. Data were treated with the GCMSsolution software version 2.5. The peaks of the chromatogram were identified based on FA molecular weights and on their retention times in the chromatographic column.

In order to present the data set in a comprehensible form, fatty acids were grouped according to their chemical structure into (a) polyunsaturated fatty acids (PUFA), compounds with two or more unsaturated bonds; (b) monounsaturated fatty acids (MUFA), compounds with one unsaturation; (c) mid-chain fatty acids (MC-FA), chain length $\leq C_{20}$; and (d) long-chain fatty acids (LC-FA), chain length C_{21}-C_{26}.

Concentrations are expressed in mg kg^{-1} DW.

2.2.3 Amino acids

Total hydrolysable amino acids (THAA) were analysed following the AccQ-Tag method by Waters which utilizes the pre-column derivatization reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) to produce fluorescently labelled amino acids for analysis. The Waters AccQ-Tag Fluor derivatization reagent kit, ref: WAT52880, was used. Approximately 15 mg of freeze-dried sediment was submitted to hydrolysis with 250 µL of hydrochloric acid 12 N and 230 µL of Milli-Q water at 100 °C for 24 h and under vacuum. Together with sediment samples, the internal standard α-amino-*n*-butyric acid (AABA) was also submitted to acidic hydrolysis. After the evaporation of the hydrolysate with a rotary evaporator, samples were derivatizated. The hydrolysate was redisolved with 300 µL of hydrochloric acid 20 mM, filtered with syringe nylon filters (pore diameter 0.45 µm) and buffered with 70 µL of a borax buffer solution. The reagent A (20 µl) was then added. After 1 min at ambient temperature and 10 min at 55 °C, the solution was mixed with 100 µL of the mobile phase and transferred to HPLC vials.

Analyses were carried out with a gradient HPLC Waters 600 coupled with a Waters Delta 600 pump and a Waters 2487 (dual absorbance detector) UV absorption detector (Ex. 250 nm, Em. 395 nm). The column was a NOVA-PAK® C_{18} 4 µm 3.9 × 150 mm part no. WAT086344. An automatic injector Waters 717 plus and an in-line degasser AF were utilized. Analyses were carried out at a temperature of 37 °C. The mobile phase consisted of three solutions, A (AccQ-Tag Eluent A), B (acetonitrile) and C (HPLC-grade water). The velocity of the flux was 1 mL min^{-1} and the pressure

2000 psi. The volume of injection was 20 µL and the run time 55 min. The Pierce (Thermo Scientific) Amino Acid Standard H, 10 × 1 mL (reference 200880) was used.

Data were treated with the JASCO ChromPass chromatography data system software version 1.7.403.1. Two subgroups of THAA, neutral THAA (alanine, valine, isoleucine, leucine and phenylalanine) and protein THAA (aspartic acid, serine, glutamic acid, glycine, histidine, arginine, threonine, alanine, tyrosine, valine, lysine, isoleucine, leucine and phenylalanine) were used as labile organic matter indicators. The aspartic acid (Asp) to β-alanine (BALA) and the glutamic acid (Glu) to γ-aminobutyric acid (GABA) ratios were utilized as degradation indexes.

Concentrations are expressed in nmol mg $^{-1}$ DW.

2.2.4 Carbohydrates

For the analysis of carbohydrates (CHO), sediment samples were prepared following the protocol of Oakes et al. (2010). A volume of 0.5 mL of concentrated (11 M) sulfuric acid was added to approximately 0.5 g of freeze-dried sediment. The mixture of sediment and sulfuric acid was mixed with the vortex, and after one hour, 4.5 mL of Milli-Q water was added to reduce the concentration of sulfuric acid from 11 M to 1 M. Samples were then hydrolysed for 1 h at 120 °C (the reaction was stopped by placing on ice the Corning Pyrex glass tubes containing the samples). After neutralization with approximately 2 g of strontium carbonate and centrifugation (15 min at 15 000 rpm), the hydrolysate was transferred to Eppendorf tubes and stored overnight at −20 °C. Before preparing the HPLC vials, Eppendorf tubes were centrifuged again (15 min at 15 000 rpm) and the supernatant was filtered with syringe nylon filters (pore diameter 0.45 µm).

Carbohydrates were analysed with a Waters 2695 52C separations module chromatograph coupled with a Waters 2414 refractive index detector (temperature 37 °C and sensitivity 16). Two columns were utilized: an Aminex HPX-87P (300 × 7.8 mm) and an Aminex HPX-87C (300 × 7.8 mm). The mobile phase consisted of Milli-Q water. The velocity of the flux was 0.6 mL min^{-1} and the pressure 1700 psi. The volume of injection was 100 µl and the run time 25 min. Standard reagents of D(+)-glucose anhydrous (reference G-8270 Sigma), D(+)-xylose (reference X1500 Sigma), D(+)-galactose (reference G-0750 Sigma) and D(+)-mannose (reference 63580 Fluka) were also analysed to obtain quantitative data. Carbohydrate peaks were identified in the chromatogram through their retention times, using the Empower software, specifically for the treatment of chromatographic data.

Due to the low quantity of sediment available from those stations sampled in spring, it has been possible to perform CHO analyses only on autumn samples.

Concentrations are expressed in mg g^{-1} DW.

3 Statistics

Univariate and multivariate statistical tests were carried out on all the analysed samples. Data were OC-normalized before performing statistical analysis by dividing the dry weight concentration by the percentage of total organic carbon in the dry weight sample (expressed as a decimal).

