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Distribution of seafloor litter and its interaction with benthic organisms in deep waters of the Ligurian Sea (Northwestern Mediterranean)

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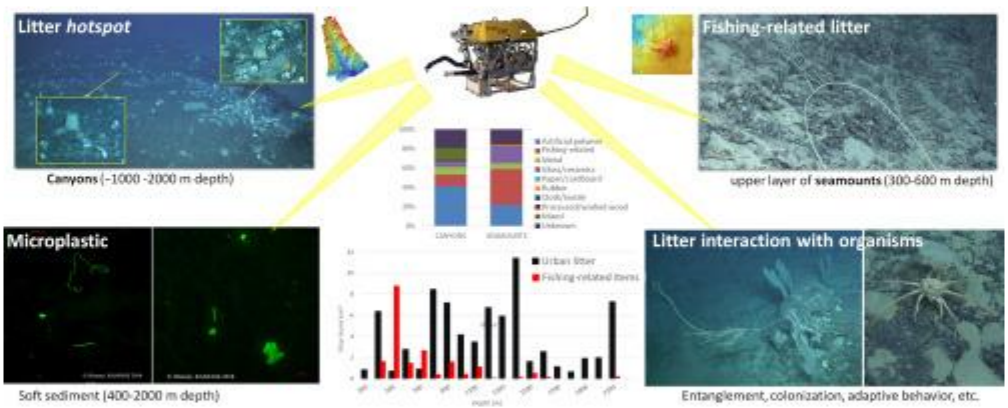
Abstract :

The Mediterranean Sea is one of the most polluted marine basins and currently serves as a hotspot for marine litter. The seafloor represents the ultimate sink for most litter worldwide. Nevertheless, the knowledge about litter distribution and its interactions with benthic organisms in deep water is poorly understood. In 2018, we investigated spatial patterns of macro- and micro-litter distribution, and their effects on benthic communities in the Ligurian Sea. An oceanographic survey was carried out with a remotely operated vehicle and a multibeam echosounder on seven seamounts and canyons, at depths ranging from 350 to 2200 m. High litter accumulations were discovered at the mouth of the Monaco canyon, where estimated densities of up to 3.8 × 104 items km-2 were found at 2200 m depth. The highest abundance of urban litter items was found on the soft substrate, at the bottom of the deeper parts of the submarine canyons, which seem to act as conduits carrying litter from the shelf towards deeper areas. In contrast, fishing-related items were most abundant in the upper layer of the seamounts (300–600 m depths). Furthermore, more than 10% of the observed deep gorgonian colonies were entangled by lost longlines, indicating the detrimental effects of this fishing gear on benthic habitats. The discovery of new litter hotspots and the evaluation of how deep-sea species interact with litter contribute to increasing the knowledge about litter distribution and its effects on the deep ecosystem of the Mediterranean basin. All the observations recorded in this study showed substantial and irreversible changes in the deep and remote areas of marine environments, and these changes were found to be caused by humans. Our

findings further stress the need for urgent and specific measures for the management of deep-sea pollution and the reduction of litter inputs in the environment.

Graphical abstract



Highlights

► Litter distribution and its interactions with organisms in the deep sea were assessed ► A litter hotspot was discovered at the base of the Monaco canyon at 2200 m depths ► Fishing-related litter was abundant in the upper layer of seamounts (300–600 m depths) ► More than 10% of observed coral colonies were entangled by longlines ► All collected sediment samples were contaminated by microplastics, mainly fibers

Keywords : ROV-imaging, Fishing impact, Microplastic, Litter hotspot, Canyon, Seamount

1. Introduction

Litter pollution has become a highly sensitive global issue, from both ecological and socioeconomic viewpoints.

The highly urbanized semi-enclosed basin of the Mediterranean Sea represents a biodiversity hotspot with high levels of endemism (Bianchi and Morri, 2000; Coll et al., 2010; UNEP-MAP RAC/SPA, 2010). It is also one of the areas most affected by litter inputs worldwide (Anastasopoulou and Fortibuoni, 2019; Galgani et al., 2019).

Waste mismanagement on land largely affects the amount and distribution of litter in the sea/ocean (Jambeck et al., 2015). In coastal areas, river inputs and activities related to fishing, as well as maritime traffic and tourism, significantly contribute to the littering of marine environments with notable temporal and seasonal variations (Lebreton et al., 2017; López-López et al., 2017; Pierdomenico et al., 2019).

The seafloor, from the intertidal zones to the abyssal depths, is a major long-term sink for marine macro- (> 5 mm) and micro-litter (< 5 mm) (Galgani et al., 1996; Gerigny et al., 2019; Kane et al., 2020; Pham et al., 2014; Sanchez-Vidal et al., 2018; Tubau et al., 2015). Near metropolitan areas in the Mediterranean Sea, macro-litter densities may exceed 10^5 items km^{-2} (Galgani et al., 2000) with large accumulations also occurring in the near-shore channels (Pierdomenico et al., 2019). Even high densities of micro-litter, including microplastics (MPs), especially fibers, have been found to contaminate benthic sediments (Kane et al., 2020; Sanchez-Vidal et al., 2018; Sleight et al., 2014). MP conglomerates, formed by biofouling and erosion (which modifies their buoyancy), determine MP sedimentation on the seafloor (Kooi et al., 2017; Leiser et al., 2020; Tu et al., 2020). Furthermore, once they sediment on the seafloor, MPs are available for many benthic species to feed on, such as detritivores and filter-feeding species. In this way, MPs can enter the deep-sea trophic web (Carbery et al., 2018; Costa et al., 2020; Setälä et al., 2018; Valente et al., 2019).

The deep sea (depths below 200 m) encompasses ~79% of the Mediterranean basin (Danovaro et al., 2010), and its biodiversity plays an important role in providing resources and ecosystem services (Danovaro et al., 2020; Manea et al., 2020). Acts of littering, dumping of industrial waste, and fishing activities are considered consistent threats to deep-sea communities. This is in addition to the release of contaminants and oil from the gas industry as well as from cargo spillage. All these factors greatly impact deep-sea species and ecosystems, potentially affecting some ecosystem functions (Danovaro et al., 2020; Ramirez-Llodra et al., 2011).

Litter reaches the seabed through oceanic and hyperpycnal currents (Galgani et al., 2000; Pierdomenico et al., 2019), together with passive sinking (Hidalgo-Ruz et al., 2012), and it may be transported to considerable distances before sinking because of fouling weight (Eriksen et al. 2014). Variations in litter transport, mainly caused by seasonal changes in river flow rate and related turbidity currents, as well as oceanic circulation patterns, explain the considerable spatial dispersion of litter with accumulation points observed in bays and canyons that are often close to large cities than in the open sea (Canals et al., 2020; Pierdomenico et al., 2019; Tubau et al., 2015).

Hydrodynamic conditions and current regimes, which are locally caused by the geomorphology, topography, and habitat heterogeneity of the seabed, influence litter distribution in the deep sea. Thus, submarine canyons with heads close to the coast, or other conspicuous topographic submarine structures (e.g., seamounts, steep or vertical walls, and channels) that funnel large masses of dense water (Canals et al., 2009; Palanques et al., 2018) can act as primary vectors of litter transport from the coast to the deep sea (Danovaro et al., 2020; Galgani et al., 1996; Tubau et al., 2015). Litter "hotspots" (i.e., large accumulations of litter) formed by mixtures of both land- and marine-sourced anthropogenic items, together with natural debris, are evidence of the efficiency of this transport system (Pham et al., 2014; Woodall et al., 2015).

Additionally, through their effect on local circulation, these submarine structures support the upwelling of nutrient-rich waters, transfer of organic matter-rich waters, and cascading events, which create more varied and complex habitats than that of the surrounding slope areas. As a result, canyons and seamounts support a greater abundance and diversity of conspicuous and vulnerable sessile organisms, such as sponges and corals (i.e., cold-water corals; Rogers et al., 2007), and provide important habitats, such as spawning and nursery areas for pelagic and demersal fish species of commercial importance (Bo et al., 2020; Fabri et al., 2019; Fanelli et al., 2018; Lo Iacono et al., 2018; Roberts et al., 2009; Würtz, 2012; Würtz and Rovere, 2015). They are also important cetacean feeding grounds because of their high levels of primary productivity (Fiori et al., 2016; Johnston et al., 2008; Morato et al., 2010; Vassallo et al., 2018; Worm et al., 2003).

The distinct combination of abiotic and biotic factors of each canyon and seamount (Würtz, 2012), determines the differences in faunal distribution, composition, and abundance (Fabri et al., 2017; Fink et al., 2015; Gori et al., 2013; Hecker, 1990; Würtz and Rovere, 2015). This complexity, which is associated with heterogeneous geomorphologic features, such as holes, cliffs, and rocks that may retain litter items, is also characterized by the patchy distribution of litter (Pham et al., 2014).

More than 500 submarine canyons and 242 seamounts and seamount-like structures characterize the continental margin of the Mediterranean Sea (Harris and Whiteway, 2011; Würtz and Rovere, 2015). Despite their widespread distribution and their function of hosting important communities, these submarine topographic structures have been largely understudied until the 1970s (Aguilar et al., 2013; Bo et al., 2011; Freiwald et al., 2011). However, recently, increasing research has focused on the role of submarine canyons and seamounts in the exchange of energy and substances between the continental shelf and the deep sea (Allen and Durrieu de Madron, 2009). This is in addition to the functioning of the benthic and pelagic ecosystems, and the impacts of human activities (Bo et al., 2020; Clark et al., 2010; Pitcher et al 2007). For instance, submarine canyons and seamounts are both hotspots for litter accumulation (Canals et al., 2020; Tubau et al., 2015) and important sites for fisheries; this is because the loss of fishing gear contributes to the accumulation of litter (Bo et al., 2020; Fanelli et al., 2018).

