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ORIGINAL ARTICLE

Quantity and biochemical composition of particulate organic matter in a highly trawled area (Thermaikos Gulf, Eastern Mediterranean Sea)

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ABSTRACT

Bottom trawling represents nowadays one of the most severe anthropogenic disturbances at sea, and determines large impacts on benthic communities and processes. Bottom trawling determines also local sediment resuspension and the effects of the injection of large amounts of surface sediments into the water column have been repeatedly investigated. Few studies have assessed the consequences of sediment resuspension caused by bottom trawling on the quantity, biochemical composition and bioavailability of suspended organic particles and how these eventually rival those exerted by natural storms. To provide insights on this poorly addressed issue, we investigated concentrations and biochemical composition of total and enzymatically digestible pools of particulate organic matter (POM) in the Thermaikos Gulf (Mediterranean Sea) under calm sea conditions, during intensive trawling activities, and after a severe storm. We show here that sediment resuspension caused by trawling can cause large effects on POM quantity, biochemical composition and bioavailability. Both during trawling and after the storm, the relative importance of the carbohydrate pools increased (in the upper water column) and the total lipid concentrations decreased (in the intermediate and bottom layers) when compared to values measured during calm conditions. These results would suggest that bottom trawling could inject in the upper water column POM pools more refractory in nature (*e.g.*, carbohydrates) than those present in calm or after-storm conditions. By contrast, we show also that the bioavailable fraction of biopolymeric C increased significantly during trawling in the upper water column of the shallowest stations and in the bottom water column layer of the deepest ones. These results provide evidence that bottom trawling can influence the overall trophic status of coastal waters, exerting effects similar or stronger than those caused by natural storms, though of variable amplitude depending on the water depth. Since bottom trawling is carried out worldwide and natural storms at sea can be frequent and intense, we claim for the need of assessing new adapting management strategies of bottom trawling in order to mitigate the synergistic impacts of anthropogenic and natural sediment resuspension on coastal biogeochemical cycles.

Key words: Particulate organic matter; bottom trawling; eutrophication; Mediterranean Sea.

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INTRODUCTION

Bottom trawling, along with dredging and dumping, represents one among the most severe physical disturbances generated by human activities at sea (Thrush and Dayton, 2002). Typically, bottom trawling is carried out using heavy ground ropes and chains to drive fish and crustaceans from the seabed into nets (Johnson *et al.*, 2015). Trawling is carried out on many types of grounds, from shallow waters down to the deep continental margins (Puig *et al.*, 2012), by small and large vessels, and for a wide range of target species, including fish and crustaceans (Hinz *et al.*, 2009). These characteristics make bottom trawling one of the preferred methods of industrial fisheries worldwide but, at the same time, one among the human activities at sea most impacting highly vulnerable benthic marine ecosystems (*e.g.*, cold-water corals or coralligenous bottoms; Fowler, 2003; Althaus *et al.*, 2009; Bruckner, 2009; Heifetz *et al.*, 2009; Bongiorni *et al.*, 2010). Recently, not irrelevant damages determined by bottom trawling have been also documented on benthic communities and processes in deep-sea soft bottoms (Pusceddu *et al.*, 2014).

Previous investigations carried out in shallow marine ecosystems have revealed that bottom trawling can generate a plethora of direct and indirect effects on pelagic and benthic environments and biota (Kaiser, 1998; Smith *et al.*, 2003; Thrush and Dayton, 2002; Queiros *et al.*, 2006; Hiddink *et al.*, 2007; Smith *et al.*, 2013). As well as having a direct impact on the stocks of the target species and the by-catch, bottom trawling can also alter the structure and physico-chemical characteristics of the trawled sediment and of the overlying water column (Jennings *et al.*, 2001; Smith *et al.*, 2003; Pusceddu *et al.*, 2005b, 2005c; Puig *et al.*, 2012). In the pelagic realm, bottom trawling can increase turbidity, internal nutrient loads, oxygen consumption, and possibly enhance phytoplankton primary production (Riemann and Hoffmann, 1991; Palanques *et al.*, 2001; Durrieu de Madron *et al.*, 2005).

Bottom trawling can play also a key role in sustaining high productivity on some continental margins by accelerating sedimentary C degradation (Polymenakou *et al.*, 2005), nutrient turnover and, thus, enhancing phytoplankton blooms (Fanning *et al.*, 1982; Christensen, 1989). One of the evident effects of bottom trawling consists in sediment resuspension which generates visible and highly turbid plumes of suspended particles, with concentrations up to several hundred mg L^{-1} near the seabed (Schoellhamer, 1996; Durrieu de Madron *et al.*, 2005). The injection of large amounts of surface sediments into the water column, particularly when trawling is carried out over soft bottoms (Black and Parry, 1994; Pilskaln *et al.*, 1998), has been hypothesized to rival storms as the main agent for sediment resuspension and transport on the middle and outer continental shelf of the Middle Atlantic Bight (Churchill, 1989). Whether indeed sediment resuspension induced by bottom trawling has more or less relevance than natural resuspension (as in the case of storms) in fuelling the water column with regenerated nutrients and suspended organic particles available as food for suspension feeders, remains to date a still largely unexplored issue.

To provide insights on this issue, we investigated concentrations and biochemical composition of both total and bioavailable (*i.e.*, enzymatically hydrolizable) pools of suspended organic particles in the Thermaikos Gulf (Mediterranean Sea) along a putative decreasing gradient of anthropogenic influence, during three periods: September 2001 (calm sea conditions and no trawling), October 2001 (trawling period), and February 2002 (no trawling, after a severe storm). More in details, we tested the null hypothesis by which the quantity, biochemical composition and bioavailability of suspended particles along the whole water column are not affected by bottom trawling or severe storms at the sea surface.

METHODS

Study area and sampling

The Thermaikos Gulf (Fig. 1) is a micro-tidal environment, located in the North Western Aegean Sea (Eastern Mediterranean), from 39°30’N to 40°38’N and 22°30’E to 23°19’E, with depths ranging from 30 to 200 m. The main circulation is characterized by more saline waters entering the outer shelf of the Gulf over the eastern part, then turning towards the northeast in the inner part; less saline waters flow southerly along the western coastline (Poulos *et al.*, 2000). The Thermaikos Gulf is characterized by an extended shelf (180 km long and 55 km wide) of very smooth relief and by meso-eutrophic conditions (Zervakis *et al.*, 2005). The area under scrutiny, in particular, is characterized in its inner part by strong anthropogenic influences, due to the Thessaloniki city’s harbour and the adjacent industrial zone. Moreover, the Gulf receives important riverine inputs from three major rivers (Axios, Aliakmon and Pinios rivers; Karageorgis and Anagnostou, 2001).