Two-way ANOVA comparisons were made to test for differences between spring (SPR) and autumn (AUTM) and between the untrawled and the trawled regions (UTR and TR, respectively), and also to verify if an interaction between the effects of trawling and season on the quality of the organic matter exists. For carbohydrates, due to the absence of spring samples, a one-way ANOVA has been performed instead of the two-way ANOVA. Normality and homogeneity of variances were tested before performing the one-way and two-way ANOVA. Normality was assessed through the Kolmogorov–Smirnov (K–S) test. The null hypothesis of the K–S test is that no difference exists between the distribution of the data set and an ideal Gaussian distribution. When the p value of the K–S test is lower than 0.05, the null hypothesis is rejected, meaning that the data did not follow a normal distribution. Measures of shape, like skewness and kurtosis, were also considered, being that a normal distribution is characterized by values of skewness comprised between −0.5 and 0.5 and values of kurtosis approximately equal to 3. When normality was violated, data were transformed before testing the homogeneity of variances (homoscedasticity). Homoscedasticity was tested though the Hartley $F_{\max}$ test (Hartley, 1950) by dividing the larger variance by the smaller one to obtain the F ratio. An F-ratio value close to 1, or higher than 1 but lower than the value in the $F_{\max}$ table, indicated homogeneity of variance. Since the number of samples was not the same within both the couple of groups compared, the degrees of freedom were calculated considering the higher number of samples for each couple of groups. When there was no normality and/or homogeneity of variance, a more conservative level of significance was considered ($p = 0.001$). Due to the uneven sample size and also to avoid problems related to normality, a generalized linear model (GLM) has been also used to compare results from the two-way ANOVA and the GLM analyses. The one-way ANOVA was performed with the Statistica software v.5.5, whereas the two-way ANOVA and the GLM were performed with the R software version 2.15.2.

Multivariate statistical analyses were also performed. Before multivariate analysis, data were pre-treated by a square root or fourth root transformation to downweight contributions from quantitatively dominant macromolecules. Euclidean distances between pairs of samples were then calculated to obtain a triangular distance matrix. Differences between SPR and AUTM as well as between UTR and TR were tested through the non-parametric analysis of similarities (ANOSIM test). ANOSIM is a resemblance-based permutation method used to test the null hypothesis of "no

differences" between a priori-defined groups of multivariate samples. Since dissimilarities are not normally distributed, ANOSIM uses ranks of pairwise dissimilarities. The null hypothesis is that the average of rank dissimilarities between objects within groups is equal to the average of rank dissimilarities between objects from different groups. The ANOSIM R statistic value is used to measure how different two groups are, and it is calculated from the average of rank dissimilarities between objects (by subtracting the average of rank dissimilarities within groups to the average of rank dissimilarities between groups). When $R = 0$, inter- and intra-group differences are equal, and when $R < 0$, intra-group differences are higher than inter-group differences, whereas when $R > 0$, inter-group differences are higher than intra-group differences (Clarke and Gorley, 2001). Primer software v.6 was utilized (Clarke, 1993; Clarke and Gorley, 2006) for multivariate statistical analyses.

The similarity percentages (SIMPER) test was carried out to quantify the contribution of biomarkers to dissimilarities within and between trawling regions and season groups. SIMPER calculates the percentage contribution of each variable (i.e. Chl *a*; MUFA: 16 : 1, 18 : 1t, 18 : 1, 18 : 1c, 20 : 1, 22 : 1, 24 : 1; protein and neutral THAA: Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Tyr, Val, Lys, Ile, Leu, Phe) to the dissimilarities within each a priori-defined group (i.e. UTR, TR, SPR and AUTM) between regions (UTR and TR) and between sampling seasons (SPR and AUTM). The average square distance is shown together with the percentage contribution of each variable to the average square distance.

Principal components analysis (PCA) was used to identify the best biomarker (among Chl *a*, 16 : 1, 18 : 1t, 18 : 1, 18 : 1c, 20 : 1, 22 : 1, 24 : 1, Asp, Ser, Glu, Gly, His, Arg, Thr, Ala, Tyr, Val, Lys, Ile, Leu and Phe) to distinguish between trawling regions and between season groups. The PCA is a multivariate technique used to reduce the multidimensionality which corresponds to the variation of a high number of correlated variables. Multi-dimensionality is reduced to two or three dimensions which correspond to a limited number of uncorrelated components, each of which is a combination of the original variables. The extracted uncorrelated components are called principal components (PC) and are estimated from the correlation matrix of the original variables. The objective of PCA is to reduce dimensionality by extracting the smallest number of components that account for most of the variation in the original multivariate data and to summarize the data with no loss of information. The first PCA accounts for as much of the variation as possible and each successive component accounts for a little less. The eigenvalues measure the amount of the variation explained by each PC and will be largest for the first PC and smaller for the subsequent PCs, whereas the eigenvectors provide the weights to compute the uncorrelated PC.

4 Results

Laboratory analysis results are shown in Supplement A, B, C and D, whereas statistical test results are shown in Tables 2–4. A significant difference between SPR and AUTM was found in the Chl *a* (SPR: 5.99 ± 5.93; AUTM: 1.84 ± 2.15) (Supplement A1, Table 2c and e) and carotenoid (SPR: 13.47 ± 7.75; AUTM: 11.99 ± 7.44) (Supplement A3 and Table 3) percentages and, as regards Chl *a* degradation products, in the sum of the percentages of the Chl *a* degradation products (SPR: 18.22 ± 11.61; AUTM: 23.97 ± 12.68) (Supplement A2 and Table 3). Based on the results of the two-way ANOVA, the Chl *a*-to-Pheo *a* ratio also showed significant inter-seasonal differences (SPR: 1.60 ± 1.86; AUTM: 0.43 ± 0.82) (Table 2c), with a p value of 0.004. Nevertheless, neither AUTM nor SPR samples showed a normal distribution (Table 2a), which is one of the assumptions for performing ANOVA. Therefore, we consider instead the results of the GLM, which showed no significant differences in the Chl *a*-to-Pheo *a* ratio between seasons (Table 2e). The two-way ANOVA evidenced a lack of interaction between trawling and season both in the case of Chl *a* and for the Chl *a*-to-Pheo *a* ratio (Table 2c).