In the last decade, the litter issue has gathered increasing concern. Thus, the attention of the scientific community is being increasingly focused on hidden sites in the deep sea, which may be significantly affected ecologically, with economic and potential human health consequences (Danovaro et al., 2020). With the technological advances and a growing accessibility to innovative and non-invasive instruments, the exploration of the marine environment is moving offshore and towards greater depths (Fanelli et al., 2017; Grehan et al., 2017; Ramirez-Llodra et al., 2011). Imaging techniques using submersibles, remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), and towed cameras (TCs), allow researchers to estimate litter distribution and abundance, whilst simultaneously detecting the visible effects of litter on the environment, such as how the litter interacts with marine organisms (Angiolillo, 2019). Entanglement, ghost fishing, ingestion, and colonization are some of the effects of litter on marine organisms that threaten biodiversity in deep-sea habitats (de Carvalho-Souza et al., 2018; Deudero and Alomar, 2015; Valente et al., 2019, 2020).

Although an increasing number of studies have focused on this issue (Anastasopoulou and Fortibuoni, 2019; Angiolillo and Fortibuoni, 2020; Galgani, 2015), the understanding of sources, patterns, and the ultimate impacts on marine ecosystems has been poorly grasped (Danovaro et al., 2020; de Carvalho-Souza et al., 2018; Ramirez-Llodra et al. 2011).

Monitoring of deep-sea areas is largely hampered by sampling difficulties and costs. Furthermore, comparable information regarding litter distribution and its interactions is limited because no harmonized monitoring protocols have been applied. This includes the lack in the definition and in the choice of sampling units, methodologies for collection, classification, the quantification of litter, and standards for the analysis and reporting of results. Moreover, studies on MPs are mainly conducted on samples from the beach or shallow sediment (Peng et al., 2020), which illustrate the importance of improving knowledge regarding MPs in deep-sea sediments.

Therefore, the collection of scientifically validated data and the development of standardized methods and harmonized protocols for long-term monitoring of the deep sea are essential to elucidate the impact of present and future human activities on deep-sea biodiversity (Danovaro et al., 2020).
Although several legal and policy frameworks have addressed this important issue and the awareness of the pressures acting on deep-sea habitats has increased, several problems remain regarding marine litter assessment and management at the Mediterranean scale.
In Europe, member states are implementing the Marine Strategy Framework Directive (MSFD, 2008/ 56/EC) to achieve or maintain the Good Environmental Status using Descriptors to assess the health of marine ecosystems (Descriptor 10 corresponds to marine litter). Additionally, measures to reduce the impacts of human activities on the marine environment are also being employed. Moreover, Marine Spatial Planning (MSP) involves implementing the ecosystem-based (EB) approach (MSPD 2014/89/EC) to achieve the goals of both the Blue Growth Strategy [COM (2012) 494] and the MSFD. Concurrently, under the framework of the Barcelona Convention, all the Mediterranean countries adopted a regional plan in 2013, which included research and monitoring of marine environment (considering the "seafloor litter" as a part of Common Indicator CI23), and are implementing MSP toward conservation efforts and sustainable development (UNEP(OCA)/MED IG.6/7, 1995). Additionally, to quantify the impact of marine litter on the biota, a new European Commission Decision proposed a further MSFD indicator concerning the entanglement of species and other adverse effects caused by marine litter. This indicator, called D10C4, describes "The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects". The development of this new indicator requires the specific data acquisition protocols and calculation methods.
Considering the trans-boundary nature of most of the deep waters, several authors (e.g., Danovaro et al., 2020; Manea et al., 2020) have suggested establishing multilateral and international agreements for the development of coordinated and harmonized marine strategies at the scale of deep-sea biogeographic areas/sub-regions in the Mediterranean to support environmental conservation.
To promote trans-boundary collaboration, in 2018 the RAMOGE (Saint-RAphaël, MOnaco, and GEnoa) international agreement (involving Italy, France, and the Principality of Monaco) organized a second campaign with the goal of studying deep-sea seamounts and canyons in the Ligurian Sea (Northwestern Mediterranean Sea) (Daniel et al., 2019). The RAMOGE area includes the maritime zones of the South Region (France), the Principality of Monaco, and the Liguria Region (Italy), which constitute a marine Mediterranean pilot zone where the three states implement integrated management actions to deal with marine pollution and protect marine biodiversity.
Here, we present the results of our investigations on i) marine litter occurrence, abundance, composition, and distribution patterns in deep seamounts and canyons of the RAMOGE pilot area; ii) the interactions between marine litter and benthic organisms observed during the surveys; and iii) the attempt to harmonize experimental protocols for MSFD monitoring of seabed litter using ROVs.

2. Materials and methods

2.1. Study areas

The RAMOGE EXPLO 2018 research cruise was undertaken on board the R/V L'Atalante (Ifremer) in the Ligurian Sea (Northwestern Mediterranean Sea) along the French, Monaco, and Italian coasts in September 2018 (Fig. 1). The Ligurian Sea coastal platform is narrow and marked by 24 major canyons; among which, 19 are along the Italian coast, one is along Monaco's coast, and four are along the French coast (Fabri et al., 2014; Würtz, 2012). Additionally, there are several seamounts in the deep basin (Würtz, 2012).

Seven focal sites were explored (Table 1): two slopes (Méjean and off Saint-Tropez), two coastal canyons (Cannes and Monaco), and three offshore seamounts (Ulysses, Janua, and Spinola).

2.2. Sampling design

Bathymetric data were collected from six sites using the hullmounted MultiBeam EchoSounder (MBES) EM122 Kongsberg of the R/V L'Atalante. MBES data were processed at a 25 m cell-size resolution (Fig. 1) using the software, GLOBE© Ifremer. The remaining site, the slope off Saint-Tropez, was not mapped during the cruise, and existing data at 100 m cell-size resolution (Loubrieu and Satra, 2010) were used for the analysis. Seven exploratory dives, one at each site, were conducted with the *VICTOR 6000* ROV at depths ranging from 350 m to 2200 m (Fig. 1, Table 2). ROV transects were carried out at each site following a depth gradient and, when possible, a linear track. The ROV was fully equipped to record images, with a 3CCD main video camera capable of zooming in, and was mounted to pan and tilt; with two controlled video cameras; a 4K video camera (Apex-4070, DeepSea Power & Ligh); and an HD digital camera (FCB-H11, Sony). A real-time chronological report was built during each dive by scientists on watch. This chronological report was georeferenced and stored together with the raw navigation files and video films. The ROV was precisely located using an ultra-short baseline (USBL) underwater positioning system (Posidonia 2, iXBlue), corrected with the inertial navigation system (INS, PHINS, iXBlue). The transponder provided detailed geographical and depth position of the ROV, which was recorded every second. Cameras continuously recorded videos onto hard disks. Specific attention was paid to maintaining constant ROV cruising speed (≈1 knot) and altitude (≈3 m from the bottom). The ROV manipulator arms and suction sampler were used to collect biological and litter samples, as well as to deploy tube cores (TCs) to collect sediment.

2.3. Data analysis

Data analyses were performed for all video transects (Fig. 1, Table 2). When ROV requirements (speed ≈1 knot, and altitude ≈3 m from the bottom) were not met, video frames were not considered in the analyses (i.e., during the ascent and descent phases of the ROV). Similarly, video footages were excluded from the analyses when the ROV was stationary (i.e., collecting samples or recording close-up images), there was poor visibility, or images were out of focus. Overall, 53 h 27 min of ROV footage was processed using the free internet VLC software. The video recordings covered a linear explored distance of ~41 km. Frame shots from principal cameras, photos from the lower camera (every second), and 4K videos were used to support video analysis, litter classification, and species identification. All macro-litter items and all macro-benthic organisms visible along each dive transect were classified at the lowest taxonomic level and counted. Samples collected by ROV equipment were used to identify the taxa. The identified samples were validated by two independent operators to minimize subjective interpretations.

Lasers allowing *in situ* measurements were not continuously recorded on videos during the survey. Therefore, densities were estimated considering the linear length of transects. The presence of litter items was evaluated both by relative occurrence (frequency of debris types, %) and linear density (items km^{-1}). The diversity of marine litter composition found at each location was evaluated according to the evenness calculation (J'). Marine litter distribution was analyzed by dividing each transect into 50 m segments, which were used as sampling units.

To collect useful and comparable data, a set of parameters was used according to the sampling protocol developed by the MSFD TG Litter Working Group (2021) "MSFD Protocol for monitoring entanglement and other interactions between litter and benthic organisms."

Marine litter items were classified in accordance with the MSFD Joint List (Fleet et al., 2021) based on their composition. These classifications included artificial polymer materials, rubber, cloth/textile, paper/cardboard, processed/worked wood, metal, glass/ceramics, chemicals, undefined, and food waste. The litter position and arrangement were classified as “laying,” when litter was settled at the bottom; “hanging,” when litter was under tension between obstacles; and “buried,” when items were partially covered by sediment. Moreover, points of accumulation (*litter hotspots*) were defined when dense litter aggregations were observed making the individual items uncountable. When this occurred, some of the items were also hidden or partially buried in the sediment or underneath larger items. In these cases, the litter abundance was recorded as the number of litter hotspots km^{-1}, and estimated according to the number of visible items on surface area (i.e., average no. of items km^{-2}).

The amount of litter was recorded in terms of seabed coverage per item, and was visually estimated and divided into four classes: Class 1 (< 1 m^2), Class 2 (1–10 m^2), or Class 3 (>10 m^2).

The interaction of litter items with benthic organisms was described based on the following categories: (i) covering, when litter completely covered or enveloped organisms and substrate portions; (ii) entanglement, when litter items entangled organisms and caused abrasions or other injuries to organisms; (iii) colonization, when fouling and other sessile organisms used litter as a substrate; (iv) refuge, when organisms used litter as a shelter; and (vi) adaptive behavior, when organisms used litter as mobile shelters. The fouling of marine litter by macro-benthic organisms was visually examined and the most common colonizing taxa were identified.

The type of substrate along each transect was determined and mapped as either soft or hard substrate. The former group included mud and sandy bottoms, while the latter included rocks, large boulders, and biogenic hard bottoms (corresponding to a predominantly rocky seafloor with evident bioconstructions) (Rouanet et al., 2019). At each sampling unit, the substratum type was assigned by the cover in higher abundance.