Five sampling stations (namely IP01, IP10, IP17, IP38, IP41 at 30, 41, 55, 51, and 54 m depth, respectively) were located along the coast and positioned along a north-to-south transect characterised by seasonally intensive bottom trawling activities (Fig. 1). At each station, water samples were collected at 2 m, 20 m and about 1 m above the bottom, in September, October 2001 and in February 2002. These periods were selected as putatively representative of calm conditions and no trawling (September 2001), calm conditions with trawling (October 2001) and no trawling after a prolonged period of severe storms (February 2002). Water samples were collected by means of a rosette sampler equipped with 15 L Niskin bottles. After pre-filtration through a 200 µm mesh net to remove larger zooplankton, the water samples were filtered onto Whatman GF/F filters (pre-combusted at 450°C, 4 h), immediately after collection. Filters were stored at -20°C, until analyses.

Data obtained from the bottom layer of the water col-

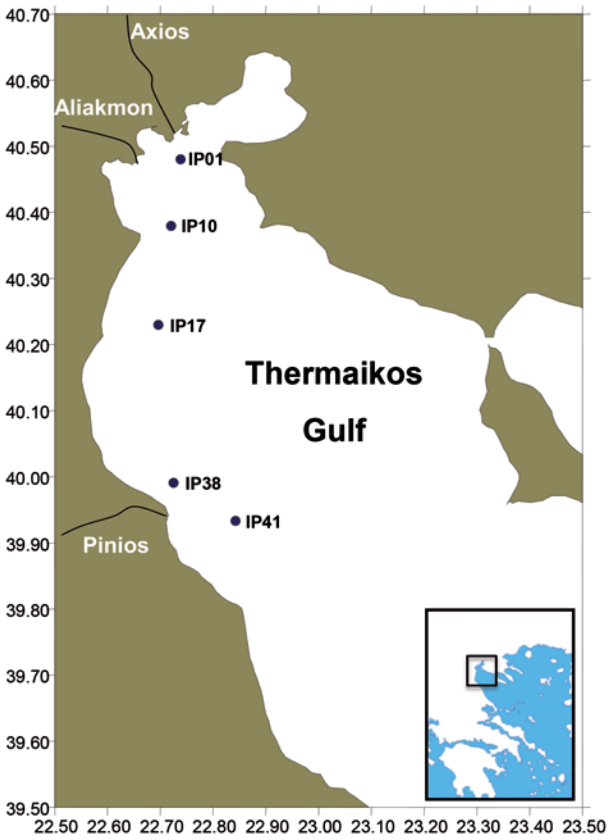


Fig. 1. Study area and location of the sampling stations.

umn have been already partially discussed elsewhere (Pusceddu *et al.*, 2005b). In this study we extended the analysis including also the data obtained from the intermediate and surface layers of the water column, to document, if any, the effects of natural and anthropogenic sediment resuspension on the quantity and food availability of (re)suspended organic particles along the entire water column.

Biochemical composition of particulate organic matter

Concentrations of total particulate proteins (TPRT), total particulate carbohydrates (TCHO) and total particulate lipids (TLIP) were determined according to Hartree (1972) modified by Rice (1982) (proteins), Dubois *et al.* (1956) (carbohydrates), and Marsh and Weinstein (1966) and Bligh and Dyer (1959) (lipids), respectively. Concentrations of the hydrolysable fractions of particulate proteins (HPRT) and carbohydrates (HCHO) were measured according to Dell'Anno *et al.* (2000), slightly modified for particulate samples (Pusceddu *et al.*, 2005c). Filters were homogenised in 0.1 M Na-phosphate buffer (pH 7.5), sonicated three times for 1 min (with intervals of 30 s) before enzyme addition. Duplicate filters from each water depth (*i.e.*, treated samples) were added to 100 μL of proteinase-K (1 mg mL^{-1}) and 100 μL of protease (600 μg mL^{-1}). An equal volume of Na-P buffer solution, without enzymes (*i.e.*, control samples), was added to another set of filters. All the samples were incubated for 1 h at 37°C, under gentle agitation; they were filtered subsequently onto GF/F filters and rinsed twice with 5 mL of cold 0.1 M Na-P buffer (pH 7.5), in order to remove the digested protein fraction and the surplus of enzymes. Filters muffled at 450°C for 4 h and processed as described above were utilised as blanks. Hydrolysable protein analyses from these samples were carried out spectrophotometrically as described above. Difference in protein concentration between the control and treated samples were assumed to represent the concentration of proteins actually hydrolysed by proteases (hydrolysed proteins, HPRT). For the enzymatic digestion of particulate carbohydrates, the filters were homogenised with 0.1 M Na-phosphate, 0.1 M EDTA (pH 5.0) and sonicated three times for 1 min (with intervals of 30 s). Replicate filters (n=3, treated samples) were added to 100 μL of α-amylase, 50 μL of β-glucosidase, 100 μL of proteinase-K and 100 μL of lipase (stock solution of all enzymes was 1 mg mL^{-1}). Another set of filters were treated by adding 0.1M Na phosphate, instead of enzyme solutions, and utilised as a control. Filters muffled at 450°C for 4 h and processed as described above, were utilised as blanks. After incubation, all the samples were centrifuged at 2000 g for 10 min and an aliquot of the supernatant was used to determine carbohydrates released from POM hydrolysis. Soluble carbohydrates were determined from the supernatant of the control sample. Carbohydrates from all the supernatants were analysed spectrophotometrically, as described above. The actual fraction of enzymatically hydrolysed carbohydrates (HCHO) was obtained on the basis of the difference between the carbohydrate concentrations determined in the supernatant of samples containing enzymes and the soluble fraction of the control. Total and hydrolysable carbohydrate and protein and total lipid concentrations were converted into carbon equivalents, using 0.40, 0.49 and 0.75 mgC mg^{-1} conversion factors, respectively (Fabiano *et al.*, 1995). The sum of the total protein, carbohydrate and lipid carbon equivalents was reported as particulate biopolymeric organic carbon (BPC, Fabiano and Pusceddu, 1998). Particulate bioavailable organic carbon (BAOC), as a proxy of the organic carbon potentially available for consumers (Pusceddu *et al.*, 2003, 2009), was defined as the sum of carbon equivalents of hydrolysable carbohydrates and proteins (Danovaro *et al.*, 2001).

Statistical analyses

To test the null hypothesis that the quantity, biochemical composition and bioavailability of suspended particles along the whole water column are not affected by bottom trawling nor by severe storms at the sea surface, three-way permutational analyses of variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) were carried out separately for each variable. The design included three orthogonal factors: period (P, 3 fixed levels: calm, trawling, storm), station (S, 5 fixed levels: IP01, IP10, IP17, IP38, IP41), and water depth (D, 3 fixed levels: surface, intermediate, bottom), with n=3 for the combination of factors. In the multivariate context, variations in the biochemical composition of suspended particles were assessed separately for the three water column layers using a 2-way design with period (P, 3 fixed levels: calm, trawling, storm), and station (S, 5 fixed levels: IP01, IP10, IP17, IP38, IP41) as orthogonal sources of variance. The analyses were based on Euclidean distances of previously normalized data, using 4999 random permutations of the appropriate units (Anderson and ter Braak, 2003). Because of the restricted number of unique permutations in the pairwise tests, p values were obtained from Monte Carlo samplings (Anderson and Robinson, 2003). Significant differences among water layers in each station and sampling period, as well as differences among sampling periods in each station and water column layer were then assessed using SNK *post-hoc* tests.

