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Nanoplastics Induce More Serious Microbiota Dysbiosis and Inflammation in the Gut of Adult Zebrafish than Microplastics

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Abstract

Microplastics (MPs) (< 5 mm) and nanoplastics (NPs) (< 100 nm) are emerging environmental pollutants and have been proved could cause a series of toxicity in aquatic organisms. In this study, the effects on gut microbiota of adult zebrafish exposed for 21 days to 10 μg/L and 1 mg/L of MPs (8 μm) and NPs (80 nm) were evaluated. We analyzed the intestinal microbial community of zebrafish using high throughput sequencing of the 16S rRNA gene V3–V4 region and also performed transcriptional profiling of the inflammation pathway related genes in the intestinal tissues. Our results showed that both spherical polystyrene MPs and NPs could induce microbiota dysbiosis in the gut of zebrafish. The flora diversity of gut microbiota significantly increased under a high concentration of NPs. At the phylum level, the abundance of Proteobacteria increased significantly and the abundance of Fusobacteria, Firmicutes and Verrucomicrobiota decreased significantly in the gut after 21-day exposure to 1 mg/L of both MPs and NPs. Furthermore, interestingly, the abundance of Actinobacteria decreased in the MPs treatment groups but increased in the NPs treatment groups. At the genus level, revealed that the relative abundance of Aeromonas significantly increased both in the MPs and NPs treatment groups. Moreover, it was observed that NPs increased mRNA levels of *il8*, *il10*, *il1β* and *tnfα* in the gut, but not in MPs exposure group, indicating that the NPs may have a more serious effect on the gut of zebrafish than MPs to induce microbiota dysbiosis and inflammation in the gut.

Keywords Microplastic · Nanoplastics · Microbiota dysbiosis · Inflammation · Zebrafish

The current statistical results show that up to 380 million tons of plastic products are produced globally per year (Nielsen et al. 2019). According to several recent studies, the presence of MPs were widely detected in seawater (Andrady 2011; Beaumont et al. 2019), estuary (Zhang et al. 2020a), bottom mud (Peng et al. 2017), soils (He et al. 2018) and freshwater lakes (Anderson et al. 2017), rivers (Wang et al. 2020), and aquaculture ponds (Lu et al. 2019). MPs were also found even in deep oceans and remote and sparsely populated areas, such as the Mariana Trench (Jamieson et al. 2019) and Tibet plateau (Ding et al. 2020). In addition, MPs can be found in almost all aquatic organisms, such as freshwater fish, marine fish, shellfish, marine turtles, grey seal and beluga whales (Ding et al. 2018; Duncan et al. 2018; Hernandez-Milian et al. 2019; Garrido Gamarro et al. 2020; Moore et al. 2020; Zhang et al. 2020b).

Microplastics (MPs) have received global concern due to its widespread contamination, hard to degrade, ingestion in aquatic organisms and the ability to cross the biological barrier (Bhagat et al. 2020). As previously reported, MPs defined as plastic particles smaller than 5 mm and nanoplastics (NPs) defined as plastic particles < 100 nm in at least one of its dimensions (Koelmans et al. 2015; Hirt and Body-Malapel 2020). Due to the inconvenient detection of

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NPs, NPs is probably the least known in the aquatic environment but potentially also the most hazardous (Koelmans et al. 2015). Previous research results show that MPs/NPs can cause a series of toxicity in aquatic organisms included developmental toxicity (Lo and Chan 2018; Malafaia et al. 2020), reproductive toxicity (Pitt et al. 2018), genotoxicity (Sokmen et al. 2020), immunotoxicity (Lei et al. 2018; Qiang and Cheng 2019), neurotoxicity toxicity (Sarasamma et al. 2020), intestinal damage (Qiao et al. 2019b; Gu et al. 2020), behavioral alteration (Limonta et al. 2019; Ma et al. 2020) and oxidative stress (Wan et al. 2019; Xia et al. 2020).

The gut microbiota is a complex and dynamic biological system composed by trillions of commensal bacteria but also including archaea, fungi, protozoa and viruses (Sekirov et al. 2010). As an integral part of organism, the microbiota plays crucial roles in host health though participating in various biological processes, such as nutrient absorption, energy metabolism and storage, drug and xenobiotic metabolism and immune responses (Jin et al. 2017; Rowland et al. 2017; Ubeda et al. 2017). In addition, gut microbial communities play crucial role for host in resistance towards environmental pollutants and some scholars claimed that the gut microbiota of aquatic organisms is a key endpoint for ecotoxicological studies (Evariste et al. 2019). Interestingly, some studies have shown that MPs could remain in the gut and serve as a distinct microbial habitat to interact with microorganisms (Grigorakis et al. 2017). So far, there have been many studies on the effects of MPs on gut microbiota. The previous results showed that the MPs could induce gut microbiota dysbiosis and inflammation both in zebrafish and mice and the size of the MPs used in these reports were all > 500 nm (Jin et al. 2018, 2019; Lu et al. 2018; Li et al. 2020). However, except two reports on large yellow croaker (*Larimichthys crocea*) (100 nm) (Gu et al. 2020) and marine medaka (*Oryzias melastigma*) (50 nm) (Kang et al. 2020), there are few studies on the effects of NPs on gut microbiota in fish. Therefore, we sought to analyze the effects of NPs (< 100 nm) on the gut microbiota in zebrafish. For this purpose, we exposed adult zebrafish to NPs (80 nm) and MPs (8 μm) under different concentrations in water for 21 days and determined whether or not they could induce microbiota dysbiosis and inflammation in the gut. The results acquired in the present study provide new information regarding NPs and MPs induced aquatic toxicity.

