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Detection of community-wide impacts of bottom trawl fishing on deep-sea assemblages using environmental DNA metabarcoding

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ABSTRACT

Although considerable research progress on the effects of anthropogenic disturbance in the deep sea has been made in recent years, our understanding of these impacts at community level remains limited. Here, we studied deep-sea assemblages of Sicily (Mediterranean Sea) subject to different intensities of benthic trawling using environmental DNA (eDNA) metabarcoding and taxonomic identification of meiofauna communities. Firstly, eDNA metabarcoding data did not detect trawling impacts using alpha diversity whereas meiofauna data detected a significant effect of trawling. Secondly, both eDNA and meiofauna data detected significantly different communities across distinct levels of trawling intensity when we examined beta diversity. Taxonomic assignment of the eDNA data revealed that Bryozoa was present only at untrawled sites, highlighting their vulnerability to trawling. Our results provide evidence for community-wide impacts of trawling, with different trawling intensities leading to distinct deep-sea communities. Finally, we highlight the need for further studies to unravel understudied deep-sea biodiversity.

1. Introduction

The deep sea (i.e. depths >200 m) comprises >60 % of the Earth's surface (Danovaro et al., 2014; Weatherall et al., 2015). As a result, the deep sea represents the largest reservoir of marine biomass and biodiversity in the world (Danovaro et al., 2008; McClain et al., 2012). Deep-sea life provides numerous ecosystem services, including provisioning (e.g. producing direct products for human use such as fisheries) and regulatory (e.g. biological waste removal) services (Thurber et al., 2014). However, ecosystem services provided by the deep sea can be geographically and temporally dissociated from the communities that provide them (Thurber et al., 2014) and as a result, these services are often undervalued.

As fish stocks become globally depleted due to improvements in fisheries technology and the gradual deepening of fishing grounds, many deep-sea fisheries have become unsustainable (Roberts, 2002; Morato et al., 2006; Clark et al., 2016). This, along with increasing anthropogenic pollution, makes deep-sea biota at risk and in need of urgent conservation measures (Danovaro et al., 2017). Many deep-sea fisheries cause unintended environmental harm through accidental bycatch and destruction of fragile benthic habitats (Clark et al., 2016). Studies have shown that sites subjected to deep-sea fishing exhibit a reduction in biodiversity, ecosystem services and sediment organic matter content (Williams et al., 2010; Pusceddu et al., 2014; Yesson et al., 2017; Amisi et al., 2018). Fishing gear modifies the benthos of the deep, altering the shape of the submarine seascape and reducing habitat complexity through destructive repeated scraping of the seafloor (Puig et al., 2012).

Habitat alteration and disturbance caused by trawling has profound impacts on sessile megafauna (Jones, 1992; Williams et al., 2010; Yesson et al., 2017; Amisi et al., 2018). Although some large and mobile fauna can recolonise regions rapidly where trawling has ceased (de Juan et al., 2007; Demestre et al., 2008; Paradis et al., 2021), ecosystem recovery in

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soft sediments can remain limited even after 30 years (de Juan et al., 2011). Mortality due to fishing can be substantial for deep-sea benthic invertebrate megafauna (Bergman and Van Santbrink, 2000), which display negative responses to trawling due to low resilience and reduced mobility (Williams et al., 2010; de Juan et al., 2011; Ingels et al., 2014; Clark et al., 2016; Yesson et al., 2017; Amisi et al., 2018). For deep-sea organisms that are long lived and grow slowly, recolonisation and recovery can be on a timescale of centuries to millennia (Jones, 1992; Clark et al., 2016; Rijnsdorp et al., 2018; Hiddink et al., 2019). Intense fishing pressure results in a reduced abundance of macro- and megafauna and an increased abundance of burrowing epifaunal scavengers and motile burrowing infauna (Jennings et al., 2002; de Juan et al., 2007). Continued disturbance is also expected to diminish the ability of highly adapted *K*-selected species, such as crinoids, to establish, instead favouring more resilient, generalist, scavenging biota (Liu et al., 2011; Mangano et al., 2013).

Meiofauna (soft-sediment organisms operationally defined based on standard sieve mesh sizes between 1000 μm and 20 μm, belonging to taxa of a wide variety of taxonomic groups) have been highlighted as a good indicator for anthropogenic impact and play key roles in the functioning of food webs and sustaining ecological services (Zeppilli et al., 2015; Carugati et al., 2015; Schratzberger and Ingels, 2018). Bottom trawling has found to reduce also meiofauna abundance by 80 % and overall nematode species richness by 25 % (Pusceddu et al., 2014). Morphological taxonomy has been the primary methodology of surveying deep-sea biodiversity (Danovaro et al., 2016). However, morphological methods can have some drawbacks, namely that they can be costly, time consuming, have a limited ability to detect rare or cryptic species or have limited scalability (Danovaro et al., 2016).

With the deep sea facing increasing anthropogenic pressures and a paucity of baseline ecosystem data, incorporating new ways to survey communities could provide greater insights into the effects of anthropogenic impacts on understudied communities. Environmental DNA (eDNA), or the DNA that can be detected in an environmental sample (Deiner et al., 2017), allows undertaking community-wide analyses at an unprecedented taxonomic coverage (Holman et al., 2021). Many different techniques have been developed for extracting and detecting eDNA (Bohmann et al., 2014; Minamoto et al., 2016; Deiner et al., 2017). Metabarcoding is one such method that, through the amplification and sequencing of DNA barcode regions, allows for the simultaneous detection of a large variety of organisms from eDNA samples (Valentini et al., 2009; Taberlet et al., 2012). This technique has vast potential for surveying deep-sea ecosystems (Dell'Anno and Danovaro, 2005; Danovaro et al., 2016), primarily due to its ability to distinguish a wide array of species and assess communities at high taxonomic coverage. It is also more suitable to large-scale standardised sampling as metabarcoding bypasses the need for time-intensive taxonomic identification (Dell'Anno et al., 2015; Danovaro et al., 2016). Surveys of deep-sea eDNA have already discovered far greater benthic biodiversity than previously accounted for in the taxonomic record, indicating our poor understanding of these ecosystems (Guardiola et al., 2015; Dell'Anno et al., 2015; Sinniger et al., 2016; Atienza et al., 2020). Recently, the development of low-cost probes that can also be used by fishing vessels (thus without associated vessel rental costs) to collect eDNA has further facilitated and expanded the use of this technique (Maiello et al., 2022). Furthermore, eDNA metabarcoding has been used in conjunction with non-molecular surveying methods to overcome limitations of visual morphological identification of deep-sea corals (Everett and Park, 2018). Studies using metabarcoding techniques have previously quantified community shifts in marine biodiversity in the vicinity of off-shore gas platforms (Cordier et al., 2019).

As all techniques, eDNA metabarcoding has its own limitations. Firstly, many taxa have an incomplete number of DNA sequences available in reference databases (Dell'Anno et al., 2015; Danovaro et al., 2016; Sinniger et al., 2016). Secondly, uncertainties surrounding the transport and residency time of eDNA remain (Deiner et al., 2017; Collins et al., 2018; Harrison et al., 2019; Holman et al., 2022). Finally, species can be missed due to low eDNA concentrations in environmental samples, and low sampling effort and/or primer biases (Cowart et al., 2018). Despite these limitations, eDNA metabarcoding has huge potential for monitoring and better understanding anthropogenic impacts at community level in the deep-sea.

1.1. Setting

Despite a global increase in research of marine ecosystems, deep-sea habitats remain largely unexplored, potentially harbouring vast undiscovered biodiversity (Danovaro et al., 2010). Submarine canyons have been highlighted as potential biodiversity hotspots due to their associated oceanographic processes and increased sea-surface primary productivity (Fernandez-Arcaya et al., 2017; Atienza et al., 2020). Furthermore, open slope communities have so far defied hypothesised patterns of diversity, suggesting greater community complexity than previously imagined (Danovaro et al., 2010). Critically, this unexplored deep-sea diversity has received little conservation or management attention compared to shallow-water systems (Danovaro et al., 2020).

The Mediterranean Sea represents only 0.8 % of the world's ocean and yet contains an estimated 8.86 % of global deep-sea canyon systems (Harris and Whiteway, 2011). The Mediterranean deep-sea sediments have previously been surveyed using eDNA metabarcoding (Guardiola et al., 2016; Atienza et al., 2020), with Metazoa, Stramenopiles and Archaeplastida being dominant taxonomic groups, and Arthropoda, Nematoda and Cnidaria being the most diverse within the metazoans (Atienza et al., 2020). Communities can be highly heterogenous, with significant differences between sampling areas, depths and seasons (Guardiola et al., 2016; Atienza et al., 2020). Even though there are gaps in the taxonomic dataset of the Mediterranean Sea deep-sea biodiversity, previous research using eDNA surveys of this region was able to accurately characterise the deep-sea communities and detect fine-scale spatio-temporal ecological shifts (Atienza et al., 2020). However, our understanding of fishing impacts of the deep sea at a community level remains limited.

