Supplementary materials

Effects of virgin microplastics on growth, intestinal morphology and microbiota in largemouth bass (*Micropterus salmoides*)

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Supplementary materials caption:

Table S1. Weight and length information of largemouth bass before and after the experiment

Table S2. Alpha diversity of intestinal microbiota

Figure S1. Identification of materials

Figure S2. The process of making fodder contained MPs

Figure S3. Villi length of largemouth bass among each group after the trial

Figure S4. Rarefaction curves

Supporting Text 1. Specific steps of PCR reactions

Supporting Text 2: Processing of raw fastq files

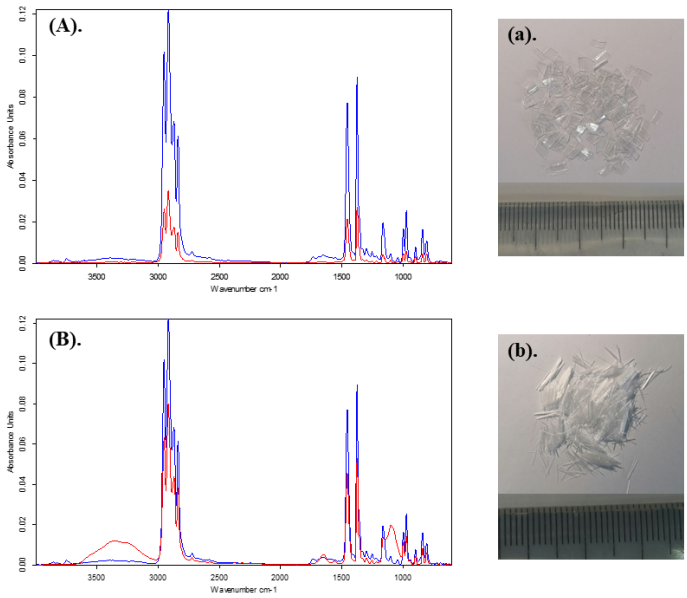
Supporting Text 3: Intestinal microbial analyses

Table S1. Weight and length information of largemouth bass before and after the experiment

	Initial weight (g)	Initial length (cm)	Final weight (g)	Final length (cm)
Group A	4.11±0.48	5.53±0.40	10.79±1.08	8.17±0.25
Group B	3.38±0.08	5.65±0.15	11.27±2.08	8.45±0.38
Group C	4.46±0.28	5.73±0.20	11.09±0.40	8.28±0.14
Group D	3.73±0.14	5.65±0.13	10.85±0.41	8.21±0.17

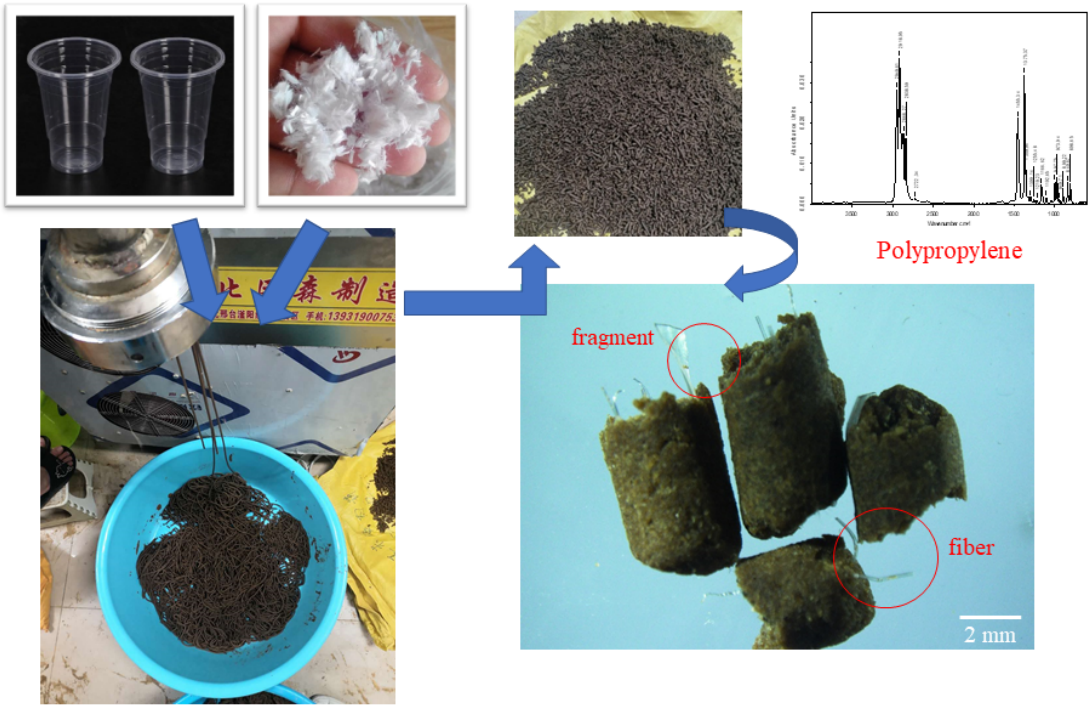
Table S2. Alpha diversity of intestinal microbiota.