Canonical analysis of principal coordinates (CAP) was used in the multivariate context to ascertain the allocation of experimental groups to those established a priori. Results from the CAP were then used to visualize, using biplots, differences among experimental groups (*i.e.*, among periods and stations). The PERMANOVA and CAP analyses were performed using the routines included in the PRIMER 6+ software (Clarke and Gorley, 2006).

RESULTS

Total protein, carbohydrate, lipid, biopolymeric C, hydrolysable protein and carbohydrate and bioavailable organic carbon concentrations at each stations, water depth and sampling period are reported in Tab. 1. The results of the PERMANOVA tests revealed a significant interaction of the three tested factors for all of the investigated variables (Tab. 2). Therefore, in order to identify the eventual significance of the major factor under scrutiny (*i.e.*, trawling *vs* storm effects), we used post-hoc SNK tests to discriminate differences in the concentration of suspended organic matter concentrations: i) among sampling depths during each period and at each station; ii) among sampling periods at each station and water column layer.

The results of the *post-hoc* tests carried out to identify changes in the vertical distribution of suspended particles in the water column during the three different conditions (Tab. 3) reveal: i) the presence during calm conditions in September of a nepheloid layer (*i.e.*, significantly higher concentrations in the bottom layer of the water column) in almost all stations; ii) a more homogeneous distribution of POM (*i.e.*, values in intermediate and surface layers higher than or similar to those in the bottom layer) during trawling activities in October and, though to a lesser extent, after-storm in February. These trends apply to almost all investigated variables and appear to be particularly evident at the shallowest stations IP01 and IP10.

The *post-hoc* tests carried out to identify variations in the concentration of suspended organic compounds among sampling periods in each water column layer and in all sampling stations (Tab. 4) reveal that: i) the stronger effects of bottom trawling - when compared to calm and after-storm conditions - are generally most evident in the intermediate and bottom layers of the water column, especially for the carbohydrate pools; ii) in some stations (*i.e.*, IP10, IP17, IP38) and pre-eminently for the protein pools, suspended particle concentrations during trawling in October are similar to those observed after-storm in February and consistently higher than those during calm conditions in September.

The results of the 2-way PERMANOVA conducted to identify variations in the biochemical composition of particulate organic matter among calm, trawling and after-storm conditions and among sampling stations in each of the three layers of the water column reveal the presence of a significant Period × Station interaction for each water column layer (Tab. 5). The bi-plots produced after the CAP analysis to better ascertain, separately for each layer of the water column, changes in the biochemical composition of particulate organic matter during the three sampling periods reveal: i) in the surface layer of the water column a clear segregation of trawling conditions at all stations, with exception of IP17 and IP38, mostly driven by increasing concentrations of carbohydrate pools, whereas storm conditions mostly overlap with calm conditions (Fig. 2A); ii) in the intermediate and bottom layers of the water column a general segregation of trawling and storm (slightly overlapped one each other) from calm conditions, mostly explained by decreasing concentrations of total particulate lipids and increasing concentrations of total and hydrolisable carbohydrate and protein pools during trawling and after storm (Fig. 2B-C); iii) in the bottom layer of the water column, the best segregation among trawling, after-storm and calm conditions in the deepest stations (*i.e.*, IP38 and IP41) (Fig. 2C).

In the surface layer of the water column of all stations, with exception of IP17 and IP38, the bioavailable fraction of particulate biopolymeric C is much higher during trawling activities than in calm and after-storm conditions (Fig. 3A). The positive effect of trawling activities on the bioavailability of particulate biopolymeric C observed in the surface layer of the water column is smoother in the intermediate layer of the water column, where it is evident only in the deepest station IP41 (Fig. 3B). A higher bioavailability of particulate biopolymeric C during trawling activities - when compared to calm and after-storm conditions - is again evident in the bottom layer of the water column, in the deepest stations IP38 and IP41 (Fig. 3C).

DISCUSSION

We show here that sediment resuspension caused by intensive bottom trawling can determine effects on particulate organic matter (POM) quantity, biochemical composition and bioavailability which are similar or even stronger than those eventually exerted by storms at the sea surface. On the one hand, this result is in accordance with previous findings from the Middle Atlantic Bight, which postulated that bottom trawling can rival natural resuspension induced by storm conditions (Churchill, 1989). Previous studies, conducted in the Thermaikos Gulf and, comparatively, in the Gulf of Lions (NW Mediterranean Sea), reported changes in the quantitative characteristics of sinking POM, resulting in increased total suspended matter concentrations and gross sedimentation rates through alternate cycles of resuspension and sedimentation associated with natural temporal variability (Grémare *et al.*, 2003; Karageorgis and Anagnostou, 2001; Fernandes *et al.*, 2009; Zeri *et al.*, 2009).

Our results show also that the bottom layer of the water column in the Thermaikos Gulf during calm conditions is characterised generally by concentrations of POM (and almost all its biochemical constituents) higher than those in the intermediate and upper layers. The presence of such a nepheloid layer indicates that natural background levels of sediment resuspension in the Thermaikos Gulf could be relatively high. In this sense, we must there-

Tab. 1. Total and hydrolizable protein, total and hydrolizable carbohydrate, total lipid, biopolymeric C (BPC), and bioavailable C concentrations at A) surface (2 m), B) intermediate (20 m) and C) bottom depths in the Thermaikos Gulf under calm (September 2001), during trawling (October 2001) and after storm (February 2002) conditions.