1.2. Objectives and hypotheses

Here, we examined the impact of deep-sea trawling on open slope regions and submarine canyons, and compared eDNA metabarcoding against taxonomic meiofaunal data from sediment communities. For the eDNA metabarcoding data, previously validated primers (COI and 18S) that target metazoans and broad eukaryote diversity were used to capture as much biodiversity of the deep-sea community as possible. We characterised the biodiversity of benthic communities found under different trawling pressures to assess whether these communities are altered by the presence of chronic fishing. We hypothesised that there would be a difference in species diversity and evenness between trawled and untrawled sites. We predicted undisturbed sites would have greater biodiversity as trawling gear smooths the deep benthos, reducing habitat heterogeneity, and therefore reduces the number of ecological niches available (Gallucci et al., 2008; Van Gaever et al., 2009; Durden et al., 2015; Zeppilli et al., 2016). Furthermore, we expected undisturbed seabed to host a larger proportion of long-lived, megafaunal benthic species due to the disproportionately adverse effect that trawling has on this type of fauna (e.g. Amisi et al., 2018). As megafauna can act as ecosystem engineers through modification of the environment (Capezzuto et al., 2018; Tavares et al., 2019), more ecological niches could be present and would further encourage greater biodiversity at untrawled sites (Gallucci et al., 2008; Bongiorni et al., 2010; Hasemann and Soltwedel, 2011; Danovaro et al., 2013; Paoli et al., 2017; Capezzuto et al., 2018; Tavares et al., 2019). We further expected a difference between communities found at the two different habitat types, open slope and submarine canyon. Finally, we expected eDNA metabarcoding and taxonomic meiofauna data to show comparable ability to distinguish

trawling impact. However, eDNA metabarcoding data are expected to provide a more holistic understanding of the community composition with greater taxonomic breath, whereas meiofauna data will provide more detailed information at species level across different sediment depth layers.

2. Data and methods

2.1. Study sites and fishing pressure

Sampling was conducted along the NW Sicilian offshore margin during the 2016 ISLAND Cruise, funded by the EU-FP7 Eurofleets2 Project (GA 31272) onboard the RV Maria Angeles Alvariño (Instituto Español de Oceanografía). Sediment samples were collected from the Arenella, Oreto and Eleuterio submarine canyons in the Gulf of Palermo and in the Castellammare open shelf, for a total of five stations characterised by putatively different levels of trawling impact (Fig. 1). Our sampling sites represent two separate habitat types. The sites in the Gulf of Castellammare were taken from open slope regions, with minimal sediment transport dynamics, whereas the Gulf of Palermo represent submarine canyon networks with a greater degree of sediment transport (Lo Iacono et al., 2014).

In the study area, where trawlers have increased their fishing efforts beyond 500 m depth since the 1980s (Lo Iacono et al., 2018; Paradis et al., 2019, 2021), we identified both undisturbed and chronically trawled sites, allowing for an assessment of the impacts of fishing activity on the deep-sea benthos. Sampling stations in both regions were deemed untrawled if the area had no known active or historic benthic fisheries. Fishing activities in the NW Sicilian margin started in late 1950s, with a continuous growth throughout the 1960s and 1970s. An industrial scale expansion occurred from the 1980s onwards, with deeper fishing grounds (up to 700 m) accessed as a result of improved trawler engine power (Paradis et al., 2019, 2021). In the Gulf of Castellammare, very high intensities of fishing effort (up to 70 ha yr^{-1}) are mainly concentrated in the western sector of the slope, between 500 m and 700 m depth (Fig. 1a). Trawlers operate parallel to the isobaths and stop fishing in the eastern slope, before crossing the most incised Castellammare Canyon (Fig. 1a). In the Gulf of Palermo, fishing intensities (up to 20 ha yr^{-1}) are much lower than Castellammare (Fig. 1b). Fishing activities are spread all around the outer shelf and on the upper slope between the Oreto and Eleuterio Canyons (Fig. 1b). The greatest fishing intensities are concentrated in the Oreto Canyon (Fig. 1b), which is the only impacted canyon of the Gulf, where bottom trawlers operate on its walls and axis, between 200 and 700 m depth (Fig. 1b).

The adjacent Gulfs of Castellammare and Palermo are located in the NW Sicilian margin (Fig. 1). The westernmost Gulf of Castellammare covers a surface of around 440 km^2. The gulf is delimited by Capes San Vito to the west and Cape Rama to the east (Fig. 1a). An almost subhorizontal 1° steep continental shelf extends in the Gulf for approximately 8 km offshore, gently sloping to the shelf break at a depth of around 140 m (Lo Iacono et al., 2014; Fig. 1a). The continental slope is around 11° steep down to 500 m water depth, and it then gradually decreases to around 1.5° at a depth of 1300 m (Lo Iacono et al., 2014). Up to 14 narrow submarine canyons incise the slope, breaching the shelf break and entering in the outer shelf up to a depth of 110 m (Lo Iacono et al., 2014; Fig. 1a). Canyons are mostly distributed in the eastern sector of the Gulf and coalesce into the Castellammare Canyon at the depth of ~970 m (Fig. 1a). In the western sector of the Gulf canyons are less incised and display a smooth morphology, probably suggesting a minor activity of sediment transport dynamics within them (Fig. 1a).

To the east of the Gulf of Castellammare, the Gulf of Palermo covers a surface of 250 km^2, and is delimited by Cape Gallo to the west and by Cape Zafferano to the east (Fig. 1b). The Palermo continental shelf presents an average width of 5 km, and is delimited by the shelf-edge at a depth of 120–130 m. The shelf edge is incised by four main submarine canyons (Addaura, Arenella, Oreto and Eleuterio Canyons), whereas the northernmost Mondello Canyon is confined to the upper slope (Fig. 1b). All five canyons display a linear to sinuous geometry with downslope gradients ranging from 6° to 13°, and their mouth connected to the intraslope basin at around 1100 m depth (Lo Iacono et al., 2011; Fig. 1b). The largest and most incised canyons in the Gulf are the Oreto and Eleuterio Canyons (Fig. 1). Oreto Canyon displays a linear to sinuous head that indents the shelf-edge at the central sector of the Gulf (Fig. 1b, Lo Iacono et al., 2011). The easternmost Eleuterio Canyon is the largest canyon of the Gulf (Fig. 1b), with its head widened by several mass wasting processes along its walls, creating an uneven and irregular seafloor morphology up to the depth of 700 m (Lo Iacono et al., 2011, 2014). The head of the Eleuterio Canyon is only ~2 km far from the shore, which favors the transport of (natural and trawling-induced) suspended sediment from the shelf into this canyon (Lo Iacono et al., 2014). The submarine canyons located in the northwestern sector of the gulf, Arenella, Addaura and Mondello canyons, are steeper and shorter (Fig. 1b; Lo

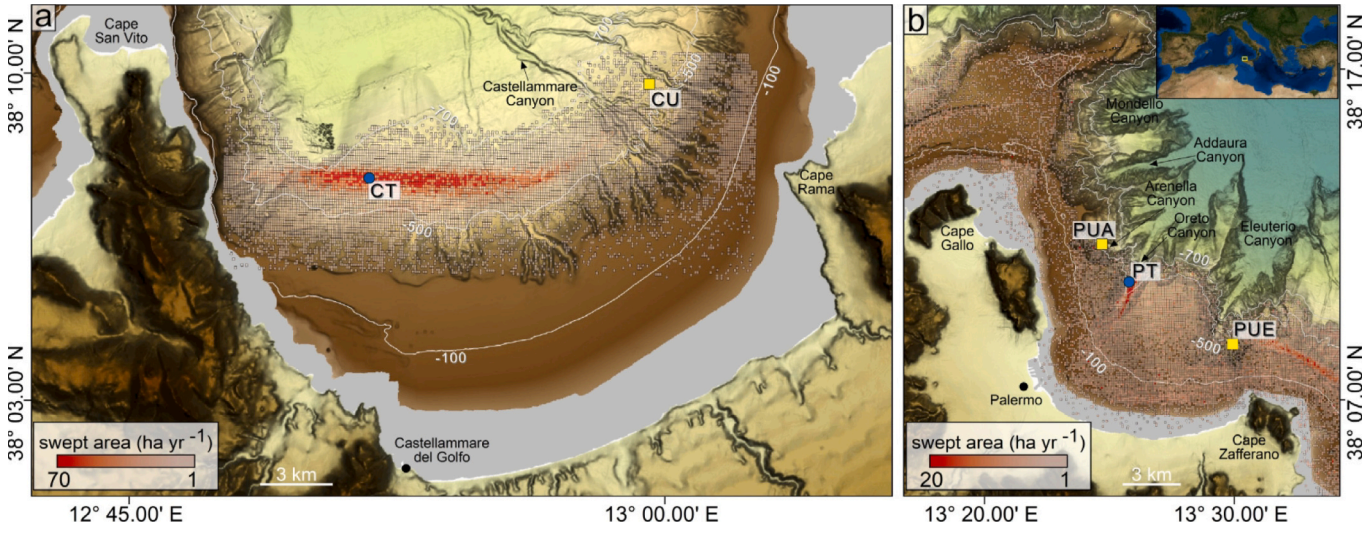

Fig. 1. Map of the study area showing the Gulfs of Castellammare (a) and Palermo (b), the distribution of the bottom trawling effort (2008–2016), and the sampling stations visited during the ISLAND Cruise. The region in the open shelf of Castellammare (a) included one trawled (CT) and one untrawled station (CU). The region in the Gulf of Palermo (b) had two untrawled stations (named PUA and PUE) and one trawled station (PT). Note the different intensity of trawling effort between the two study sites At each station, three replicate multi-core deployments were performed.

Iacono et al., 2011), and their evolution is mainly controlled by mass wasting events retrograding from the base of the slope to shallower domains (Lo Iacono et al., 2011).

Water masses circulation on the northern Sicilian shelf and upper slope is controlled by the Modified Atlantic Water (MAW), which flows from west to east through cyclonic geostrophic currents parallel to the coastline, with average speed of 0.1–0.2 ms^{-1} (Pinardi and Masetti, 2000; Sarà et al., 2006; Caruso and Cosentino, 2008). Both Palermo and Castellammare Gulfs present a micro-tidal regime (Istituto idrografico della Marina, 1982).