The total concentration of FA varied from ~ 1.4 mg kg^{-1} DW (at NF-3 station, from 4 to 5 cm depth) to ~ 43.1 mg kg^{-1} DW (at SF-5 station, from 0 to 1 cm depth) (Supplement B). PUFA and MC-FA concentrations were not significantly different, neither between seasons nor between trawling regions, whereas significant differences in MUFA concentrations were found between SPR and AUTM (Table 3). Also LC-FA concentrations were significantly different between sampling seasons (Table 3).

The total concentration of THAA varied from ~ 11 nmol mg^{-1} DW (at NF-4 station, from 3 to 4 cm depth) to ~ 32 nmol mg^{-1} DW (at SF-3 station, from 2 to 3 cm depth) (Supplement C). No significant differences in the concentration of total, protein and neutral THAA were found between SPR and AUTM, whereas significant differences between UTR and TR were found in the concentrations of total, protein and neutral THAA (Table 3). No significant differences in the Asp-to-BALA and the Glu-to-GABA ratios were found between SPR and AUTM or between UTR and TR (Table 2c and e). The two-way ANOVA evidenced a lack of interaction between trawling and season both in the case of the Asp-to-BALA ratio and in the case of the Glu-to-GABA ratio (Table 2c).

Carbohydrate results are shown in Supplement D. The total concentration of carbohydrates varied from ~ 0.3 mg g^{-1} DW (at SF-4 station, from 2 to 3 cm depth) to ~ 1.1 mg g^{-1} DW (at SF-3 station, from 2 to 3 cm depth) (Supplement D). No significant difference between UTR and TR was found for xylose, rhamnose or for the sum of the labile sugars glucose and mannose (Tables 2d and 3).

Based on the SIMPER analysis, homogeneity was similar in the untrawled and in the trawled region (average square

Table 2a. Results of the Kolmogorov–Smirnov test. Normality distributions are marked in bold.

Variable	Transformation	K–S	Skewness		Kurtosis	
		p value	Skewness	Std. Err. Skewness	Kurtosis	Std. Err. Kurtosis
% Chl *a* AUTUMN	–	< 0.10	0.953	0.403	−0.246	0.788
% Chl *a* SPRING	–	**> 0.20**	0.414	0.580	−1.220	1.121
% Chl *a* TRAWLED	–	< 0.10	1.341	0.427	0.914	0.833
% Chl *a* UNTRAWLED	–	**> 0.20**	1.161	0.524	0.364	1.014
% Chl *a* AUTM trawled	–	< 0.10	0.990	0.512	−0.285	0.992
% Chl *a* AUTM untrawled	–	**> 0.20**	0.849	0.597	−0.537	1.154
% Chl *a* SPR trawled	–	**> 0.20**	0.022	0.687	−1.741	1.334
% Chl *a* SPR untrawled	–	**> 0.20**	0.477	0.913	−3.086	2.000
Chl *a*-to-Pheo *a* ratio AUTUMN	–	< 0.01	2.492	0.409	5.534	0.798
Chl *a*-to-Pheo *a* ratio SPRING	–	**> 0.20**	0.991	0.580	−0.300	1.121
Chl *a*-to-Pheo *a* ratio TRAWLED	Log $(x+1)$	< 0.05	1.248	0.427	0.166	0.833
Chl *a*-to-Pheo *a* ratio UNTRAWLED	Log $(x+1)$	< 0.15	1.571	0.536	1.530	1.038
Chl *a*-to-Pheo *a* ratio AUTM trawled	Log $(x+1)$	< 0.10	2.061	0.512	3.495	0.992
Chl *a*-to-Pheo *a* ratio AUTM untrawled	Log $(x+1)$	< 0.20	1.924	0.597	3.657	1.154
Chl *a*-to-Pheo *a* ratio SPR trawled	–	**> 0.20**	1.076	0.687	0.572	1.334
Chl *a*-to-Pheo *a* ratio SPR untrawled	–	**> 0.20**	0.733	0.913	−2.262	2.000
Asp-to-BALA ratio AUTUMN	–	**> 0.20**	0.963	0.434	1.326	0.845
Asp-to-BALA ratio SPRING	–	**> 0.20**	0.542	0.661	0.364	1.279
Asp-to-BALA ratio TRAWLED	–	**> 0.20**	0.991	0.472	1.142	0.918
Asp-to-BALA ratio UNTRAWLED	–	**> 0.20**	1.623	0.564	4.587	1.091
Glu-to-GABA ratio AUTUMN	–	**> 0.20**	1.324	0.448	1.702	0.872
Glu-to-GABA ratio SPRING	–	**> 0.20**	2.154	0.661	5.691	1.279
Glu-to-GABA ratio TRAWLED	–	**> 0.20**	1.641	0.481	3.574	0.935
Glu-to-GABA ratio UNTRAWLED	–	**> 0.20**	1.176	0.580	0.800	1.121
Xylose TRAWLED	–	**> 0.20**	−0.390	0.524	−0.581	1.014
Xylose UNTRAWLED	–	**> 0.20**	1.802	0.616	3.598	1.191
Rhamnose TRAWLED	–	**> 0.20**	0.238	0.524	1.379	1.014
Rhamnose UNTRAWLED	–	**> 0.20**	−0.168	0.616	−0.164	1.191

Table 2b. Results of the Hartley F_{max} test. Homogeneity of variance is marked in bold. Transformed data are marked with an asterisk.

