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Negative food dilution and positive biofilm carrier effects of microplastic ingestion by *D. magna* cause tipping points at the population level☆

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ABSTRACT

Ingestion of microplastics by aquatic organisms is often harmful due to the dilution of their regular food with low-calorie microplastic particles, but can also be beneficial if nutritious biofilms are present on the microplastic surface. This begs the question: is ingestion of microplastic harmful or beneficial and can the net effect of the two mechanisms be quantified? Here, we quantified these harmful and beneficial effects on *Daphnia magna*, using dose-response tests with clean and biofouled microplastic respectively, and determined the trade-off between these counteracting effects. A population model was developed to calculate the isoclines for zero population growth, separating the regime where adverse food dilution dominated from that where the beneficial biofilm vector mechanism dominated. Our results show that the organisms grew better when exposed to biofouled microplastic compared to pristine microplastic. Very good model predictions ($R^2 = 0.868–0.991$) of the effects of biofouled microplastic were obtained based on literature parameter values, with optimization required only for the two sub-model parameters driving the dose-effect relationships for pristine microplastic. These results contradict previous sudies were only pristine microplastic were used and demonstrate that the ruling paradigm of unambiguously adverse microplastic effects is not ecologically justifiable.

1. Introduction

In recent years there is increasing scientific, public, and regulatory interest in the potential adverse effects of microplastic on aquatic ecosystems. Ingestion of low-caloric microplastic has been argued to dilute the nutritional value of food, leading to adverse effects (Burns and Boxall, 2018; de Ruijter et al., 2020). It has been emphasized recently that many microplastic effect studies lack ecological relevance (Connors et al., 2017; Koelmans et al., 2016; Phuong et al., 2016). For example, microplastic studies lack relevance in terms of the size, shape, and concentrations of tested microplastic, and lack agreement between the type of microplastic tested in the laboratory and those detected in the field (de Ruijter et al., 2020; Koelmans et al., 2020). In addition, the vast majority of studies have investigated the impact of microplastic using short-term experiments, while there is much less insight into the chronic effects of microplastics on organisms (SAPEA, 2019). Reduced feeding or food dilution due to microplastic ingestion has been proposed to be the dominant effect mechanism for microplastic (de Ruijter et al., 2020; Rauchschwalbe et al., 2021).

Several studies have also shown the presence of a microbial biofilm consisting of bacteria, algae and fungi, which form a matrix that remains on the surface of the microplastic (the plastisphere) in nature (He et al., 2022; Wright et al., 2021; Amaral-Zettler et al., 2020; Rummel et al., 2017; Kooi et al., 2017; Kaiser et al., 2017; Vroom et al., 2017), which has been shown to enhance their ingestion (Vroom et al., 2017; Zimmermann et al., 2020) and which is believed to reduce the relevance of tests that use pristine particles only (de Ruijter et al., 2020). Biofilms like those on microplastic constitute a food source for primary consumers and improve their growth (Fernandes da Silva et al., 2008; Tang et al., 2021). Moreover, biofilms are generally a food source (Siehoff et al., 2009; Horváthová and Bauchinger, 2019). This raises the question of how the effects of food dilution and nutritious biofilm on microplastic intake compare and whether the tipping points between their respective negative and positive effects can be quantified. An assessment of such

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tipping points is critical for our understanding of the resilience of ecosystems with respect to changing levels of nutrients and contaminants such as microplastics (Kong and Koelmans, 2019). However, to our best knowledge this work is the first quantitative assessment of this interaction.

Here, we aim to assess the trade-off between negative effects of microplastic ingestion and beneficial effects of microplastic-associated biofilms as food source for *Daphnia Magna* (*D. magna*) empirically, as well as by population modelling. We choose *D. Magna*, because species within this order are likely to ingest microplastic (Besseling et al., 2014; Bosker et al., 2019; Canniff and Hoang, 2018; Chen et al., 2020; Colomer et al., 2019; De Felice et al., 2019; Eltemsah and Bohn, 2019; Fueser et al., 2019; Jemec et al., 2016; Redondo-Hasselerharm et al., 2018; Rehse et al., 2016; Rist et al., 2017; Sun et al., 2019) and because of the evidence that ingestion of biofouled microplastic can have beneficial effects (Rummel et al., 2017; Bukovinszky et al., 2012; Jaikumar et al., 2019; Ogonowski et al., 2016; Scherer et al., 2017). Polyethylene (PE) irregularly shaped fragments with size ranging from 10 to 50 μm were selected to represent the fraction of environmental microplastic that is bioavailable to *D. Magna* (Koelmans et al., 2020; Xu et al., 2020). The microplastics were aged in natural water samples with different bacterial and organic load up to 3 weeks to allow the formation of a substantial biofilm, resembling realistic conditions. We then explored the effects of water type on biofilm formation and extent, as well as effects on *D. magna* growth and mortality by assessing dose-effect relationships for pristine and biofouled microplastic. Daphnids were exposed for 14 days to pristine and biofouled PE microplastic at concentrations of 10–200 mg/L. The doses were based on measured environmental concentrations in freshwater systems, which can range up to 10^8 #/L (Koelmans et al., 2019) while food conditions were tuned to those of oligotrophic waters (Ogonowski et al., 2016). Based on earlier work (Kupryianchyk et al., 2012), we developed a theoretical model to quantify the trade-offs, in terms of biomass changes, between the negative effects of microplastic ingestion and the beneficial effects of biofilm as food source on populations of *D. magna*. The model simulates population growth by incorporating dose-effect relationships for ingestion of microplastic and ingestion of food, with part of the food being biofilm from biofouled microplastic. We parametrized the model using our 14-day experimental bioassays data and plotted isoclines for zero population growth, separating microplastic concentration and food concentration combinations that support *D. Magna* population growth from the combinations that jeopardize population growth due to ingestion of microplastic.