At each sampling station (Fig. 1), three replicate deployments of a K/C Denmark A/X six-tube multicore (inner diameter 9.4 cm) were performed at a depth between 516 m and 568 m (Table 1). For each successful deployment, 50 cm deep sediment cores were collected and seawater was gently syphoned off the top of the core. Two grams of sediment for eDNA analysis was collected from the surface 1 cm of each of the three corer deployments. Sediment samples were kept independent, generating three ecological replicates per site. The sediment was taken from the centre of the core avoiding the edges and stored in a plastic sterile syringe at −20 °C during the cruise and at −80 °C when the samples arrived in the laboratory until DNA extraction. Cores from the Castellammare region also underwent taxonomic analysis for meiofauna, where vertical 15 cm slices of sediment samples remained at −20 °C until analysis. Sediment organic matter data was collected and recorded as described in Paradis et al. (2019). Full details of the corers can be found in.

2.2. Fishing effort calculation

Distribution of bottom trawling effort on the study sites is based on data from the vessel monitoring system (VMS), a tracking device mandatory for fishing vessels with a minimum length over of 15 m (European Commission, 2003), sending to the national coast guard position, speed, and heading of the vessels at 1–2 h intervals. To overcome the low sampling frequency, VMS data were interpolated using the R package VMSbase (Russo et al., 2014), which takes into account the vessels' speed, heading and its relative drift. After the identification of fishing events (hauls), which were isolated from each whole fishing trip by removing steaming and other non-fishing behaviours, the vessel-specific gear width was estimated using the approach described in Eigaard et al., 2016. Then, the values of the swept area corresponding to each haul and vessel were computed and aggregated at a grid resolution of 100 m^2. Finally, trawling intensity, expressed as mean swept area in hectares per year, was estimated using VMS data from 2008 to 2016 and represented (Fig. 1).

2.3. Environmental DNA extraction

Environmental DNA was extracted in a PCR-free clean room separate from laboratories containing post-PCR or high copy number DNA samples. DNA extraction proceeded using the Qiagen DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) as manufacturer instructions. For each sediment sample four replicate subsamples of 250 mg were processed, for a total of 1000 mg processed per sample. These subsample extractions were pooled after DNA extraction, and the pooled DNA sample used for all further processes. To test for PCR inhibition a Primer Design Real-Time PCR Internal Control Kit (PrimerDesign, Southampton, UK) was used as manufacturer recommended with reaction volume and eDNA quantity as detailed for metabarcoding PCR reactions.

2.4. Metabarcoding library construction

Metabarcoding proceeded using two sets of previously validated metabarcoding primers targeting metazoans and broad eukaryotic diversity. These primers targeted a 313 base pair (bp) region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) (Leray et al., 2013) and a variable length region of the V4 nuclear small subunit ribosomal DNA (18S) (Zhan et al., 2013). The preparation of Illumina sequencing libraries proceeded using a 2-step PCR approach as in Holman et al. (2019). Briefly, this approach involves an initial amplification with primers that consist of the target region and a unique tail sequence in the first PCR. This tail acts as a target for the second PCR, which uses pairs of primers that have unique indices and sequencing regions so that each sample has a unique pair of indices (Brennan et al., 2019) to avoid cross-talk associated with combinatorial indexing (MacConaill et al., 2018).

The first PCR was prepared in a clean laboratory separated from post-PCR work. No high copy DNA samples or post-PCR products are permitted in this laboratory. The first PCR consisted of 10 μl Amplitaq GOLD 360 2X Mastermix (Applied Biosystems, California, USA), 0.8 μl (5 nmol ml^{-1}) of each forward and reverse primer and 2 μl of undiluted environmental DNA extract in a total reaction volume of 20 μl. PCR conditions consisted of an initial denaturation step at 95 °C for 10 min followed by 20 cycles of 95 °C for 0:30, variable annealing temp (46 °C for COI, 50 °C for 18S) for 30 s, and extension at 72 °C for 1 min. A final extension at 72 °C was performed for 10 min.

For each sample three replicate first PCR reactions where performed, these where then pooled and cleaned using AMPure XP beads (Beckman Coulter, California, USA) at a 0.8 beads:sample volume ratio as manufacturer's instructions. The second PCR consisted of 10 μl Amplitaq GOLD 360 2X Mastermix, 0.5 μl (10 nmol ml^{-1}) of both forward and reverse primers and 5 μl of pooled undiluted cleaned PCR product from the first reaction in a reaction volume of 20 μl. Samples were then cleaned with the AMPure XP beads as above and normalised using the NEBNext Library Quant qPCR kit (New England Biolabs, Massachusetts, USA) under manufacturer's instructions. These normalised samples were then equimolarly pooled along with control samples and subject to sequencing on an Illumina MiSeq Instrument (Illumina, San Diego, USA) with a V3 2x300bp kit.

2.5. Bioinformatics

The Illumina MiSeq control software (v.2.6.2.1) was used to demultiplex samples. To analyse the demultiplexed data a custom pipeline written in R programming language (R_Core_Team, 2018) was used (Holman, 2019: https://doi.org/10.5281/zenodo.3336241). Using the -fastq_mergepairs option of USEARCH v.10.0.240 (Edgar, 2013), forward and reverse paired end reads were merged with a maximum difference of 15, a percent identity of 80 % and a maximum expected error threshold of 1 in the quality filter. Cutadapt v.1.16 (Martin, 2011)

Table 1
Characteristics of the sampling sites and the sample types collected via the sediment multi-cores. Codes in brackets refer to the core labels in Figs. 1, 4 and 7. Untrawled refers to site with no known active or historic benthic fisheries.

Region	Trawling intensity	Meiofauna sampling	eDNA sampling	Latitude (N) – WGS1984	Longitude (E) – WGS1984	Core depth (m)
Castellammare	Trawled (CT)	Y	Y	38.1305	12.8667	568
	Untrawled (CU)	Y	Y	38.1657	12.9887	542
Palermo	Trawled (PT)	N	Y	38.1753	13.428	560
	Untrawled – Arenella (PUA)	N	Y	38.1949	13.409	544
	Untrawled – Eleuterio (PUE)	N	Y	38.147	13.4924	518

was used to ensure only sequences containing both forward and reverse primer regions were retained and sequences were outside of the defined length boundary of 303–323 bp (COI) or 375–450 bp (18S) were discarded. Selected sequences were pooled, singletons discarded and quality filtered with a maximum expected error of 1 using the -fastq_filter option of VSEARCH v.2.4.3 (Rognes et al., 2016). We then used the unoise3 algorithm in USEARCH to denoise and to filter out chimeras. Resulting amplicon sequence variants (ASVs) were curated using the default parameters of the LULU R package v.0.1.0 (Frøslev et al., 2017). A sample table of ASVs was produced by mapping merged and trimmed reads against ASVs using USEARCH (with parameters *-id 0.97-maxaccepts* 8 *-maxrejects* 256). The sample table was filtered to eliminate spurious results using R. Criteria for filtering include setting any record with fewer than three raw reads as zero and removing any ASV that did not appear in more than one sample. Any ASVs found in negative control replicates were removed from further analysis. Breakdown of the number of ASVs removed from the filtering process can be seen in Table S2.

2.6. Taxonomic assignment

Initial taxonomic assignments for each ASV were performed by querying in a BLASTn (v.2.6.0+) search with '-num_alignments' set to 200 against the entire *nt* database from NCBI (downloaded on Sep 1st 2020). These results were then parsed using a custom R function (*-ParseTaxonomy* DOI: https://doi.org/10.5281/zenodo.3336241) to return a taxonomic assignment for each ASV. Hits above 97 % identity, contingent on a minimum coverage of 80 %, were annotated as *high* quality. In cases were more than one taxon was found to have a *high*-quality assignment to an ASV, a lowest common ancestor algorithm was annotated to the lowest common taxonomic rank to the taxa.

2.7. Meiofauna analyses

Once in the laboratory, sediment samples were thawed and the sediment was divided into five depth layers: 0–1 cm, 1–3 cm, 3–5 cm, 5–10 cm and 10–15 cm. To separate the meiofauna, sediment was sieved through a 1000 μm mesh, followed by a 20 μm mesh. The sediment retained in the 20 μm sieve was re-suspended and washed three times in Ludox HS40 colloidal silica (density 1.31 g cm^{-3}) following previous work (Heip et al., 1985; Higgins and Thiel, 1988; Danovaro et al., 2010). Animals retained in the supernatant were once again sieved through a 20 μm mesh, washed using tap water, and stained with 0.5 g L^{-1} rose Bengal solution. These were then sorted under a stereomicroscope (magnification 40-62×), according to Danovaro et al. (2010). As meiofauna is an operational group including small organisms with a size assumed to be related to their intermediate trophic significance between micro- and macroscopic organisms, meiofaunal taxa belong to different taxonomic levels, spanning from phylum (e.g., Nematoda, Loricifera), to class (Polychaeta), sub-class (e.g., Copepoda), and so on. Abundances of each taxon (no unclassified taxa) and their sum (total meiofaunal abundance) were expressed as number of individuals 10 cm^{-2}.

2.8. Statistical analyses

All the below statistical analyses were performed on both the COI and 18S eDNA datasets. For alpha diversity proxies (ASV numbers, Shannon diversity and Evenness) data was non-normally distributed and could not be transformed to obtain normality. Due to the limited sample size, both parametric (linear models) and non-parametric (Kruskal-Wallis) analyses were performed to overcome the statistical limitations of each model. For the linear models, three maximal linear models were run which included the effect of trawling intensity, sample site, site depth and organic matter [protein (mg C/g sed), carbohydrate (mg C/g sed), and lipids(mg C/g sed)] and their interaction with ASV number, Shannon diversity index and species evenness index. The maximal model was evaluated to determine the minimum adequate model by removing non-significant terms. For all analyses, trawling condition was treated as a categorical variable (trawled/untrawled).