Macromolecule	Differences to test	Indicator	Variance AUTM	Variance SPR	Variance TRAW	Variance UNTRAW	*F* ratio	df	No. of groups
PIGMENTS	BETWEEN SEASONS	% Chlorophyll *a*	4.62	35.17			7.61	33	2
		Chl *a*-to-Pheo *a* ratio	0.67	3.46			**5.16**	32	2
	BETWEEN TRAWLING REGIONS	% Chlorophyll *a*			23.81	6.35	3.75	29	2
		% Chl *a* SPR			44.22	13.84	**3.20**	9	2
		% Chl *a* AUTM			5.66	3.46	**1.64**	19	2
		Chl *a*-to-Pheo *a* ratio*			0.0576	0.0625	**1.09**	29	2
		Chl *a*-to-Pheo *a* ratio SPR			2.47	6.00	**2.43**	9	2
		Chl *a*-to-Pheo *a* ratio AUTM*			0.0361	0.0225	**1.60**	19	2
AMINO ACIDS	BETWEEN SEASONS	Asp-to-BALA ratio	68.72	15.05			4.57	28	2
		Glu-to-GABA ratio	78.68	62.09			**1.27**	26	2
	BETWEEN TRAWLING REGIONS	Asp-to-BALA ratio			47.20	69.56	**1.47**	23	2
		Glu-to-GABA ratio			55.65	105.88	**1.90**	22	2
CARBO-HYDRATES	BETWEEN TRAWLING REGIONS	Xylose			9.852	70.232	7.13	18	2
		Rhamnose			103.665	230.600	**2.23**	18	2

distance: 18.31 and 21.12, respectively), and amino acids (total, protein and neutral THAA) contributed with low percentages to the average square distance within UTR and TR (Table 4a). Homogeneity was similar also in AUTM and in SPR (average square distance: 20.77 and 17.76, respectively), and in both cases, Chl *a* and MUFA showed low contributions

Table 2c. Results of the statistical test two-way ANOVA for pigments (Chl *a* and Chl *a*-to-Pheo *a* ratio) amino acids (Asp-to-BALA and Glu-to-GABA ratios) and carbohydrates (xylose and rhamnose). Significant differences are in bold.

Indicator	Effect to test	DF	SS	MS	*F* value	Pr (> *F*)
Chl *a*	TRAWLING	1	23.8	23.85	1.803	0.18547
	SEASON	**1**	**149.6**	**149.57**	**11.306**	**0.00149**
	TRAWLING : SEASON	1	13.2	13.18	0.997	0.32294
	Residuals	50	661.5	13.23		
Chl *a*-to-Pheo *a* ratio	TRAWLING	1	0.24	0.238	0.155	0.69613
	SEASON	**1**	**14.39**	**14.393**	**9.351**	**0.00378**
	TRAWLING : SEASON	1	1.65	1.653	1.074	0.30577
	Residuals	44	67.72	1.539		
Asp-to-BALA ratio	TRAWLING	1	20.0	20.01	0.350	0.558
	SEASON	1	68.7	68.71	1.201	0.280
	TRAWLING : SEASON	1	1.6	1.59	0.028	0.869
	Residuals	36	2059.2	57.20		
Glu-to-GABA ratio	TRAWLING	1	55.3	55.33	0.738	0.396
	SEASON	1	89.6	89.56	1.195	0.282
	TRAWLING : SEASON	1	69.6	69.56	0.928	0.342
	Residuals	34	2548.8	74.97		

DF: degrees of freedom; SS: sum of squares; MS: mean squares, obtained dividing the sum of squares by the degrees of freedom; *F* represents the result of the *F* test and is obtained dividing the mean squares of the inter-group variability by the mean squares of the intra-group variability.

Table 2d. Results of the statistical test one-way ANOVA for carbohydrates (xylose and rhamnose). Significant differences are in bold.

Macromolecule	Differences to test	Indicator	Variability	SS	DF	MS	*F*	*p* value
CARBOHYDRATES	BETWEEN TRAWLING REGIONS	Xylose	Inter	80.775	1	80.775	2.376	0.134
			Intra	1020.122	30	34.004		
		Rhamnose	Inter	5.485	1	5.485	0.036	0.852
			Intra	4633.175	30	154.439		

DF: degrees of freedom; SS: sum of squares; MS: mean squares, obtained dividing the sum of squares by the degrees of freedom; *F* represents the result of the *F* test and is obtained dividing the mean squares of the inter-group variability by the mean squares of the intra-group variability.

Table 2e. Results of the generalized linear model (GLM) for pigments (Chl *a* and Chl *a*-to-Pheo *a* ratio) and amino acids (Asp-to-BALA and Glu-to-GABA ratios).

Formula	Deviance residuals						***Coefficients***			
	Min	1Q	Median	3Q	Max		Estimate	Std. Error	*t* value	Pr (> |*t*|)
ASPBALA ∼ TRAWLING × SEASON	−12.8018	−4.3935	−0.9087	3.0020	23.5412	(Intercept)	24.4198	1.7826	13.699	7.55×10^{-16}
						TRAWLING	−1.0020	2.8945	−0.346	0.731
						SEASON	-2.5512	3.5653	−0.716	0.479
						TRAWLING : SEASON	−0.9029	5.4177	−0.167	0.869
GLUGABA ∼ TRAWLING × SEASON	−10.764	−5.959	−2.076	3.256	24.007	(Intercept)	11.5215	2.1646	5.323	6.54×10^{-6}
						TRAWLING	0.6098	3.3912	0.180	0.858
						SEASON	−5.7052	3.9236	−1.454	0.155
						TRAWLING : SEASON	6.1644	6.3993	0.963	0.342
CHLA ∼ TRAWLING × SEASON	−6.0580	−1.8845	-0.8236	2.0574	11.1820	(Intercept)	1.8845	0.8133	2.317	0.0246
						TRAWLING	−0.1059	1.2675	−0.084	0.9337
						SEASON	**4.1735**	**1.2424**	**3.359**	**0.0015**
						TRAWLING : SEASON	−2.2621	2.2659	−0.998	0.3229
ChlPheoRATIO ∼ TRAWLING × SEASON	−2.1306	−0.4409	−0.3827	0.1495	3.3054	(Intercept)	0.44095	0.27741	1.589	0.1191
						TRAWLING	−0.03857	0.44199	−0.087	0.9309
						SEASON	0.88825	0.48050	1.849	0.0712
						TRAWLING : SEASON	0.83997	0.81062	1.036	0.3058

Table 3. Results of the multivariate statistical test ANOSIM for pigments (Chl *a* degradation products and carotenoids), fatty acids (PUFA, MUFA, MC-FA and LC-FA) amino acids (total THAA, protein THAA and neutral THAA) and carbohydrates (sum of glucose and mannose). Significant differences are shown in bold.