Sample	Shannon	Simpson	Ace	Chao	Coverage
Group A	2.73±0.93	0.21±0.15	546.59±136.88	540.57±138.96	1
Group B	2.80±1.26	0.24±0.15	573.81±218.04	569.23±230.23	1
Group C	1.08±0.38	0.24±0.19	555.72±50.92	554.63±51.25	1
Group D	1.63±0.18	0.35±0.08	380.98±134.89	367.32±140.84	1



Compound Name: Polypropylene
Molecular Formula: C22H42O3
Molecular Weight: 354.56708
CAS Registry Number: 9003-07-0

Figure S1. Identification of materials



Supplementary Figure S2. The process of making fodder contained MPs

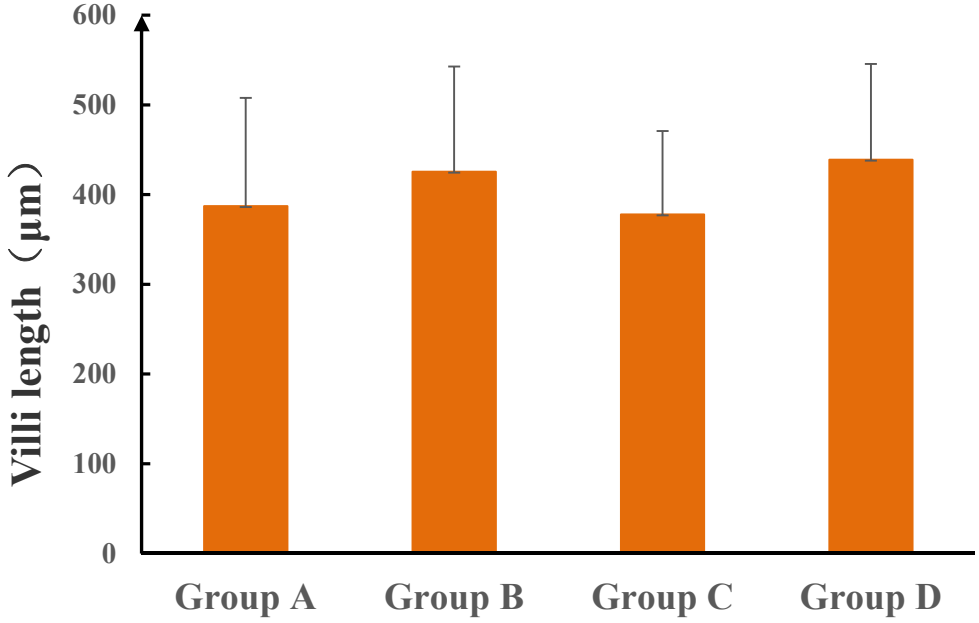


Figure S3. Villi length of largemouth bass among each group after the trial

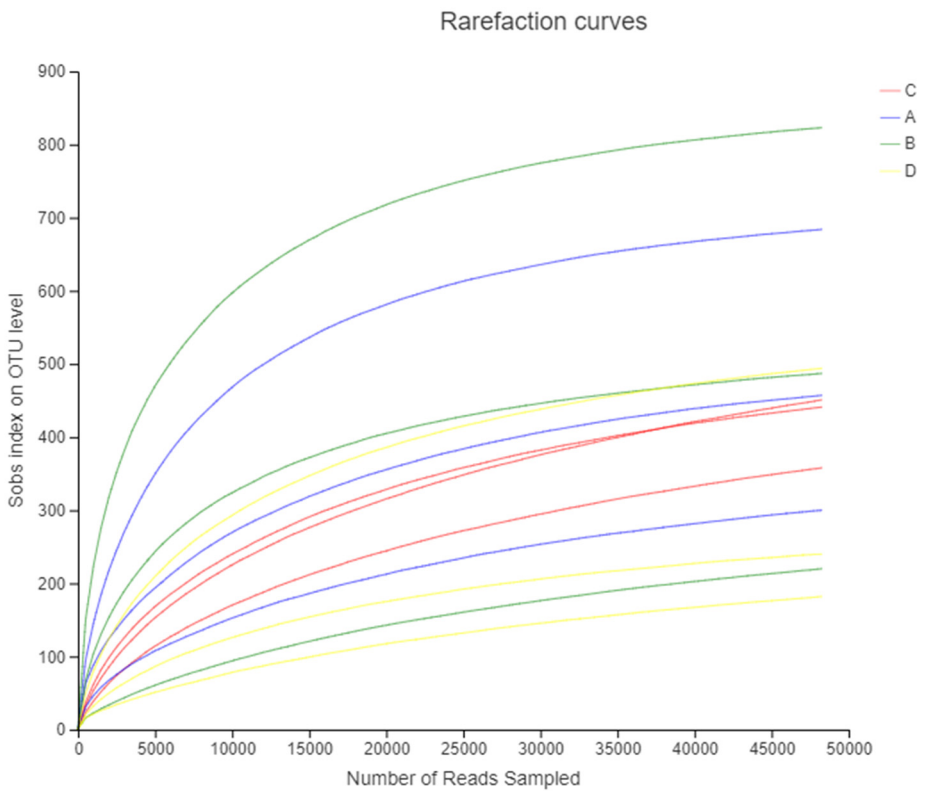


Figure S4. Rarefaction curves

Supporting Text 1. Specific steps of PCR reactions

The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles of 30 s at 95 °C, 30s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Supporting Text 2: Processing of raw fastq files

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. (iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence.

Supporting Text 3: Intestinal microbial analyses

For intestinal microbial analyses, operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (http://www.arb-silva.de) database using confidence threshold of 70%. Alpha diversity analyses were calculated using the Mothur software. Co-occurrence network analysis were calculated using Networkx software. Other analyses of intestinal microbiota were conducted using the R package.