Two main groups of marine litter were distinguished to investigate the relationships between possible sources, and relative spatial and bathymetric distribution. The first group is i) Fishing-related items (FG) namely “abandoned, lost, or otherwise discarded fishing gear (ALDFG).” This includes all materials associated with fishing activities, such as artificial polymers (e.g., lines, ropes, nets, sticks, buoys) and ceramics (brick links to ropes), because of their documented significant presence in seamounts and on rocky substrates (Angiolillo 2019; Angiolillo and Fortibuoni, 2020; Galgani et al., 2018). The other group is ii) General Wastes (GW), including all the remaining materials from domestic and recreational activities.

A set of seafloor characteristics was derived from bathymetric data at each location. Slope was calculated in degrees using the Spatial Analyst Tool in ArcMap 10.7 (ESRI). Curvature and bathymetric position indexes (BPI) were calculated using the Benthic Terrain Modeler (Walbridge et al., 2018). A fine, intermediate, and broad scale BPI was calculated at each location. Fine scale BPI was calculated with a 9-cell radius using 25 m bathymetric maps, corresponding to a 225 m resolution, and with a 3-cell radius using 100 m bathymetric maps, corresponding to a 300 m resolution. Intermediate scale BPI was calculated with a 33-cell radius using 25 m bathymetric maps, corresponding to an 825 m resolution, and with a 9-cell radius using 100 m bathymetric maps, corresponding to a 900 m resolution. Broad scale BPI was calculated with a 65-cell radius using 25 m bathymetric maps, corresponding to a 1625 m resolution, and with a 17-cell radius using 100 m bathymetric maps, corresponding to a 1700 m resolution. A preliminary correlation test of BPIs showed that the three values were correlated. However, because the broad-scale BPI explained 89% of the FG distribution variability (the main contributor of the first PCA axis regarding FG distribution), it was the only one retained for further statistical analyses. The working matrix was composed of seven locations and seven seafloor characteristics (depth, distance to the coast, slope, curvature, BPI, linear density of soft substrate, and linear density of hard substrate). Distance from the coast was measured in kilometers (km). Statistical analyses were performed using XLSTAT (Addinsoft, 2019). A non-parametric Mann-Whitney comparison test that was applied between mean depths of FG and GW occurrences at each site. Additionally, to determine if they were similarly distributed among the seven locations, the Monte Carlo simulation method was used for the validation, with 10,000 permutations. Another test used was a principal component analysis (PCA) that was computed based on Spearman's rank correlation to examine similarities between locations based on their distance to the coast and their seafloor characteristics derived either from bathymetric data (mean depth, slope, curvature, BPI) or data extracted from the video (linear density of soft or hard bottom). The linear densities of FG and GW at each location were considered supplementary variables. A cross-check was also performed against the results of hierarchical classification using simple linking based on Spearman's rank correlation similarity matrix.

Thereafter, to analyze marine litter distribution, the following variables were considered for each sampling unit: mean depth; mean slope; prevalent type of substrate; as well as the number of FG items, GW items, and marine litter (ML = FG + GW) items. Moreover, the frequency of occurrence (FO) was computed as the percentage of sampling units, where at least one item was observed. To identify accumulation points, the average number of observed ML items (± SD), GW items (± SD), and FG items (± SD) were computed considering only sampling units of marine litter. Differences among locations were tested using hurdle models for data regression. The relationship between marine litter distribution and depth, as well as with the type of substrate and slope was investigated using generalized linear mixed-effects models (GLMMs) for the negative binomial family, with location set as random effect (Millar and Anderson, 2004). The best fitting model for each response variable (ML, GW, and FG) was selected, according to the information-theoretic approach, by choosing the model with the lowest AIC (Akaike's Information Criterion; Akaike, 1974; Zuur et al., 2009). Statistical analyses were performed with R 3.6.1 (R Core Team, 2019) using the packages "pscl" (Jackman, 2017; Zeileis et al., 2008), "lme4" (Bates et al., 2015), and "DHARMa" (Hartig, 2020). Graphical outputs were produced using "visreg" (Breheny and Burchett, 2017) and "Cairo" (Urbanek and Horner, 2015).

2.4 Sample processing

2.4.1 Meiofauna on litter samples

Along the transects, 10 litter samples were collected (Table SM1). Whilst on board the vessel, samples were preserved in plastic flasks and fixed with artificial seawater containing paraformaldehyde (3%). In the laboratory, samples were rinsed several times with Milli-Q® water, before being rinsed with 100% ethanol solution, and dried at 40°C. Thereafter, samples were coated with gold-palladium and observed at 3–5 kV using a JEOL JSM-6010LV multiple touch panel scanning electron microscope to detect the colonization of biomineralizing organisms on litter samples. The limited material and paucity of species-level information in the literature on deep-water Mediterranean meiofauna meant that the identification of organisms in litter samples was limited to broad taxonomic groups.

2.4.2 Microplastics in the sediment

To collect MP samples, 11 sediment cores were opportunistically sampled at two stations along each transect (Fig. 1, Table SM2). Notably, because of a core problem, samples TC11 and TC12 were mixed together in the slope off Saint-Tropez, whereas in the Cannes and Monaco canyons, only one core tube was sampled at each location.

The assessment of MPs (< 5 mm) was performed by imposing an operational lower size limit of 300 µm. MPs were quantified using the fluorescent dye method, which uses the ability of artificial polymers to fluoresce with Nile Red (Erni-Cassola et al., 2017; Maes et al., 2017; Shim et al., 2016).

While on board, the first 5 cm of sediment core samples were preserved in glass flasks and fixed with formalin solution (30%). In the laboratory, processing and analysis were conducted with the use of nitrile gloves and protective cotton in all steps to limit contamination. The samples were processed under an air extractor, with blanks to monitor potential contamination, as recommended by other authors (Frias et al., 2018; Fu et al., 2020; Galgani et al., 2013). For each core sample, 100 g of sediment was removed and cleaned with H_2O_2 solution (30%) to limit fluorescence from organic matter. MPs found at the surface were extracted and filtered on glass microfiber membranes (Whatman GF/A®; pore size = 0.45 µm). The second step included a density separation where a solution of high-density zinc chloride ($ZnCl_2$, 62.5%; density = 1.814 g cm^{-3}; ratio = 3:1) was added, mixed under agitation, and passed under an ultrasonic bath. Subsequently, the MPs were extracted and filtered (Cadiou et al., 2020; Vermeiren et al., 2020). The extraction steps were repeated three times (Besley et al., 2017). Finally, the sediments were cleaned twice with Milli-Q® water and the supernatants containing the MPs were filtered.

All filters were analyzed under a fluorescent stereomicroscope (Karakolis et al., 2019) with Nile Red staining (10 µg/mL). Even the remaining sediment in the beaker bottom was stained with Nile Red, and MPs were counted and added to the results obtained from the first filters.

3. Results

3.1 Characterization of macro-litter

Litter items were recorded in all transects but they were not uniformly distributed as there were differences in composition and abundance with dives. Overall, 575 litter items were counted, excluding the items found in the accumulation points recorded in the Monaco canyon (Fig. 2A-B, 3). In this area, litter was composed of a mixture of light and heavier items, mainly items of urban origin and organic debris. Given the high number of these items (up to thousands of pieces) and the fact that some were buried in the sediment, it was not possible to count each item.

Litter type composition varied among the explored areas (Fig. 2, 4) with the highest evenness found in Spinola (J' = 0.89), followed by Janua (J' = 0.83), Saint-Tropez (J' = 0.81), Monaco (J' = 0.80), and then Méjean (J' = 0.69), Ulysses (J' = 0.67), and Cannes (J' = 0.65).

Artificial polymers represented 56.6% of the overall identified items, ranging from 32.7% (Janua seamount) to 76.2% (Ulysses seamount). These consisted of plastic bags (8.5%), bottles (8.2%), fragments (2.7%), plastic glasses (2.7%), forks (1.0%), food sacks (0.7%), and unidentifiable pieces of plastics (17.3%) (Table SM3, Fig. 2A-D). Fishing-related items represented a conspicuous part (52.4%) of artificial polymers, particularly in the shallower areas, such as on the top of the Ulysses and Janua seamounts, and the Méjean shoal, where fishing-related items contributed 49.4%, 21.2%, and 58.5% of recorded items (Fig. 2K-Q, 4), respectively. Fishing-related items were predominantly longlines (39.1%) and ropes (5.1%), followed by irrelevant portions of nets (2.8%). Artisanal accessories for fisheries, contributing 4.8% of the artificial polymer category, were found exclusively on the Ulysses seamount and consisted of small plastic sticks and a few plastic bottles used as signal flags and buoys for longline fishing (Fig. 2K). Furthermore, glass/ceramics and metal contributed 11.3% and 6.8%, respectively, to the overall amount of litter (Fig. 2D, G-H, 4). In particular, at Janua and Spinola, numerous glasses, jars, and glass bottles (i.e., beer and fruit juice bottles) were found. Moreover, four old amphorae were recorded at the slope off Saint-Tropez and the Janua spur (Fig. 2I-J). In addition to this, an important contribution was represented by mixed items (6.1%), especially on the Cannes (6.8%) and Monaco (26.0%) canyons, where large accumulation points of litter were detected (Fig. 2A-B, 3).

Although litter of each size class was present at all study sites, the most common dimensional classes were Class 1 (< 1 m^2), mainly consisting of plastic fragments, bags, and food packaging. The largest classes were mostly related to longline fisheries that were observed over an extended portion of the substratum (Fig. 2L).

The major portion of litter items was observed laying on the bottom (55.6–96.7% of the total number of observed items). However, many buried items were observed in the Monaco canyon (44.4% of litter) and occasionally at the Méjean shoal (10.8% of litter). Hanging items were mainly found at the Méjean shoal (13.8% of litter), and the Ulysses (7.1% of litter) and Janua (3.1% of litter) seamounts, where lost fishing gear was often observed under tension between rocks.

3.2 Distribution of macro-litter

Mean depths and linear densities of FG items and GW at the seven locations were calculated (Table 3). Cannes canyon was the location where the highest density of litter (26.5 items km^{-1}) was observed, essentially composed of GW (25.6 items km^{-1}). Meanwhile, Spinola spur was the location with the lowest litter density (3.5 items km^{-1}). The highest density of FG (9.8 items km^{-1}) was observed on the Ulysses seamount, whereas the lowest density of FG (0.2 items km^{-1}) was observed on the Spinola spur and on the slope off Saint-Tropez (0.5 items km^{-1}).

