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Exposure to virgin and marine incubated microparticles of biodegradable and conventional polymers modulates the hepatopancreas transcriptome of *Mytilus galloprovincialis*

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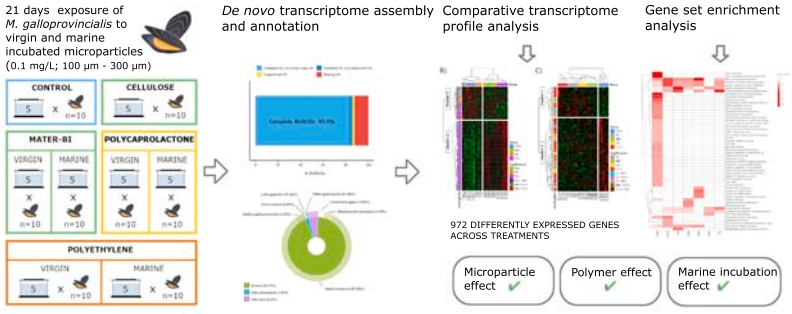
HIGHLIGHTS

- *M. galloprovincialis* hepatopancreas *de novo* transcriptome sequencing was performed.
- All microparticle (MPs) treatments significantly alter the mussel transcriptome profile.
- The marine incubation of MPs increases the transcriptional effects of all polymers.
- Biopolymers (cellulose and Mater-Bi) exposure causes lower transcriptional alteration.
- Innate immunity and lipid metabolisms processes are the most affected by MPs exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradable polymers have been proposed as an alternative to conventional plastics to mitigate the impact of marine litter, but the research investigating their toxicity is still in its infancy. This study evaluates the potential ecotoxicological effects of both virgin and marine-incubated microparticles (MPs), at environmentally relevant concentration (0.1 mg/l), made of different biodegradable polymers (Polycaprolactone, Mater-Bi, cellulose) and conventional polymers (Polyethylene) on *Mytilus galloprovincialis* by using transcriptomics. This approach is increasingly being used to assess the effects of pollutants on organisms, obtaining data on numerous biological pathways simultaneously. Whole hepatopancreas *de novo* transcriptome sequencing was performed, individuating 972 genes differentially expressed across experimental groups compared to the control. Through the comparative transcriptomic profiling emerges that the preponderant effect is attributable to the marine incubation of MPs, especially for incubated polycaprolactone (731 DEGs). Mater-Bi and cellulose alter the smallest number of genes and biological processes in the mussel hepatopancreas. All microparticles, regardless of their polymeric composition, dysregulated innate immunity, and fatty acid metabolism biological processes. These findings highlight the necessity of considering the interactions of MPs with the environmental factors in the

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marine ecosystem when performing ecotoxicological evaluations. The results obtained contribute to fill current knowledge gaps regarding the potential environmental impacts of biodegradable polymers.

1. Introduction

Marine ecosystems are particularly sensitive to the accumulation of plastic waste due to the multiple processes which can cause plastic litter dispersion from marine, coastal and land sources in the marine environment. Furthermore, the physical properties of plastics, such as their durability and buoyancy, allow them to persist in the environment for decades, if not centuries [1]. Biodegradable polymers have been recently proposed as a potential solution to the problem of plastic litter as alternatives to conventional plastics [2]. A wide range of biodegradable plastics are available on the market, mainly designed for industrial and home composting, or soil degradation (e.g. for agricultural and horticultural uses) [3]. Few efforts have focused, instead, on the use of biodegradable polymers that involve the marine ecosystem as aquaculture and fishery activities [4,5]. For instance, mussel farming industry significantly contribute to the issue of marine litter, particularly in the Mediterranean Sea, due to the dispersion of mussels nets (namely "socks") and their subsequent accumulation on beaches or on the seabed [6,7]. Only in recent years, aquaculture industry is attempting to become more sustainable, testing the possibility of using alternative biodegradable or compostable materials for mussel sock to replace traditional non-biodegradable materials, such as polypropylene (PP) or polyethylene (PE) [8,9].

Biodegradable polymers can undergo a variety of transformation routes in the environment, including biodegradation, photodegradation, and hydrolysis [10], resulting in the release of potentially harmful degradation products [11]. However, research investigating the acute and chronic toxicity of polymers is still in its infancy and contrasting results exist in the literature depending on the biological model, the tested material and the exposure parameters [12–15]. Moreover, studies on the toxicity of degradable polymers in the marine environment focused primarily on the evaluation of the effects of potential degradation byproducts, recalcitrant residues, and/or additives [16] rather than in the microparticles themselves. Venancio et al. [17] have recently reviewed the available literature on this topic, underling that the few studies on the effects on marine organisms available are limited to Polylactic acid (PLA) and effects related to the exposure to bioplastics are similar to those to polyolefins.

Thus, a comprehensive understanding of the environmental fate and behavior of biodegradable polymers, as well as their potential toxicity to marine organisms in comparison with conventional plastics and natural materials is important to promote their use and commercialization a large scale.

Amongst methodologies to detect the effects of pollution in marine organisms, the transcriptomic profiling is a comprehensive and powerful methodology which could allow to determine a wide array of effects on many biological pathways at once, efficiently providing relevant data on substance-induced molecular perturbations from the toxicological perspective, and detect a significant fraction of a pathway's response to pollutant exposure [18].

Studies on mussel exposure to several contaminants by using a transcriptomic approach have successfully shown the suitability of these organisms to understand the toxicity of ocean acidification, legacy and emerging contaminants [19,20]. Recently, transcriptomic analysis have also been applied to understand the molecular mechanism of MPs toxicity in model species, such as mice [21,22] and zebrafish [23,24]. The same approach has been successfully applied in mussels as well, detecting alterations in the expression of genes involved in apoptotic processes, immunity and detoxification pathways [25,26]. However, still very few data are available on microplastics, especially on the ecotoxicological effects of biodegradable polymers in bivalve species. Previous studies have shown that mussels, being suspension-feeding bivalves, are capable of accumulating microplastics and related toxic compounds in their tissues, which can have negative implications for the health of both the mussels and the consumers [27–29]. Moreover, mussels can serve as vectors for the transport of microplastics to other organisms higher up the food chain, further exacerbating the impacts of plastic pollution on marine ecosystems [30].

The aim of this study was to evaluate the potential ecotoxicological effects of microparticles (MPs), at environmentally relevant concentration (0.1 mg/l), made by different biodegradables polymers (polycaprolactone, Mater-Bi, cellulose) and conventional polymers (polyethylene) on *Mytilus galloprovincialis* by using a transcriptomic approach and simulating an environmentally realistic scenario.

Mussels were exposed to both virgin and marine-incubated biodegradable polymers and PE, and a comprehensive analysis was performed to investigate the entire transcriptome of the exposed individuals. This approach aims at providing a more in-depth comparative understanding of the potential ecotoxicological effects of biodegradable polymers and conventional polymers in the marine environment and also to select specific endpoints in the target species to be used in further field studies. It offers insights into the suitability of different biodegradable polymers for their use in marine applications.

2. Material and methods

2.1. Particle preparation and marine incubation

The selection process identifies four distinct polymers, each one chosen for specific purposes. These polymers are: cellulose (CE), serving as the natural control polymer, Mater-Bi (MB), a bio-based biodegradable polymer, polycaprolactone (PCL), a fossil-based biodegradable polymer, and polyethylene (PE), a conventional non-biodegradable polymer. For the scope of the ecotoxicological evaluation, all polymers' grades were selected as pure, without additives or products added during manufacturing.

Cellulose used in the present study was pure cellulose microcrystalline powder (Merck). Mater-Bi (EF51L Novamont, IT) is a biodegradable polyester-biobased polymer. It complies with the standard EN 13432 that defines requirements for packaging recoverable by means of organic recycling. The material was assessed for biodegradability at 28 °C and showed to be intrinsically biodegradable when exposed to microorganisms under mesophilic conditions. All polyesters used in Mater-Bi are made with monomers that biodegrade in soil [31]. Mater-Bi is designed to degrade in a range of environments, including marine environments, and its ecotoxicity in the marine environment has been preliminary explored [16]. Polycaprolactone (PCL) (CapaTM 6800, Perstorp, SE) is considered a biodegradable and high flexible linear polyester, having biocompatible nature with many polymeric and thermoplastics materials [32]. PCL has several uses which include tissue engineering, drug release, and packaging [33]. Despite several studies on its degradation and behavior in several matrices, its ecotoxicity in marine environment remains poorly studied [34]. Polyethylene (Lupolen 1800, Lyondell Basell, NL) is a conventional fossil-based polyolefin known to be recalcitrant to biodegradation and prone to the formation of persistent microplastics in the event of discharge in the environment [35].

Following the selection, the pellets of these chosen polymers were subjected to cryogenic grinding with liquid nitrogen and sieved using two sieves with mesh size of 100 and 300 μm, the resulting particles (size range 100 – 300 μm) were of irregular fragment shape, mimicking microplastics as one of the most commonly observed in the

environment.

In addition, to investigate the impact of microparticles after their exposure in the marine environment, 50 g of grinded PE, PCL, and MB, were placed directly below the sea surface using specially designed containers with a 100 μm mesh, retaining the particles while allowing the entry of seawater. Incubation was conducted in the Gulf of La Spezia, (44.079452 N, 9.868460 E; Italy), which is an area recognized for its thriving mollusk aquaculture activity alongside a pronounced anthropogenic port-related activity, in order to simulate the natural exposure. After a 60-day incubation period, the microparticles were stored at − 80 °C until exposure experiment and further analysis. Cellulose was also exposed in the marine environment, but the particles formed indissoluble aggregates which clogged the containers net and it was not possible to use the MPs in the laboratory experiment.