Macromolecule	Differences to test	Transformation	Indicator	Global R	P
PIGMENTS	BETWEEN SEASONS	Square root	**% Chl *a* degradation products**	**0.212**	**0.002**
		Square root	**% Carotenoids**	**0.194**	**0.004**
	BETWEEN TRAWLING REGIONS	Square root	% Chl *a* degradation products	0.036	0.199
		Square root	% Carotenoids	−0.043	0.862
FATTY ACIDS	BETWEEN SEASONS	Fourth root	PUFA	0.063	0.085
		Square root	**MUFA**	**0.792**	**0.001**
		Square root	MC-FA	−0.028	0.584
		Square root	**LC-FA**	**0.427**	**0.001**
	BETWEEN TRAWLING REGIONS	Fourth root	PUFA	0.039	0.140
		Square root	MUFA	0.067	0.078
		Square root	MC-FA	−0.011	0.530
		Square root	LC-FA	−0.044	0.829
AMINO ACIDS	BETWEEN SEASONS	Square root	TOTAL THAA	−0.076	0.920
		Square root	PROTEIN THAA	−0.077	0.919
		Square root	NEUTRAL THAA	0.001	0.447
	BETWEEN TRAWLING REGIONS	Square root	**TOTAL THAA**	**0.409**	**0.001**
		Square root	**PROTEIN THAA**	**0.272**	**0.001**
		Square root	**NEUTRAL THAA**	**0.260**	**0.001**
CARBOHYDRATES	BETWEEN TRAWLING REGIONS	Square root	GLUCOSE + MANNOSE	−0.020	0.576

to the intra-group distances (Table 4a). Inter-group distances were higher than intra-group distances (Table 4a and b). Similar average square distances were found between UTR and TR (average square distance: 47.54) and between SPR and AUTM (average square distance: 48.47) (Table 4b). Based on the intra-group distances, as expected, amino acids contribute with high percentages to the average square distance between UTR and TR, whereas the contribution of Chl *a* and MUFA was high in the case of the seasonal inter-group distances (Table 4b).

Based on the PCA, PC1 accounts for the 27.4 % of the total variation and explains the distribution of total, protein and neutral THAA between trawling regions (Fig. 2a), whereas PC2 accounts for the 17.4 % of the total variation and explains the distribution of Chl *a* and MUFA between sampling seasons (Fig. 2b).

5 Discussion

The main objective of this study is to characterize the organic matter (OM) present in sediments from two regions of a submarine canyon that are affected and unaffected by bottom trawling. We found substantial differences in the quality of the organic matter between these two regions. In particular, the organic matter was more degraded in the trawled than in the untrawled region.

In the sediment column, OM preservation is controlled by several interrelated factors, including oxygen penetration, bioturbation, sediment density and grain size. The monomeric components of biomolecules degrade at similar rates under oxic and anoxic conditions (Henrichs and Doyle, 1986), but macrobiomolecule degradation is faster in oxic conditions than in anoxic compact sediments, where benthic organisms cannot survive and cannot participate, together with bacteria, in OM degradation (Sun et al., 1993; Bianchi et al., 2000). Also protozoa, like benthic organisms, need oxygen to survive, contributing to the higher degradation rates measured in oxic than in anoxic sediments (Williams, 1981).

It has been stated that bottom trawling affects the physical properties of the seafloor, altering grain-size distributions and sediment porosity (Jones, 1992; McConnaughey et al., 2000, and references therein). In addition, the pulling of the bottom trawl gears along the seafloor is deemed responsible for resuspension of surface sediment, which is in this way oxygenated and may lose its finest fraction (Palanques et al., 2001). By removing bioturbators and at the same time artificially mixing sediments, bottom fishing gear is expected to deeply affect benthic community composition and metabolism and hence the biogeochemical characteristics of the affected sediments (Duplisea et al., 2001, and references therein). These environmental factors influence OM preservation and can be responsible for making trawling regions different in terms of the quality of the OM present in the

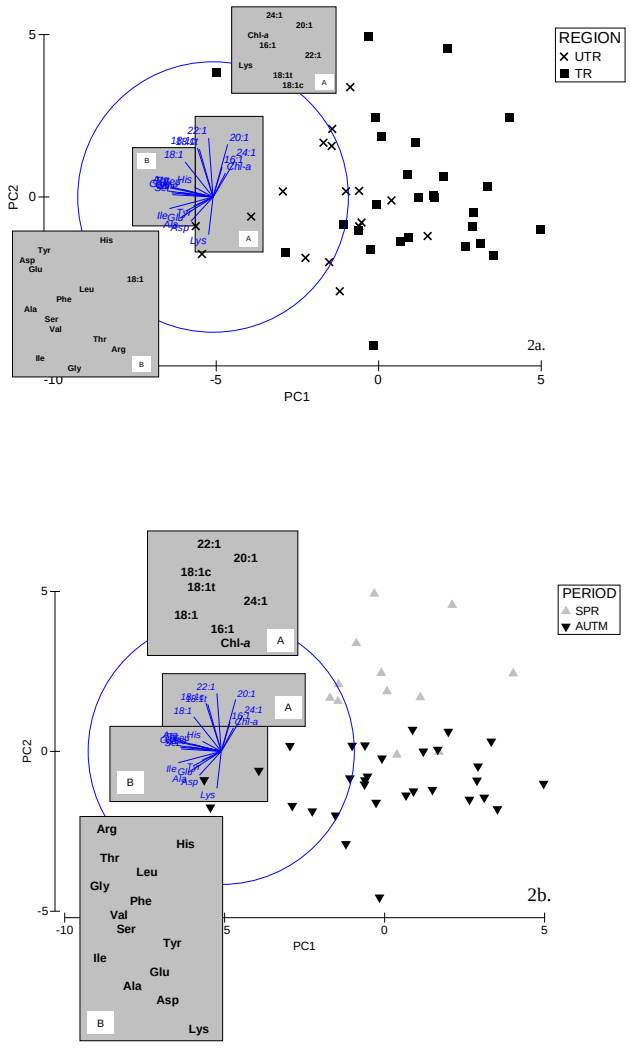

Fig. 2. Representation of the multivariate statistical test PCA carried out on those biomarkers which better evidenced differences between sampling seasons (Chl *a* and MUFA) and between trawled and untrawled regions (protein and neutral THAA). In **(a)**, stations located in the untrawled and the trawled regions are evidenced, whereas in **(b)**, stations are distinguished based on the sampling season – spring or autumn. Biomarkers are shown in both plots **(a)** and **(b)**.