			Protein				Carbohydrate				Total lipid		Biopolymeric C		Bioavailable C		
	Station	Condition	Total		Hydrolizable		Total		Hydrolizable								
			µg L^{-1}	SD	µg L^{-1}	SD	µg L^{-1}	SD	µg L^{-1}	SD	µg L^{-1}	SD	µgC L^{-1}	SD	µgC L^{-1}	SD	% of BPC
A)	IP01	Calm	83.7	1.3	20.4	1.5	67.7	1.9	14.7	0.1	23.1	0.0	85.4	1.4	33.2	0.8	38.8
		Trawling	100.2	0.5	51.3	4.0	80.0	5.4	28.2	0.3	23.2	3.3	98.5	4.9	53.8	4.5	54.6
		Storm	117.8	7.5	8.8	0.1	106.7	3.2	8.5	2.1	23.2	3.3	117.8	7.5	25.1	3.4	21.3
	IP10	Calm	38.1	2.1	2.1	0.3	32.3	1.4	0.0	0.0	10.1	0.0	39.1	1.6	8.6	0.2	21.9
		Trawling	99.6	8.4	66.6	12.2	126.2	16.3	38.4	2.0	9.4	4.7	103.8	15.7	52.5	11.8	50.6
		Storm	108.5	6.6	3.8	0.1	107.1	4.9	1.0	0.0	9.3	4.7	100.5	10.2	6.8	5.1	6.7
	IP17	Calm	51.0	0.3	11.2	1.7	29.1	1.6	6.5	1.7	14.3	2.4	47.3	2.6	18.8	3.3	39.7
		Trawling	59.5	2.0	0.6	0.0	67.0	10.8	0.0	0.0	7.2	1.4	61.3	6.3	5.7	1.0	9.3
		Storm	80.4	4.4	1.0	0.0	95.9	4.5	6.9	2.4	7.2	1.4	83.1	4.9	8.6	2.0	10.4
	IP38	Calm	55.5	0.3	9.4	0.4	46.9	7.4	33.1	0.3	11.3	2.4	54.4	4.9	26.3	2.1	48.3
		Trawling	65.9	2.3	10.0	0.6	80.9	22.4	11.6	0.0	7.2	0.0	70.0	10.1	14.9	0.3	21.3
		Storm	132.1	8.7	83.3	4.7	48.4	2.1	1.0	0.0	3.1	1.7	86.4	6.4	43.5	3.6	50.4
	IP41	Calm	57.6	6.2	16.0	0.5	38.6	2.2	4.9	0.8	14.1	2.1	54.3	5.5	20.3	2.1	37.5
		Trawling	76.1	16.8	23.2	4.1	98.8	23.4	54.8	0.4	3.1	1.7	79.1	18.9	35.6	3.5	45.0
		Storm	84.0	18.2	26.5	2.2	77.2	10.4	12.3	3.3	5.0	0.0	75.8	13.1	21.6	2.4	28.5
B)	IP01	Calm	128.4	31.9	46.8	0.8	71.3	2.4	5.2	0.1	30.9	2.9	114.7	18.7	48.2	2.6	42.1
		Trawling	132.7	0.5	45.0	0.2	91.3	3.1	0.0	0.0	21.8	1.4	117.9	2.5	38.4	1.2	32.6
		Storm	126.1	2.3	19.8	3.2	97.4	1.4	1.0	0.0	21.8	1.4	117.1	2.8	26.5	2.7	22.6
	IP10	Calm	48.6	0.7	1.9	0.3	36.5	2.8	0.6	0.0	8.9	1.0	45.0	2.2	7.8	0.9	17.4
		Trawling	77.7	1.0	48.5	3.3	113.6	18.6	0.0	0.0	7.3	1.0	89.0	8.7	29.3	2.4	32.9
		Storm	84.4	0.9	1.0	0.0	74.8	3.2	1.0	0.0	7.3	1.0	76.8	2.5	6.4	0.7	8.3
	IP17	Calm	37.0	6.4	0.0	0.0	27.3	3.6	5.7	0.6	13.7	1.0	39.3	5.3	12.6	1.0	32.0
		Trawling	72.3	0.7	18.7	0.6	84.8	2.1	0.0	0.0	3.6	2.4	72.1	3.0	11.9	2.1	16.5
		Storm	70.3	3.3	7.8	0.4	93.5	1.2	32.5	3.5	3.6	2.4	74.6	3.9	19.5	3.4	26.2
	IP38	Calm	81.0	2.8	18.9	6.6	48.3	4.1	11.9	0.7	15.8	2.0	70.9	4.5	25.9	5.1	36.6
		Trawling	61.4	5.4	3.2	0.2	104.1	8.8	67.8	1.9	5.0	1.4	75.5	7.3	32.4	1.9	42.9
		Storm	105.5	1.3	47.1	12.7	65.1	2.2	21.1	0.1	3.1	2.4	80.1	3.3	33.8	8.0	42.2
	IP41	Calm	113.2	3.7	0.0	0.0	49.5	2.9	0.0	0.0	16.6	1.0	87.7	3.7	12.4	0.8	14.2
		Trawling	63.8	4.7	22.2	5.5	115.0	9.7	50.6	14.0	3.1	2.4	79.6	8.0	33.4	10.1	42.0
		Storm	70.9	9.1	23.8	0.3	93.1	1.3	1.0	0.0	2.1	1.2	73.5	5.9	13.6	1.0	18.5
C)	IP01	Calm	173.3	6.1	76.7	3.7	150.6	5.8	73.5	5.3	40.4	1.0	175.4	6.0	97.3	4.7	55.4
		Trawling	135.1	10.3	18.8	1.1	123.4	17.6	42.0	0.9	22.2	1.9	132.2	13.5	42.6	2.3	32.2
		Storm	185.9	5.2	46.0	1.9	151.5	11.0	67.6	4.1	22.2	1.9	168.3	8.4	66.2	4.0	39.3
	IP10	Calm	84.9	8.0	12.9	0.7	85.6	2.2	39.3	1.9	29.9	0.5	98.3	5.2	44.5	1.4	45.3
		Trawling	108.2	1.4	23.8	5.7	153.0	36.1	40.2	9.3	10.0	2.9	121.7	17.3	35.3	8.7	29.0
		Storm	105.2	7.5	28.4	4.9	105.1	2.5	1.0	0.0	10.0	2.9	101.1	6.8	21.8	4.5	21.6
	IP17	Calm	76.0	0.9	21.2	0.7	84.1	2.5	48.9	8.2	27.6	0.0	91.6	1.5	50.7	3.7	55.3
		Trawling	105.9	6.6	21.1	2.8	98.1	14.1	27.3	6.1	1.3	0.8	91.7	9.8	21.8	4.7	23.8
		Storm	135.1	13.1	50.0	0.8	149.0	4.2	1.0	0.0	4.0	0.4	128.7	8.4	27.9	0.7	21.6
	IP38	Calm	103.5	0.5	26.2	9.6	74.3	8.0	44.0	1.0	17.5	2.9	93.5	5.6	43.6	7.2	46.6
		Trawling	112.8	4.2	64.0	6.2	174.5	4.6	109.8	19.3	4.0	0.4	128.1	4.2	78.2	11.0	61.0
		Storm	128.4	6.6	53.0	1.3	114.6	5.6	79.6	5.4	6.0	0.0	113.3	5.5	62.3	2.8	55.0
	IP41	Calm	88.9	14.6	1.0	0.1	65.9	0.2	25.7	5.5	34.3	3.8	95.7	10.1	36.5	5.1	38.1
		Trawling	77.3	4.7	16.1	1.4	139.0	7.5	97.0	7.5	6.0	0.0	98.0	5.3	51.2	3.6	52.2
		Storm	100.5	5.6	39.7	8.0	57.4	3.4	7.5	0.6	6.7	1.9	77.2	5.5	27.5	5.6	35.6

SD, Standard deviation (n=3).