Rarefaction curves and species accumulation curves were generated for both datasets to ensure adequate sequencing depth and environmental sampling, respectively. Data were rarefied to the minimum number of reads per sample (COI: 31,305, 18S: 32,441) using the *rarefy* function in the vegan R package v.2.6–2 (vegan: Community Ecology Package). Multifactorial Permutational Analyses of Variance (PERMANOVA) were then performed for both datasets using the vegan R package function *adonis* to test for differences in community composition between trawling impact, sampling region, and their co-interactions. Multifactorial two-way PERMANOVAs were used to test for differences between levels of the following fixed and orthogonal factors: sampling region (Palermo canyons vs. Castellammare open slope) and trawling intensity (untrawled vs. trawled). The Jaccard diversity metric was used to reduce the weighting of ASV abundance in the results. Sampling stations were deemed untrawled if the area had no known active or historic benthic fisheries. When a significant result was found, multivariate dispersion (PERMDISP) tests were conducted using the vegan R package (function *betadisper*) to identify if significance arose due to discrete multivariate means or due to varying heterogeneity between groups.

For the meiofauna data, a maximal linear model was run to test whether the effects of trawling impact, sediment layer, and sediment organic matter (protein, carbohydrate and lipids) had a relationship with Shannon diversity index values. Sediment layer depths were counted as categorical factors. Sediment organic matter data was only available for depths up to 10 cm, therefore all values for the 10–15 cm sediment layer were excluded from the initial maximal linear model. The maximal model was evaluated, and non-significant terms were removed to determine the minimum adequate model. A subsequent maximal linear model examining the relationship of all sediment depth layers to trawling impact and Shannon diversity index scores was performed, which was evaluated, and a minimum adequate model was produced. An Analysis of Variance (ANOVA) was run to test meiofauna abundance from trawled and untrawled sites against sediment layer depth. Abundance was non-normally distributed and was log transformed to obtain normality. Two-way PERMANOVAs were conducted to assess the effect of trawling intensity (untrawled and trawled) and sediment layer depth on meiofaunal community composition using a Bray-Curtis diversity metric. All the above-described tests were conducted using R.

3. Results

3.1. Alpha diversity – eDNA metabarcoding

ASV taxonomic assignments were substantially different between the COI and 18S datasets. 7 super-groups were detected in the COI dataset, and 6 super-groups in the 18S data (Fig. 2). In total, there were 7501 18S ASVs and 17,698 COI ASVs. Of the 17,698 COI ASVs, 1533 could be assigned to a super-group (8.66 % of total ASV number) with 611 assigned to Metazoa (3.45 % of the total number of ASVs). For the 18S dataset, 3789 of the 7501 ASVs could be assigned to a super-group (50.51 %), with 843 of those ASVs being assigned to Metazoa (11.24 %). Therefore, more ASVs could be assigned as metazoan in the 18S dataset, both in percentage and absolute number, than the COI dataset (Fig. 2).

From our rarefaction curves we can be confident that the majority of the ASVs present in our samples were captured (Fig. S1), as plateau was achieved at ca. 20,000 reads for both datasets, with our mean number of reads per sample being 156,515 (COI) and 200,334 (18S). To assess completeness of environmental sampling effort, a species accumulation curve was plotted (Fig. S2). Species accumulation curves for COI and 18S datasets showed that both Castellammare and Palermo sampling

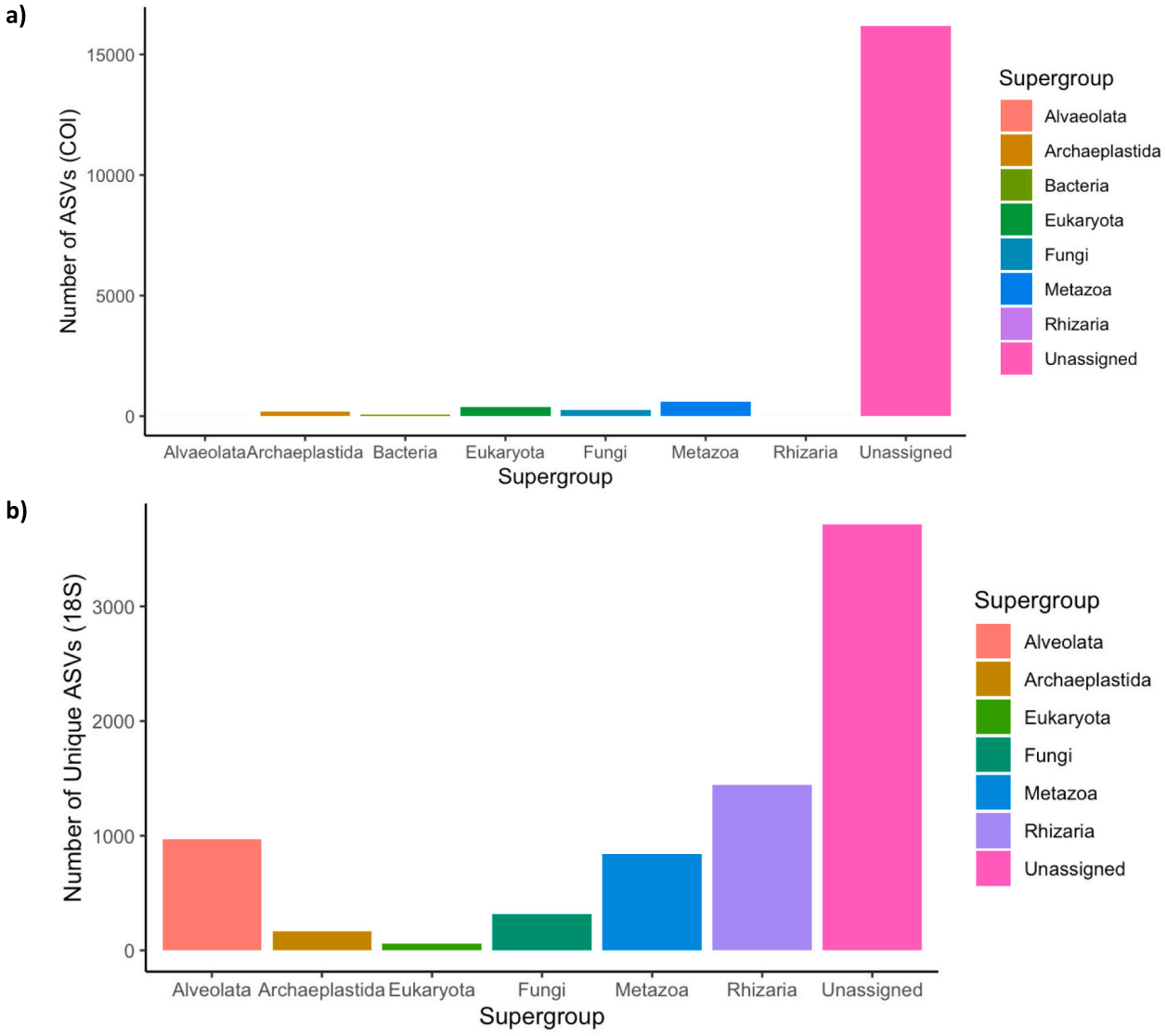

Fig. 2. Amplicon sequence variants belonging the super-groups (i.e. the broad category of Eukaryota) detected in a) COI and b) 18S datasets.

stations plateaued in ASV numbers between 6 and 9 samples respectively. However, neither reached asymptote. Therefore, more ASVs could be obtained through a greater sampling effort, although we expected the broad-scale diversity patterns to be captured, in line with previous similar studies (Guardiola et al., 2016; Atienza et al., 2020).

Through evaluating the minimum adequate models, sample site, core depth and organic sediment matter were found to have a non-significant contribution and were removed from the models examining ASV number, Shannon diversity and species evenness. For all diversity proxies, the results of both parametric (Table S3) and non-parametric tests (Table S4) did not identify any significant difference between trawling intensities (Fig. S3). While non-significant, Castellammare untrawled did show the largest range across all diversity indices (Fig. S3). Subsequent taxonomic community analysis is presented for 18S data only. All 18S figures have equivalent figures for COI, which can be found in the supplementary material.

3.2. Alpha diversity – meiofauna data

The meiofaunal community found in the sediment samples were represented by five taxa; Amphipoda, Gastrotricha, Copepoda, Polychaeta, and Nematoda. Total meiofauna abundance (number of individuals per 10 cm^{-2}) ranged between 2 and 8.4 at the untrawled site and 1.4 and 22 at trawled sites (Table S5). The maximal linear model examining effect of trawling impact, layer depth (excluding layer 10–15 cm), and organic sediment components (protein, carbohydrates, lipids and biopolymeric carbon) on Shannon diversity was evaluated and non-significant terms were removed to produce a minimal adequate model. The only significant term was the interaction between sediment lipid concentration and Shannon diversity score ($R^2 = 0.21$, $t = -0.163$, d.f. = 22, $p = 0.015$; Fig. 3). The broad pattern is that sediment lipid concentration is positively correlated with diversity scores, with diversity increasing with lipid concentration (Fig. 3). It is worth noting that not only does sediment layer depth appear to relate to lipid concentration, but also that trawled layers had consistently lower lipid concentrations and diversity scores compared to their untrawled counterparts (Fig. 3). For the maximal model examining Shannon diversity scores across all sediment layer depths and across trawling impact, influence of sediment layer was found to have a non-significant impact on the model and was removed. The minimum adequate model found that trawling had a significant impact on Shannon diversity (one-way ANOVA, $F_{1,28} = 6.86$, $p = 0.014$; Fig. S4).