The accumulation points recorded in the Monaco canyon were not considered in the density calculations. A total of six accumulation points (approx. 0.001 litter hotspots km^{-1}) were identified along the video transect in the Monaco canyon, occupying estimated areas ranging between 3 m^2 and 300 m^2 (Fig. 3, 5). Counting only visible items in small patches where laser beams were visible ($n = 10$; 2–6 m^2), litter ranged from 60 items to 873 items. If we extrapolate the abundance, we would obtain minimum estimated abundance values of 6,140 items km^{-2} and maximum estimated values of 38,560 items km^{-2} for the accumulation points. However, these estimates should be considered with caution because of the impossibility to count every object as many were partially buried or hidden underneath larger objects. Moreover, at the accumulation point the ROV camera did not record a constant field of view. Therefore, it was not always appropriate to count all objects within litter-accumulation areas.

Mean FG and GW depths were statistically different (p-value < 0.05) at Cannes (p-value = 0.047), Monaco (p-value = 0.004), Ulysses (p-value = 0.008), Janua (p-value = 0.017), and the Méjean shoal (p-value = 0.017). At the Spinola spur and on the slope off Saint-Tropez, no statistical comparison could be applied because only one FG item was observed during the entire dive. Generally, FG was observed at depths that are shallower than GW at each location (Fig. 5, 6, SM1).

The PCA results showed that the first two axes represented 80% of the variability (Fig. 7). The main axis, accounting for 57% of the variability, represented soft bottoms, broad scale BPI, and curvature. Meanwhile, the second axis, accounting for 23% of the variability, represented the distance from the coast and the depth. FG and GW densities were added as supplementary variables. A cross-check against the results of hierarchical classification was made by superimposing the two groups that had 50% similarities (Fig. 7). The first group was composed of the Cannes and Monaco canyons, where marine litter was observed at 100% soft substrate, with a broad scale BPI showing negative values (< -25), which was typically valleys and areas located near the coast (less than 20 km) and at depths of approximately 1300 m. This group was composed of geomorphologic features that showed the highest GW density (10.9–25.6 items km^{-1}) and important accumulation points. The second group was composed of the Méjean shoal, and the Ulysses and Janua seamounts, the characteristics of which were intermediate depths (-516 m, -588 m, and -920 m, respectively) with intermediate values of BPI and curvature, indicating convex geomorphology. This group was composed of features that showed the highest FG densities (6.6 items km^{-1}, 9.2 items km^{-1}, and 1.9 items km^{-1} for the Méjean shoal, and the Ulysses and Janua seamounts, respectively). Two locations were isolated: the Spinola spur and the slope off Saint-Tropez, both of which had almost no litter and no relevant FG items (only one).

Hurdle models for count data regression also exhibited significant differences in marine litter distribution among locations (Table SM4a). The hurdle component, which models zero counts, depicted Cannes (FO_{ML} = 53.9%) and Ulysses (48.7%) as the most affected sites, followed by Monaco (39.2%), Saint-Tropez (33.3%), Méjean (32.9%), and Janua (32.7%). The results indicated Spinola was the least affected location (16.3%). In contrast, the truncated count component for positive counts showed a higher abundance of marine litter

items in Monaco (AB_{ML} ± SD = 9.9 ± 23.0) because of the presence of the accumulation points. Moreover, marine litter was more abundant in Cannes (2.5 ± 2.3) than in Ulysses (1.9 ± 1.2); while Méjean (1.7 ± 0.9), Saint-Tropez (1.6 ± 1.3), Janua (1.4 ± 1.0), and Spinola (1.1 ± 0.3) had the lowest abundances.
Similar results emerged when considering only GW (Table SM4b). In this case, the binomial part of the model showed that Cannes (FO_{GW} = 51.7%) was the most affected location (followed by Monaco at 36.8%, Saint-Tropez at 33.3%, Ulysses at 28.6%, Janua at 25.2%, Méjean at 16.4%, and Spinola at 15.1%). Count data highlighted GW accumulation in Monaco (AB_{GW} ± SD = 7.4 ± 21.2) and Cannes (2.5 ± 2.3), whereas the other locations had values that were either lower or similar (Ulysses 1.6 ± 0.9, Saint-Tropez 1.5 ± 1.1, Janua 1.4 ± 1.1, Méjean 1.4 ± 0.8, Spinola 1.1 ± 0.3).
Finally, regarding FG items, the highest FOs were found in Ulysses (32.3%), Méjean (21.9%), and Janua (9.3%). Because of the low number of FG items detected in the other locations (FOs_{FG}: Cannes 4.5%; Monaco 3.2%; Saint-Tropez 2.2%; and Spinola 1.2%), the count model coefficients were computed considering only these three sites. The results showed a slightly higher accumulation of FG items in Méjean (AB_{FG} ± SD = 1.5 ± 0.6) and Ulysses (1.4 ± 0.8) than in Janua (1.1 ± 0.2).
The output of the GLMMs showed that the best fitting models that described the ML distribution included depth and slope as fixed effects (Table SM5a), with increasing ML abundance correlating to increasing depth and decreasing slope (Table SM6a; Fig. SM2a, b). Considering GW, a significant difference was observed between hard and soft substrate types (Table SM5b), with soft substrates holding most of the litter (Table SM6b; Fig. SM2c-e). In contrast, regarding the FG distribution, substrate was not selected as a predictor (Table SM5c), the slope was not significant, and the number of items decreased with increasing depth (Table SM6c; Fig. SM2f). Diagnostic plots for the graphical validation of GLMMs are shown in Fig. SM3. The relationship between marine litter distribution and depth, as well as with the slope and the type of substrate, may partially explain the patchy distribution highlighted in Fig. 5.

3.3 Litter interaction with organisms

Overall, 70% of the litter items were observed to be in contact with marine organisms. The most evident and common interaction was the use of litter as substratum by several sessile invertebrates (59.4% of recorded interactions). Porifera, Hydrozoa, Actinaria, Alcyoniidae, Scleractinia, Zoantharia, Serpulidae, Bryozoa, and the species *Amphianthus dohrnii*, were the most frequent and identifiable taxa colonizing litter. Different fouling levels ranged from 10% to complete coverage of litter items (Fig. 2P). Meiofauna, consisting of Scyphozoan polyps (chitinous envelope) (n = 6), Brachiopods (n = 10), Radiolarians (n = 1), Foraminiferans (n = 37), and Bryozoans (n = 5), was also detected by scanning electron microscopy of litter samples (Fig. SM4). Moreover, Bacillariophyceae trapped in sediment, Chelae spicule clusters, Polychaeta Serpulidae tubes, and other spicules of unidentified Porifera were also recorded.
The use of litter as refuge by megafauna (i.e., *Munida* spp., *Paromola cuvieri, Lepidion lepidion*, crustaceans, and unidentified fish) was rarely observed (1.1% of recorded interactions, Fig. 2R). No events of ghost fishing were recorded. However, two crabs of *Paromola cuvieri* (0.4% of recorded interactions, Fig. 2S) at Saint Tropez and the Ulysses seamount were observed exhibiting adaptive behaviors, during which they carried plastic sheets instead of common sponges or coral colonies.
Entanglement of fauna in litter items was frequent (8.7% of recorded interactions) in the Ulysses and Janua seamounts, and in the Méjean shoal. Several taxa were observed entangled by items that caused abrasions (e.g., Fig. 2N-O) at the Ulysses seamount, including 15 colonies of *Callogorgia verticillata* (10.4% of the surveyed specimens; n = 144), one *Dendrophyllia cornigera* (33.3%, n = 3), one colony of *Anthipates* sp., as well as some scleractinians and several sponges. *Callogorgia verticillata* was observed to have formed *facies* in the upper layer (400–700 m) of the Ulysses seamount. Some colonies were also colonized by the Zoanthid parasite *Isozoanthus zibrowii* and by the anemone *Amphianthus dohrni*. Moreover, four dead colonies of *C. verticillata* were recorded. At the Janua seamount, the entangled species included two

colonies of the rare Atlantic bamboo coral *Chelidonisis aurantiaca*, observed in the upper layer (700–800 m), as well as a few individuals of *Farrea bowerbanki* and one Litistidae sponge. The Méjean shoal is characterized by the wide distribution of massive and encrusting sponges. In the shallow areas, colonies of the small white gorgonian *Muriceides lepida* were observed. However, only eight unidentified sponges, four Litistidae sponges (*Leiodermatium* cf. *pfeifferae*), and two colonies of *M. lepida* were observed entangled by lost lines. Considering the litter types, the items most frequently entangling sessile organisms were exclusively fishing-related litter, mainly longlines, lines, and ropes.

3.4 Microplastic in the sediment

MPs were widely distributed and found in 100% of the 11 samples. The median density of MPs was 0.32 item g^{-1} and the average density was 0.45 (± 0.28) item g^{-1}, ranging from 0.12 item g^{-1} (bathyal slope off Saint-Tropez) to 1.04 item g^{-1} (Méjean - TC11; Fig. 8A). The bathyal slope off Saint-Tropez presented the lowest MP concentration, but it should be noted that only one sample was collected. Méjean was the most contaminated area with an average density of 1.04 item g^{-1} and 0.83 item g^{-1} found in TC11 and TC10, respectively; followed by Cannes canyon with 0.70 item g^{-1} in TC9. The other seamounts and canyons appeared to be contaminated in a similar way. Except for Janua (0.43 item g^{-1}–TC10) and Ulysses (0.41 item g^{-1}–TC10), the other MP concentrations were below 0.32 item g^{-1}.
MPs were composed of 80.0% fibers, 17.0% fragments, and 2.2% pellets, with the remainder of the typologies accounting for less than 1.0% (0.6% foams and 0.4% films; Fig. 8B). Pellets were found only in the Spinola spur and the Janua seamounts. Meanwhile, films were found only in the bathyal slope off Saint-Tropez and the Méjean shoal (only in the TC10 sample), and foams were found in Ulysses and Méjean (only in the TC10 sample).
The correlation between ML and total MPs (including fragments, fibers, pellets, and foam) was only 0.35, but this value would increase up to 0.59 if the only MPs considered were fragments.