Tab. 2. Results of 3-way PERMANOVA testing for differences in the investigated variables among sampling periods, stations and sampling depths.

	Source	DF	MS	F	P
Total protein	Period (P)	2	8.2	134.9	***
	Station (S)	4	12.1	199.4	***
	Depth (D)	2	14.1	232.4	***
	P x s	8	1.5	24.2	***
	P x D	4	1.3	21.7	***
	S x D	8	1.2	19.1	***
	P x S x D	16	0.6	9.9	***
	Residual	90	0.1		
Hydrolysable protein	Period (P)	2	4.0	117.6	***
	Station (S)	4	5.7	165.0	***
	Depth (D)	2	4.5	131.1	***
	P x s	8	5.1	148.0	***
	P x D	4	1.3	38.4	***
	S x D	8	1.1	31.8	***
	P x S x D	16	2.3	66.9	***
	Residual	90	0.0		
Total carbohydrate	Period (P)	2	21.4	292.7	***
	Station (S)	4	2.0	26.7	***
	Depth (D)	2	17.3	236.3	***
	P x s	8	2.9	39.8	***
	P x D	4	0.4	5.8	**
	S x D	8	1.1	14.6	***
	P x S x D	16	0.5	7.4	***
	Residual	90	0.1		
Hydrolysable carbohydrate	Period (P)	2	7.1	270.2	***
	Station (S)	4	4.5	173.7	***
	Depth (D)	2	19.7	755.9	***
	P x s	8	2.9	109.4	***
	P x D	4	0.7	25.2	***
	S x D	8	1.7	66.0	***
	P x S x D	16	1.3	49.6	***
	Residual	90	0.0		
Total lipid	Period (P)	2	21.2	397.8	***
	Station (S)	4	14.1	264.8	***
	Depth (D)	2	4.0	75.2	***
	P x s	8	0.4	8.5	***
	P x D	4	3.2	60.8	***
	S x D	8	0.4	7.7	***
	P x S x D	16	0.2	3.3	**
	Residual	90	0.1		
Biopolymeric C	Period (P)	2	4.9	66.8	***
	Station (S)	4	11.6	156.8	***
	Depth (D)	2	20.9	282.8	***
	P x s	8	1.5	19.7	***
	P x D	4	1.2	15.7	***
	S x D	8	0.8	11.4	***
	P x S x D	16	0.4	5.6	***
	Residual	90	0.1		
Bioavailable organic C	Period (P)	2	2.0	39.3	***
	Station (S)	4	9.3	183.6	***
	Depth (D)	2	19.6	387.0	***
	P x s	8	2.4	47.8	***
	P x D	4	1.1	21.5	***
	S x D	8	0.6	12.8	***
	P x S x D	16	1.3	25.2	***
	Residual	90	0.1		

DF, degrees of freedom; MS, mean square; F, F value; ***P<0.001; **P<0.01.

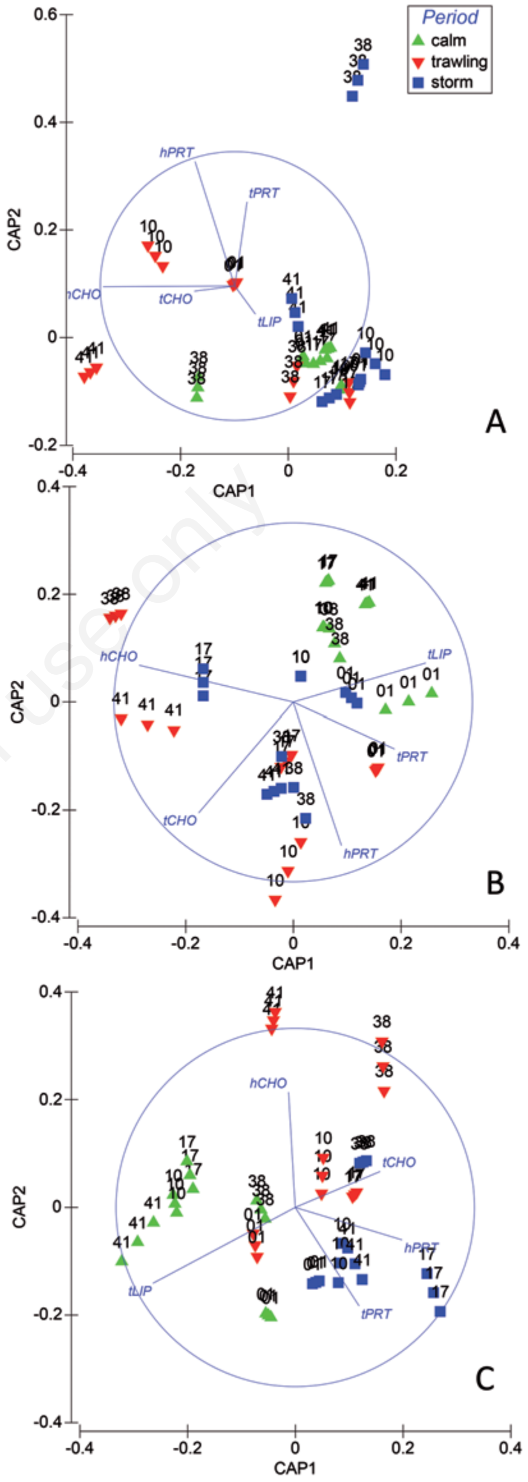
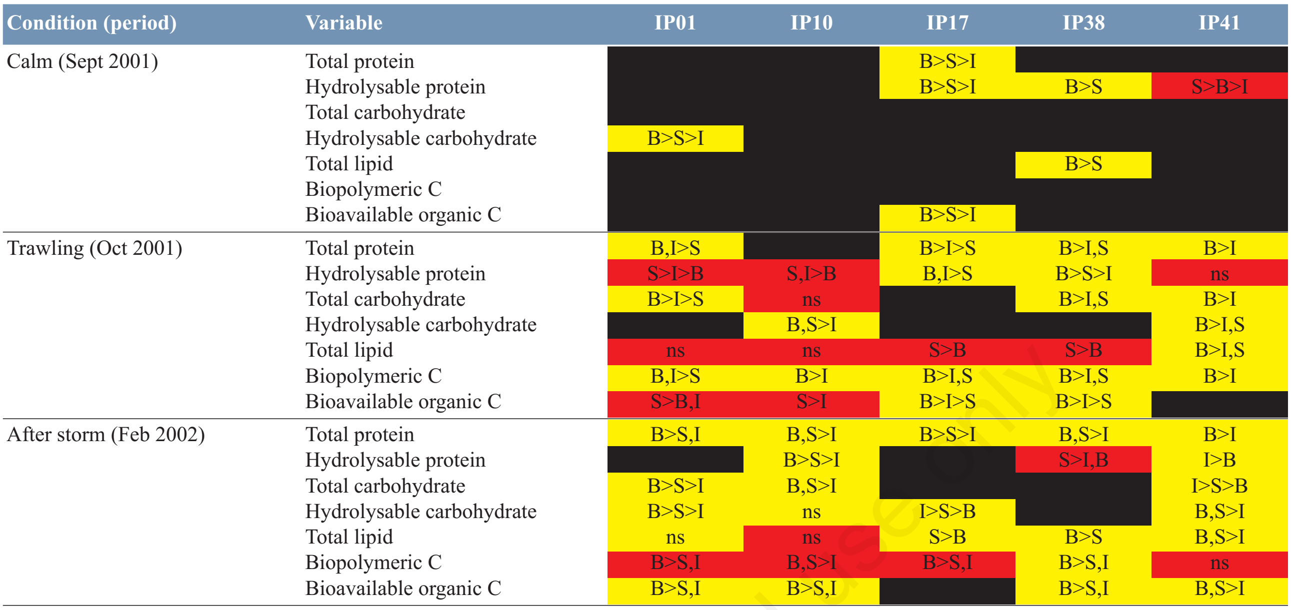