Meiofauna abundance was significantly associated with sediment layer depth (one-way ANOVA, $F_{2,19} = 11.21$, $p < 0.05$). However, a post-

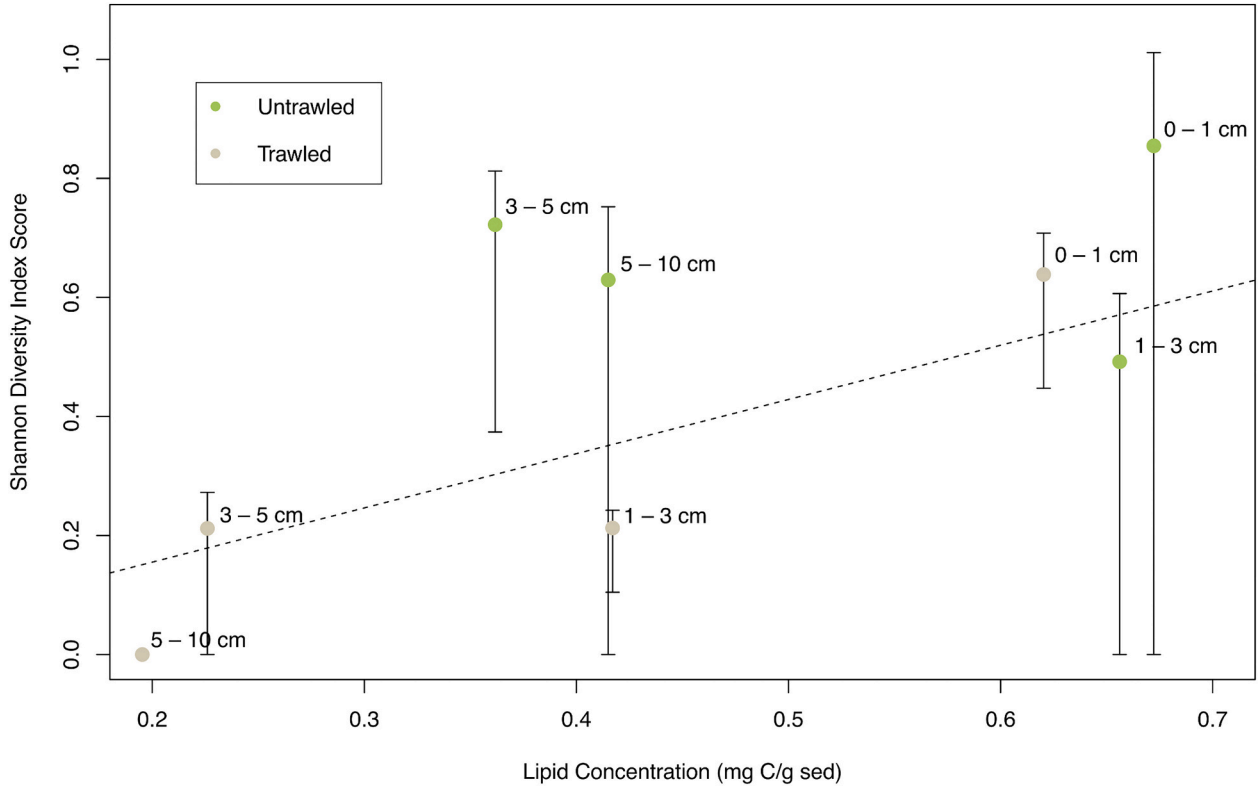

Fig. 3. Relationship between meiofauna Shannon diversity score and sediment lipid concentration. Layer depth is indicated by the labels. Points indicate median diversity score, whiskers highlight the range of data. Dotted line indicates trend from linear regression model examining the relationship between Shannon diversity scores and lipid sediment concentration.

hoc Tukey test showed that when equivalent sediment layer depths were compared between trawled and untrawled conditions, no significant difference was observed between meiofauna abundance ($p \geq 0.05$). While not significant, meiofauna abundance suggests patterns of divergence between untrawled and trawled sites (Fig. S5). Untrawled and trawled sites had meiofauna abundance peak at the 1–3 cm sediment depth layer. For the 0–1, 1–3 and 3–5 cm depth layers, the trawled site had greater meiofauna abundance compared to the untrawled site. This pattern is reversed in the deepest sediment layers (5–10 and 10–15 cm).

3.3. Beta diversity and community composition – eDNA metabarcoding

For the COI and 18S datasets, PERMANOVA tests showed that community composition was significantly different between trawling intensity, across sampling regions, and that the impact of trawling varied between regions ($p < 0.05$, Table 2). PERMDISP tests showed non-significant differences in heterogeneity, indicating no detected difference in dispersion around the multivariate centroid of trawled and untrawled sites.

Patterns displayed from both 18S and COI datasets are highly comparable, therefore the COI nMDS is presented in the supplemental material (Fig. S6). The non-metric MDS (nMDS) plot has a low stress value, indicating the ordination is likely to be a good representation of the data. The patterns displayed in Fig. 4 reflect the results of the PERMANOVA and PERMDISP. Stark differences between Castellammare (open slope habitat) and Palermo (submarine canyon habitat) are apparent, with no overlap between the two regions. Within sampling region, untrawled replicates (triangles, Fig. 4) are comparatively more distinct from one another than their trawled counterparts (circles, Fig. 4), with trawled replicates from both regions clustering tightly. This suggests a greater variety of communities are present within untrawled sites compared to trawled sites. At Palermo, some untrawled replicates are closer to trawled replicates than other untrawled sites, indicating some comparable community composition across trawling intensity. Castellammare, however, has highly distinct separation between trawled and untrawled samples (Fig. 4).

Table 2
Results of permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP) analyses on trawling impact and sampling region. Results for COI and 18S genomic data are presented. Significant results are highlighted in bold. Results in bold indicate significant differences ($p < 0.05$).

Dataset	Explanatory variable	Statistical test	Degrees of freedom	F value	*p* Value
COI	Trawling impact	PERMANOVA	1,14	1.42	**0.003**
		PERMDISP	1,13	1.95	0.19
	Sampling region	PERMANOVA	1,14	1.54	**0.001**
		PERMDISP	1,13	0.07	0.80
	Trawling impact:	PERMANOVA	1,14	1.36	**0.009**
	sampling region	PERMDISP	3,11	2.51	0.11
18S	Trawling impact	PERMANOVA	1,14	1.40	**0.003**
		PERMDISP	1,13	2.56	0.13
	Sampling region	PERMANOVA	1,14	1.58	**0.001**
		PERMDISP	1,13	0.85	0.37
	Trawling impact:	PERMANOVA	1,14	1.32	**0.13**
	sampling region	PERMDISP	3,11	3.41	0.057

A breakdown of the 18S metazoan phyla community composition for each sampling station can be seen in Fig. 5. In total, 17 phyla were identified in the 18S dataset. Nemotoda was the phylum with the largest number of unique ASVs across all stations, contributing the majority of ASVs at each site. Bryozoa was the only phylum to be present at all untrawled sampling stations but absent at all trawled stations. Loricifera was unique to the trawled Castellammare sites. All Palermo sites had a higher number of ASVs than that of Castellammare sites. Similar to the nMDS plots, Castellammare (open slope habitat) and Palermo

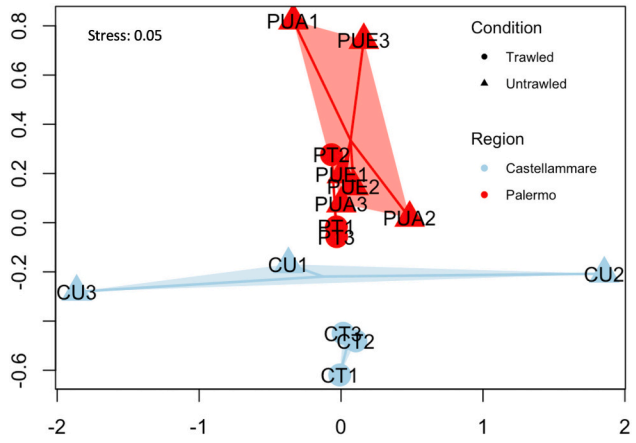

Fig. 4. Non-metric multidimensional scaling plot using Jaccard dissimilarity of amplicon sequence variant number by sample station (colours) and impact level (shapes) for 18S dataset. CT = castellammare trawled, CU = castellammare untrawled, PT = palermo trawled, PUA = Palermo untrawled arenella, PUE = palermo untrawled eleuterio. Numbers indicate replicates.

(submarine canyons) appear to display different responses to trawling. At Palermo, the trawled site had the largest number of ASVs, indicating more species, however, the greater number of ASVs is almost entirely due to a greater number of Nematoda ASVs (PT = 325 Nematoda ASVs, PUA = 181, PUE = 237). Platyhelminth ASVs also appeared more numerous at Palermo trawled, with 10 assigned ASVs compared to 4 at Arenella and 2 at Eleuterio. At Castellammare trawled, there were fewer Nematoda ASVs (CT = 134, CU = 199) and Mollusca (CT = 6, CU = 11), but a greater number of Arthropoda ASVs (CT = 26, CU = 9).

3.4. Beta diversity and community composition – meiofauna data

For meiofauna data, PERMANOVA tests were significant for both the effect of trawling impact and sediment depth layer ($p < 0.05$; Table 3). For sediment depth layer, PERMDISP tests showed non-significant differences in heterogeneity, suggesting that the effect of sediment depth layer was not due to dispersion levels. Comparatively, trawling impact did return a significant PERMDISP result ($p < 0.05$), indicating the dispersion around the centroid of trawled and untrawled conditions was different.