4 Discussion

4.1 Abundance and distribution of macro-litter

RAMOGE EXPLO 2018 collected important data for describing the abundance and distribution of marine litter in canyons and seamounts of the deep waters of the Ligurian Sea. This data was also useful for highlighting the interactions occurring between marine litter and benthic organisms.
The present work highlighted the pivotal role of canyons in the transport of solid domestic litter from shallow coastal waters to deeper waters. It also revealed the sensitivity of seamounts, which are attractive areas for offshore fishing. Several authors have highlighted the importance of submarine canyons as vectors for marine litter (Galgani et al., 1996; Mordecai et al., 2011; Pierdomenico et al., 2019; Tubau et al., 2015). Through turbidity, hyperpycnal currents, and strong bottom currents generally flowing from the surface, canyons carry light urban litter towards the deepest part, together with organic and inorganic sediments (Paull et al., 2018; Pierdomenico et al., 2020). The substantial amount of anthropogenic debris recorded at the accumulation points of the Monaco canyon could not be precisely assessed; however, it covered a large portion of the seafloor at the canyon base and numbered in the thousands (estimated abundance up to 38,560 items km^{-2}) at very low depths (-2200 m). This accumulation area hosted a multiplicity of urban solid waste mainly composed of single-use items (e.g., plastic cups and glasses, bottles, and plastic packages), champagne glasses, wine buckets, and toys, mixed with coarse sediment (Fig. 2A-B, 3). This differed from the heavier materials found in the strait of Messina at 300–600 m depths by Pierdomenico et al. (2019). The poor preservation and partial burial status of some litter items did not

allow for detailed identification of their origin and composition, but some items of brands that have not been distributed since the 1990s remained un-degraded.

The accumulation of debris in the Mediterranean Sea likely results from a large input related to significant pressure brought on by human presence, combined with the hydrodynamics of this semi-enclosed basin (Suaria et al., 2016). Worldwide, river inputs represent one of the main sources of marine litter, which can transport large amounts of litter into the sea according to specific seasonal regimes (Lebreton et al., 2017; Rech et al., 2014; Schirinzi et al., 2020). Wind, terrestrial runoff, or dumping directly on beaches or in the sea are other vectors of litter that uses canyons as natural conduits to reach the bathyal depths (Rech et al., 2014; Tubau et al., 2015). In the proximity of the Monaco canyon there are two river mouths, a large urbanized area, and two large industrialized cities (Nice and Genoa). Moreover, the popularity of the Monaco Principality as a tourist attraction, which experiences a significant increase in tourism and recreational boating during summer helps explain the amount and variety of litter encountered in its submarine canyon. Even the main circulation pattern from east to west and from south to north along the Tyrrhenian Sea (Abella et al., 2008; El-Geziry and Bryden, 2010; Fossi et al., 2018) favors the transport of lighter litter items in this area. Other way that may favor the transport of litter is the Deep/Bottom Mediterranean Water (> 1500 m), formed by the Tyrrhenian Dense Water (TDW), flowing northwards along the western side of Corse Island; along with the Western Deep Mediterranean Water (WDMW), originating both in the Ligurian Sea and in the Gulf of Lion during winter (El-Geziry and Bryden, 2010; Millot, 1999; Millot and Taupier-Letage, 2005; Robinson et al., 2001).

No other accumulation points were detected in the study area, but this does not exclude the possibility of other litter hotspots, which were not in the ROV's transect routes, because hotspots of marine litter have become a common occurrence in the Mediterranean Sea (Galgani et al., 1996; Pierdomenio et al., 2019, 2020; Tubau et al., 2015). A large amount of domestic waste has been recorded in the Cannes canyon, followed by that found in the Saint Tropez slope. Plastic was by far the most common material among the urban litter items. Most items were small in size and included plastic bags and bottles, as well as glass items, food packaging, toys, clothes, and other light objects that are used daily. Similar items have been found in most shelf and slope environments of both the Atlantic Ocean (e.g., Maes et al., 2018; Moriarty et al., 2016; van den Beld et al., 2017) and the Mediterranean Sea (e.g., Fabri et al., 2014; García-Rivera et al., 2018; Gerigny et al., 2019; Spedicato et al., 2019).

Marine litter in the Cannes canyon was previously investigated in 2009 between depths of 180 m to 692 m on its flanks (Fabri et al., 2014), and the GW composition was essentially made of plastic and metal with a mean linear density of 3 items km^{-1}, in addition to a few FG (1 item km^{-1}). In 2018, a deeper part of the canyon was investigated during the RAMOGE expedition, and a higher linear density of GW (25 items km^{-1}) was found, along with the same linear density of FG (1 item km^{-1}) (Table 3).

As already reported in the western Mediterranean Sea, coastal canyons of the Ligurian Sea accumulate more debris and marine litter than canyons located further away, like those in the Gulf of Lion, because of the narrow continental shelf (Fabri et al., 2014).

Evidence of this difference was also observed when comparing the studied seamounts located far from the coast that were found to harbor less GW (3 items km^{-1} to 9 items km^{-1}) than the canyons of Monaco and Cannes (15 items km^{-1} to 25 items km^{-1}) (Table 3). Moreover, an overall increase in litter abundance was observed within each canyon that had its head located very near to the coastline and had increasing depth, which, in turn, is associated with a decrease in slope gradients and higher sedimentation.

The major abundance of domestic items, coupled with terrestrial organic debris (Fig. 3), in a depression area at the base of the canyon, could lead to the assumption that the majority of items originate from land-based sources. This idea also supported by the lower number of fishing-related items in the surveyed canyons. However, this is difficult to confirm because a marine-based source cannot be excluded. The large abundance of plastic most likely reflects its near null degradability in the deep-sea environment, which is characterized by low temperatures, low oxygen, absence of light, and a low energy regime (with respect to shallower environments) (Napper and Thompson, 2019). Furthermore, fishing gear and fishing-related items are mainly made of artificial materials. Materials such as nylon degrade very slowly and persist in the environment for centuries (Thompson et al., 2004; Watters et al., 2010). Therefore, the increasing amount of litter is more likely to accumulate on the seafloor over time (Gerigny et al., 2019; Tekman et al., 2017), with the consequences to the marine environment not yet understood (Deudero and Alomar, 2015; Gall and Thompson, 2015).

The major extension of soft substrate in the canyons and the presence of some commercial species, such as the Norway lobster (*Nephrops norvegicus*) and the giant red shrimp (*Aristaeomorpha foliacea*) (Rouanet et al., 2019), lead to the assumption that other fisheries exploit the area, using gear such as those used in trawling. However, there was no direct evidence of fishing scars on the seafloor and the French Riviera does not have favorable conditions for this type of fishing activity.

A high abundance of fishing-related items was recorded mainly in the upper layer of the investigated seamounts (300–600 m), where coral assemblages were observed. This observation not only confirms the importance of seamounts for enhancing biodiversity, but also highlights the vulnerability of naturally occurring species to the direct and indirect effects of fishing (Bo et al., 2011; Würtz and Rovere, 2015). Seamounts are recognized as an important hotspot for benthic and pelagic biodiversity, and as fishing grounds for commercial species (Bo et al., 2011, 2020a, b; Würtz and Rovere, 2015). In particular, when seamounts are close to the coastline, they may become spots for artisanal fisheries and be affected by the cumulative impact of a large amount of lost fishing gear (e.g., longlines, trolling lines, trammel nets, pots, and other gear) (Lastras et al., 2016). Numerous deep longlines were recorded on the Ulysses and the Janua seamounts, as well as at the Mejean shoal. Even if Italian seamounts are distant from the coastline, they have been important areas for artisanal and recreational fishing since the 1970s (Bo et al., 2020; Orsi Relini and Relini, 2014), targeting pelagic (such as the swordfish *Xiphias gladius*), demersal, and benthic species, such as *Pagellus bogaraveo*, *Merluccius merluccius*, *Polyprion americanus*, *Pagellus acarne*, *Epigonus telescopus*, and *Palinurus mauritanicus* (Orsi Relini and Relini, 2014; Würtz and Rovere, 2015). The high abundance of the deep longlines reflects the pressure imposed by these specific fishing fleets, which are also common in other Mediterranean seamounts. This is confirmed by the recent explorations on the Ulysses and Janua seamounts carried out by Bo et al. (2020a, b), which revealed traditional fishing practices and their long-term effects, particularly on the Ulysses seamount. Gear can be lost while being caught or snagged on submerged features such as the roughest and steepest areas of the seamount or in arborescent species and coral framework present at the summit of the seamount. Furthermore, the bottom currents on seamounts may favor entanglement during hauling and retrieving operations.

Numerous reports regarding this issue have highlighted the detrimental effects caused by a large number of lost fishing gear on the benthic realm (Angiolillo and Fortibuoni, 2020). For instance, Fabri et al. (2014) reported up to 4 fishing gear km^{-1}, which was mainly concentrated at a depth of 250–350 m in the adjacent Gulf of Lion. Additionally, Cau et al. (2017) recorded fishing-related item abundances ranging from 2.3 items km^{-2} to 5.8 items km^{-2} along the upper Sardinian slope (Central Western Mediterranean).

At the current stage, further studies on deep water currents and hydrological dynamics, and their association in the identification of litter sources are essential to identify new accumulation patterns and hotspot areas that are yet to be discovered (Galgani et al., 2015). This information is also useful for more efficient litter management practices, to avoid continuous litter input, and to preserve the marine environment (Schneider et al., 2018; Tunca Olguner et al., 2018).

4.2 Litter interaction with benthic organisms

In the study area, we detected different types of interactions between litter and benthic organisms. The most detrimental interaction in the benthic realm was entanglement, mainly caused by longline fishing, particularly for arborescent erect species. The Ulysses and Janua seamounts, as well as the Méjean shoal, were the most affected areas. In these regions, deep longlines were recorded mainly at 300–600 m depths and were almost the only discarded fishing gear observed. The most affected species were massive sponges (e.g. Lithistidae) and deep colonial anthozoans, such as large gorgonians, antipatharians, and scleractinians. Because of their arborescent and massive morphology, these taxa are easily entangled in fishing lines (Bo et al., 2014a; Valisano et al., 2019; Yoshikawa and Asoh, 2004). These taxa play a key ecological role, particularly in the deep sea and on seamounts. This is because their complex morphologies enhance the tridimensional structure of the substrate, creating niches and refuges for several associated species, and therefore increasing biodiversity levels (Freiwald et al., 2009; Ingrosso et al., 2018; Rossi et al., 2017).