2.2. Mussels' exposure

For the exposure in the laboratory, adult mussels (*Mytilus galloprovincialis*) were collected in a mussel farm in La Spezia. To minimize potential variations in biological responses, the mussels were chosen based on a constant shell length (5.82 ± 0.43 cm). After selection, the organisms were acclimated to laboratory conditions for 10 days and then randomized between 40 aquaria (10 individuals per aquarium), each containing 10 L of synthetic sea water adequately oxygenated with aerators. Five aquaria were assigned to each experimental group, one control (CTRL) which was fed only with algal food (Coral Fito Complex Concentrate) and seven experimental groups fed with algal food supplemented with microparticles of each pure polymer free of additives (0.1 mg/l, 15.37 ± 3.70 MPs/l) both virgin and marine incubated (Fig. S1). Samples were coded as follows: Virgin Mater-Bi (MBV), incubated Mater-Bi (MBI), virgin polyethylene (PEV), incubated polyethylene (PEI), virgin polycaprolactone (PCLV), incubated polycaprolactone (PCLI), virgin cellulose (CE). Mussels were exposed to MPs for 21 days. During the experiment, the water was changed entirely every 3 days before feeding. At the end of the 21-day exposure period, the mussels were sacrificed. Morphometric data of the specimens such as total length (cm), total weight (g), shell weight (g) and soft tissue weight (g) were measured and used for the calculation of the Condition Index (CI) [36] (Table S1). The hepatopancreas of 3 individuals per each tank replicate (15 per treatment) was submerged in RNA Later and stored at − 20 °C until RNA extraction.

2.3. RNA extraction and sequencing

Total RNA was isolated from approximately 30 mg of hepatopancreas for each mussel (n = 120, 15 per treatment), using the Aurum™ Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad), following the manufacturer instructions. Potential gDNA contamination was removed by on-column DNase I digestion during the extraction process. All RNA samples were quantified using the BR Assay Kit of the Qubit 4 fluorometer (Thermo Fisher Scientific) and 4 μg of RNA from three mussels from each tank were pooled together, obtaining a total of 40 RNA pools (5 per treatment). RNA pools were quantified, and quality tested by Agilent 2100 Bioanalyzer RNA assay (Agilent technologies, Santa Clara, CA) and Caliper (PerkinElmer, Waltham, MA. The TruSeq Stranded mRNA kit (Illumina, San Diego, CA) was used for library preparation, following the manufacturer's instructions. The final libraries quality was also checked with both Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA), Agilent Bioanalyzer DNA assay and Caliper, and then sequenced in paired-end 150 bp mode on NovaSeq 6000 system (Illumina, San Diego, CA) at IGAtech (Udine, Italy). Base calling and demultiplexing was performed with the Illumina software Bcl2Fastq (ver. 2.20), adapters were masked from raw reads using Cutadapt1 (ver. 1.11; [37]). The sequencing output was analyzed using FastQC (ver. 0.11.9; [38]), checking Illumina quality metrics (per base sequence quality and per sequence quality scores), N content, GC content, adapters content and overrepresented sequences. The reads were quality trimmed removing bases below a quality score of 20 at the beginning or at the end of the reads and using a sliding window of 4 bases. Residual adapters were removed by using Trimmomatic (ver. 0.39; [39]).

2.4. Transcriptome assembly

The *M. galloprovincialis* reference genome (GCA_025277285.1), available on the National Center for Biotechnology Information (NCBI) do not include a functional annotation of transcripts, and more importantly, high genomic variability was observed between *M. galloprovincialis* populations, with approximately 20000 dispensable genes subject to presence-absence variation [40]. For these reasons, attempting to minimize mismatches with the reference transcriptome, we opted for a de novo transcriptome assembly and annotation, using sequencing data from the specimens used in this experiment, belonging to the control group (CTRL, n = 5). Sequences from the control mussels were selected for the transcriptome reconstruction, aiming to detect easily quantifiable genes both in treated and control mussels, employable as molecular biomarkers, rather than discover rarely expressed transcripts. Trimmed reads were prefiltered against ribosomal and mitochondrial sequences using bbduk (bbmap suite, ver. 35.x; Bushnell B., unpublished, available at sourceforge.net/projects/bbmap/) using sequences from *M. galloprovincialis* and *M. coruscus* as a reference. Reads from the 5 replicates were then combined and normalized using bbnorm (bbmap suite, ver. 35.x; Bushnell B., unpublished, available at sourceforge.net/projects/bbmap/), targeting a k-mer depth of 200. The transcriptome was assembled *de novo* and functionally annotated using the TransPi pipeline (ver. 1.0.0; [41]). Briefly, reads are assembled using 5 different assemblers and multiple k-mers to obtain a largely redundant trascriptome that was then reduced to a consensus, non-redundant, transcriptome using EvidentialGene [42]. ORFs were identified using Transdecoder (ver. 5.5.0; HaasBJ, unpublished, available at https://github.com/TransDecoder/TransDecoder) and Trinotate (ver. 3.2.0; [43]) was used to provide functional annotation of transcripts. The first raw assembly of 207,772 contigs was refined by removing short contigs (<200 bp), and redundant sequences using CD CD-HIT-EST (ver. 4.8.1; [44]) at 95% similarity. Finally, the reads were mapped back onto the assembly using Bowtie2 (ver. 2.4.5;[45]) with 76.99% overall alignment rate, to remove scarcely represented contigs (<5 reads aligned across all samples. The quality and completeness of the final assembly was assessed by Benchmarking Universal Single-Copy Orthologs (BUSCO ver. 5.4.3; [46]), against the Mollusca dataset and rnaQUAST (ver. 2.2.4; [47]).

2.5. Differential expression analysis and gene set enrichment analysis

The trimmed reads were mapped on the assembled transcriptome using Bowtie2. Default settings were used after testing the performance of different software preset. On average, 59.7% of the reads per sample mapped correctly on the assembled transcriptome, with no significant difference between mapping rates across treatments (Table S2). The lower mapping rate compared to the re-mapping performed during the assembly reconstruction (76.99%), is justified as the reads were filtered for mitochondrial and ribosomal RNAs, which are therefore not present in the assembled transcriptome. The samtools suite (ver. 1.16.1; [48]) was used to retrieve the mapping stats and reads counts from the alignments files. Detailed mappings stats are reported in supplementary information Table S2. The differential expression analysis was carried out using the R package DESeq2 (ver. 1.38.1; [49]) on the raw count matrix. The differentially expressed genes (DEGs) were selected based on an adjusted *p*-value threshold (false discovery rate) below 0.05. Data was elaborated and graphically represented using R studio v.2022.12.0 and packages: ggplot2 (ver.3.4.2), webr (ver. 0.1.5) and UpSetR (ver. 1.4.0), heatmaps and hierarchical clustering of DEGs were generated using the package pheatmap (ver. 1.0.12). To investigate the most

informative genes according to experimental factors, a random forest algorithm was employed using the R package randomForest (ver. 4.7–1.1). Gene set enrichment analysis of biological processes (BP) was done on the list of significantly differentially expressed genes for each treatment separately, using the R package TOPGO v2.50.0 [50], while the whole transcriptome assembly gene ontology (GO) annotation was used as background reference. The *p*-value for terms enrichment was calculated using fisher statistic and a weighted algorithm which considers the neighboring terms and their relationships [51].

2.6. Validation of RNAseq results by qRT-PCR

Among the differentially expressed genes, three were selected for PCR validation of transcriptomic results using quantitative Real Time-PCR (qRT-PCR): Tumor protein p53 inducible protein 3 (TP53I3), ATP binding cassette subfamily A member 1 (ABCA1) and Peroxisomal Biogenesis Factor 19 (PEX19). The selection was made based on DESeq2 *p*-value and number of reads mapped, targeting highly expressed genes and genes with low variance across replicates. The house-keeping gene (HK), tyrosine aminotransferase (TAT), was selected among the most stable genes across experimental conditions, ranking all transcripts by their coefficient of variation. Specific primers were designed on the sequences using the online tool Primer3web (ver. 4.1.0; [52]) paying attention to amplicon lengths, absence of secondary structures and/or primer dimers. The specificity of the primers and the correct amplicon length were confirmed by standard PCR followed by agarose gel electrophoresis. The iScript ™ gDNA Clear cDNA Synthesis Kit (Bio-Rad) was used for cDNA synthesis from 1 μg of total RNA from 3 samples per group (total n = 24), each from a different biological replicate. The primers efficiency was calculated over a five-points serial dilution curve, from 1:1 to 1:625. The oligos used, melting temperature, and efficiency are reported in Table S3. qRT-PCR was performed using the SsoAdvanced Universal SYBR Green Supermix kit (Bio-Rad) and the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad) according to the method described in Limonta et al., 2021 [53]. Gene expression was quantified according to the ΔΔCt method [54] normalizing the results over the expression of the HK gene (TAT). To compare RNAseq and qRT-PCR, fold change of expression was calculated from normalized read counts.