sediment column. To study the effects of chronic trawling disturbance, we will now discuss the results obtained from the analysis of several groups of biomarkers, describing the information associated with each of them.

5.1 Pigments

The distribution of Chl *a* in SPR and AUTM samples (Fig. 2b) suggests that this pigment, which is present in all photosynthetic algae and higher plants, can be used as an indicator of the seasonal fresh OM input to the seafloor also in the trawled submarine canyon flanks. In spring, high percentages of Chl *a* were found not only at UTR but also at TR (Supplement A1), indicating that the information associated with this tracer (presence of very fresh OM; Wakeham et al., 1997) is prevalent in the entire study area and hence not masked by the long-terms effects of bottom trawling. This agrees with previous work carried out in the southwestern canyons of the Gulf of Lion, in which high quantities of fresh organic matter were found in spring sediment trap samples (Pasqual et al., 2011). In contrast, no significant differences in the Chl *a* percentage were observed between UTR and TR (Table 2c and e). The highly labile Chl *a* biomarker is useful for detecting the subtle OM quality differences between SPR and AUTM sediments but probably too sensitive for evidencing alterations in the quality OM related to trawling activities.

Chl *a* is degraded to Pheo *a* by loss of Mg; to pyropheophorbide *a* by loss of Mg, the phytyl chain and carbomethoxy; and to pheophorbide *a* by loss of the phytyl chain and oxidation (Louda et al., 2008). Chl *a* degradation products can be used as OM quality indicators (Lee et al., 2000), such as the Chl *a*-to-Pheo *a* ratio and the sum of the Chl *a* degradation products (Lee et al., 2004). However, when using these pigment degradation indexes, it should be considered that the formation of such degradation products depends not only on those environmental factors which enhance Chl *a* degradation but also on the initial concentration of Chl *a*. Low percentages of pheophytin *a*, pyropheophorbide *a* and pheophorbide *a* can be related to Chl *a* preservation and/or to an initial low availability of Chl *a*. In spite of these considerations, the Chl *a* degradation products confirmed the presence of fresher OM in spring than in autumn samples (Table 3) suggested by the Chl *a* (Table 2c and e).

Another group of pigments is represented by carotenoids, which are characterized by a higher stability than chlorophylls (Reuss, 2005) and may represent the majority of pigments in sediment samples (Repeta and Gagosian, 1987). The distribution of carotenoid percentages in this study suggested higher amounts of refractory OM in spring than in autumn (Supplement A3 and Table 3), but this must be interpreted with caution. In the study area, the relevance of lateral transport of particulate matter (Martín et al., 2006) could mask the signal of the vertical deposition of refractory OM particles onto the seafloor. Labile compounds are also laterally transported, but since they degrade with time, once they settle on the seafloor it is possible to distinguish between the fresh forms, representative of the vertical component of the OM input, and the degraded forms, which are the result of degradation processes during lateral transport. In addition, the presence of carotenoids is not influenced by those environmental factors related to trawling or seasonality which influence the biochemical quality of sediments, and therefore their distribution in the study area cannot help us understanding the effects of these two external factors.

Table 4a. Results of the multivariate statistical test SIMPER carried out on those biomarkers which better evidenced differences between sampling seasons (Chl *a* and MUFA) and between trawling regions (protein and neutral THAA). Intra-group distances within the a priori-defined groups UTR, TR, SPR and AUTM.

	INTRA-GROUP DISTANCES			
Group	UTR	TR	SPR	AUTM
Average square distance	**18.31**	**21.12**	**17.76**	**20.77**
Variable contribution (%)	**Tyr (14.12)**	**Thr (6.05)**	Leu (6.86)	Thr (5.83)
	Leu (9.11)	Chl *a* (5.95)	**18 : 1*c* (6.73)**	**16 : 1 (5.71)**
	Phe (7.57)	18 : 1*t* (5.88)	Phe (6.59)	Gly (5.45)
	Val (7.34)	**Ala (5.75)**	Val (6.21)	His (5.34)
	20 : 1 (6.62)	**His (5.69)**	**22 : 1 (6.14)**	Asp (5.26)
	18 : 1*c* (5.40)	16 : 1 (5.56)	Ala (5.81)	Ser (5.19)
	24 : 1 (4.88)	22 : 1 (5.41)	Glu (5.30)	Arg (5.12)
	22 : 1 (4.42)	18 : 1 (5.34)	Arg (5.07)	Glu (5.09)
	16 : 1 (4.27)	**Lys (5.30)**	Ser (4.93)	Ile (5.01)
	Asp (4.21)	**Ile (5.29)**	Ile (4.58)	Lys (4.83)
	Lys (4.10)	**Gly (5.09)**	His (4.49)	Val (4.75)
	Gly (4.10)	**Arg (4.90)**	Gly (4.17)	Ala (4.68)
	His (3.91)	**Asp (4.15)**	Asp (4.09)	**24 : 1 (4.65)**
	Arg (3.73)	18 : 1*c* (4.15)	**20 : 1 (4.06)**	Phe (4.63)
	Chl *a* (3.49)	24 : 1 (3.92)	**24 : 1 (4.03)**	Leu (4.55)
	Glu (3.28)	**Phe (3.70)**	**16 : 1 (3.29)**	**18 : 1 (4.52)**
	18 : 1 (2.67)	20 : 1 (3.53)	Thr (2.79)	**20 : 1 (3.70)**
	Ile (2.35)	**Glu (3.29)**	Lys (2.44)	**18 : 1*t* (3.69)**
	18 : 1*t* (2.22)	**Leu (2.78)**	**18 : 1*t* (2.29)**	**Chl *a* (2.94)**
	Ser (1.13)	**Val (1.97)**	**18 : 1 (1.00)**	**18 : 1*c* (1.67)**
	Thr (0.94)	**Tyr (0.00)**	Tyr (0.01)	**22 : 1 (0.54)**

Table 4b. Inter-group distances between UTR and TR and between SPR and AUTM.