Meiofaunal community patterns across sediment depth layers and trawling conditions are presented, complementing the findings of the PERMANOVA and PERMDISP (Fig. 6). The plot had a low stress value, indicating good representation of the patterns in two dimensions. The patterns displayed show that regardless of trawling condition, shallower sediment layer depths have clearly segregated communities whereas deeper sediment communities are more homogenous. This pattern is most apparent within trawled communities, which has a much greater distinction between surface and deeper layers, with a higher degree of similarity between layers 5–10 and 10–15 cm. Both trawled and untrawled communities had sediment layer 1–3 cm as its most distinct, being furthest away from all other layer depths within their respective

Table 3
Results of permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP) testing for trawling impact and sediment depth layer on the composition of meiofauna community composition. Results deemed significant when $p < 0.05$.

Explanatory variable	Statistical test	Degrees of freedom	F value	*p* Value
Trawling impact	PERMANOVA	1,29	5.73	**0.013**
	PERMDISP	1,28	8.00	**0.01**
Sediment depth layer	PERMANOVA	8,29	7.45	**0.001**
	PERMDISP	9,20	0.28	0.97

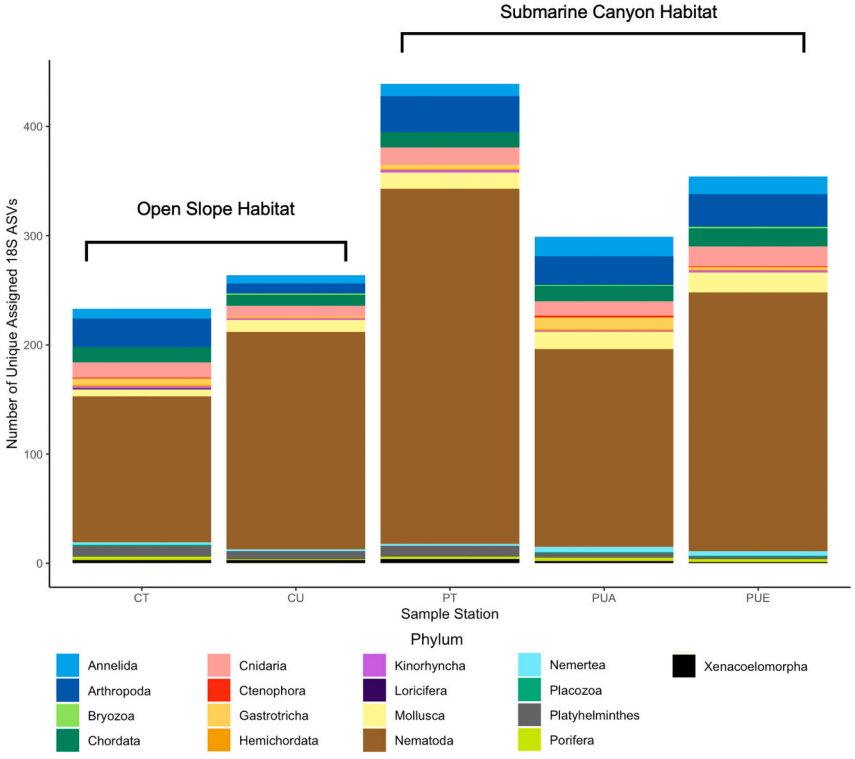

Fig. 5. Metazoan phylum composition of amplicon sequence variants (ASVs) for the 18S dataset. Excludes unassigned ASVs. CT = castellammare trawled, CU = castellammare untrawled, PT = palermo trawled, PUA = palermo untrawled arenella, PUE = palermo untrawled eleuterio.

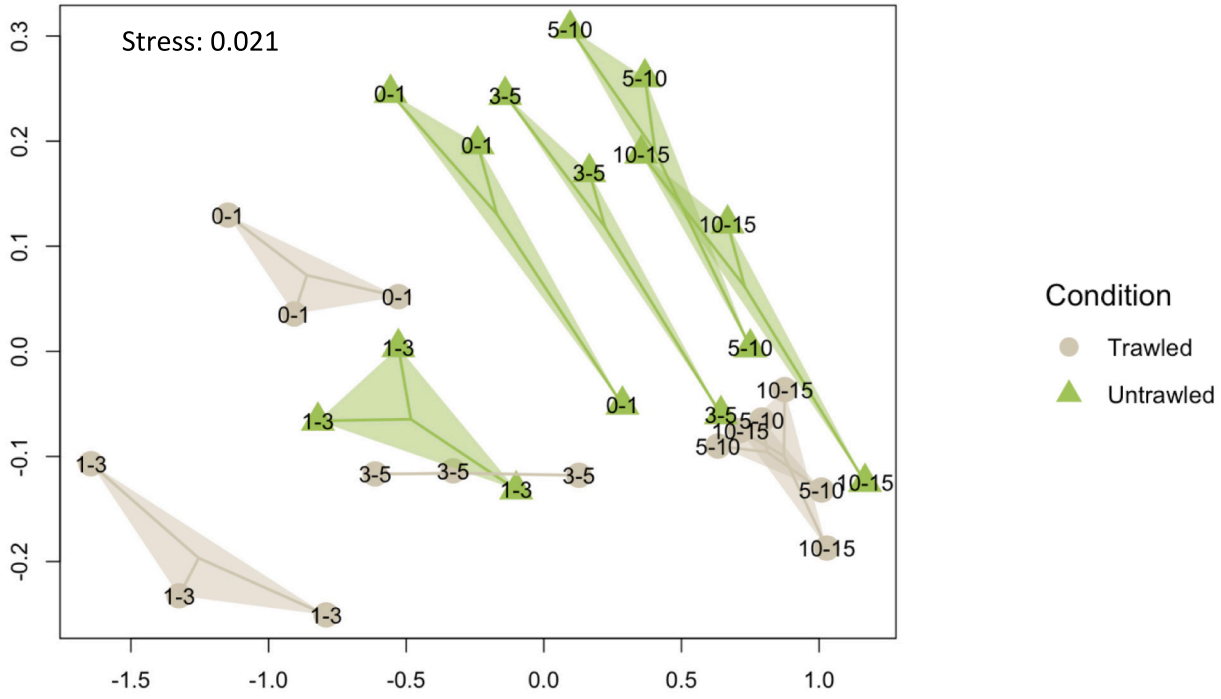

Fig. 6. Non-metric multidimensional scaling plot using Bray-Curtis dissimilarity of meiofauna community by sediment layer depth within trawling conditions at Castellammare.

trawling condition. When comparing across trawled and untrawled conditions, respective communities of individual layer depths remain distinct across the entire sediment profile.

For community composition, replicates were summed and total community taxonomic composition for each sediment layer depth is presented in Fig. 7. Both untrawled and trawled sites have greatest community diversity in their shallower sediment layers, however the untrawled site retained greater taxa diversity throughout all depth profiles compared to the trawled communities. Nematoda contribute overwhelmingly (> 75 %) to meiofaunal communities at each sediment depth layer regardless of trawling impact. In trawled sediment deeper than 5 cm, meiofauna were solely represented by Nematoda. Copepoda are present in all untrawled sediment layers, however they are absent in layers 5–10 cm and 10–15 cm at the trawled site.

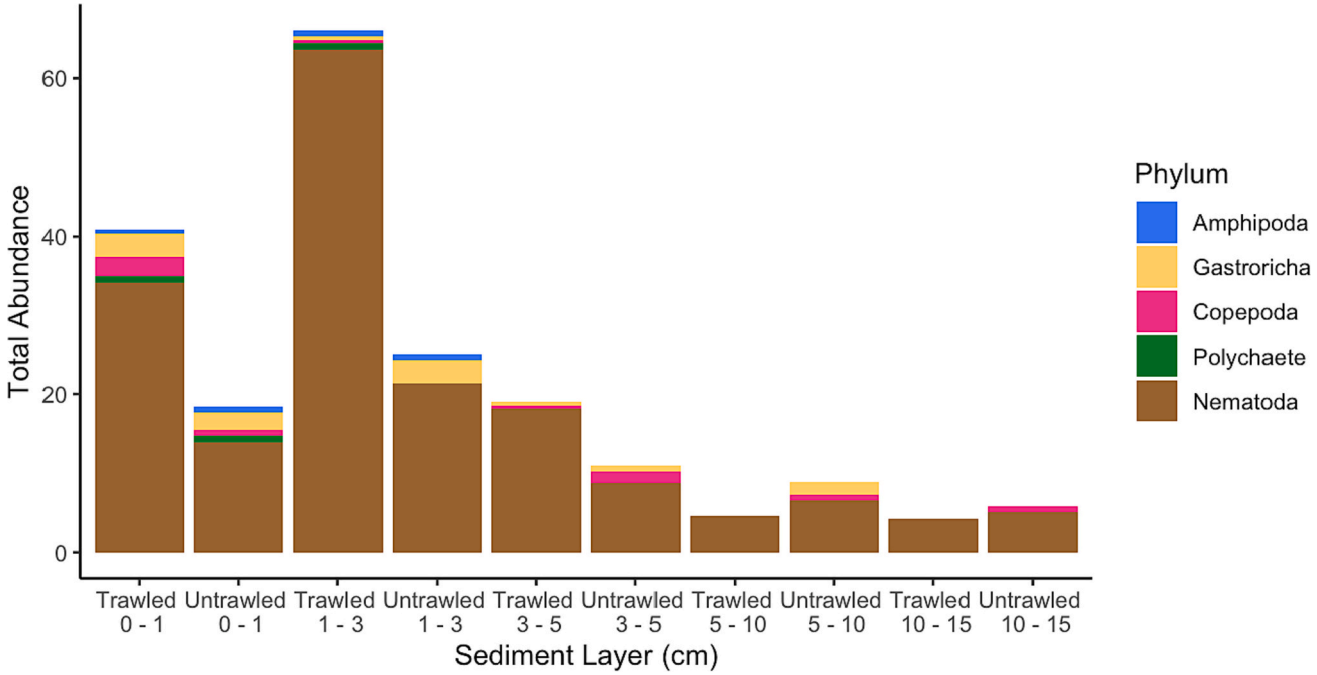

Fig. 7. Meiofauna taxonomic composition for each sediment depth layer for trawled and untrawled sites at Castellammare. Abundance values for all replicates summed to create total community composition for each sediment layer. Abundance calculated as number of individuals per 10 cm^{-2}. No meiofauna data available for Palermo.