Fig. 2. Bi-plots after CAP analysis illustrating spatial (among stations) and temporal (among calm, trawling and after-storm conditions) variations in the biochemical composition of particulate organic matter in the Thermaikos Gulf, in the superficial (A), intermediate (B) and bottom (C) water layers. Vectors are proportional to the correlation of variables with the two major axes. tPRT, total proteins; tCHO, total carbohydrates; tLIP, total lipids; hPRT, hydrolysable proteins; hCHO, hydrolysable carbohydrates.

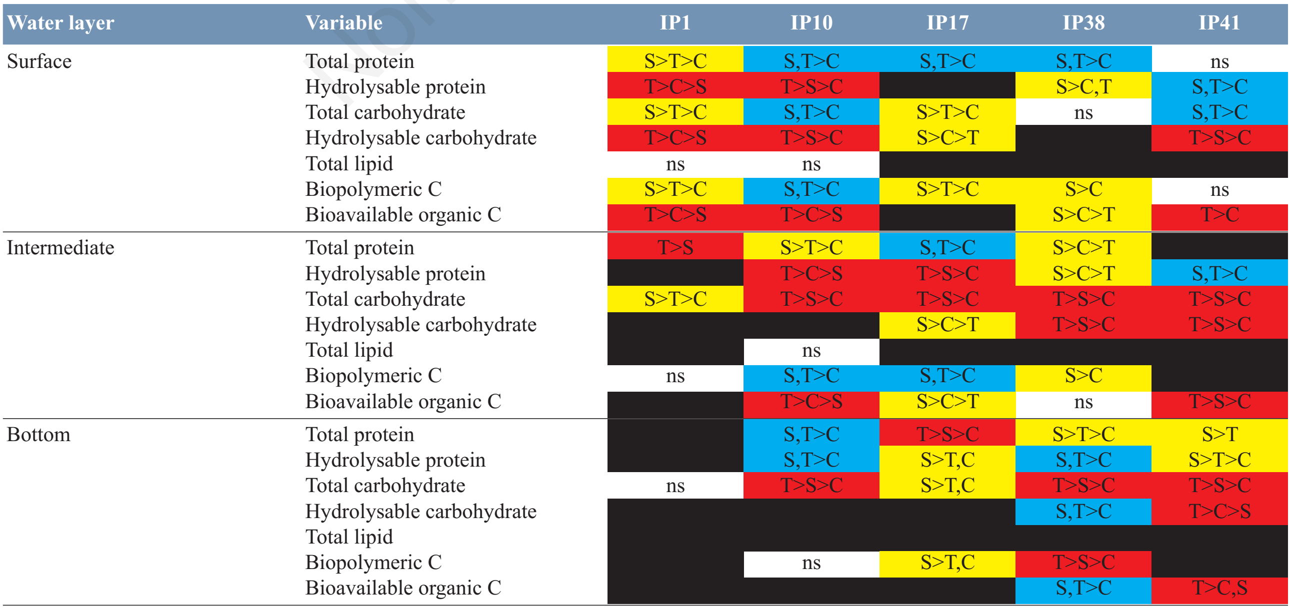
Tab. 3. Visual representation of *post-hoc* tests carried out to ascertain variations in the distribution of particulate organic compounds in the water column of the Thermaikos Gulf during calm (September 2001), trawling (October 2001) and after storm (February 2002) conditions. Green, yellow and red cells indicate missing, weak and strong signatures, respectively, of sediment resuspension in the upper water column layer.



Condition (period)	Variable	IP01	IP10	IP17	IP38	IP41
Calm (Sept 2001)	Total protein			B>S>I		
	Hydrolysable protein			B>S>I	B>S	S>B>I
	Total carbohydrate					
	Hydrolysable carbohydrate	B>S>I				
	Total lipid				B>S	
	Biopolymeric C					
	Bioavailable organic C			B>S>I		
Trawling (Oct 2001)	Total protein	B,I>S		B>I>S	B>I,S	B>I
	Hydrolysable protein	S>I>B	S,I>B	B,I>S	B>S>I	ns
	Total carbohydrate	B>I>S	ns		B>I,S	B>I
	Hydrolysable carbohydrate		B,S>I			B>I,S
	Total lipid	ns	ns	S>B	S>B	B>I,S
	Biopolymeric C	B,I>S	B>I	B>I,S	B>I,S	B>I
	Bioavailable organic C	S>B,I	S>I	B>I>S	B>I>S	
After storm (Feb 2002)	Total protein	B>S,I	B,S>I	B>S>I	B,S>I	B>I
	Hydrolysable protein		B>S>I		S>I,B	I>B
	Total carbohydrate	B>S>I	B,S>I			I>S>B
	Hydrolysable carbohydrate	B>S>I	ns	I>S>B		B,S>I
	Total lipid	ns	ns	S>B	B>S	B,S>I
	Biopolymeric C	B>S,I	B,S>I	B>S,I	B>S,I	ns
	Bioavailable organic C	B>S,I	B>S,I		B>S,I	B,S>I

B, bottom; I, intermediate; S, surface.

Tab. 4. Visual representation of *post-hoc* tests carried out to ascertain variations in the concentration of particulate organic compounds in the Thermaikos Gulf during calm (September, C), trawling (October 2001, T) and after storm (February 2002, S) conditions in the three water column layers. Green/white, yellow, light blue and red cells indicate missing effects of both trawling and storm, stronger effects of storm, similar effects of storm and trawling, stronger effects of trawling, respectively.