	INTER-GROUP DISTANCES	
Groups	UTR and TR	SPR and AUTM
Average square distance	**47.54**	**48.47**
Variable contribution (%)	**Glu (5.69)**	**22 : 1 (8.28)**
	Val (5.66)	**18 : 1*c* (7.08)**
	Tyr (5.44)	**18 : 1*t* (5.71)**
	24 : 1 (4.89)	**Chl *a* (5.48)**
	Asp (4.89)	**20:1 (5.46)**
	20 : 1 (4.76)	**18 : 1 (5.07)**
	Ala (4.73)	Lys (4.56)
	Leu (4.70)	**24 : 1 (4.51)**
	18 : 1*c* (4.62)	Ala (4.24)
	Ile (4.54)	Leu (4.21)
	Arg (4.49)	Phe (4.17)
	18 : 1 (4.44)	Val (4.11)
	Phe (4.43)	Ile (4.08)
	Thr (4.35)	Glu (3.90)
	Gly (4.28)	Arg (3.90)
	18 : 1*t* (4.17)	Asp (3.90)
	Lys (4.14)	Ser (3.85)
	Ser (4.13)	His (3.76)

5.2 Fatty acids

Like Chl *a*, also the labile monounsaturated fatty acids (MUFA) indicated an efficient input of fresh OM (Haddad et al., 1992) to the seafloor following the spring phytoplankton bloom. The lack of significant differences in the concentration of polyunsaturated fatty acids (PUFA) between SPR and AUTM samples (Table 3) may be related to the low number of PUFA compounds found in the study area (only two: FA 22 : 2 and FA 22 : 6; see Supplement B1), which indicates a rapid degradation of this labile group of FA before accumulating in the sediment column (Haddad et al., 1992; Sun and Wakeham, 1994). The degradation of FA, aliphatic hydrocarbon chains with a carboxylic group at one extremity, is selective and depends on the number of carbons and double bonds of the chain. PUFA, FA with a high number of double bonds, represent the most labile group of FA, and their low concentration even in SPR, when high Chl *a* percentages and MUFA concentrations were found (Supplement A1 and B2), suggests a prior degradation in the water column and/or after deposition on the seafloor (Sun et al., 1997; Wakeham et al., 1997). The selective degradation of PUFA can be particularly intense at the sediment–water interface (Laureillard et al., 1997) and prevents their use as tracers of fresh OM in this region.

Mid- and long-chain FA (MC-FA and LC-FA, respectively) represent the most refractory fatty acid groups. MC-FA did not evidence differences in the quality of the OM in the study area, neither between seasons nor between trawled/untrawled regions, whereas LC-FA evidenced seasonal differences (Table 3). Nevertheless, the distribution of LC-FA in the study area suggested high quantities of refractory material in spring. This further limits the utility of refractory compounds (i.e. carotenoids, MC-FA and LC-FA) as biomarkers in the present study.

To summarize, the fatty acid group includes compounds characterized by a wide range of different labilities. Depending on its lability, each compound behaves in a different way (Fig. 3). At the two extremes, the FA with highest (PUFA) and lowest lability (MC-FA and LC-FA) did not trace differences in the quality of the OM, neither between seasons nor between trawling regions. PUFA are too labile and probably degrade before accumulating in the sediment column, whereas MC-FA and LC-FA are too refractory to be used as biomarkers. MUFA are able to detect differences between sampling seasons.

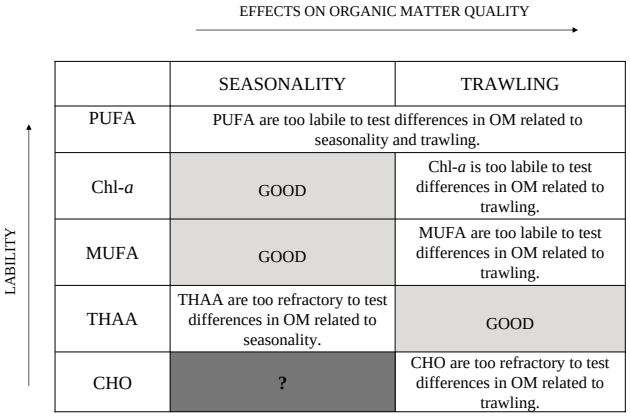

Fig. 3. Schematic representation of the results obtained in this study by using a pool of biomarkers with different lability to investigate on the effects of seasonality and sediment reworking by trawling on the quality of the OM. Due to the unavailability of spring samples, we did not test differences in CHO between seasons.