4. Discussion

Our eDNA metabarcoding data did not detect trawling impacts or habitat differences using alpha diversity indexes. In contrast, we found in the meiofauna data a significant impact of trawling on Shannon diversity scores, as well as a significant interaction between sediment lipid concentration and diversity scores. There was consensus between the eDNA and meiofauna data when examining the analyses of beta diversity, which revealed distinct communities across habitat types and trawling intensity. PERMANOVAs conducted on both COI and 18S datasets suggested that trawling and sampling region had significant impacts on community structure. The nMDS visualisation showed divergent regional patterns, suggesting distinct communities between regions, and varying responses to trawling within regions. This is further supported by the ASV taxonomic assignments. Although meiofauna data is only available for the Castellammare region, the results of this analysis were complementary to our eDNA findings. PERMANOVA procedures found significant differences between meiofauna communities found in untrawled and trawled sites within the Castellammare region. Furthermore, these differences extended throughout sediment layers. Although total meiofaunal abundance was not significantly different between the trawled and untrawled sites, patterns suggested that trawled communities had greater meiofauna abundance in the top five centimetres of sediment compared to untrawled communities, while untrawled had greater abundance in deeper sediment layers.

4.1. Trawling effects on community composition

Bryozoa were only detected using eDNA at untrawled sites of both Palermo and Castellamare and absent at all trawled sites suggesting that this taxonomic group may be a good indicator of absence of anthropogenic impacts. This finding is widely supported in the existing literature, as deep-sea benthic invertebrate megafauna are particularly vulnerable to benthic trawling due to their fragile structures and slow recovery times (Bergman and Van Santbrink, 2000; Williams et al., 2010; de Juan et al., 2011; Wood et al., 2012; Ingels et al., 2014; Clark et al., 2016; Yesson et al., 2017; Amisi et al., 2018). Bryozoans can act as secondary space providers, modifying the habitat and providing refuge for greater numbers of species (Cocito, 2004; Paoli et al., 2017). Therefore, damage to bryozoans is likely to have broader ecological consequences, and their absence from trawled sampling stations is indicative of anthropogenic displacement.

Based on the nMDS plots, untrawled sampling sites had a greater dispersion of community types compared to the tightly clustered trawled sites. Castellammare untrawled had the greatest dispersion on the nMDS plot, along with the largest range in ASV numbers and diversity indices. This suggests that there was greater variation in the communities found within untrawled sites and more homogenous communities at trawled sites. Repeated trawling events modify the seafloor, smoothing it with successive scraping from fishing gear (Puig et al., 2012). This reduces the habitat complexity, destroys biogenic constructors, and reduces small-scale heterogeneity, all of which represents a strong driver of biodiversity change (Gallucci et al., 2009; Van Gaever et al., 2009; Bongiorni et al., 2010; Hasemann and Soltwedel, 2011; Durden et al., 2015; Zeppilli et al., 2016). Sediment cores from untrawled survey sites have been observed to have distinct diversity patterns at scales as small as $\leq$10 cm (Gallucci et al., 2009; Hasemann and Soltwedel, 2011). Therefore, the greater dispersion of community types found in untrawled sample sites suggests fine-scale patterns of diversity within sample sites.

The meiofauna data supported the findings of the eDNA metabarcoding data in relation to trawling impacts, which led to more homogenous communities. The meiofauna nMDS showed that for the 5–10 cm and 10–15 cm layers, trawled communities were much more tightly clustered compared to those from untrawled sites. Although shallower sediment layer (0–5 cm) communities were broadly similar, trawled communities had a much higher abundance of nematodes contributing to total meiofauna abundance. At sediment depths between 5 and 15 cm, trawled communities solely comprised of nematodes compared to untrawled communities which were a mix of Nematoda, Copepoda and Gastroricha. Copepods are very sensitive to changing environmental conditions (Zeppilli et al., 2015), and so their exclusion in deeper sediment depths of trawled sites could indicate displacement from trawling.

Sediment nutrient composition, specifically lipids, had a significant interaction on meiofaunal species diversity. Sediment analyses of the trawled Castellammare region found that surface layers (0–2 cm) of trawled sites were equally rich in organic matter compared to untrawled areas (Paradis et al., 2019). In deeper layers, Castellammare sediment organic matter content from trawled sites was highly compacted, century-old material, comprised of degraded and less labile organic matter (Paradis et al., 2019). The nutritionally rich surface sediment is caused by input of fresh organic matter from the upper water column (Paradis et al., 2019). This fresh nutrient layer could be supporting a diverse meiofaunal community, which, due to the continuous reproduction strategy of taxa such as Nematoda, would be able to recolonise recently trawled sites rapidly (Vranken and Heip, 1986). However, the sudden transition into more degraded and less labile sediment would prevent a gradient of diversity across all sediment depth layers (Raghukumar et al., 2001; Sañé et al., 2013; Pusceddu et al., 2014). Our meiofauna data is closely aligned with these results, as untrawled sediment layers had consistently higher lipid concentrations and diversity scores compared to their trawled counterparts. Furthermore, the lipid concentration and the diversity score of the trawled 0–1 cm layer is comparable to that of the 0–1 and 1–3 cm untrawled layer, suggesting a nutrient rich surface layer at the trawled site. At deeper sediment layers effect of nutrients is more apparent. The trawled 1–3 cm sediment layer has a much lower lipid concentration to that of its untrawled equivalent, instead being comparable to lipid concentrations of the 5–10 cm untrawled layer. The diversity score mirrors this, with trawled 1–3 cm layer having a lower median diversity score than that of all untrawled sediment layers. Therefore, our data suggest that modifications to sediment composition as a result of chronic trawling are degrading nutrient availability for meiofaunal communities and reducing their diversity.

4.2. Regional variation

Our findings appear to add to the idiosyncratic response of meiofauna to trawling disturbance. Castellammare and Palermo showed divergent responses to trawling, specifically regarding Nematoda. At Palermo, the trawled site had substantially more ASVs assigned to Nematoda; 325 Nematoda ASVs compared to 181 at untrawled Arenella and 237 at Eleuterio. This is compared to Castellammare, where trawling appeared to reduce Nematoda ASV numbers, with the trawled site having 134 and untrawled having 199. Other phyla appear to be displaced in one sample site due to trawling but display no impact at the other; there were more than double the Platyhelminth ASVs at Palermo trawled compared to the untrawled sites (10 vs 4 vs 2), but no noticeable difference at Castellammare. Conversely, at Castellammare Mollusca ASVs were nearly halved (11 vs 6) at the trawled site but Arthropoda more than doubled (26 vs 9). The meiofauna data found that overall meiofauna diversity was lower at the trawled Castellammare site compared to the untrawled site. However, despite reducing meiofauna diversity, the taxonomy data appears to also suggest that trawling at Castellammare increases the abundance of nematodes. Trawling has been found to have no effect on, or even increase the abundance of, meiofauna compared to untrawled sites (Liu et al., 2011; Amisi et al., 2018). Other studies, however, reported a severe meiofaunal abundance drop in chronically trawled deep-sea sediments (Pusceddu et al., 2014).

One explanation for the divergent response of trawling could reside in the far greater intensity of the fishing effort in the Castellammare area

(Fig. 1), stressing the differences between highly impacted trawled areas and untrawled areas. At Castellammare, trawling intensity is 70 ha yr^{-1}, whereas Palermo trawling is limited to 20 ha yr^{-1}. With moderate levels of trawling, small polychaetes have been found to proliferate quickly, but their biomass and production fell when disturbance became more intense (Jennings et al., 2001; Jennings et al., 2002). Similarly, nematode assemblages had a significantly fewer species number, diversity and richness at sites of high trawling, but moderate trawling was insufficient to cause long-term changes (Schratzberger and Jennings, 2002). The different responses of meiofauna to trawling intensity and frequency has been attributed to meiofauna's small size, where trawling disturbance has led to resuspension in the water column rather than being killed by the fishing gear (Lampadariou et al., 2005; Costa and Netto, 2014).

A further explanation of differing responses to trawling pressure could relate to the different sedimentary settings of the two areas: open slope systems in Castellammare vs submarine canyons in Palermo. eDNA metabarcoding studies have already found this to be a driver of community composition, with comparable studies of the Mediterranean finding distinct communities between canyons and open slope habitats (Atienza et al., 2020). Based on the eDNA data, Catellammare had greater differentiation between the communities from the trawled and untrawled sites compared to Palermo, which had more overlap between trawled and untrawled sites. Castellammare sampling sites were in open slope regions and devoid of channelised fluxes (Fig. 1a). In this area, sediment resuspended by bottom trawlers is likely swept away by bottom currents and does not accumulate in the proximity of sampling locations, resulting in the trawled and untrawled sampling sites having more distinct communities.