Materials and Methods

Polystyrene Microplastics (MPs) and nanoplastics(NPs) was obtained from Big Goose Technology Co., LTD (Tianjin, China). Particle shape and morphology of the MPs and NPs were characterized by Biological Microscope (Nikon, ECLIPSE E200) and Transmission Electron Microscope (SEM, Hitachi TEM), respectively (Fig. S1). All other reagents were of analytical grade.

The parents of the zebrafish (AB strain) were purchased from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). The zebrafish were maintained a constant temperature of 28 °C under a light: dark photoperiod of 14 h: 10 h and were fed with fairy shrimp twice a day. For fish exposure, total 120 healthy adult zebrafish (four-month-old) were bred and cultured in our own laboratory. The adult zebrafish were exposed to two different concentrations (10 μg/L and 1 mg/L) of MPs (8 μm) and NPs (80 nm) for 21 days (3 glass beakers/replicates per treatment, 8 fish per beaker, 15 beakers in total and 400 mL test solution in each beaker).

Microbial community genomic DNA was extracted from intestines using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions. The quality of DNA was checked by agarose gel electrophoresis and measuring the optical density at 260/280 nm using a NanoDrop2000 (Thermo Scientific, Wilmington, USA).The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F and 806R (Table s2) by an ABI GeneAmp® 9700 PCR thermo cycler (ABI, CA, USA). The PCRs were performed in a 20 μl final reaction volume with 29 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a single extension at 72 ℃ for 10 min, and end at 4 °C. Equal quantity of three PCR reactions per sample was further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Then, purified amplicons were sequenced using an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

After sequencing, the 16S rRNA raw sequencing reads were filtered and trimmed by Trimmomatic and merged by FLASH with the criteria as previously described (Fan and Li 2019; Fan et al. 2019). Operational taxonomic units (OTUs) were defined using 97% sequence similarity and then clustered using UPARSE (version 7.1, http://drive5.com/uparse/). Database Program (RDP) classifier (http://rdp.cme.msu.edu/) was used to assign taxonomic categories to all OTUs against the 16S rRNA database (Silva SSU132) using confidence threshold of 0.7 (Quast et al. 2012; Amato et al. 2013). To determine the abundance of communities and sequencing data of each sample, the rarefaction curves were plotted for each sample (Amato et al. 2013). Alpha diversity indexes were measured using the level of OTUs, including Chao, ACE, Shannon and Simpson were calculated using the Mothur software (Schloss et al. 2011). Principal coordinate analyses (PCoA) based on OUT level were performed using QIIME. Other analyses were visualized

with the R package software. All the data were analyzed on the free online platform of the Majorbio Cloud Platform.

Total RNA was extracted from the intestine using AG RNAex Pro Reagent (Accurate Biology, Hunan, China). All the samples were homogenized in 600μL AG RNAex Pro Reagent by using a frozen grinding mill (Jingxin, Shanghai, China). Subsequently, the RNA is extracted according to the manufacturer's recommendations. Finally, RNA samples were dissolved in total 30μL DEPC treated water and checked the quality by 1% agarose gel electrophoresis. The optical density was measured at 260/280 nm by using a VWR® mySPEC spectrophotometer (VWR, Germany). Then, approximately1μg of total RNA of each sample was transcribed to cDNA using the Evo M-MLV RT Kit with gDNA Clean for qPCR II (Accurate Biotechnology, Hunan, China) according to the instructions. Six genes related to innate immune system including *il6, il8, il10, il1β, tnfα, ifn-phi1* were detected in this experiment. The specific primers used for the real-time PCR were shown in Table s2 and the primers were referred to Qian et al. (Qian et al. 2019). The RT-qPCRs were performed using the SYBR Green Premix Pro Taq HS qPCR Kit Mix (Accurate Biotechnology, Hunan, China) and BIO-RAD CFX Connect™ Real-Time System (Bio-Rad, USA). The amplification was set at 10 μl containing 5 μl of SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Hunan, China), 0.2 μl of each of the forward and reverse primers (10 μM), 4 μl of the dilute cDNA and 0.6 μl of nuclease free water. Real-time PCR program was 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s. Each run included blank controls and cDNA controls. Each sample was performed in triplicate. The data were analyzed according to the $2^{-\Delta\Delta CT}$method (Livak and Schmittgen 2001). The β-actin was used as the internal control (Jin et al. 2018).

All statistical analyses were conducted using R package software. Differences between populations were analyzed using a one-way ANOVA. The relative expression levels of the immune-related genes analyzed by qPCR were presented as the fold increase or decrease of the fish that are exposed the MPs/NPs compared to that of the control fish. The one-way ANOVA was used to evaluate statistical difference between the exposed and control fish. The value of $p < 0.05$ was set for statistical significance and $p < 0.01$ for extreme significance.