Water layer	Variable	IP1	IP10	IP17	IP38	IP41
Surface	Total protein	S>T>C	S,T>C	S,T>C	S,T>C	ns
	Hydrolysable protein	T>C>S	T>S>C		S>C,T	S,T>C
	Total carbohydrate	S>T>C	S,T>C	S>T>C	ns	S,T>C
	Hydrolysable carbohydrate	T>C>S	T>S>C	S>C>T		T>S>C
	Total lipid	ns	ns			
	Biopolymeric C	S>T>C	S,T>C	S>T>C	S>C	ns
	Bioavailable organic C	T>C>S	T>C>S		S>C>T	T>C
Intermediate	Total protein	T>S	S>T>C	S,T>C	S>C>T	
	Hydrolysable protein		T>C>S	T>S>C	S>C>T	S,T>C
	Total carbohydrate	S>T>C	T>S>C	T>S>C	T>S>C	T>S>C
	Hydrolysable carbohydrate			S>C>T	T>S>C	T>S>C
	Total lipid		ns			
	Biopolymeric C	ns	S,T>C	S,T>C	S>C	
	Bioavailable organic C		T>C>S	S>C>T	ns	T>S>C
Bottom	Total protein		S,T>C	T>S>C	S>T>C	S>T
	Hydrolysable protein		S,T>C	S>T,C	S,T>C	S>T>C
	Total carbohydrate	ns	T>S>C	S>T,C	T>S>C	T>S>C
	Hydrolysable carbohydrate				S,T>C	T>C>S
	Total lipid					
	Biopolymeric C		ns	S>T,C	T>S>C	
	Bioavailable organic C				S,T>C	T>C,S

ns, not significant.

fore acknowledge that a certain (unknown) proportion of variance among calm, trawling, and after-storm conditions is most likely associated to natural temporal variability in the POM quantity and biochemical composition in the Thermaikos Gulf. Indeed, variations in the concentration of POM along the water column, as well as in its biochemical composition, can be the result of biological processes, including among the others primary productivity and particle consumption (Fabiano and Pusceddu, 1998; Fabiano *et al.*, 2001). Moreover, concentration and composition of POM can be affected, in particular along the continental shelf, by rivers discharge (Goñi *et al.*, 2013). This latter, however, was most likely not the case as the analysis of meteorological data in the region during the study period revealed a very dry season in autumn 2001, accompanied by very low levels of river discharge (Tragou *et al.*, 2005)

Differences in the environmental characteristics among the sampling periods appeared to be particularly relevant between September-October 2001 and February 2002. In fact, the hydrological characteristics of the Thermaikos Gulf did not change markedly from September (calm conditions) to October 2001 (during trawling), when pronounced thermoclines and haloclines were recorded at ca. 35-40 m depth in the whole study area (Tragou *et al.*, 2005; Zervakis *et al.*, 2005). On the other hand, a series of cold fronts that passed over the region in late January 2002 completely homogenised the thermohaline structure of the water column in February 2002 (Tragou *et al.*, 2005; Zervakis *et al.*, 2005). Moreover, during this period, a low salinity, low temperature water front occurred along the western boundary of the Gulf, characterised by strong, vertically homogeneous southward velocities of up to 20 cm s^{-1} (Zervakis *et al.*, 2005). These large differences would make in principle difficult interpreting the differences in POM characteristics among the September-October 2001 and February 2002 periods. However, the lack of significant variations in the physico-chemical characteristics from calm to trawling conditions, at least, allows us to corroborate the hypothesis by which bottom trawling is a major factor stimulating sediment resuspension and, as a consequence, increasing POM concentrations in the water column as well as significantly modifying its biochemical composition and bioavailability for consumers. This result is indeed consistent with previous investigations carried out by means of both correlative and manipulative approaches, which generally demonstrated that bottom trawling can significantly inject large amounts of sediments and associated POM into the overlying water column (Durrieu de Madron *et al.*, 2005; Pusceddu *et al.*, 2005a, 2005c; Martín *et al.*, 2014b). Our results paradigmatically demonstrate that along with the well-known mechanisms of pelagic-benthic coupling associated with sedimentation processes of POM (Graf, 1992), the reverse exchange of material (*i.e.*, benthic-pelagic coupling, *sensu* Marcus and Boero, 1998) stimulated by sediment resuspension caused by anthropogenic and, to a lesser extent, natural processes can have important consequences on POM stocks in the water column.

We report here also that the transition from calm to trawling and to after-storm conditions is characterised by clear changes in the biochemical composition of POM. In particular, we show that both during trawling in October and after-storm in February, the relative importance of total and hydrolysable carbohydrates increases significantly when compared to values measured during calm conditions. At the same time, suspended POM during trawling and after the storm is also characterised by lipid

Tab. 5. Results of PERMANOVA analysis testing for differences in the biochemical composition of particulate organic matter considering all investigated variables in the three layers of the water column.

	Source	DF	MS	F	P
Surface	Period	2	34.302	85.383	***
	Station	4	15.271	38.012	***
	Period x Station	8	9.7823	24.349	***
	Residual	30	0.40175		
	Total	44			
Intermediate	Period	2	27.632	102.07	***
	Station	4	21.758	80.37	***
	Period x Station	8	8.6978	32.128	***
	Residual	30	0.27072		
	Total	44			
Bottom	Period	2	28.789	106.5	***
	Station	4	21.519	79.606	***
	Period x Station	8	8.5294	31.553	***
	Residual	30	0.27032		
	Total	44			

DF, degrees of freedom; MS, means square; F, F value; ***$P<0.001$.

concentrations significantly lower than those in calm conditions. The gross biochemical composition of POM is the result of a complex multiple-source array of biotic and abiotic factors (Danovaro *et al.*, 2000) and variations in the relative importance of protein, carbohydrate and lipid contents can be highly indicative of changes in the labile *vs* refractory nature of particles (Fabiano *et al.*, 2001). Carbohydrates are usually associated with organic matter pre-eminently refractory in nature (Grémare *et al.*, 2003; Pusceddu *et al.*, 2009), so that our results strongly suggests that sediment resuspension caused by trawling and, though to a lesser extent, by storms can lower the food availability of suspended organic particles. This hypothesis is also corroborated by the significant decrease, during trawling, in the concentration of particulate lipids, whose major fraction is generally highly labile (Carreira *et al.*, 2010). Altogether these results would suggest that OM particles resuspended after bottom trawling could more refractory in nature than those present in the water column in calm or after-storm conditions. Nevertheless, we report here that, in contrast with what hypothesized from the gross biochemical composition only, the bioavailable fraction of particulate biopolymeric C increases significantly during trawling, though not consistently at all stations and water layer. This apparent incongruence is due to the fact that while total protein, lipid and carbohydrate pools include generally a very heterogeneous complex of organic compounds which are not