3. Results and discussion

3.1. Sequencing output and transcriptome assembly

The RNA sequencing yielded 30.35 ± 3.86 million (mean±sd) reads per sample. The full sequencing output is available in the NCBI Sequence Read Archive (SRA) under the BioProject ID: PRJNA1013380. On average 1.28% of the reads from each sample were removed during quality trimming, obtaining a mean sequence quality score above Q30 (Illumina quality score) for more than 98% of the generated reads. The quality assessment by FastQC is reported in Fig. S2. The final transcriptome assembly is composed by 107,294 contigs, with an average sequence length of 806.8 bp per transcript and an N50 of 1226. Among the assembled contigs, 53,478 sequences were determined to be putative protein-coding genes. Of these, 50,432 (94.3%) were functionally annotated through BlastX and BlastP of translated nucleotide sequences using the UniProtKB/Swiss-Prot and UniProtKB/TrEMBL databases. The top Blast matches were with sequences of closely related Bivalvia species (92.97%) and gastropods (1.90%), and only small portion of sequences (5.13%) were annotated from other taxa (Fig. 1 A). Species wise, the assembled sequences matched *Mytilus coruscus* (87.85%), *Mizuhpecten yassoensis* (4.38%), *Mytilus galloprovincialis* (4.09%), and *Crassostrea gigas* (3.04%) protein entries. The completeness of the assembly was evaluated using the BUSCO single-copy orthologs molluscan database, 85.9% of the BUSCO entries (n = 5295) were present in single-copy in the assembly, showing good level of representativeness for a transcriptome assembly of a non-model organism [41] and a low duplication level was observed (2.6%) (Fig. 1 B).

3.2. Differently expressed genes after MPs exposure

After 21 days of exposure to 0.1 mg/L of MPs, alterations in the hepatopancreas transcriptomes were evaluated for each MPs typology, for both virgin and marine incubated particles. The full list of differentially expressed genes (FDR<0.05) for each MPs exposure (CE, MBV, MBI, PEV, PEI, PCLV, PCLI) versus the control group (CTRL) is reported in supplementary materials (Table S4). For results interpretation and the evaluation of transcriptional effects only protein coding genes with a log2fold change of at least ± 0.5 in comparison with the control mussels were included. The number of significantly up- and down-regulated genes for each experimental condition was evaluated, providing a first

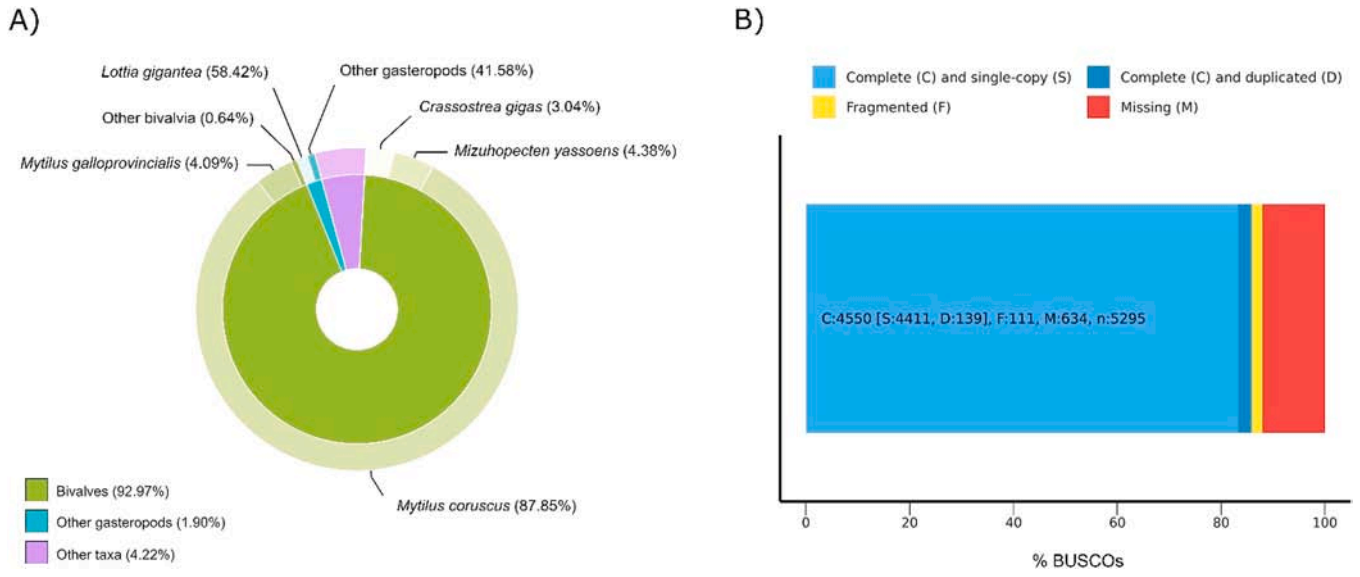

Fig. 1. Functional annotation of the *M. galloprovincialis* transcriptome assembly. A) Taxonomic classes abundance of functional annotations is reported in the inner donuts, the outer donuts represent the most represented species in each taxonomy class. B) Assembly completeness by BUSCO single-copy orthologs assessment, showing the percentage of BUSCO Terms in the Mollusca database present in the assembly.

indication of the magnitude of transcriptional alterations induced by different MPs (Fig. 2). In total, 972 genes were found differentially expressed in at least one experimental group. Polycaprolactone (PCL) MPs, both virgin (PCLV) and marine incubated (PCLI), exerted the highest effects on the mussel's digestive gland transcriptomic profile, 731 DEGs were found after exposure to PCLI MPs and 183 after exposure to PCLV MPs.

A lower number of genes compared to PCL, is modulated by Polyethylene (PE) MPs: 137 genes were differentially expressed after exposure to marine incubated PE microparticles (PEI) and 108 after virgin PE microparticles (PEV). Microparticles made of bio-based polymers, Mater-Bi and cellulose, seem to cause the least transcriptional effects in terms of number of number of DEGs: incubated Mater-Bi (MBI) modulate the expression of 102 genes, cellulose (CE) 98 genes, and virgin Mater-Bi (MBV) only 49 genes. In general, MPs incubated at sea for 60 days, affect the expression of a higher number of genes than the respective virgin particles, suggesting that particles modifications which occur during marine incubation have a direct impact on the transcriptome profile of the exposed mussels. This tendency was observed for every polymer tested, however, the increase in the DEGs number was different according to the polymer. Marine incubation of PE induced only a 1.2-fold increment in DEGs compared to the virgin PE, while for Mater-Bi the increment was of 2-fold and a striking 4-fold increase in DEGs was observed in mussels exposed to incubated polycaprolactone MPs (PCLI) in comparison to PCLV. This result suggest that PCL MPs are particularly susceptible to the incubation in the marine environment. The observed effect could be related to the adsorption of chemicals/microorganisms or to an alteration of the polymeric structure/stability due to ongoing biodegradation processes, however further investigation are needed to clarify which is the most relevant mechanism which drive these alterations. Despite PCL is recognized as a safe and biocompatible polymer when it is used for surgical implants [55], in this study, PCL, when incubated at sea for 60 days, causes strong transcriptional alteration in the exposed mussels, likely due to modifications occurring in the marine environment. Overall, the majority of the DEGs found after any MPs exposure, are down-regulated compared to the control. This is in accordance with numerous previous transcriptomic studies on MPs' effects [21–23,56]. The only exception is represented by marine incubated polycaprolactone (PCLI), which induces the up-regulation of 506 out of 731 differentially expressed genes. The high number of down-regulated genes raise also questions regarding the mechanism of gene expression dysregulation caused by MPs. Gene silencing for instance, is a common

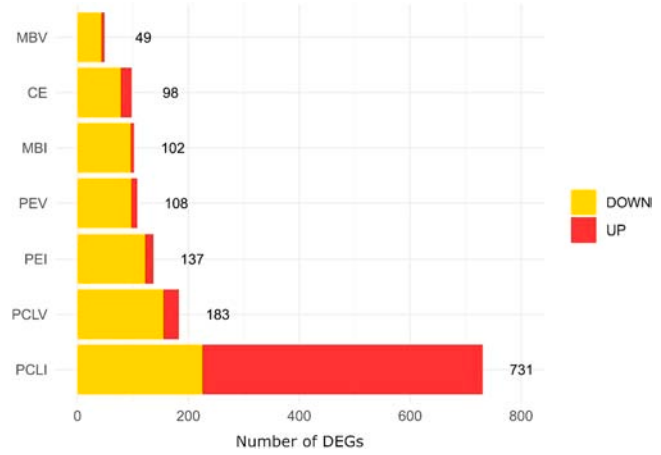

Fig. 2. Number of differentially expressed genes (DEGs) detected in each experimental condition (n = 5) compared to the control group (n = 5), up-regulated (red) and down-regulated (yellow) genes. Virgin Mater-Bi (MBV), incubated Mater-Bi (MBI), virgin polyethylene (PEV), incubated polyethylene (PEI), virgin polycaprolactone (PCLV), incubated polycaprolactone (PCLI), virgin cellulose (CE).

outcome of DNA methylation on CpG islands, although few studies have yet investigated the relationship of MPs and effects on DNA methylome, there is evidence that MPs may affect DNA methylation thus modulating the expression of methylated genes [57].

3.3. Validation of trascriptomic data by qRT-PCR

To validate the results of the RNAseq experiment, we performed a qRT-PCR analysis on 3 genes selected among the whole set of DEGs: Tumor Protein 53 (TP53), Peroxisomal Biogenesis Factor 19 (PEX19) and ATP-Binding Cassette A1 (ABCA1). The expression value obtained though qRT-PCR follows the same trend of RNAseq data across all 8 experimental groups, with a greater fold change of expression based on qRT-PCR, confirming that the data obtained with the two techniques are consistent. For detailed results of qRT-PCR and RNAseq data comparison see supplementary information Fig. S3.