Results and Discussion

We assessed the gut microbiota of zebrafish under MPs/NPs exposure using high throughput sequencing of the V3–V4 regions of the bacterial 16S rRNA gene through an Illumina MiSeq platform. The results showed that the total 785,503 optimized sequencing reads were obtained from 15 samples belonging to five groups (C, 81, 810, 801 and 8010) (Table S1). The Good's coverage of all samples were all > 99.8% and could represent most of the bacteria in each sample (Table S1). In addition, a total of 133 OTUs were shared among the five groups (Fig. 1).

The bacterial diversity and richness were calculated via by the Shannon and Simpson indices and the ACE and Chao indices, respectively. As shown in Fig. 2 and Table S1, the ACE and Chao indices have no significant differences between the MPs/NPs treatment groups and the control group. However, the Simpson indices increased and the Shannon indices decreased in the MPs/NPs treated group compared with the control group except the T801group (80 nm 1 mg/L). On the contrary, the Simpson indices significantly decreased and the Shannon indices significantly increased in the T801group.

The beta diversity analysis was performed to explore the changes of microbial community of gut microbiota under MPs/NPs exposure. The hierarchical clustering tree showed that with the higher exposure concentration and the smaller particle size, the farther the sample is from the control group (Fig. 3A). Additionally, the results of the PCoA analysis was consistent with the hierarchical clustering tree, the samples in the same group were also clustered closer (Fig. 3B). And,

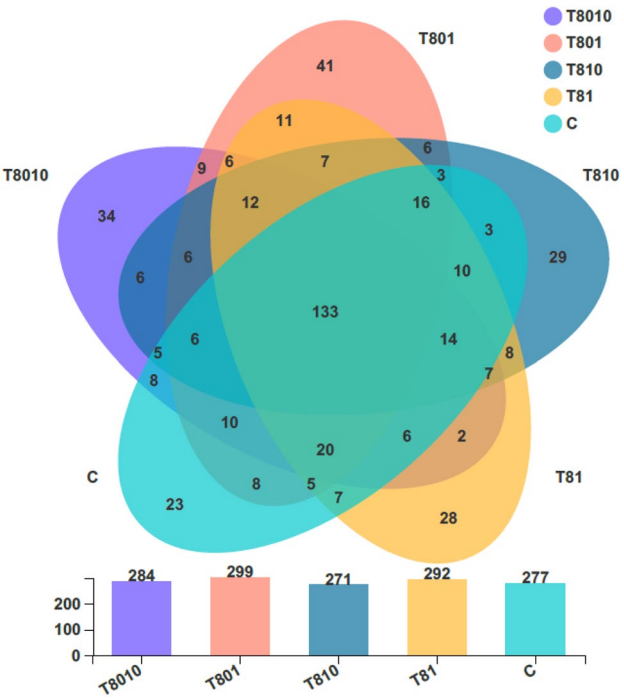

Fig. 1 Venn diagram for the comparison of the MPs/NPs exposed groups and the control group from zebrafish. C stands for the control group, T81 stands for the 1 mg/L MPs (8 μm) exposure group, T810 stands for the 10 μg/L MPs (8 μm) exposure group, T801 stands for the 1 mg/L NPs (80 nm) exposure group, T8010 stands for the 10 μg/L NPs (80 nm) exposure group