5.3 Amino acids

Like the other macromolecules studied, also amino acids are partially degraded in the water column, but a fraction of the amino acid pool reaches the seafloor and is incorporated in the sediment column (Wakeham et al., 1997; Moore et al., 2012), allowing for the use of these compounds as biomarkers of the quality of the OM. When compared with other results from the NW Mediterranean continental shelf at ~ 300 m depth (Grémare et al., 2002, 2005), THAA showed relatively high values in the study area, which can be related to the high downward flux of biogenic particles in La Fonera Canyon (Martín et al., 2006). Amino acids are also considered fresh OM indicators (Lee et al., 2004) and the distribution of THAA values in the study area, with higher mean concentrations in the control area ($\sim 22 \pm 3$ nmol mg^{-1} DW) than in the trawled area ($\sim 17 \pm 4$ nmol mg^{-1} DW), confirmed the presence of more labile material at UTR (Fig. 2a), as previously suggested by MUFA biomarkers. Statistical tests indicated a clear separation between trawled regions when using THAA as biomarkers, more evident than the separation observed with MUFA as tracers (Table 3). The presence of more labile OM in the untrawled than in the trawled region is evidenced also by the distribution of neutral and protein THAA (Fig. 2a). Neutral THAA are not adsorbed onto clay minerals and are more susceptible to degradation than charged ones. Regarding protein THAA, their concentrations depend on the phytoplanktonic production and, like neutral THAA, can be used as fresh OM indicators. Other THAA – like β-alanine (BALA), which originates from aspartic acid (Asp), and γ-aminobutyric acid (GABA), which originates from glutamic acid (Glu) – are associated with degradation processes and may be used as degradation indexes (Ingalls et al., 2003), but the absence of significant differences in the Asp-to-BALA and Glu-to-GABA ratios between UTR and TR (Table 2c and e) supports the doubts expressed by other authors on the validity of these ratios as OM quality indicators (García and Thomsen, 2008). The different degradation pathways and/or rates of Asp and Glu to BALA and GABA, respectively, can be responsible for the inconsistence of the Asp-to-BALA and Glu-to-GABA ratios as organic matter degradation indexes (Lee et al., 2000).

Based on the distribution of THAA in the trawled areas, we might expect higher concentrations of neutral and protein THAA in SPR than in AUTM samples. Nevertheless, no differences were found in the distribution of these THAA indicators between spring and autumn sediments (Table 3). Amino acids are considered less susceptible to degradation than Chl *a* and MUFA (Wakeham et al., 1997) and this characteristic could make them less adequate than the other two biomarkers for tracing seasonal differences in the nutritional quality of the sedimentary OM. As mentioned before, based on our results, we hypothesize differences in the "freshness" of the OM related to seasonality to be subtle and for this reason detectable by very labile and sensitive OM indicators like Chl *a* and MUFA but not by less reactive biomarkers like amino acids (Fig. 3).

5.4 Carbohydrates

The absence of significant differences between UTR and TR in the concentration of the labile sugars xylose (Opsahl and Benner, 1999), glucose (Hedges et al., 1988; Hofmann et al., 2009) and mannose (Kerhervé et al., 2002) (Table 3) can be related to the low lability of these compounds if compared with that of MUFA or THAA. In spite of the relative lability of xylose, glucose and mannose, CHO compounds are in fact considered macromolecules with a low nutritional

quality (Handa and Tominaga, 1969), characteristic of regions where the supply of fresh OM is limited, like deep-sea habitats (Danovaro et al., 1993) and oligotrophic environments (Rodil et al., 2007), and more resistant to degradation than pigments, fatty acids and amino acids (Wakeham et al., 1997). In fact, carbohydrates did not detect differences among trawled and control areas. The possible seasonal influence on carbohydrate distribution could not be assessed due to the fact that only autumn samples were available.

5.5 Organic matter differences between trawled and untrawled regions

As expected, environmental factors related to the stirring and resuspension of sediments caused by the passage of bottom trawl gears, such as deepening of the oxygen penetration depth or the loss of the sediment finest fraction (Jones, 1992; McConnaughey et al., 2000; Palanques et al., 2001), do not favour the preservation of organic matter, making trawled regions different in terms of the quality of the organic matter present in the sediment column. Our hypothesis that fresher organic matter would be present in the untrawled rather than in the trawled region was confirmed. Nevertheless, as expected, only some of the analysed compounds provided good evidence of this hypothesis. The best biomarker in this geographical region to trace the effects of sediments reworking by trawling on the biochemical quality of sediments was represented by amino acids (Table 4b). The higher concentrations of total, neutral and protein THAA found in the untrawled than in the trawled region were supported by multivariate statistical tests like ANOSIM (Table 3) and PCA (Fig. 2a).

The use of four groups of biomarkers differently susceptible to degradation also allowed for us to gain qualitative information about the potential effects of trawling on the quality of the OM. The similar behaviour of Chl *a* and MUFA – which appeared as good biomarkers for seasonality but too labile to trace differences in the quality of the OM related to long-term chronic perturbations such as bottom trawling, and the opposite behaviour of the less labile THAA, which appeared better suited to trace changes associated with trawling but were too refractory to trace differences related to seasonality – suggested that the impact exerted by trawling on the biochemical composition of sediments is higher than the natural variation related to seasonality (Table 4b and Fig. 3).

Since deep-sea trawling is not exclusive to the study area but widely extended throughout the world's oceans (Bensch et al., 2009; Puig et al., 2012), our results suggest that commercial bottom trawling may have produced changes in the nutritional quality of deep-sea sediments at large spatial scales.

6 Conclusions

Bottom trawling in the flanks of La Fonera Canyon has caused an alteration of the quality of the organic matter in surface sediments (upper 5 cm), as has been evidenced by the distribution of amino acids in the study area, which can be interpreted as the result of sediment reworking caused by repeated bottom trawling in the northern flank and in the inshore southern flank of the canyon.

The fact that differences between spring and autumn sediments were detected by the most labile biomarkers, Chl *a* and MUFA, together with the high percentage contribution to the average square distance between SPR and AUTM for Chl *a* and MUFA, as well as the high percentage contribution to the average square distance between UTR and TR for the majority of amino acids, suggests that alterations in the quality of the organic matter caused by trawling can be considered relatively high if compared with the effects of seasonality. Nutritional value of sedimentary organic matter is crucial for benthic communities; therefore, the changes produced by repeated trawling might imply profound changes on the ecosystem as a whole.

Supplementary material related to this article is available online at http://www.biogeosciences.net/10/8093/2013/bg-10-8093-2013-supplement.pdf.

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