Generally, a monofilament fishing line is considered to have a minor impact on benthic communities, especially when compared to trawls or other destructive fishing methodologies (Macfadyen et al., 2009). Nevertheless, frequent hook-and-line fishing is the main cause of entanglement events in the Mediterranean reefs, rocky areas, and seamounts (Galgani et al., 2018; Angiolillo, 2019), similarly to what is observed in other parts of the world (Chiappone et al., 2005). For instance, several authors have documented the destructive effects of ALDFG, mainly on benthic habitat-forming species (Angiolillo and Fortibuoni, 2020; Bo et al., 2014a; Galgani et al., 2018), making these ecosystems highly vulnerable to fishing activities. The abrasive action of entangling fishing gear can lead to multiple consequences on organisms, ranging from tissue loss, broken branches, parasite infection, and death (Angiolillo et al., 2015; Bavestrello et al., 1997; Bo et al., 2014a; Consoli et al. 2019). This, in turn, leads to the impoverishment of marine animal forests and facilitates progressive habitat degradation (Bo et al., 2014a, b). In addition to this, some of the affected species are fragile and can form *facies*, such as the primoid gorgonian *Callogorgia verticillata*. Indeed, broken branches of this species are often recorded on the seabed, particularly at the base of entangled colonies in intensely exploited fishing grounds of the Mediterranean Sea (Angiolillo et al., 2015; Bo et al., 2014a, b). The presence and vulnerability of *C. verticillata* is known as far as the Ulysses seamount because of by-catches of its colonies in longlines, which is due to the popularity of this seamount for semi-professional and recreational longliners (Bo et al., 2020a; Orsi Relini and Relini, 2014). Although a relatively low number of colonies was affected by entanglement in the study area, the observation of dead colonies and epibiontic species confirmed the detrimental effects of lost gear on the benthic realm at great depths.

The most frequent form of interaction described in this study was the use of litter as a substratum for several encrusting and sessile taxa. Almost all litter items were covered by fouling. The presence of fouling provides evidence of the permanence of litter in the sea, considering that at the deepest depths, fouling colonization is probably very slow (Breen, 1990; Saldanha et al., 2003).

Some specimens of squat lobster, *Munida* spp., were observed using a plastic bottle as a refuge and some fishes were attracted to a ski laying on the seafloor. Other authors have already observed similar behavior (Pierdomenico et al., 2018; Tubau et al., 2015); however, larger domestic objects (e.g., washing machines and bins), which are usually used as a refuge, were rarely observed in this study. In soft sediments or in a degraded environment where there is a scarcity of natural structures functioning as shelters, the artificial three-dimensional structures are usually used as a refuge (Angiolillo, 2019). These types of interactions (colonization and shelter) are often considered neutral or even positive because these new artificial substrates can enhance species richness and biodiversity. In the sea, within a short time, any anthropogenic artifact is usually colonized and reused (Angiolillo and Fortibuoni, 2020). Nevertheless, these artifacts modify sea bottom complexity, altering the natural environment, community structure, and consequently ecosystem functioning (Angiolillo, 2019; de Carvalho-Souza et al., 2018; Saldanha et al., 2003).

Among the four observed specimens of the crab *Paromola cuvieri,* two carried plastic on their backs, confirming the adaptive behavior of this species, as has already been observed by other authors in other Mediterranean sites (Angiolillo, 2019; Mecho et al., 2018; Pierdomenico et al., 2019; Taviani et al., 2017).

All the observations recorded in this study showed substantial and irreversible changes that humans are causing to marine environments, even in deep and remote areas. The accumulation points of litter found at the Monaco canyon extended across large areas of the sea bottom, where the original communities were completely covered by a large amount of waste, preventing gas exchange and oxygenation. The litter cover also decreased the mobility and feeding capacity of the fauna (Kühn et al., 2015), and impeded recolonization (Galgani et al., 2015). At the Monaco canyon, the layer under the litter deposit appeared to be decomposed, probably because of the presence of organic debris. However, no information is available on the rate of decomposition of different materials in the deep sea, nor on the possible release of toxic chemicals into the environment (Bergmann et al., 2015; Ramirez-Llodra et al., 2011).

In our study, we reported the macroscopic interactions between litter and benthic organisms, which was collected and analyzed using ROV cameras. Other indirect aspects of litter effects include the transport of invasive alien species (Kühn et al., 2015) as well as micro-litter ingestion by marine organisms and their subsequent entrance into the trophic web (Corcoran et al., 2014; Gall and Thompson, 2015). It is important to note that these aspects, each, have their relative detrimental consequences (e.g., the release of xenobiotics and toxic chemicals with sublethal and chronic effects), which could not be addressed in this work.

However, the large amount of recorded waste could lead to serious consequences for the marine environment and potentially impact human economy and health (Danovaro et al., 2020). This is in light of the fact that for a very long time the sea was used as an unlimited natural dump for many kinds of waste (Angiolillo, 2019; Ramirez-Llodra et al., 2011), without anticipating the depletive effects of this practice on marine resources.

4.3 Microplastic

Although the assessment of MP contamination in sediment is not the main focus of the cruise, the collected data offer some interesting insights. The MP data collected in this study have the limitations of an opportunistic approach. Obviously, while MP sampling requires many samples at comparable depths and replicates, the limited accessibility and high costs associated with this work in the deep sea have hindered sampling strategy. Moreover, the assessment used here is mainly dedicated to large particles (300–5000 µm) using an existing method implemented for monitoring (Galgani et al., 2013). Consequently, the results should be regarded as a snapshot of MP contamination, rather than data from an extensive study.

Nevertheless, all collected sediment samples contained MPs and were mainly contaminated with fibers and fragments, as recorded in previous studies of Mediterranean deep-sea sediments (Danovaro et al., 2020; Kane et al., 2020; Sanchez-Vidal et al., 2018; Van Cauwenberghe et al., 2013; Woodall et al., 2014). Pellets, foams, and films were rarer.

A few studies have assessed MP contamination in sediments collected from the deep sea (Van Cauwenberghe et al., 2013; Woodall et al., 2014; Sanchez-Vidal et al., 2018; Danovaro et al., 2020; Harris, 2020; Kane et al., 2020; Peng et al., 2020). Previous studies of deep waters indicated average (± SD) concentrations of 34 ± 10 items kg^{-1} in the Baltic Sea (Zobkov and Esiukova, 2017); a range of 0 items kg^{-1} to 200 items kg^{-1} in the Central Arctic Basin (Kanhai et al., 2019); 7 items kg^{-1} to 25 items kg^{-1} in China (Zheng et al., 2019); and 2.8 items kg^{-1} to 1,188.8 items kg^{-1} in the coastal areas of the Southern North Sea (Lorenz et al., 2019). The Mediterranean Sea is known to be a hotspot for the accumulation of floating MPs (Cincinelli et al*.,* 2019), but recently several studies have also recognized Mediterranean sediments as long-term sinks with the potential to accumulate MPs. Martellini et al. (2018), in a recent review, evaluated the occurrence of MPs in different sediment types (beach, lagoons, estuaries, and off-shore areas affected by the contribution of rivers) providing MP contamination values; however, there was no homogeneity in the methods used or in the expression of the results. Cutroneo et al. (2020) in the Gulf of Tigullio, a close and complementary area to our study in the Ligurian Sea, found mean MP concentrations of 1.5–1.6 item cm^{-3}

in sediments sampled between 5 m and 50 m depths. However, all these values are difficult to compare with those found in this study because they were collected from beach or shallow sediment or used different units of measurement. Nevertheless, a recent study in the deep Mediterranean Sea reported up to 190 MPs 50 g^{-1} or 3,800 items kg^{-1} (Kane et al., 2020). If the values presented here are normalized to the same unit, they correspond to a mean of 450 items kg^{-1} of sediment, a minimum of 120 items kg^{-1}, and a maximum of 1,040 items kg^{-1}. In light of the results from these previous studies, the values in our study appeared to be important, indicating that sediments in Mediterranean canyons and seamounts are highly contaminated by MPs. This is so considering that small particles, less than 300 µm, were also observed but not counted.

While MPs have been found to contaminate all canyons and seamounts, it was difficult to determine which factors influenced their distribution. Although sampling was not homogeneous and did not account for the heterogeneity of the distribution patterns, no clear trend could be observed, and neither depth nor geomorphology (canyons, seamounts), and distance to the coast were explanatory factors for MP distribution. Kane et al. (2020) did not find a relationship between the MP concentrations and the distance from terrestrial plastic sources. While several studies (Iwasaki et al., 2017; Liubartseva et al., 2018) have shown that MPs are controlled by vertical movements (surface currents, waves) and settling, Kane et al. (2020) demonstrated that the spatial distribution and fate of MPs could be strongly controlled by bottom currents. In the RAMOGE sediment sampling area, the currents are mainly oriented east to west from the surface to the bottom (Millot, 1999; Millot and Taupier-Letage, 2005). This suggests that the potential sources of MPs could be the Tyrrhenian Sea, particularly from Corsica and Italy, with contributions from major rivers (i.e., Arno, Tevere, Volturno, and Golo), port areas (i.e., Genova, Livorno, Civitavecchia, Bastia, and Porto Vecchio), and wastewater treatment plants along the coast.

Although the MP types seem more diversified on seamounts (where we found pellets and foam as well as fibers and fragments), it was also not possible to demonstrate a link with the location of the samples, or the geomorphology of the seamounts (compared to that of canyons).