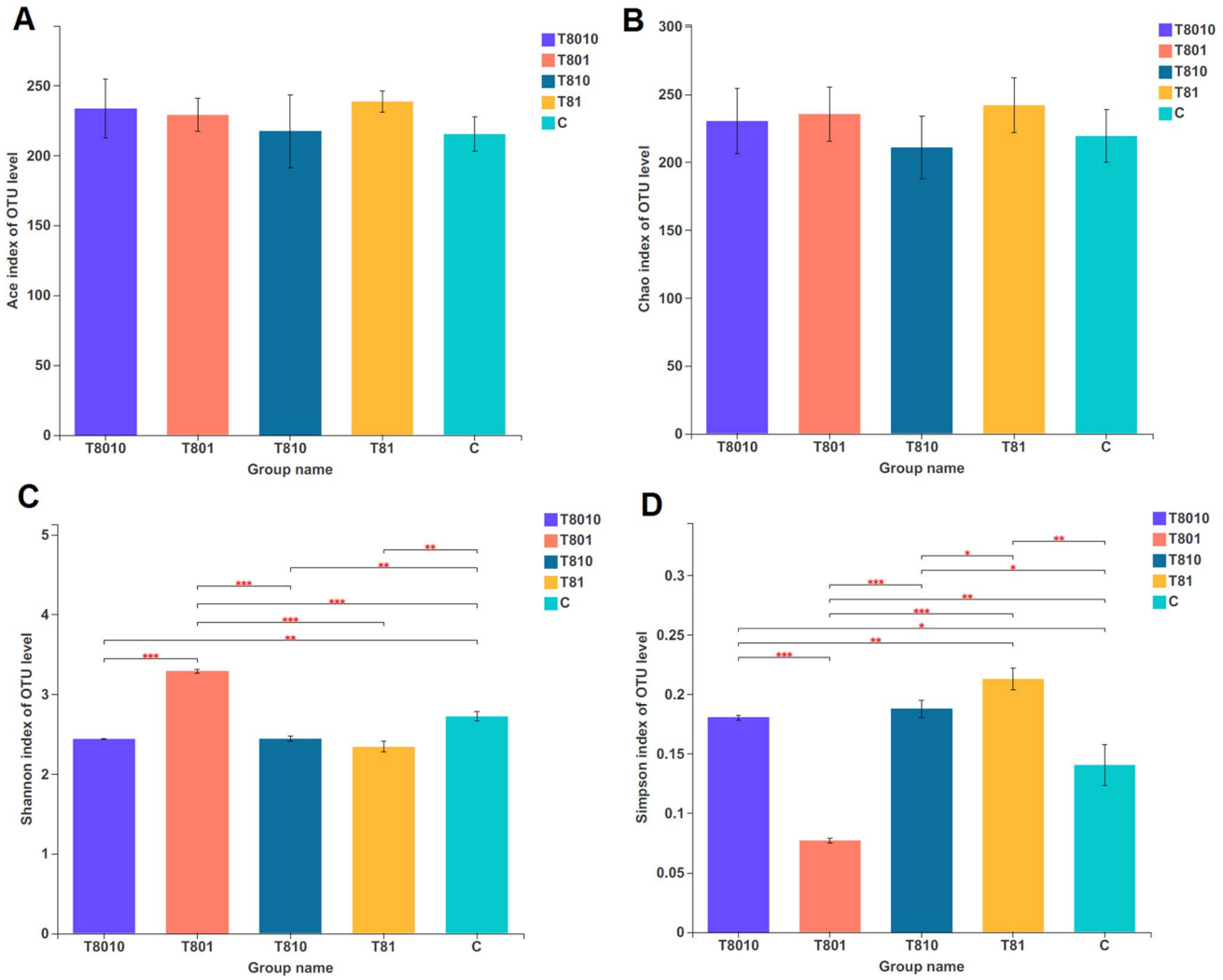

Fig. 2 Richness and diversities of bacterial species in the five groups according to the Ace (**A**), Chao (**B**), Shannon (**C**) and Simpson (**D**) indexes of OTU level. *$0.01 < p \leq 0.05$ and **$0.001 < p \leq 0.01$***$p \leq 0.001$

the higher exposure concentration treatment groups (T81 and T801) were clustered separately stay away from the control group. Moreover, two low concentration treatment groups (T8010 and T810) tended to cluster closer with the control group (Fig. 3B).

The difference of the intestinal microbiota compositions between control groups and MPs/NPs treatment groups at phylum and genus levels were showed in Fig. 4. At the phylum level, the composition of gut microbiota changed significantly after 1 mg/L 80 nm and 8 μm polystyrene NPs/MPs exposure for 21 days (Fig. 4A). Exposure to MPs/NPs (1 mg/L) increased the abundance of Proteobacteria (C 29.33%, T81 57.08% and T801 51.19%) in the intestine of zebrafish but resulted in a decrease of the abundance of Fusobacteria (C 31.02%, T81 20.72% and T801 4.47%), Firmicutes (C 15.39%, T81 8.04% and T801 10.45%) and Verrucomicrobiota (C 6.38%, T81 3.01% and T801 0.76%) (Fig. 4A and Table S2). Furthermore, interestingly, the abundance of Actinobacteria decreased in the MPs treatment groups (C 17.34%, T81 10.8% and T810 10.45%) and increased in the NPs treatment groups(C 17.34%, T801 32.46% and T801 33.82%). The results of the relative abundance of intestinal microbiota comparisons between the control groups and MPs/NPs treatment groups through one-way ANOVA were showed in Fig. S1. For Proteobacteria, its composition increased significantly in both 1 mg/L 80 nm and 8 μm polystyrene MP-treated groups. In addition, the composition of Fusobacteria, Firmicutes and Verrucomicrobiota decreased significantly in the 1 mg/L polystyrene MPs/NPs treatment groups.

Furthermore, a hierarchically clustered heatmap analysis of the top 30 abundant genera was performed (Fig. 4B and Table S3). The results showed that the relative abundance of Aeromonas significantly increased both in the MPs and