3.4. Differences in the transcriptome profiles

The differences in the hepatopancreas transcriptome profile of *Mytilus galloprovincialis* after exposure to different MPs was visualized through a correlation heatmap build on variance stabilized read counts of all significantly DEGs, detected in at least one experimental condition (n = 972), using hierarchical clustering (Fig. 3 A). The results show that the 5 biological replicates of control mussels (CTRL) cluster neatly together, while the MPs exposed mussel cluster mainly according to the "Incubation" factor (virgin or marine incubated MPs). From this first analysis emerges that the effect of marine incubation is stronger than specific effect of the MPs polymer. Indeed, previous studies reported the capability of microplastics to act as vectors of contaminants due to the adsorption of contaminants having higher affinity with the plastic particles than with the surrounding water [58]. Chemicals can then be released inside the organism due to different chemical-physical conditions, potentially causing harmful effects [59,60]. Separate gene expression heatmaps were also built on virgin and marine incubated MPs treatments (Fig. 3 B, C). In mussels exposed to marine incubated MPs, samples do not clearly separate based on the polymer (Fig. 3 B). However, a similar expression profile is noticeable in all incubated MPs (Fig. 3 B), especially for mussels exposed to incubated biodegradable polymers (MBI and PCLI), that cluster tighter than those exposed to incubated polyethylene. Mater-Bi and polycaprolactone, although having different chemical composition, share some similarities, they are both biodegradable polyesters, and the degradation process could enhance the formation of biofilm furnishing additional carbon sources to microorganisms [61,62]. The increase in surface roughness can also facilitate the adsorption of contaminants [63,64]. PCL in particular, is employed in the biomedical field as a drug delivery material, and it has been reported to absorb contaminants such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals [65]. On the other hand, mussels exposed to virgin MPs, clearly groups together based on the polymer type, suggesting the presence of a specific transcriptional modulation induced by different polymers (Fig. 3 C). Overall, the most substantial differences observed in the transcriptome profiles are attributable to the marine incubation of the particles. When the "incubation" effect is removed a clear effect of the polymer type is observable. These findings highlight the importance of comprehensive ecotoxicological evaluation, including the interactions of the tested materials with the natural environment.

3.5. DEG driven by the "particle effect"

To investigate the presence of common modulated genes among different MP treatments, all DEGs detected were visualized through an UpSet plot (Fig. 4). Most of the differently expressed transcripts are significantly modulated in a treatment specific manner, while few genes are shared among different experimental conditions. Interestingly, 5

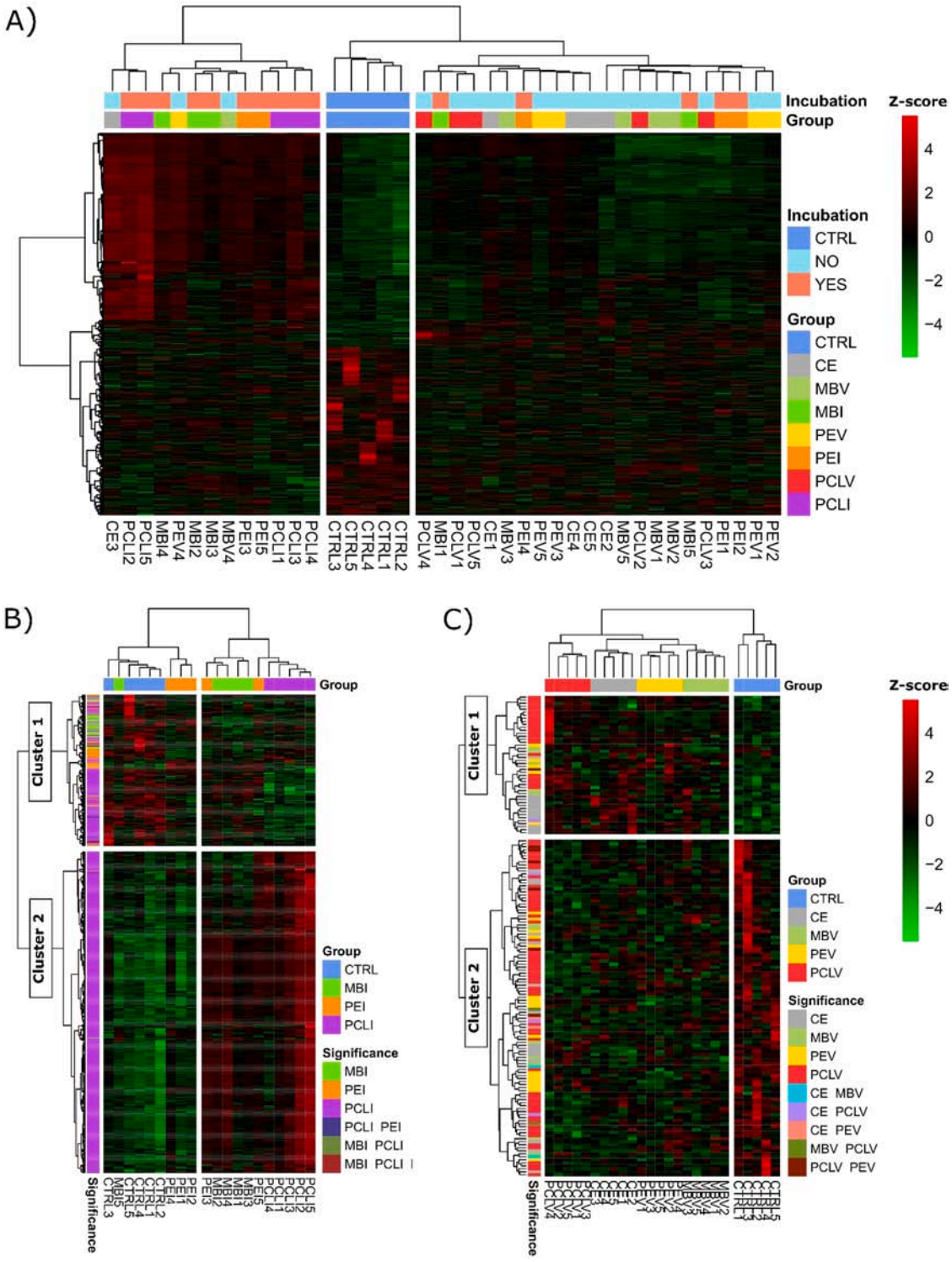

Fig. 3. Gene expression heatmaps and hierarchical clustering of normalized reads counts. A) Expression heatmap of all DEGs (FDR<0.05) found in at least one experimental group. B) Expression profile of DEGs (FDR<0.05) found in mussels exposed to marine incubated MPs. C) Expression profile of DEGs (FDR<0.05) in mussels exposed to virgin MPs.

transcripts were found differently expressed in all MP treatments, all of them are down-regulated compared to the control: cluster of differentiation 56 (CD56), Septin (SEPT), AIG1-type G protein, and 2 other transcripts only partially annotated (Zona pellucida and immunoglobulin-like domains). In mammals, the CD56 gene encodes for proteins which are specifically expressed on the surface of immune cells. CD56 is a classical marker for Natural Killer cells, although its expression has been reported on T cells and dendritic cells as well, and it is believed to have an important anti-tumor immune effect [66]. These immune cells do not have an exact correspondence in the simpler immune system of mollusks, however, an NK-like cytotoxic activity has been found in bivalve species, and molluscan immunocytes react with mouse antibodies for CD56 suggesting similar mechanisms and effectors to those present in vertebrates [67]. Besides its loose linking to natural

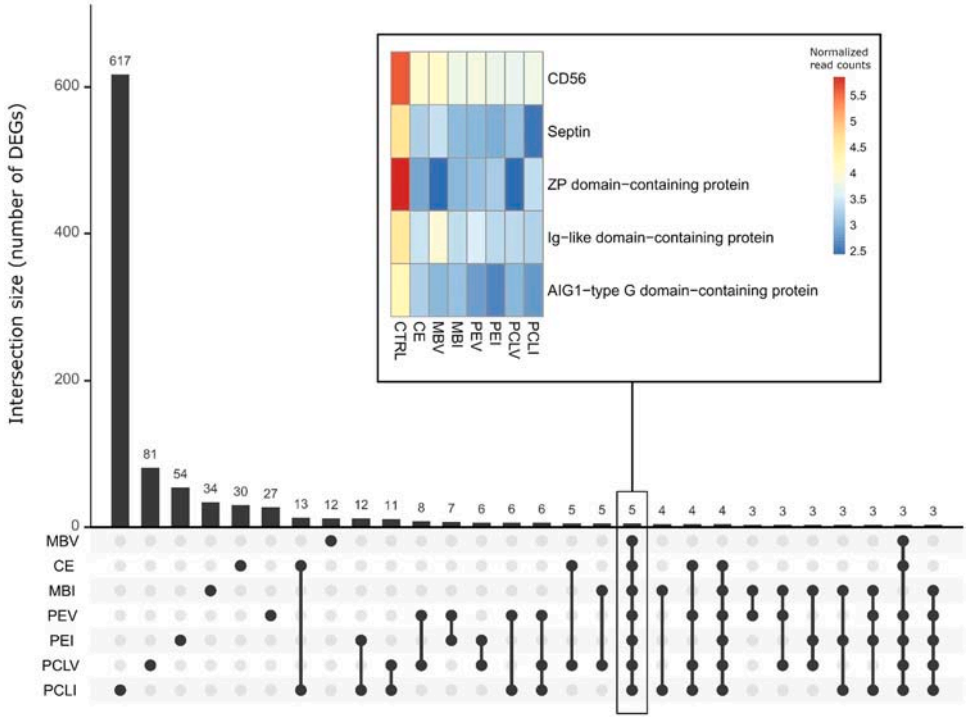

Fig. 4. UpSet plot of the number of differentially expressed genes specific for each condition (single dots) and DEGs shared between different MPs treatments (linked dots). Inside the box the expression heatmap of the DEGs shared between all MPs treatments.