Samples in the Gulf of Palermo were collected from three submarine canyons, whose sedimentary dynamics, mainly consisting of channelized fluxes, contrast with the Castellammare open slope sites. The Palermo canyons are in close proximity to one another, and while Arenella and Eleuterio Canyons are not subject to trawling pressure, the outer shelf regions and the slope sectors surrounding them are impacted by trawling activities (Fig. 1b). These activities are likely to cause resuspension of sediment, which is eventually funnelled along the axes of the above untrawled canyons (Paradis et al., 2021; Palanques et al., 2022). Submarine canyons are known to be preferential pathways of transport and accumulation of particulate sediment (de Stigter et al., 2011; Liu et al., 2016; Maier et al., 2019). This has been specifically suggested for the canyons of the Gulf of Palermo, where trawling activities on the outer shelf and the open slope sectors enhance sediment resuspension specifically with the boost of industrial fishing activities during the 1980s (Paradis et al., 2021). Such processes could minimize the difference of sedimentary dynamics within the trawled and untrawled sampling sites, resulting in more homogenised benthic communities across all trawling conditions at Palermo.

4.3. Methodological comparisons

The present work showed how eDNA metabarcoding detected whole community shifts in response to trawling. When we compared the eDNA datasets, the 18S dataset consistently yielded more ASVs that were successfully taxonomically assigned compared to the COI dataset (Fig. 5), however, COI data included a greater number of ASVs in total. This is supported by previous benthic marine sediment metabarcoding studies, which found similar assignment rates and ASV detections between 18S and COI primers (Guardiola et al., 2016; Clarke et al., 2017; Cordier et al., 2019; Dopheide et al., 2019; Tytgat et al., 2019; Wood et al., 2019; Atienza et al., 2020). Nematoda was severely underrepresented in the COI data; nematodes account for 90 % of meiofaunal abundance, and meiofauna account for up to 80 % of deep-sea biodiversity at 2000 m (Danovaro et al., 2010, 2013; Pusceddu et al., 2014). While Nematoda accounted for 70 % of ASVs found in our 18S dataset, they contributed to only 4.4 % of the COI data. Poor COI genetic reference databases are likely to contribute to this as they currently underrepresent the nematodes and platyhelminths (Sinniger et al., 2016). However, the absence of any nematode assignments suggests that primer bias is likely to further exacerbate this genetic underrepresentation. Nematoda were poorly represented in the initial COI primer development, with two taxa of nematode being tested, of which only one successfully amplified (Leray et al., 2013). This is important for taxonomic characterisation of deep-sea communities, as primer selection will bias the biodiversity detected, misrepresenting the proportions of different taxa, and further highlights the substantial gap in our understanding of these ecosystems. However, in comparisons between morphological identification of deep-sea nematodes and metabarcoding matches using 18S primers, results were comparable down to the order-family level (Dell'Anno et al., 2015). Furthermore, it is worth noting that both 18S and COI datasets presented comparable patterns when examining effects of trawling and sampling region on ASV numbers, diversity and evenness indexes, as well as observed MDS patterns.

Metabarcoding techniques appear to have several advantages over meiofauna sampling alone. Both 18S and COI data consistently identified a greater number of phyla present through metabarcoding compared to meiofaunal sampling, whereas meiofauna data remained constrained to the infaunal communities. Meiofaunal analysis is further constrained by the need for taxonomic experts to perform time consuming identification, experts who may only be specialised on certain groups (Danovaro et al., 2016). In addition to this, microscopic analysis has failed to detect differences in diversity or community structure between sites due low abundance of meiofauna when compared to eDNA (Kitahashi et al., 2020). By using eDNA metabarcoding, our data can better represent all organisms in the community, generating a more holistic representation of the impacts of trawling than any one visual methodology can capture. eDNA surveys are commonly conducted alongside visual methodologies, and while concordance between metabarcoding and other datasets is strong, eDNA often reveals new insights previously unseen (Everett and Park, 2018; Fediajevaite et al., 2021; Holman et al., 2021).

The limitations of our eDNA data primarily stem from a lack of reference databases, which will only improve with time. Reanalysing old metabarcoding datasets is unlikely to take the same labour hours as the initial analysis but will produce results with many more taxonomic assignments. In contrast, reanalysing meiofauna data to a greater taxonomic resolution would, at the very least, require the same person hours to complete as the samples would have to be manually taxonomically reanalysed and reclassified, assuming the sample has been preserved (Danovaro et al., 2016). Metabarcoding datasets will continue to have relevance into the future as genomic databases improve, offering potential future insights.

Questions remain regarding the distance eDNA can be transported in oceanic systems (Laroche et al., 2020). Deep-sea sediments are rich in concentrations of eDNA and represents the largest source of oceanic eDNA in the world (Dell'Anno and Danovaro, 2005; Atienza et al., 2020). This makes deep-sea sediments incredibly powerful for eDNA metabarcoding, however, if sediment is transported across sampling sites, such as it is at Palermo, it might have the effect of mixing eDNA of two separate communities. The result of this could be more homogenous communities being reported. However, as previously stated, if sediment is being transported then the two sites may have similar sedimentary dynamics and selection pressures, therefore resulting in more comparable communities. Despite this uncertainty, eDNA decay rates have been estimated to be as quick as 48 h (Holman et al., 2022), distinct communities have been detected at spatial scales as small as 5 km (O'Donnell et al., 2017; Jeunen et al., 2019), and that eDNA studies of the deep-sea sediments can identify highly heterogenous communities across sampling areas and seasons (Guardiola et al., 2016; Atienza et al., 2020). Cumulatively this evidence supports the use of sediment eDNA metabarcoding to evaluate local changes in community composition.

Non-molecular methodologies do offer advantages over current

eDNA-based methods that should not be discounted. Of the five taxa observed in the meiofauna, three (Polychaeta, Copepoda and Amphipoda) were not detected in the metabarcoding data, and act as important data to ground truth the conclusions drawn from metabarcoding which can suffer from primer biases. The meiofauna data further offered insights into the effect sediment composition, modified by trawling, had on community diversity. Furthermore, taxonomic analyses offer important abundance data. Currently eDNA metabarcoding data lacks substantial evidence to make inferences of abundance from sequence read numbers (Deiner et al., 2017), although progress is being made (Di Muri et al., 2020; Li et al., 2021; Pukk et al., 2021; Laporte et al., 2021). While we showed that whole community metabarcoding analyses are a powerful tool for assessing anthropogenic impacts, combining molecular and non-molecular methodologies remain the most holistic way to evaluate such impacts.

4.4. Applying whole community analyses

Ecological research has primarily assumed that as species richness increases, the ecosystem services, productivity and stability of that community increases in tandem (Tilman et al., 2014). However, given that eDNA derived alpha biodiversity indexes were similar across all sampling stations of this study, but community structure differed between trawling impact and across regions, it is apparent that alpha biodiversity indexes alone are not representative of all ecological patterns (Tavares et al., 2019). Instead, trait-based biodiversity assessments, which looks at the traits of the organisms within an ecosystem and attempts to assess the ecosystem services, seeks to assess how changes to a community affects its function (Tavares et al., 2019). For example, benthic trawling can alter the trophic structure of molluscan communities, as sediment plumes are more likely to adversely affect herbivorous and suspension feeder species compared to scavenging and carnivorous molluscs (Dimitriadis et al., 2014). Without knowing whether the functional role of certain specific species has been lost, conclusions that can be drawn regarding changes in ASV numbers between trawled and untrawled sites are limited. Therefore, understanding the extent to which community divergence is driven by changes in functionally different species is essential for describing the true impact trawling has on deep-sea benthic communities. While community insights into the broad impacts of trawling can still be made at the phylum level with the available data, important community differences at lower taxonomic orders might be being masked within each phylum and within the anonymous unassigned ASVs.

4.5. Conclusions

Our metabarcoding eDNA data revealed distinct deep-sea communities at both trawled and untrawled regions despite no differences in ASV numbers, species diversity or evenness between sampled areas or trawling impact. This is demonstrated by the results of our PERMANOVAs, which found significantly different communities between regions and across trawling impact. Taxonomic assignment further supported this, with bryozoan ASVs only found in untrawled sites of both regions, contributing to the well-described vulnerability of fragile sessile megafauna. The eDNA results were supported by an analysis of meiofauna samples from the Castellammare region. Meiofauna communities of Castellammare were found to be distinct both between trawling impact and across sediment depth layers, with untrawled communities maintaining greater diversity to deeper depth layers. Our analysis did not exclude the possibility of further trawling impacts being masked by poor taxonomic assignment of ASVs, due to limited deep-sea species reference databases, nor did it capture three of the five taxa reported in the meiofauna data. For taxonomy-based metabarcoding approaches to be improved in the future, gaps in reference databases need to be filled to improve the completeness of taxonomic assignment. Furthermore, meiofauna data offered valuable depth profiles of communities across sediment layers and abundance data not currently possible through eDNA analysis alone. Despite these limitations, eDNA metabarcoding was shown to be a powerful tool for assessing community responses to deep-sea benthic trawling, and thus complemented existing taxonomic methods.

Credit authorship contribution statement

M.R. and C.L.I designed the sample collection. C.L.I. collected the samples, L.E.H. conducted the laboratory work and ran the bioinformatic analyses, E.G. analysed the data, prepared all figures except Fig. 1, and wrote the first draft of the paper. A.P. provided the meiofaunal data. C.L.I and T.R. contributed Fig. 1. E.G., L.E.H. M.R. and C.L.I. substantially contributed to revised drafts.

Declaration of competing interest

The authors have no competing interests to declare.

Data availability

DeepSeaCanyonDNA (Original data) (GitHub)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114062.

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