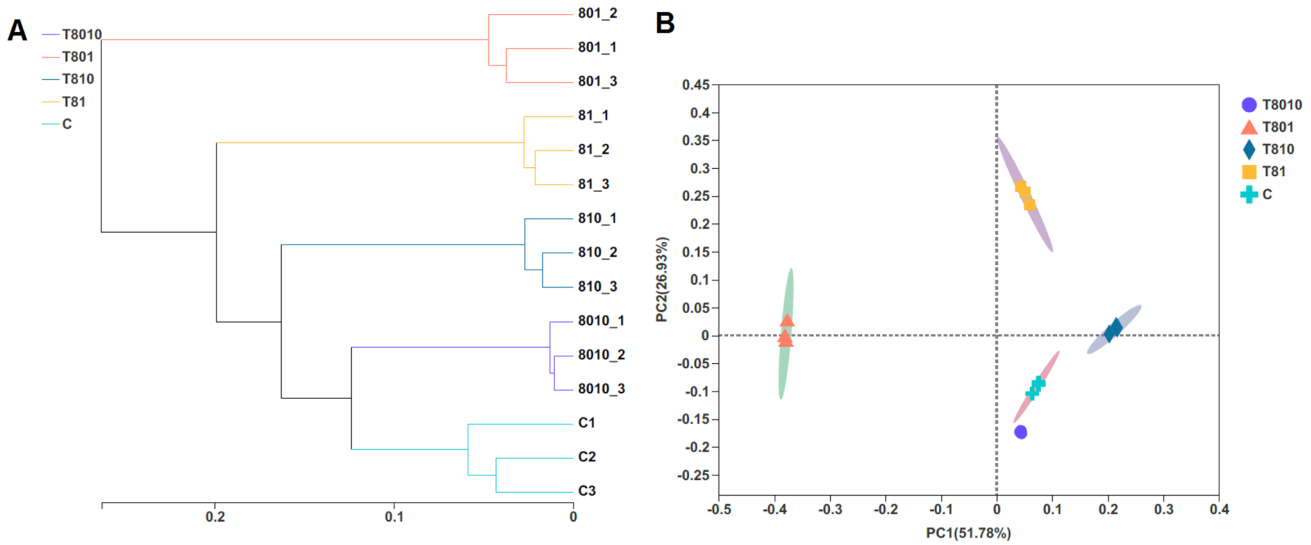

Fig. 3 Hierarchical clustering tree (**A**) and principal co-ordinates analysis (PCoA) of the bacterial community (**B**) on the OUT level. C1-3 stand for the control groups, T81 1–3 stand for the 1 mg/L MPs (8 μm) exposure groups, T810 1–3 stand for the 10 μg/L MPs (8 μm) exposure groups, T801 1–3 stand for the 1 mg/L NPs (80 nm) exposure groups, T8010 1–3 stand for the 10 μg/L NPs (80 nm) exposure groups

NPs treatment groups (Fig. 4B). In addition, the relative abundance of Cetobacterium, Protochlamydia, Legionella, Phreatobacter and Enterococcus significantly decreased in the MPs /NPs treatment groups. What's more, compared with the control group, the relative abundance of Defluviimonas, Ralstonia, Rhodobacter, Mycobacterium, Rhodococcus, Bosea and Staphylococcus were only significantly increased in the high exposure concentration NPs treatment group (T801). Moreover, the relative abundance of other two genera Lactococcus and Enterococcus was significantly increased in the low concentration exposure MPs /NPs treatment groups (T8010 and T810).

The results of the transcriptional expression of intestinal inflammation related genes, including *il6*, *il8*, *il10*, *il1β*, *ifnphi1* and *tnfα* in zebrafish were shown in Fig. 5. The transcription of *il8*, *il10*, *il1β* and *tnfα* were significantly upregulated in the 1 mg/L NPs treatment group (T801) compared to the control group in zebrafish ($p < 0.05$). Moreover, the results of the correlation between intestinal microbiota composition and immune-related genes showed that the alteration of intestinal microbiota comparisons were positively interacting with the mRNA levels of genes related to the innate immune system (Fig. 6).

Gut microbial communities are closely related to the host physiological functions as well as in resistance towards environmental pollutants (Evariste et al. 2019). Previous studies have shown that the gut microbiota was highly susceptible to environmental pollutants and could lead to alteration of host health (Chen et al. 2018). In this study, the results of the richness of the gut microbiota showed no significant difference between the NPs/MPs exposure groups and the control groups. This means that NPs/MPs exposure had no effect on gut microbial species. However, the diversity of the gut microbiota was increased significantly in zebrafish under a high concentration of NPs exposure. Similar to our results, Li et al. used the PE MPs (10–150 μm) fed each mouse 600 μg every day, Jin et al. exposed adult zebrafish in 1 mg/L of polystyrene MPs (0.5 μm and 50 μm) for 14 days and Feng et al. exposed marine medaka in polystyrene MPs (2 μm and 200 μm) for 28 days also showed an increase in intestinal microbial diversity (Jin et al. 2018; Feng et al. 2020; Li et al. 2020). The increase of the diversity of the gut microbiota indicated that the abundance of original dominant bacterial flora in zebrafish gut was reduced. With no doubt this will induce microbiota dysbiosis and due to the increase of the abundance of harmful flora. In addition, on the contrary, the diversity of the gut microbiota was decreased under a lower exposure concentration of NPs or exposed to MPs in our study. Qiao et al. and Huang et al. obtained similar results in zebrafish (50–500 μg/L MPs 5 μm) (Qiao et al. 2019b) and Juvenile guppy (*Poecilia reticulata*) (1 mg/L MPs 32–40 μm) (Huang et al. 2020). Their results showed that the evenness of Shannon even index significantly decreased under MPs exposure group. Why do different researchers get opposite results? There need more in-depth research to analyze the reasons, including the effects of MPs particle size, exposure concentration, exposure time and experimental species.