immunity, further studies are needed to elucidate the role of this protein in bivalves and its relations with MPs exposure. Septins are a family of conserved GTP-binding proteins, that interact with the cytoskeleton, take part in many cellular functions such as cytokinesis and vesicles transport and were found to be implicated in the cellular-autonomous immune response as well [68]. More recently, the septins' role in the biogenesis and functioning of cilia was uncovered [69,70] linking their down-regulation with histological characteristics of the mussels digestive gland and the ciliated epithelium of the digestive tubules, a potential target of microparticles exposure. The hypothesis that septins expression may be affected by MPs is supported by another study on the bivalve *Dreissena polymorpha,* which found the protein septin-2 silenced in Polystyrene MPs exposed bivalves compared to the control [71]. The down-regulation of AIG1-type G domain containing protein further indicates that the main response to MPs is related to the immune processes, as the AIG genes (GTPase of the immunity associated protein family) in gastropods are known to be responsive to immune challenges [72]. Less information is available for the other two transcripts: ZP and Ig-like domain containing proteins. These protein families are too numerous and functionally diverse to allow a discussion on the potential biological implications of their down-regulation. The structure and sequence of these proteins should be further investigated using molecular and computational approaches, to understand their role and biological function. In summary, regardless of polymeric composition or marine incubation, MPs induce a shared transcriptional response, primarily characterized by the modulation of immune related genes, suggesting that the physical interaction between microparticles and the mussels' tissues, may be responsible for the observed effects. Shared DEGs between all MPs treatments, may also be deployed as a molecular marker of exposure to MPs to be applied in field studies, although, considering the complex multiple stressors interactions to which organisms are exposed in the natural environment, further research in this direction is necessary.

Overall, mussels exposed to Polyethylene (PE) and Polycaprolactone (PCL) MPs share the highest number of DEGs (12 between PEI-PCLI, 8 between PEV-PCLV, 6 between PCLI-PEV, 6 between PCLV-PEI, 5 between PCLI-PCLI-PEV), highlighting some similarities in the biological response to these two typologies of MPs. Among interesting dysregulated transcripts is Prostaglandin-D synthase (PGDS), which is involved in many inflammatory conditions though the arachidonic acid (AA) pathway [73], and is up-regulated in mussels exposed to PEI and PCLI. The arachidonic acid metabolism was already indicated as a target of PS-MPs in mussels and zebrafish [74,75]. PGDS expression is increased in PEV and PCLV treated mussels as well, although the alteration is not statistically significant. The lysozyme (LYS) expression was found down-regulated in PEI and PCLI treated mussels. Lysozymes are important components of the innate immune response, and one of the first cellular defense against infections, which may get activated against microorganisms adhered to the microparticle surface during their incubation at sea. Previous studies have reported the down-regulation of LYS expression after exposure to virgin microplastics, in Chinese mitten crab (*Eriocheir sinensis)* [76,77] and Pacific White Shrimp (*Litopenaeus vannamei*) [78] hepatopancreas.

3.6. DEGs driven by MPs' marine incubation

To better understand the transcriptional effect of MPs after incubation in the marine environment, a new DESeq2 model was built, including the polymer as confounding factor, and testing for "incubated" vs "virgin" MPs exposed mussels. The top 40 most significant genes based on the FDR value were extracted from the model and reported in an expression heatmap (Fig. 5).

Up-regulated genes in mussels exposed to marine incubated MPs include guanylate cyclase (GUCY), an enzyme which converts guanosine triphosphate to cyclic guanosine monophosphate. This enzyme is specifically activated by nitric oxide (NO), an important mediator and regulator of inflammatory responses, produced also by activated macrophages, in response to pathogens [79,80]. The heat shock protein 70 family protein member 12 A (HSPA12A) is also up-regulated in the incubated MPs groups, and it is known to be up-regulated by heavy metal contamination, with strong evidence indicating its protective role against lead, cadmium, copper and zinc [81–83]. Heat shock proteins

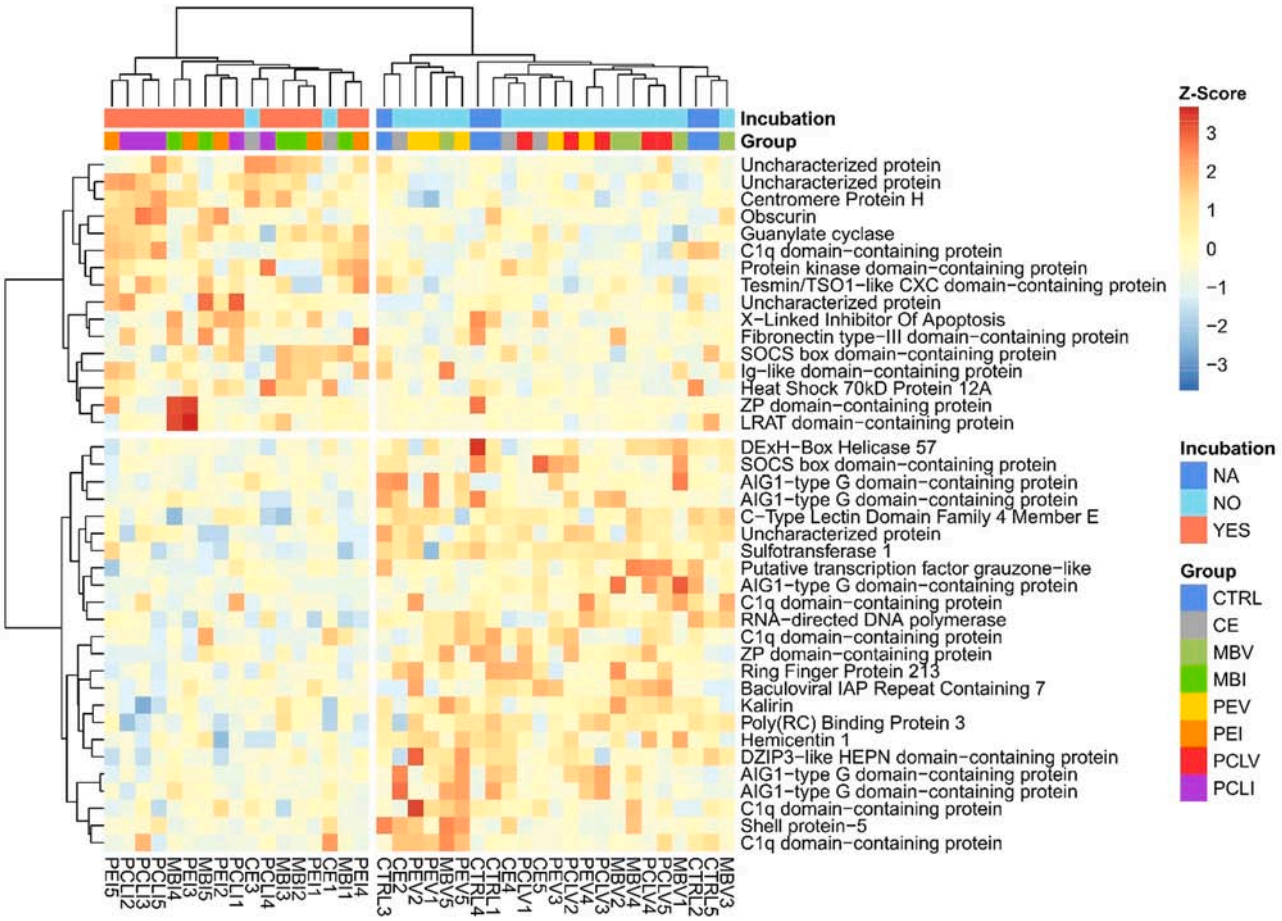

Fig. 5. Heatmap of normalized read counts for the 40 most significant genes based on FDR value, comparing mussels exposed to incubated MPs vs virgin MPs.

are molecular chaperones, that assist newly formed proteins in the folding process, and their expression can be up-regulated by oxidative and cellular stress [84]. According to several studies, nitric oxide is also strongly linked with the expression of the Heat Shock Protein 70 (HSP70), which plays a role in nitric oxide homeostasis and protects the cell from NO toxicity [85].

Among up-regulated genes is Tesmin (TSO1) also known as metallothionein like 5 protein, involved in reproductive functions, it is thought to be necessary for germinal cells genesis, but its expression is responsive to heavy metals contamination as well, such as high levels of cadmium and zinc [86]. Scarce information is available regarding its heavy metal binding capacity; however, it is possible that this protein represents another marker for heavy metal contamination associated with MPs. The up-regulation of Lecithin retinol acyltransferase (LRAT) may be linked to presence of xenobiotic substances which may have been absorbed to MPs. This enzyme is involved in the esterification of retinol to produce fatty acid esters [87] and its activity is regulated by the nuclear receptors RXR that, as other nuclear receptors, represent a major targets for endocrine disrupting chemicals [88]. A transcript annotated as shell protein 5 appears also down- regulated by exposure to incubated MPs. This protein is still poorly characterized, and further studies are needed to understand its nature and biological function. Notably, in this experiment the condition index (Table S1) of mussels exposed to incubated MPs was slightly lower than those exposed to virgin polymers, raising question on the potential adverse impact on mussel's growth by incubated MPs, which may involve the modulation of shell proteins expression.