Interestingly, the correlation values between macro-litter and MP fragments suggests that a significant portion of MP fragments is more likely a result of the fragmentation of large items from the same area than transported items from remote areas. Pellets are not often found in marine litter, and foam degrades quickly (Pedrotti et al., 2016). However, more data are needed to confirm this hypothesis. In the literature, MPs in deep sediments are mainly fibers and fragments, with the majority being fibers (Abidli et al, 2018; Cutroneo et al, 2020; Kanhai et al, 2019; Martellini et al, 2018; Mendoza et al, al, 2020; Sanchez-Vidal et al, 2018; Woodall et al, 2014); however, this varies depending on the study area (Frère et al., 2017). Synthetic textile clothing is the main source of fibers (CIRFS, 2019) that are then transported to the marine environment through wastewater treatment plants (Browne et al., 2011; Napper and Thompson, 2019). Fragments are derived from the decomposition of macro-plastics which are, in turn, highly dependent on the number of inhabitant, human activities, tourism, and the presence of ports, as well as fishing and maritime activities (all highly developed activities in the Mediterranean; UNEP/MAP, 2015a; UNEP, 2016). These two typologies (fiber and fragments) correspond to the secondary MPs which result from the degradation of macro-plastics; therefore, MPs may be found far from their emission sources. However, it is difficult to relate the observed element types and concentrations to specific local human activities (Classens et al, 2011).

4.4 Monitoring tools and implications for marine environmental policies and management

Considering the ubiquity of litter and its impact, global interest in this issue has increased remarkably over the last decade and several projects on marine litter have been financed in Europe in the last few years (Maes et al., 2019). The MSFD (2018) in Europe and the Barcelona Convention EcAp process at the

Mediterranean level (UNEP-MAP, 2017) address the reduction of litter into oceans, thereby promoting remedial actions. Therefore, monitoring activities are regularly conducted in many European countries (e.g., OSPAR/ICES/IBTS, ICES, 2012; and MEDITS programs, Fiorentino et al., 2013) to detect baseline levels of litter accumulated on the seafloor. However, while the majority of the studies and the monitoring activities assess litter distribution and abundance, little attention has been paid to study the effects of macro- and micro-litter on the seafloor and on marine organisms, especially in deep sea. This is because of two main reasons: the major portion of studies was conducted using trawling (e.g., Valente et al., 2019, 2020) because of the applicability, greater availability, and affordability of this method. However, this method does not allow the investigation of the effects of litter. Furthermore, the MSFD and EcAp indicators that are used to assess the litter interaction with marine organisms (D10C4 of the MSFD and 24 of the EcAp) are not mandatory. The increasing availability of non-destructive sampling technologies in the last decade - such as seafloor imagery technology (e.g., SCUBA, ROVs, AUVs) are applicable at various depths and all sea bottom types. As a result, this technology has allowed the collection of information on the effects of litter and its abundance, particularly in the deep sea. This is because it has been coupled with research activities or monitoring programs aimed at studying marine biodiversity. However, in the Mediterranean Sea, limitations brought on by the overall costs of instrumentation and equipment confined these techniques to the Western basin (Angiolillo and Fortibuoni, 2020). Moreover, the absence of specific protocols led to studies conducted with different strategies for data collection (e.g., sampling methodologies, unit of measures, parameters). As a consequence of the collection of heterogeneous data, it is often not comparable in a robust way (Angiolillo and Fortibuoni, 2020).

Therefore, harmonized procedures using common templates, joint item categories, types of impacts, and adequate data management are urgently needed to collect comparable data on the temporal and spatial distribution of marine litter and its effects.

In this study, we tested the sampling protocol developed by the MSFD TG Litter Working Group (2021), which proposed guidelines for the assessment of marine litter interaction and entanglement on benthic organisms using visual methods that were applicable on soft and hard seafloors. This protocol defined harmonized procedures for collecting and reporting marine litter data (distribution, occurrence, abundance, litter typology, and impact categories) that are gathered for use in conjunction with biodiversity surveys, as an opportunistic approach.

Our results, collected within the framework of an international multidisciplinary campaign, showed that the tested protocol can be suitable for collecting data on litter and its interactions with biota in the deep benthic environments, and can also be used with ROVs. This allowed the gathering of efficient data by means of a non-destructive sampling method. Nevertheless, the application of the protocol is limited by the availability of specific equipment. For example, in this study, the discontinuous recording of the laser beams did not allow the measurement of the width of the field of view nor did it allow us to provide data related to a specific surface (square kilometers). This is a general problem that reduces the possibility of comparing litter abundance values among different studies. In this way, only estimates of global litter abundance can be attained, while assessment is not possible.

The problem of quantification also occurs in the accumulation areas. However, even if the amount of litter at accumulation points is not always quantifiable, ROV-imaging has allowed the discovery and description of the striking situation in some areas of the Mediterranean Sea (i.e., Monaco canyon, as seen in this study and Messina Strait, as seen in Pierdomenico et al., 2019). For instance, ROV-imaging has allowed the collection of important information on the distribution of deep-sea litter and has defined specific interaction types between marine organisms and the environment. Regarding this topic, recent interest has focused mainly on entanglement because of the high level of impact it is reported to have on vulnerable species (Angiolillo and Fortibuoni, 2020; Claro et al., 2019; Kühn et al., 2015), and to design a monitoring procedure that could be applied to the MSFD (Attia El Hili et al., 2018). The prevalent number of coral taxa affected by entanglement in the present work confirmed the importance of using Cnidarians as indicators for entanglement events, as proposed by Galgani et al. (2018). Cnidarians are the most affected taxa (Anastasopoulou and Fortibuoni, 2019; Angiolillo and Fortibuoni, 2020; de Carvalho-Souza et al., 2018) as their sessile characteristics, significant distribution and vulnerability, and the possibility of obtaining an accurate location of the entanglement event, make them the most suitable taxa to be used as indicators for detecting and monitoring entanglement on the seafloor (Galgani et al., 2018).

The protocol used here for MP extraction was developed for the analysis of MPs in the beach sand (Besley et al., 2017), which often have a larger grain size than deep-sea sediments. This is important because a correlation between MP concentration and sediment grain size has been identified by Zobkov and Esiukova (2017). The authors consider that this correlation plays a role in extracting MPs. It would be interesting to conduct further studies to determine whether this step (considering pellets) is necessary to determine recommendations for protocols, particularly in the context of future monitoring. This could be the case in the MSFD, which has defined deep sediment as an MP indicator (D10C2 – microplastics in sediment). This work could then, in the long term, contribute to the improvement of future extraction methods and the standardization of measurements to obtain comparable data.

Although several legal and policy frameworks have been established to tackle the litter issue, problems still exist in relation to marine litter assessment and management in the Mediterranean deep sea. Therefore, it is important to promptly define and implement common procedures with cross-country collaboration to collect a large series of consistent data, essential to help define measures for the protection of deep-sea ecosystems (Manea et al., 2020).

At present, the priority is to stop the mismanagement of solid waste, which is the main cause of litter (UNEP-MAP 2015a). A preventative approach in the management of human activities, and of fisheries in particular, is needed to reduce the amount of marine litter input in the environment (UNEP-MAP 2015a, 2015b).

Some actions have already been promoted in this sense, such as the already ratified ban on all types of plastic bags or other single-use plastics (e.g., European Commission, 2019). Initiative to remove objects from the seabed are currently underway; however, measures to retrieve them are complex and expensive, especially with increasing depths (Iñiguez et al., 2016).

5 Conclusions

1) This study clearly presents evidence of the pivotal role of canyons as vectors for the transport of solid municipality waste from land sources, and the role of seamounts as hotspots for fishing-related litter. The new hotspot of litter found in the Northern Mediterranean Sea suggests that other accumulation points are distributed in the depths of the Mediterranean Sea.

2) The Mediterranean Sea acts as a sink for plastic pollution; therefore, the identification of the source of litter distribution and transfer and accumulation patterns and mapping of hotspots are crucial for efficient litter management measures, which aim to limit environmental risks (Galgani et al., 2019).

3) Microplastics were found in all sampled stations, with a significant portion of fragments likely originating from larger items distributed locally at the sites.

4) In this study, the most frequent sign of interaction between litter and benthic species observed was the entanglement of colonies of *Callogorgia verticillata*. However, the large amount of litter recorded in the Monaco canyon suggests that the Mediterranean deep sea may be significantly affected by litter that could impact different ecological compartments, and consequently, human health, with potentially severe economic consequences.

5) The use of oceanographic campaigns as opportunistic approaches using standardized non-invasive methodologies is the tool of choice to obtain sufficient amount of comparable long-term data. This data will be useful in supporting the assessment of litter distribution and abundance, and determining its impacts on species, particularly those in the deep sea where monitoring is expensive. The application of the proposed protocol was particularly effective for studies of litter interactions on deep seafloors.

6) The litter concentrations observed at the Monaco canyon were comparable to garbage dumps on land and warrant reflections on specific additional policy needs for mitigation. Clean up of seafloor litter can only be efficiently applied in select areas and does not represent a sustainable and operational solution. Therefore, a preventative approach is needed to reduce the amount of marine litter in the environment. This represents one of the main challenges to future management (UNEP-MAP, 2015a).

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Author Contributions

FG, MA, and OG conceived the idea of the paper and MA wrote the manuscript. MA, OG, FG, MCF, ER, ET, AV, and LT collected the data. MCF analyzed the multibeam data, processed the seafloor parameter statistical analyses, and wrote the portions of the manuscript concerning the results from these analyses. MA and OG processed the video data and OG analyzed the sediment samples. MA, OG, TV, and MCF analyzed and interpreted the data. OG interpreted the data on microplastics and wrote the related parts of the manuscript. Furthermore, ET analyzed the meiofauna in the litter samples. All authors contributed critically to the drafts and gave final approval for publication. AV was the coordinator of the project. AV and LT were the project administrators for each country in the RAMOGE Agreement, and BD was the PI of the cruise.

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Tables

Table 1 – Description of each focal site.

Table 2 – List of ROV dives analyzed in the present study, with geographical coordinates (start and end), depth range, and total length.

Table 3 – Count of marine litter (ML) observed on video, reported by location and separated into two groups "fishing-related items" (FG) and "general waste" (GW) with their number (nb), linear density (items km^{-1}), mean depth (m), and standard deviation of mean depth. The seafloor characteristics used for the PCA include: the slope in degrees, curvature, broad scale BPI (resolution approximately 1.7 km), the linear density (items km^{-1}) of hard and soft substrate, observations extracted from video when litter was observed, as well as the distance to the coast (km) calculated for each location. * The accumulation points were not considered in the calculation of the abundance value.