The previous studies have showed that polystyrene MPs/NPs could induce intestinal inflammation, oxidative stress,

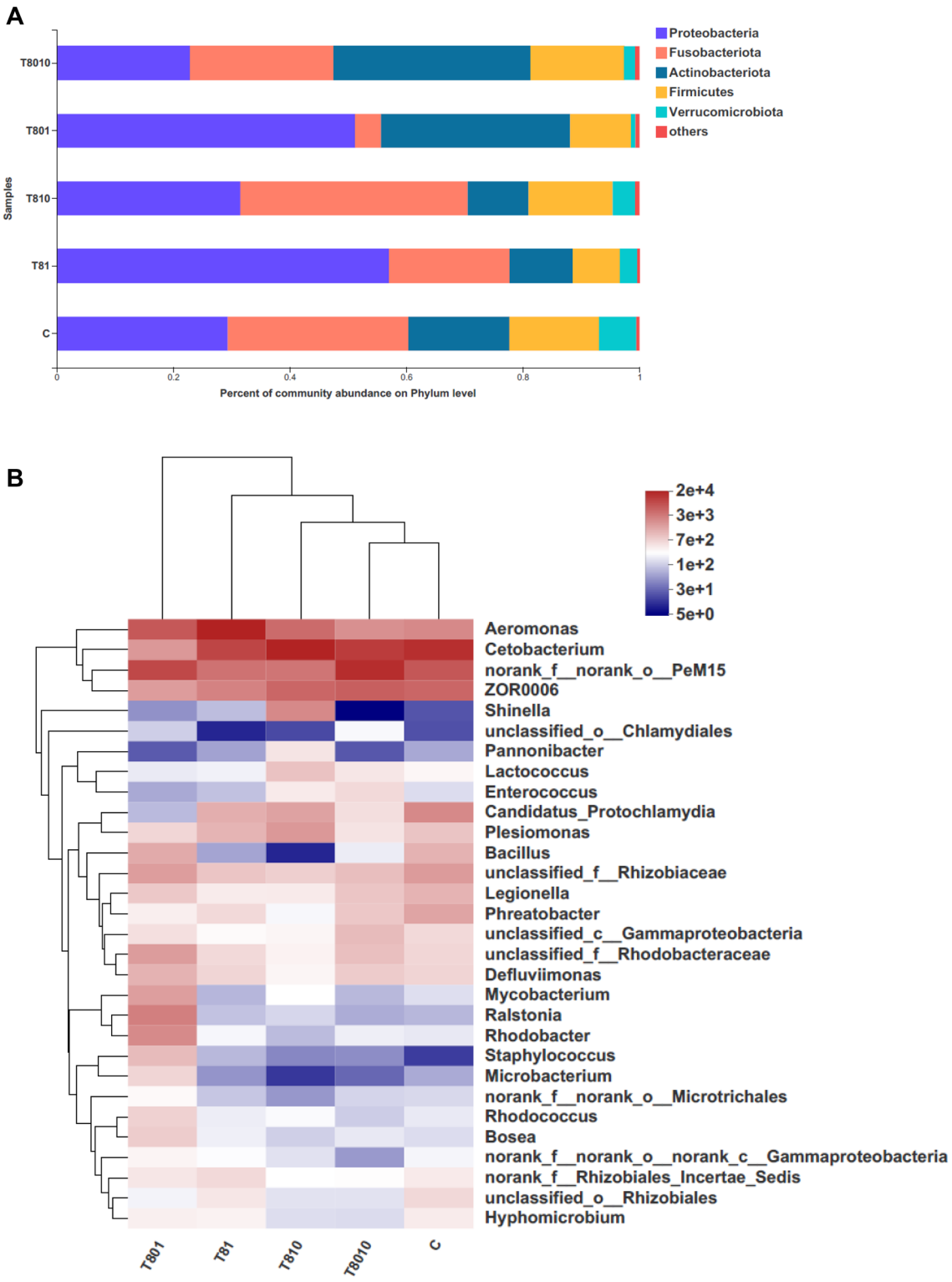

Fig. 4 Effects of polystyrene MPs/NPs on the composition of microbiota in the gut, as detected by 16S rRNA gene sequencing. (**A**) Gut microbiome composition profiles at the phylum level in the control groups and polystyrene MPs/NPs-treated groups, respectively. (**B**) Changes in the gut microbiota at the genus level; heat map of specimens showing relative abundance of the main identified bacteria at the genus taxonomic level