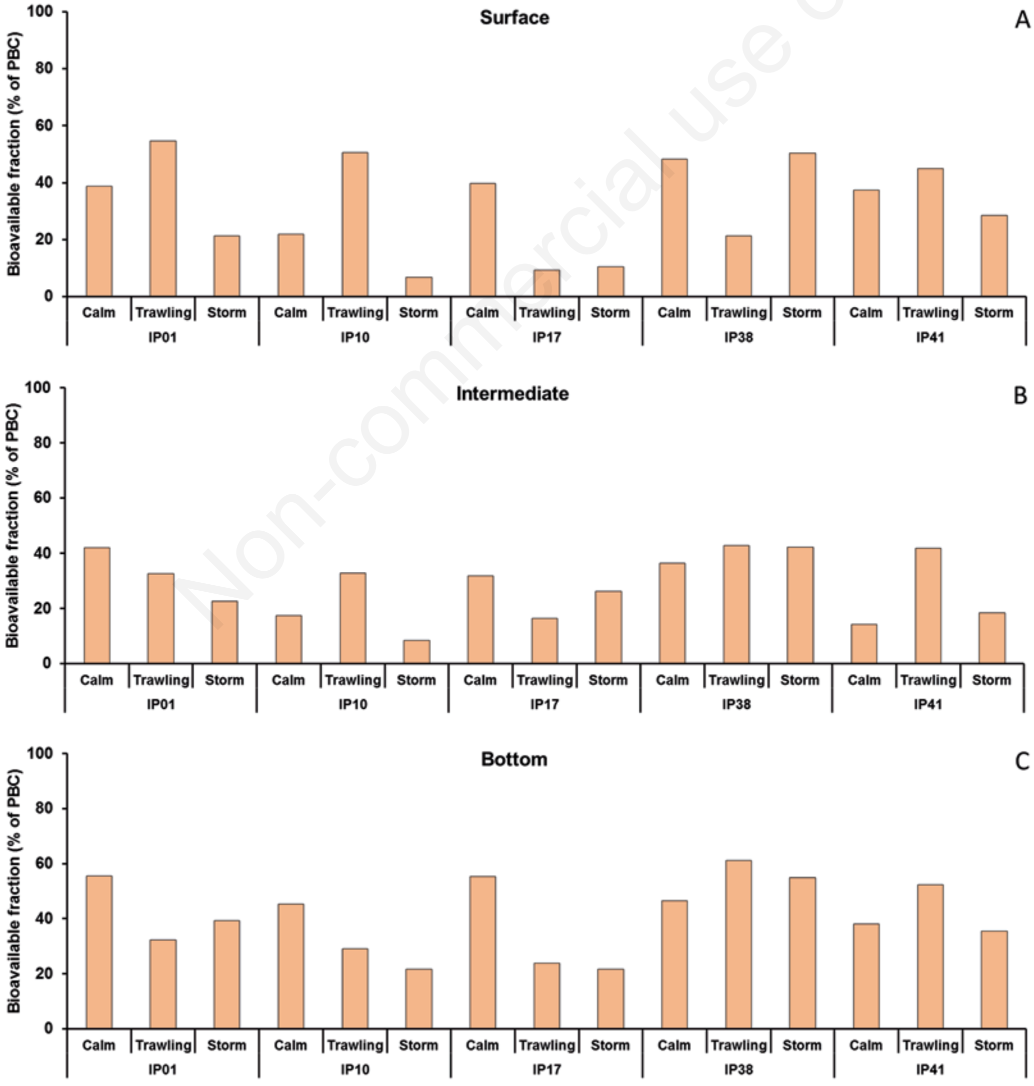


Fig. 3. Variations in the bioavailable fraction of particulate organic matter (in terms of percentage fraction of biopolymeric C enzymatically digestible) in the Thermaikos Gulf during calm (September 2001), trawling (October 2001) and after-storm (February 2002) conditions.

equally reactive to degradation (Pusceddu *et al.*, 2009), the enzymatically digestible fractions of protein and carbohydrate pools (*i.e.*, the bioavailable fraction of organic C; Dell'Anno *et al.*, 2000; Pusceddu *et al.*, 2003) are more reliable descriptors of organic matter food availability to consumers than the gross biochemical composition alone (Fabiano and Pusceddu, 1998; Pusceddu *et al.*, 1999; Danovaro *et al.*, 2001).

The apparent positive effect of bottom trawling on the bioavailability of suspended POM is however, most clearly confined to the bottom layer of the deepest stations investigated in this study. This result does not allow us concluding that sediment resuspension induced by trawling can cause the injection of labile organic compounds in the whole water column, nor that the faster mobilisation rates of organic C buried in the sediment after trawling in coastal sediments is a major factor contributing to coastal eutrophication (Polymenakou *et al,*. 2005; Pusceddu *et al.*, 2005c).

Previous studies carried out in the field and under laboratory conditions have demonstrated that sediment resuspension caused by storms or trawling activities can enhance either suspended or sedimentary POM quantity and bioavailability (Pusceddu *et al*., 2005a, 2005b, 2005c). In particular, benthic microbes exposed to O_2-rich waters caused by sediment disturbance induced by trawling can stimulate a faster mobilisation of organic C buried in the sediment, injecting more labile molecules into the system (Polymenakou *et al*., 2005). Other studies have also demonstrated that bottom trawling in coastal waters creates plumes of resuspended sediments which last just a few hours in the water column (Schoellhamer, 1996; Palanques *et al.*, 2001; Durrieu de Madron *et al.*, 2005). Nevertheless, volumes moved from the sediment to the water column can be huge if trawling activities are chronic as observed in several regions of the Mediterranean Sea (Martín *et al.*, 2014a). In such conditions we can hypothesize that the overall enhancement of C cycling in the water column and in the upper layers of the sediment exposed to chronic bottom trawling might stimulate an increase of available nutrients able, in turn, to sustain increased levels of primary productivity, ultimately leading to a potential internal eutrophication process (Polymenoakou *et al.*, 2005).

Overall, the results of this study are very different from what, instead, observed in deep-sea sediments exposed to chronic trawling activities, where the lack of a conspicuous re-deposition from the water column determines a dramatic lowering of organic C sedimentary contents and turnover rates (Pusceddu *et al.*, 2014).

CONCLUSIONS

Although limited to a very short-term analysis, likely biased by the uncontrolled seasonal variations in the quantity and biochemical composition of POM, our results corroborate the most recent literature which demonstrates that bottom trawling represents a major threat not only for commercially exploited target species and the associated by-catch, but can have also important consequences on the biogeochemical cycling of organic C. Our results have also shown that the effects of intensive trawling activities can rival those (temporarily) exerted by natural events (*e.g.*, storms). Bottom trawling represents the most common fishing practice worldwide, it is being carried out at progressively deeper depths (Puig *et al.*, 2012) and is exerting severe consequences on the submersed seascape (Martín *et al.*, 2014a) as well on the biodiversity and functioning of marine ecosystems (Pusceddu *et al.*, 2014). Since bottom trawling is carried out worldwide and natural storms at sea can be frequent and intense, we claim for the need of assessing new adapting management strategies of bottom trawling in order to mitigate the synergistic impacts of anthropogenic and natural sediment resuspension on coastal biogeochemical cycles..

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