Figures

Figure 1 – Location of RAMOGE bathymetric data (25 m cell-size), ROV exploration dives, and core tubes dedicated to microplastic analysis. All data were collected in the Ligurian Sea in 2018. The slope off Saint-Tropez bathymetry was a 100 m cell-size resolution (Loubrieu and Satra, 2010). The general bathymetry map was obtained from GEBCO (GEBCO, 2020).

Figure 2 – Examples of marine litter in the canyon and seamounts of the Ligurian Sea: (A-B) accumulation points of different litter typologies found in the Monaco canyon at 2194 m depths; (C-D) examples of artificial polymer items frequently observed on the seafloor, a plastic glass and a plastic bag, respectively. A can is present near the bag; (E-F) example of rubber items, a ball and a Carnival mask, respectively; (G-J) examples of glass/ceramic items, a glass bottle, a cup, and two ancient amphorae; (K) a fishing-related plastic item used as the float flag of a longline frequently observed at the Ulysses seamount; (L-M) fishing gear laying on the seafloor, a lost longline and a net, respectively; (N) a fishing longline entangled in a colony of *Callogorgia verticillata*, abrading its branches (white arrows), (O) a line entangled on several sponges and other organisms; (P) a longline completely tangled up and covered by Zoanthids; (Q) a lost net with some deflated balls probably used as floats; (R) litter items attracting a squat lobster *Munida spp.*; (S) the crab *Paromola cuvieri*, carrying plastic on its back (white arrow), instead of the usual sponges/gorgonians.

Figure 3 – Litter hotspots of the Monaco canyon: A) Bathymetry map of the Monaco Canyon with ROV track (red line) and litter accumulation points (colored triangles). Litter hotspots were recorded at the bottom of the canyon. B) Close-up of wider litter hotspots and indication of their approximate extent. The dashed area highlights a depression which we assume is most likely full of debris. 1) Area 1 is approximately 6 m long and 1 m wide. Terrestrial/vegetal and anthropogenic debris seemed to be trapped by a relief in the bottom. 2) Area 2 is approximately 60 m long and 5 m wide. It is composed of a mat of terrestrial/vegetal debris and very dense anthropogenic litter cover. It is located in a small depression, which probably accentuated the tunnel effect of the canyon. 3) Area 3 is approximately 20 m long and 3 m wide. It is composed of patches of terrestrial/vegetal debris and anthropogenic litter. C) Close-up of denser spots of area 2. D) Close-up of less dense spots of area 3. The general bathymetry map was obtained from GEBCO (GEBCO, 2020).

Figure 4 – Percentage composition of each litter category (according to the MSFD Commission Decision 2017/848) in the seven areas explored in the Ligurian Sea. Fishing-related items are considered separately and the category "unknown material" was added when litter was not identifiable.

Figure 5 – Box-plots representing the depth distribution of fishing-related items (FG) and general waste (GW) observed at each site. Red dots correspond to minimum and maximum depth values. Blue dots and horizontal lines in the box correspond to average-depth and median values, respectively. Grey dots are outliers. A depth comparison was performed at each site, using the Mann-Whitney bilateral test and 1000 Monte-Carlo simulations. Saint-Tropez and Spinola could not be compared because only one FG item was reported. Cannes, Monaco, Ulysses, Janua, and Méjean showed significant p-values (*) indicating that mean depths of FG and GW were different at each site.

Figure 6 – Bathymetric profile and the occurrence of fishing-related items (FG, blue triangles), general waste (GW, orange circles), and litter accumulation points (red asterisks). The distribution on the X axes indicates the distance (in meters) along the track from the origin of the transect, whereas the Y axes indicate the depth.

Figure 7 – PCA ordination of locations explored during the RAMOGE 2018 expedition. Spearman's similarity coefficients of seafloor characteristics were used for the ordination. Clusters formed at the 50% similarity level were superimposed on the PCA plot (Group 1 and Group 2).

Figure 8 – A) Densities of microplastics (item g^{-1}) extracted from sediment collected with the tube cores (TCs) along each ROV transect, and gathered according to microplastic typologies (fiber, fragment, pellet, foam, and film); B) Overall percentage of the different types of microplastics collected in the study areas.

Author Contributions

FG, MA, and OG conceived the idea of the paper and MA wrote the manuscript. MA, OG, FG, MCF, ER, ET, AV, and LT collected the data. MCF analyzed the multibeam data, processed the seafloor parameter statistical analyses, and wrote the portions of the manuscript concerning the results from these analyses. MA and OG processed the video data and OG analyzed the sediment samples. MA, OG, TV, and MCF analyzed and interpreted the data. OG interpreted the data on microplastics and wrote the related parts of the manuscript. Furthermore, ET analyzed the meiofauna in the litter samples. All authors contributed critically to the drafts and gave final approval for publication. AV was the coordinator of the project. AV and LT were the project administrators for each country in the RAMOGE Agreement, and BD was the PI of the cruise.

Declaration of interests

x The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Tables

Table 1 – Description of each focal sites

Sites	Description
Slope off Saint-Tropez	The bathyal slope off Saint-Tropez was chosen because it was previously described to be an area hosting the *Isidella elongata facies* (Fredj, 1964) belonging to the “Deep muds” biocoenosis.
Méjean shoal	The Méjean shoal, which rises from the bottom at 2255 m up to 361 m depth, was chosen because previous exploration described unusual sponge grounds (Fourt and Goujard, 2012). The shoal is aligned with the Cannes canyon (Fig. 1). The upper part is about 10 nm from the coast.
Cannes Canyon	This canyon, located at about 1.3 nm from the French coast, has a NW-SE orientation and is from Messinian origin. Its head begins at 180 m depth and the deeper part is at 2000 m depth. It is characterized by V shape and its walls are gently slope and muddy, forming terraces. Some parts are characterized by conglomerates with high sedimentation rate from torrential valleys and paleo-deltas dating from the Messinian (J. Mascle, *comm. pers.*).
Monaco Canyon	This canyon, at about 1.6 nm from the coast, has a NS orientation and is from Messinian origin. Its head is divided into two parts and the two gullies, made of mud and very high conglomerate walls, descend gently from 150 m to about 1700 m depth, merging at about 11 nm from the coast. At 16 nm from the coast the canyon reaches a depth of 2200 m.
Ulysses Seamount	This seamount is an old volcano (~18 MA) located about 28.6 nm from South of Genoa, is an 18 million years old volcano that has a minimum depth at 397 m (Würtz and Rovere, 2015). Made of lava rocks, it is a very popular long-line fishing area, in particular for semi-professional and recreational fishermen (Orsi-Relini and Relini, 2014).
Janua Seamount	This seamount is an old volcano (~18 MA) located about 35 nm south of Genoa, characterized by lava rocks and arising from the bottom around 820 m in depth. The Janua Seamount is known as a swordfish fishing area (Würtz and Rovere, 2015).
Spinola Spur	This seamount is a composite volcanic structure located about 85 nm south of Genoa, is a rocky elevation SW-NE oriented, arising from the bottom around 2150 m depth and rising the shallow pick at about 1970 m depth. It is the deepest mount of the Ligurian basin, characterized by low silting levels and the presence of *ripple marks*, probably caused by strong bottom currents. Agglomerations of volcanic rocks and numerous pebbles are also observed. These rocky outcrops and bioconstructions are strongly covered by a Fe-M (Würtz and Rovere, 2015).

Table 2 – List of ROV dives analyzed in the present study, with geographical coordinates (start and end), depth range, and total length.

Date	Dive ID	Location	Lat (start)	Long (start)	Lat (end)	Long (end)	Depth range (m)	Transect length (m)
18/09/2018	710-02	Slope off Saint-Tropez	43.188125	6.821732	43.2022632	6.8328471	787–1070	2,208
19/09/2018	711-03	Cannes Canyon	43.423862	7.0428358	43.4477796	7.0490354	945–1443	4,450
20/09/2018	712-04	Monaco Canyon	43.566877	7.4771399	43.6097028	7.4846362	1291–2194	6,233
21/09/2018	713-05	Ulysses Seamount	43.935049	8.8859918	43.9295309	8.9310371	397–1234	9,422
22/09/2018	714-06	Janua Seamount	43.716111	8.8192509	43.7657247	8.7815693	790–1118	11,284
23/09/2018	715-07	Spinola Spur	43.391562	8.7333031	43.3846827	8.7598704	1934–2129	4,271
24/09/2018	716-08	Méjean shoal	43.394549	7.0030633	43.3920025	7.0242557	358–918	3,613

Parameters	Slope off Saint-Tropez	Cannes Canyon	Monaco Canyon	Ulysses Seamount	Janua Seamount	Spinola Spur	Méjean shoal
ML- nb	**25**	**118**	**100***	**172**	**104**	**15**	**41**
ML - Linear density	11.3	26.5	16.0	18.3	9.2	3.5	11.3
ML - Mean depth	914	1338	1839	588	920	2029	516
FG - nb	1	4	4	87	22	1	24
FG - Linear density	0.5	0.9	0.6	9.2	1.9	0.2	6.6
FG - Mean depth	973	1164	1459	535	856	2031	460
FG - Standard deviation	0 (1 item)	185	66	132	80	0 (1 item)	109
GW - nb	24	114	96	85	82	14	17
GW - Linear density	10.9	25.6	15.4	9.0	7.3	3.3	4.7
GW - Mean depth	911	1345	1858	656	937	2029	607
GW - Standard deviation	72	122	287	245	125	56	223
Distance to the coast	12	11	20	52	80	120	11
Slope	20	12	17	19	11	24	21
Curvature	-0.14	-0.17	0.09	0.42	0.06	0.99	0.49
Broad scale BPI	-25	-47	-40	146	82	162	166
Hard substrate	0	0	0	12.1	2.3	3.0	4.2
Soft substrate	10.9	22.0	14.0	5.1	6.6	0.2	6.6

Table 3 – Count of marine litter (ML) observed on video, reported by location and separated in two groups "fishing-related items" (FG) and "General Waste" (GW) with their number (nb), linear density (items km^{-1}), mean depth (m) and standard deviation of mean depth. The seafloor characteristics used for the PCA include: the slope in degrees, curvature, broad scale BPI (resolution around 1.7 km), the linear density (items km^{-1}) of hard and soft substrate, observations extracted from video when litter was observed, as well as the distance to the coast (km) calculated for each location. *The accumulation points were not considered in the calculation of the abundance values.