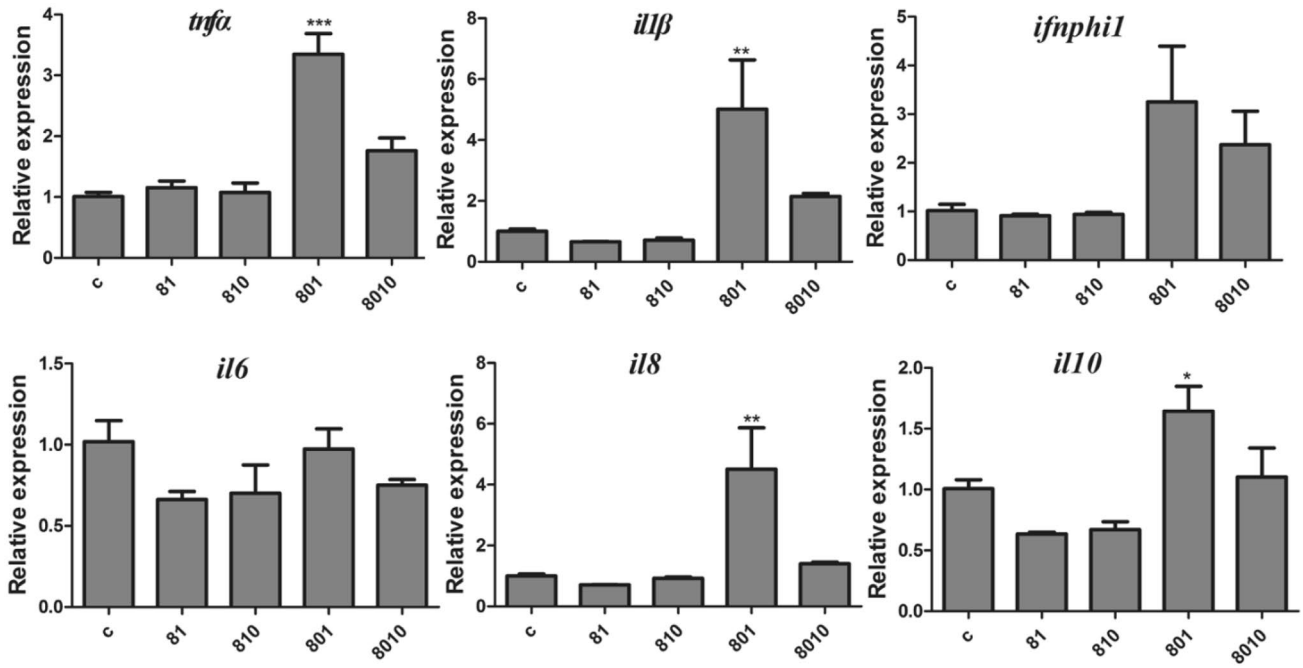

Fig. 5 Effects of polystyrene MPs/NPs exposure on the mRNA expression of several cytokines in the guts of adult zebrafish. Data are expressed as the mean ± SEM of 3 parallel samples. The asterisk represents a statistically significant difference when compared with the control. *$0.01 < p \leq 0.05$ and **$0.001 < p \leq 0.01$ ***$p \leq 0.001$

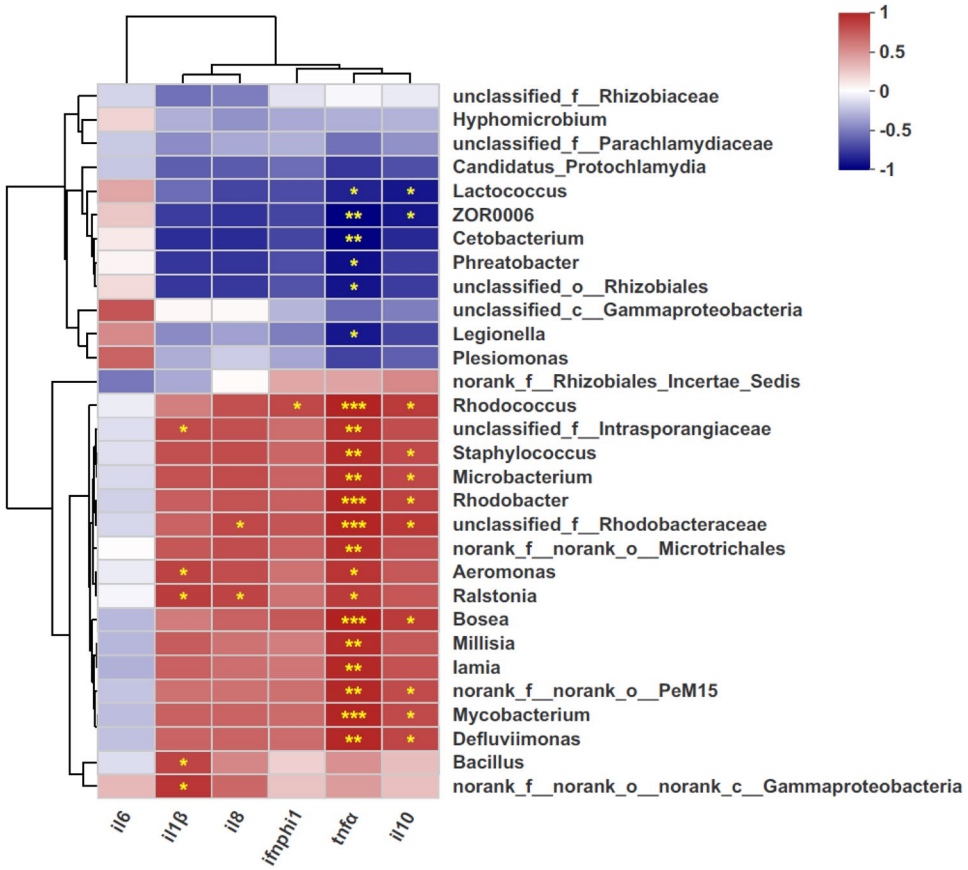

Fig. 6 Correlation between the immune defense system related genes and the relative abundance of intestinal microtia incultivation groups