There is very few data in the literature regarding the transcriptomic profiles of mussels exposed to marine MPs, but from our results, we can infer that the distinct alteration in the transcriptome related to the marine incubation of MPs could associated with a mixture of chemical contaminants, pathogens and/or harmful algae that can be transferred to the mussels from the marine environment using MPs as carriers [89]. Therefore, additional analysis, such as multi -omics approaches are warranted to elucidate the multiple interactions between gene expression, potential pathogens, microorganisms, and chemical contamination.

3.7. DEGs driven by MPs' polymeric composition

The detection of polymer specific effects proved challenging on the whole dataset due the preponderant effect of MPs marine incubation. For this reason, the potential effect driven by MPs' polymer was investigated only in mussels exposed to virgin particles. All DEGs detected in at least one virgin MPs treatment were used to build a random forest model to identify the most informative genes, which were included in a gene expression heatmap, where samples clearly cluster according to the MPs' polymer (Fig. 6).

Regarding PEV modulated genes, a CARD domain containing protein is significantly up-regulated in mussels exposed to virgin polyethylene (PEV); this domain, known as caspase activation and recruitment domains (CARDs), is typically present in proteins involved in apoptotic and inflammatory processes [90]. Pro-apoptotic processes have been also linked to the intraflagellar transport 57 protein (IFT57), which is also up-regulated after PEV exposure. This protein is essential for the formation of cilia, but can also bind to caspases promotor regions, regulating their transcription [91]. The up-regulation the intraflagellar transport proteins by polyethylene MPs was also reported at the protein level, in a proteomic study conducted on the bivalve *Scrobicularia plana* [92]. PEV exposed mussels are characterized by the down-regulation of

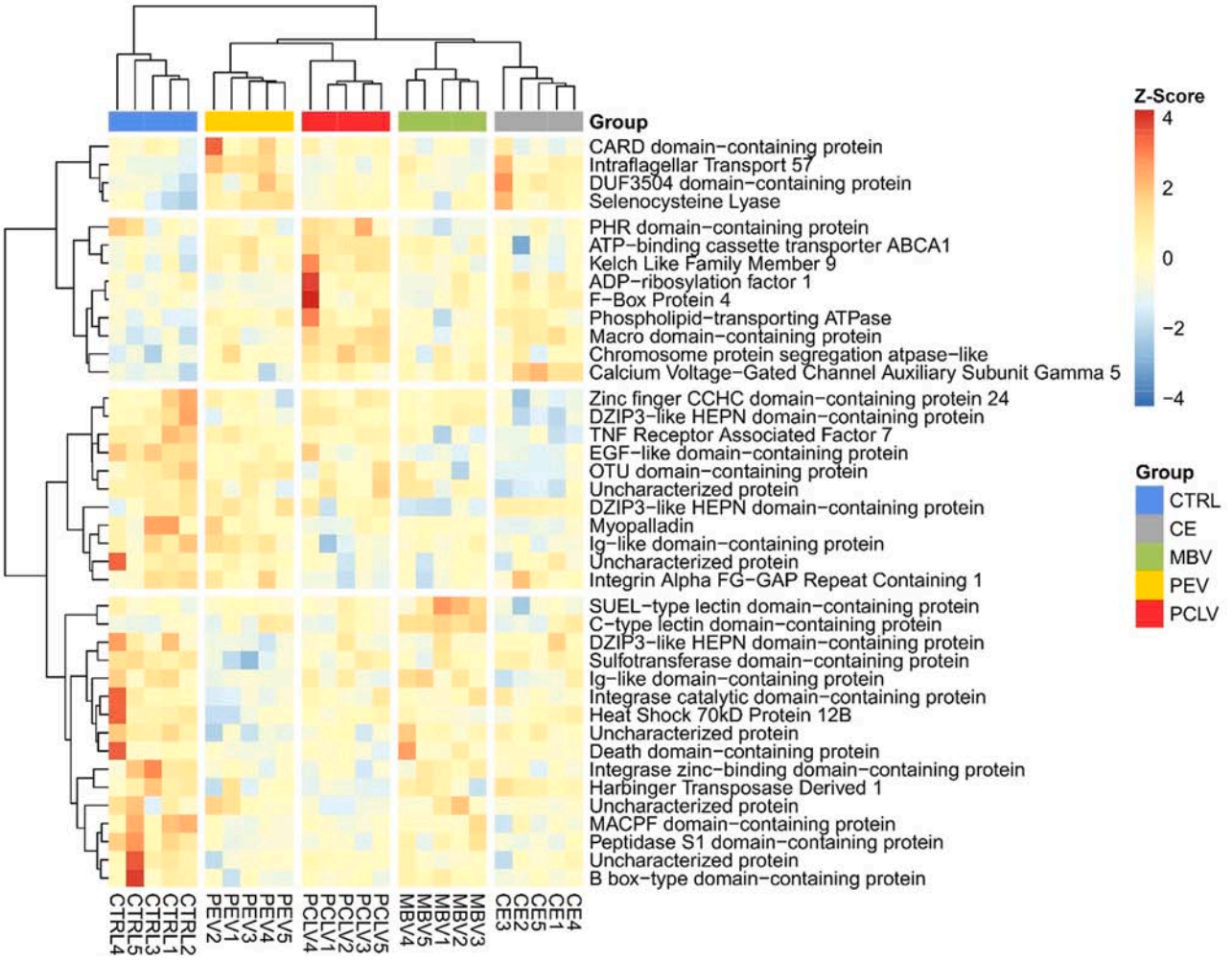

Fig. 6. Heatmap of normalized read counts for the 40 most informative genes according to differences between mussels exposed to virgin microparticles made of different polymers (CE, MBV, PCLV, PEV, CTRL).

HSPA12B, an HSP70 isoform which has a different biological role than HSPA12A, that, instead, is up-regulated in mussels exposed to incubated MPs compared to virgin particles. It has been proposed that HSPA12B may play a role in regulating macrophage inflammatory responses, exerting an inhibitory effect on the Nf-Kb signaling pathway [93]. The downregulation of HSP70 by PS and PE MPs exposure, was also reported on zebrafish [23], and *Daphnia magna* exposed to a mix of MPs [94]. In the giant river prawn (*Macrobrachium rosenbergii*) fed with polystyrene microspheres, HSP70 was up-regulated in the hepatopancreas but down-regulated in the muscle [95]. It appears unlikely that HSP70 represent a specific marker for PE, but this study confirms that microplastic mediated stress response in mussels may be linked to HSP70 isoforms gene expression and their involvement in the regulation of the inflammatory response.

Mussels exposed to MBV MPs show few strongly up-regulated transcripts, in particular proteins containing lectin domains: a SUEL-type lectin domain and C-type domain lectin containing proteins. A deeper characterization of these proteins would be interesting for further studies, as c-type lectins are known to be widely expressed in the immune system and have a role in inflammation, innate and adaptive immunity [96].

Two transcripts are specifically down-regulated in the CE group: a zinc finger CCHC domain and a DZIP3 HEPN domain containing protein. Further characterization of the modulated proteins is needed to investigate the biological meaning of these results. Interestingly, both MBV and CE are characterized by the down-regulation of TRAF7, one of the last discovered Tumor Necrosis Factors, and a recent study reported its anti-apoptotic processes, through degradation of p53 [97]. At the same time, the expression of P53 is slightly increased in MBV and in most of the MP treatments. Therefore, the reduced expression of TRAF7, may be indicative of active apoptotic processes though the TNF pathway and the activation of P53 in MP treated organisms.

The exposure to MPs in polycaprolactone (PCLV) causes the up-regulation of membrane transporters of the ABC family, ATP binding cassette A1 (ABCA1) and the up-regulation of the ATPase transporter for phospholipids. ABC transporters present a conserved nucleotide binding site, which hydrolyze ATP to activate the molecular transport. Most of the ABC transporters in eukaryotes are effluxes, actively involved in the removal of toxic and xenobiotic substances from the cell [98]. However, although some studies have linked ABCA1 with drugs and heavy metal resistance, its main function is related to lipid homeostasis and cholesterol transport [99,100]. The alteration of cholesterol and phospholipids efflux is linked to inflammation, playing a role in the triggering of inflammatory responses, accumulating for instance in macrophages and other immune cells [101] and thus suggesting the role of the cellulose MPs as inflammatory agents as well despite their natural origin.

Overall, although each polymer is characterized by a distinct transcriptome profile, most of the dysregulated gens are involved in stress and inflammatory responses, highlighting similarities in the biological response regardless of polymer type.

3.8. Gene set enrichment analysis (GSEA)

Although the analysis of the transcripts expression can offer valuable insights regarding the organism's response to MPs, it may not provide an easily interpretable picture of the biological effect. The analysis of gene ontology (GO) terms enrichment can provide a useful tool for the identification of broader biological processes significantly altered under the experimental condition studied. In this case we performed a separate GSEA analysis for each treatment with MPs which will be described in detail in the following paragraphs. The significantly enriched biological processes (p-value<0.05) were reported in a heatmap for an easier comparison between different experimental groups (Fig. 7).