and disorder of metabolism and gut microbiota in zebrafish and other species (Jin et al. 2017; Lu et al. 2018; Gu et al. 2020; Li et al. 2020). In our study, after 21 days exposure to polystyrene MPs/NPs under different concentrations, the composition of adult zebrafish gut microbiota changed at both the phylum and genus level. At phylum level, the most abundant phylum was Proteobacteria, Fusobacteria, Actinobacteria and Firmicutes in the intestine of zebrafish, and these results are consistent with our previous results in zebrafish (Xie et al. 2020). Furthermore, our data suggest that the high concentration MPs/NPs-treated groups had a substantial increase in Proteobacteria and a reduction in Fusobacteria, Firmicutes and Verrucomicrobiota. Huang et al. and Qiao et al. also got the similar results that MPs could increase the abundance of Proteobacteria, respectively in juvenile Guppy and zebrafish (Qiao et al. 2019a; Huang et al. 2020). Proteobacteria is the most dominant group in the fish gut microbiome (Tyagi et al. 2019) and an increased abundance of the bacterial phylum Proteobacteria is a microbial signature of inflammation in the gut (Shin et al. 2015). Therefore, the high concentration of polystyrene MPs/NPs could induce intestinal inflammation due to disorder the gut microbiota in zebrafish. In addition, according to a previous study, increased Firmicutes/Bacteroidetes was associated with metabolic disorders and the development of obesity (Ley et al. 2006). Therefore, these changes in relative abundance of microbiota implied that MPs exposure might affected the energy metabolism in fish, as previously reported in juvenile guppy and marine medaka (Feng et al. 2020; Huang et al. 2020). Moreover, the abundance of Actinobacteria decreased in the MPs treatment groups and increased in the NPs treatment groups. Similar to our results the polystyrene MPs (20–100 μm) induced a diminution in the Actinobacteria under 10 μg/L of MPs exposure for 21 days (Qiao et al. 2019a). A study has shown that there was an overall loss in diversity with enrichment of specific groups within the Proteobacteria and Actinobacteria between healthy and early stages of enteritis (Legrand et al. 2018). However, why the abundance of Actinobacteria increased in the NPs treatment groups, it need more research.

At the genus level, the NPs/MPs treatment increased the abundance of Aeromonas, which is one of the fish pathogens that causes several fish diseases, including enteritis, septicemia, ulcer disease, and carp erythrodermatitis (Yu et al. 2019). In addition, the relative abundance of Defluviimonas, Ralstonia, Rhodobacter, Mycobacterium, Rhodococcus, Bosea and Staphylococcus were significantly increased in the high exposure concentration NPs treatment group. Some research showed that the Staphylococcus, Ralstonia, Mycobacterium, Rhodobacter, and Rhodococcus were closely related to fish disease and inflammation (Muñoz Perdiguero et al. 2011; Hashish et al. 2018; Jin et al. 2018). Moreover, the relative abundance of Cetobacterium, Protochlamydia, Legionella, Phreatobacter and Enterococcus significantly decreased in the MPs /NPs treatment groups. Previous studies showed that some of them are probiotics in the gut (Banerjee and Ray 2017). These finding suggests that NPs/MPs exposure may increase the number of harmful bacteria and reduce the number of beneficial bacteria.

According to previous studies, analysis of the expression levels of cytokines can be considered as effective biomarkers of inflammation in zebrafish (Jin et al. 2015). To further investigate the effects of NPs/MPs exposure on the gut microbiota, six genes related to innate immune system including *il6, il8, il10, il1β, tnfα* and *ifnphi1* were detected in this experiment. Here, pro-inflammatory cytokine *il1β, il8, il10* and *tnfα* were significantly up-regulated in the 1 mg/L NPs treatment group. In general, *il1β* is produced by activated macrophages as inflammatory responses to exogenous stimuli (Kim 2004) and *il8* plays an important role as a mediator of inflammation by activating leukocytes. In addition, the *il10* inhibit the excessive production of pro-inflammatory cytokines to protect organism (Sapan et al. 2016). Similar to the *il10*, the *tnfα* is produced as a critical cytokine to activate T cells and macrophages for suppressing inflammation (Beutler and Cerami 1989). According to our results, there is no doubt that NPs activates the inflammatory response in zebrafish. Similar to our results, Jin et al. reported that 0.5 μm polystyrene MP not only increased mRNA levels of IL1α, IL1βand IFN but also their protein levels in the gut. More recently, Huang et al. reported that MPs stimulated the expression of immune cytokines (TNF-α, IFN-γ, TLR4 and IL-6) in juvenile guppy (Huang et al. 2020). Moreover, the results of the correlation between intestinal microbiota composition and immune-related genes showed that the alteration of intestinal microbiota comparisons was positively interacting with the expression of immune cytokines. To sum up, the NPs could induce microbiota dysbiosis and inflammation in the gut of adult zebrafish.

In summary, our results indicate that NPs and MPs exposure could both significantly modulate the intestinal microbial community at both the phylum and genus levels. Additionally, NPs can induce significant upregulation of the expression of the inflammation-related genes and cause intestinal inflammation in zebrafish. These results indicated that the NPs may have a more serious effect on the gut of zebrafish than MPs. Finally, we found a few difference in the intestinal bacterial composition between the NPs exposure and MPs exposure. More studies are needed to elucidate the underlying mechanism of differences in intestinal microflora composition caused by NPs and MPs.

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Authors' Contributions SX, JZ, GX, and AZ: Writing-Conceptualization, Original draft, Funding acquisition, Formal analysis, Methodology and Writing-review & editing; SX, TW, SL, and BY: Resources, Formal analysis, Data curation, Software, Visualization.

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Data Availability The datasets used and/or analyzed under the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval All protocols were in accordance with the National Institutes of Health guide for the care and use of laboratory animals and performed under ethic approval from the Animal Experiments Ethical Committee of South China Agricultural University.

Consent to Participate Not applicable (This study does not contain any individual person's data in any form).

Consent to Publish Not applicable (This study does not contain any individual person's data in any form).

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