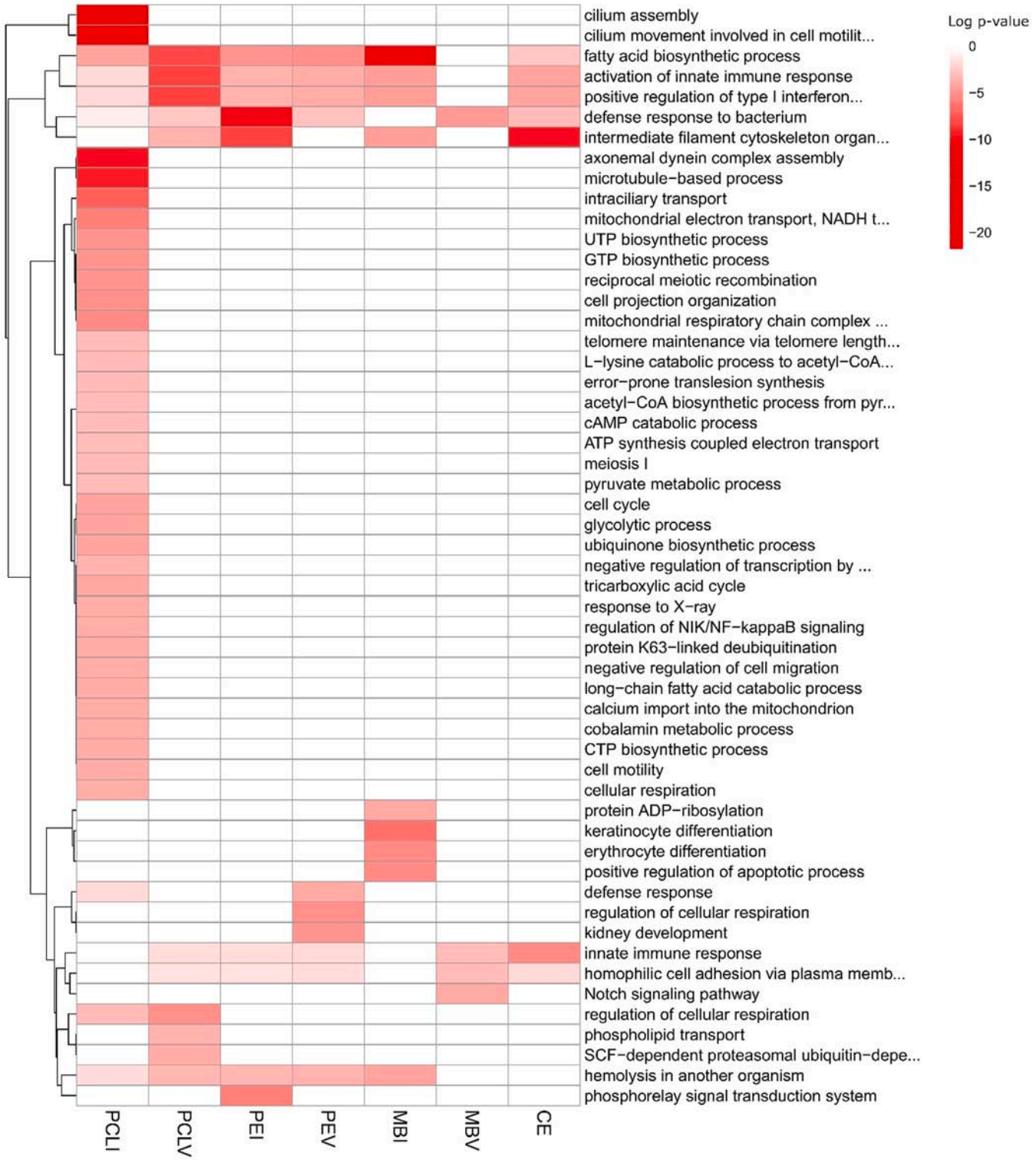

Fig. 7. Biological processes (BP) significantly enriched (p-value<0.05) in the sets of DEGs (FDR<0.05) found in each group of mussels exposed to MPs. The color scale represents the significance level (log of the Fisher's test p-value).

3.8.1. Enrichment of innate immunity processes

As already seen from the shared DEGs between all MPs treatments, GSEA results clearly indicate that mussels exposed to all MPs typologies are characterized by the alteration of a few shared biological processes. MPs regardless of the polymeric composition and marine incubation, significantly enrich GO terms connected to innate immunity, such as "activation of innate immune response" (GO:0002219) and "innate immune response" (GO:0045087) (Fig. 7). Innate immunity is the primary defense mechanism in mussels, and these immune processes include any activation of "non-specific" responses to foreign antigens and pathogen [102]. For instance, the activation of NOD (Nucleotide--binding oligomerization domain) and TLR (Toll-like receptor) signaling pathways are part of the innate immune response, leading to inflammatory reactions, production of cytokines and antimicrobial peptides [102]. One of the key mechanisms of MPs-mediated innate response involves interferon, as has been reported repeatedly in the literature, both in laboratory studies and in the wild [23,103]. However, the mechanism is not fully elucidated yet. Some studies report an inhibition of IFN production upon MPs exposure [23,103], whereas others point to IFN induction [103–105]. In this study we report the down-regulation of Stimulator of interferon genes (STING), transmembrane proteins that play an important role in innate immunity stimulating the production of Type 1 interferons [106], in all mussels exposed to MPs (apart from MBV). These proteins are also subject to an auto-inhibitory mechanism [72], which could explain the fluctuation of IFN genes expression levels reported in literature. Our results also indicate that the innate immune response to MPs is mediated by the intracellular TIR-IRAK signaling pathway. Two of the genes consistently modulated by MPs exposure are part of this pathway, namely the Toll-Interleukin 1 receptor (TIR) and the interleukin 1 receptor associated kinase 1 (IRAK1). The TIR is downregulated by MPs exposure, while the associated kinase is up-regulated (Fig. 8). When specific exogenous ligands or endogenous pro-inflammatory molecules interact with the TIR receptor, an intracellular signaling cascade is activated and through the phosphorylation of IRAK, transcription factors like NF-Kb are activated, producing cytokines, including interleukin 1 [107]. The mechanism contributes to inflammation homeostasis, prolonged exposure to pro-inflammatory cytokines can in fact lead to the down-regulation of TIR receptors. In particular, IRAK has been proposed as inhibitor of the TLR/IL-1R signaling in mammals [108].

Furthermore, anti-microbial peptides (AMPs), which are an important components of the bivalve's immune system, tend to be up-regulated in MPs exposed mussels. Up-regulated AMPs include two transcripts for lysozyme and a C-type lysozyme, as well as other peptides belonging to the defensin category: Gallicin and the antimicrobial peptide MGD2b (Fig. 8). In bivalves, these peptides play a fundamental role in the innate defense of the organism, being their main function the host defense against pathogenic microorganisms, including bacteria, viruses, fungi or other parasites. However, it has been proposed that these molecules may be involved in a broader range of biological functions, including immune regulation, angiogenesis, anti-tumor activity and tissue repair [109]. Thus, it is interesting to observe a direct relationship

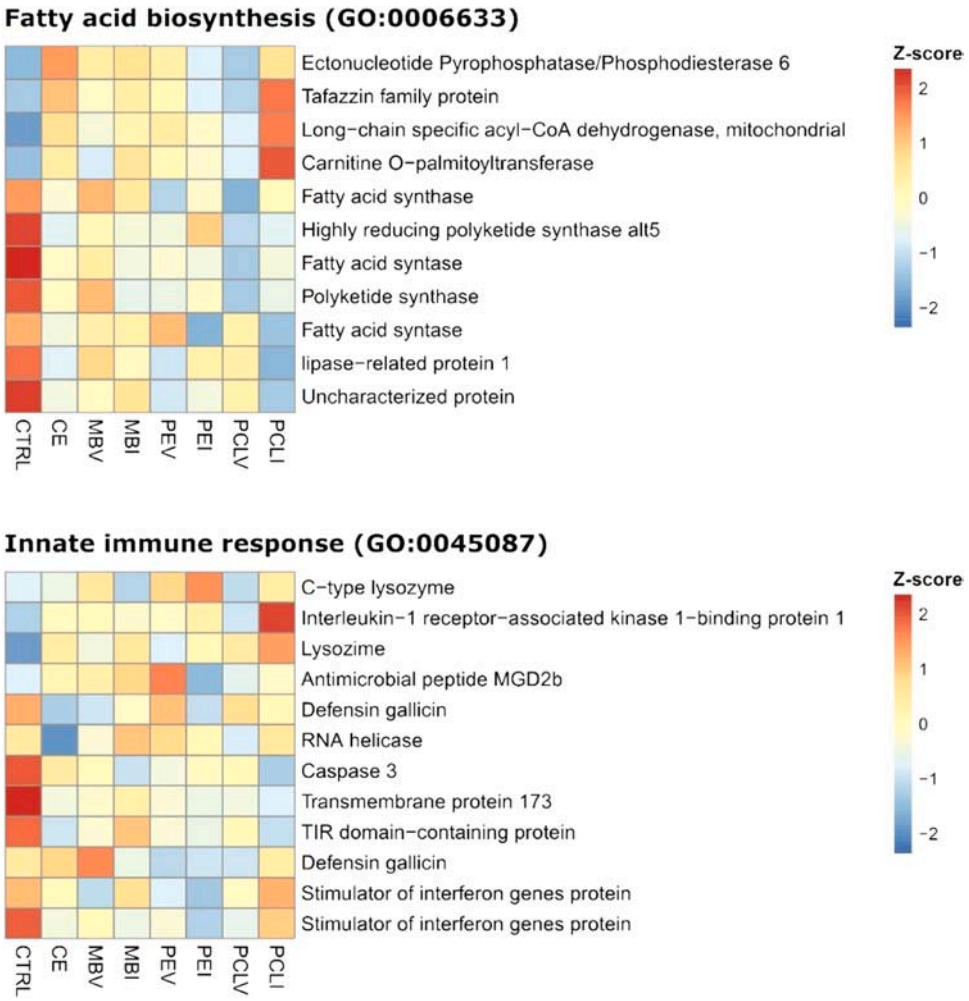

Fig. 8. Normalized expression (average expression value of 5 biological replicates) of key genes involved in significantly enriched biological processes (GO Terms) shared between all MPs treatments: innate immunity (GO:0045087) and fatty acid biosynthesis processes (GO:0006633).

between transcriptional up-regulation of AMPs and MPs exposure in mussels. Previous studies have reported the modulation of lysozyme gene expression in the crab *Eriocheir sinensis* after MPs exposure in conjunction with alterations in the microflora composition [76].

Finally, caspase 3 (CASP3) expression is down-regulated in MPs exposed mussels. This protease is one of the main executioner caspases involved in apoptotic processes [110]. The modulation of CASP3 expression levels by MPs have already been reported in the literature, confirming that this protein is part of the organism's response to MPs. A review on MPs effects in bivalves describe instances of up-regulation or no significant induction of CASP3 [111]. However, a study in conducted on *E. sinensis* reported that CASP3 expression was first increased and then significantly decreased after a long term exposure to PS microspheres [76]. The down-regulation of CASP3 mRNA levels may be due to modulation through the inflammation signaling pathway that leads to Nf-Kb activation, inhibiting CASP3 activity, while at the same time caspase-3 can inhibit innate immune response [112].

Our results suggest that the mussel's immune response to MPs is likely not depending on the polymeric composition or the chemical additives of the polymers, since both cellulose, biodegradable polymers or polyethylene cause a similar response. The effects may be more likely due to physical interactions of microparticles with the organism's tissues, or the colonization of biodegradable MPs by microorganisms or sorbed contaminants that can interact with the physiological microbiota of the mussels.

3.8.2. Enrichment of fatty acid biosynthetic processes

All MPs treatments, except for virgin Mater-BI (MBV), are characterized by the significant enrichment of the biological process "Fatty acid biosynthetic process" (GO:0006633) (Fig. 8). The effect of MPs on the lipid metabolism has been observed in multiple studies [113–115]. In this study, we identified some of the key molecular mechanisms activated by MPs exposure in mussels, leading to the alteration of fatty acid metabolic processes. Mussels exposed to MPs (especially to PCLI) show an up-regulation of the enzyme Acyl Co-A dehydrogenase (ACADs) (Fig. 8). ACADs plays a crucial role in fatty acid beta-oxidation, catalyzing the initial step of the process by removing hydrogen atoms from the acyl-CoA molecules. At the same time the fatty acid synthase (FAS) and the polyketide synthase (PKS) are down-regulated by MPs (Fig. 8). FAS is the main enzyme of fatty acid synthesis, while PKS is responsible for the synthesis of polyketides, lipid molecules whose synthesis resemble that of fatty acids. Both biosynthetic processes use acyl-CoA as substrate. More importantly, fatty acid synthesis by FAS and fatty acid oxidation by acyl-CoA dehydrogenase are tightly interconnected processes and the inter-regulation of these pathways is fundamental to maintain lipid homeostasis [116]. We can hypothesize that mussels exposed to MPs are subject to higher energy demand, due to the need to activate protective responses, in this circumstance fatty acids can undergo beta-oxidation involving acyl-CoA dehydrogenase to produce ATP. The active process of fatty acids metabolism in MPs exposed mussels is supported also by the up-regulation of Carnitine O-palmitoyltransferase (CPT) (Fig. 8), a crucial enzyme that enables the transport of fatty acids across the mitochondrial membranes, where they can undergo beta-oxidation.

3.8.3. Other significantly enriched processes

Other enriched biological processes have been identified through GSEA analysis. In mussels exposed to MBV MPs the Notch signaling pathway (GO:0007219) results significantly enriched, mainly driven by the down-regulation of E3 ubiquitin protein ligases. These enzymes are fundamental for the stability and the activity of the Notch receptors, which are modulated by unbiquination [117]. For instance, the E3 ligase can promote an ubiquination-mediated degradation of notch receptors [118], regulating the signaling pathway. It is worth mentioning that in MBV exposed mussels, some lectin-domain containing proteins are significantly up-regulated, and Notch receptors are themselves characterized by lectin domains [119]. It is therefore possible that down-regulation of Ubiquitin E3 may be linked to the up-regulation of notch receptors and activation of the Notch pathway, as was reported for polystyrene MPs in mouse organoids [120], but further studies are needed in this regard.

Exposure to incubated PCL (PCLI), induced the significant enrichment of numerous biological processes connected to cytoskeletal functions, including "cilium movement involved in cell motility" (GO:0060294), "cilium assembly" (GO:0060271), "axonemal dynein complex assembly" (GO:0070286), "microtubule-based processes" (GO:0007017), "intraciliary transport" (GO:0042073). The cytoskeleton and cilia are fundamental to provide structural support for the cell and for many vital functions such as cell motility, cell polarity, intracellular organelles organization and vesicles transport [121] and they can directly interact with MPs. Strong evidences suggest that the cytoskeleton is a regulator of cell signaling pathways [122], and it is deeply linked to gene expression and chromatin remodeling [123], controlling also cell growth and differentiation [124]. PCL is a synthetic polyester produced from ε-caprolactone derived from fossil carbon, and both the polymer itself and some of its degradation products (6-Hydroxycaproic acid, ε-caprolactone) were found to reduce offspring and increased mortality in *Daphnia magna* [125]. Given that, Incubated polycaprolactone can induce profound transcriptional alterations in cytoskeleton and cilia related genes. It must be mentioned that cilia are present in the digestive tubules of the hepatopancreas, representing the cellular structures that are exposed in the first instance to ingested microparticles. Whether the biological effect of incubated PCL is due to the ongoing biodegradation of the polymer, adsorbed chemical or leached additives is not easily understandable.

4. Conclusions

This study represents one of the first assessment of the biological effects of biodegradable MPs in comparison with conventional polymers in a marine key species. Polyethylene (PE) was chosen as the most diffused conventional polymer. Three types of biodegradable polymers were selected: Cellulose (CE), a Mater-Bi (MB) and polycaprolactone (PCL). Both virgin MPs and marine incubated MPs were used, to simulate modifications that particles could undergo in nature. The effects were evaluated through differential gene expression analysis on a *de novo* assembled and annotated *Mytilus galloprovincialis* transcriptome, allowing to obtain robust DEGs and GESA data.

The hepatopancreas RNA sequencing suggests that all types of MPs, regardless of polymeric composition or marine incubation, affect some signature biological processes. The exposure caused the enrichment of fatty acid metabolism and innate immunity processes, altering the expression of genes involved in fatty acids synthesis and metabolization, anti-microbial peptides, caspase3 and cytokines.

Through the comparative analysis of transcriptomic profiles, emerges that the preponderant effect is attributable to the marine incubation of MPs, underlining that a key factor affecting mussel response is the permanence of these particles in the environment rather than the polymer itself. The permanence at sea (60 days) cause modifications in the MPs, likely through the adsorption of chemicals and the fouling by microorganisms, leading to significant alterations in the expression profile of genes linked with nitric oxide homeostasis and the response to heavy metals and chemical compounds.

Regarding the polymer type, PCL causes the strongest transcriptional response, especially after marine incubation, altering the expression of a striking number of genes, involved in inflammation and metabolic pathways, but also cytoskeletal and cilia structure and biogenesis. Exposure to the bio-based biodegradable polymer Mater-Bi (both virgin and marine incubated) affects a fewer number of genes compared to polyethylene and polycaprolactone. In its virgin form, Mater-Bi exerts effects similar or even less than virgin cellulose MPs, with the exception of the enrichment in the Notch signaling pathway.

This study is one of the first to investigate by a transcriptomic approach the effects of several conventional and biodegradable polymers in mussels, contributing to fill the current knowledge gaps regarding the ecotoxicological effects of microparticles and biodegradable polymers in the marine environment, and offering a first indication that bio-based biodegradable polymers may represent a promising alternative to conventional polymers, especially for their use in marine application.

Environmental implication

Non-biodegradable microparticles are considered hazardous material by a large amount of scientific publications worldwide, but hazardousness of biodegradable polymers in the marine environment is an open question. The present work helps to understand their ecotoxicological effects on sentinel species by comparing the entire trascriptome of mussels exposed to environmentally relevant concentrations of both virgin and marine incubated microparticles of natural polymers (cellulose), biodegradable polymers (Polycaprolactone, Mater-Bi) and conventional polymers (polyethylene). The concentration used and the incubation of the microparticles at sea make the experiment extremely realistic. The results contribute to fill current knowledge gaps regarding the impacts of biodegradable polymers.

CRediT authorship contribution statement

Baini Matteo: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Limonta Giacomo:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Panti Cristina:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Fossi Maria Cristina:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization. **Nardi Francesco:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.133819.

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