2. Materials and Methods

2.1. Aging of microplastics

Irregular PE fragments (density 0.94 kg/L, $\leq$ 50 μm, Sigma Aldrich) were used in this study as a proxy for environmentally realistic microplastic. Full microplastic characterization is provided as Supplementary data, SD (Fig. S1 and Table S1). Prior to the aging experiments and bioassays, microplastics were washed with methanol (three 1h-cycles, under orbital shaking), rinsed with deionized water (DW), filtered with a 10 μm mesh, and finally dried for 2 day at room temperature (Redondo-Hasselerharm et al., 2018). Biofouled microplastics were obtained by adding the preconditioned microplastics (70 mg/L) into 2 L glass beakers containing natural waters collected along an urban-rural gradient (river downstream, river upstream, effluent and influent of a municipal WWTP) in Arnhem (Nederrijn river, Netherlands). The beakers were covered and incubated at 25 °C, under 18/6 light cycles for 1, 2, and 3 weeks, respectively. Sterile DW was used as negative control for biofilm formation. The incubation experiments were conducted in triplicate. After completing the aging assays, the microplastics were retrieved from the beakers using a 10 μm mesh, gently rinsed with DW to remove planktonic cells and re-suspended in 100 mL of DW. The mesh was weighted before and after filtration, in order to verify the real exposure concentration of microplastic. Prior to bioassays and biofilm characterization, microplastic suspensions were gently sonicated (BRANSON Digital Sonifier®) for 10 s to disperse possible aggregates (Fig. S1-panel D). Only cotton lab coats were used and all materials (instruments, vessels, work surfaces) were thoroughly cleaned in order to avoid microplastic contamination (de Ruijter et al., 2020).

2.2. Microplastic characteristics and water quality data

Biofilm formation was quantified by optical density measurements of bacteria, algae and organic substances at OD_{600}, OD_{750} and OD_{260}, respectively, using an UV/Vis spectrophotometer (UV-1800 Schimadzu) (Stevenson et al., 2016; Trabelsi et al., 2009). The optical density of pristine microplastic was used as background for the calculations. Confocal laser microscopy (CLSM, Zeiss LSM 510 microscope) was performed to visualize the formation of biofilms. The cell bodies were observed with FilmTracer FM 1–43 Green Biofilm Cell Stain at $\lambda_{em/ex}$ of 472/580 nm. The extracellular polymeric substances (EPS) were examined using FilmTracer SYPRO Ruby (Molecular Probes, Invitrogen) at $\lambda_{em/ex} = 450/610$ nm. Main water quality parameters were examined for all water matrices before and after the aging experiments. pH and conductivity were determined with a multimeter (Orion Star A329, Thermo Scientific). Total organic carbon (TOC) was measured using a Shimadzu TOC-VCSH analyser.

2.3. Bioassays

D. magna specimens were supplied by the Laboratory of Aquatic Ecology and Water Quality Management, Wageningen University (The Netherlands). The organisms were cultured in standard medium (11.76 g $CaCl_2 2H_2O$/L; 4.93 g MgSO4 $7H_2O$/L; 2.59 g $NaHCO_3$/L and 0.23 g KCl/L), at 20–22 °C, and 16:8 h light photoperiod for10 days (Canniff and Hoang, 2018; Ogonowski et al., 2016; Scherer et al., 2017). Semi-sterile cultured *Scenedesmus obliquus* algae were used to feed the daphnids. The chronic toxicity test with microplastic followed previously published procedures with some modifications (Canniff and Hoang, 2018; Rehse et al., 2016; Ogonowski et al., 2016). Three replicates, with 5 daphnids per replicate, were each exposed to 10, 25, 50, 100, or 200 mg/L (8.54×10^5, 2.13×10^6, 4.27×10^6, 8.57×10^6, 1.71×10^7 #/L) of pristine (maintained in DW) and biofouled microplastic suspended in 25 mL of medium in glass tubes. In addition, tubes containing only nutritive medium were used as zero microplastic treatments. The medium was not renewed during microplastic exposure. The test tubes were maintained at same conditions as used for culturing. The organisms were fed with a constant amount (0.0125 mg-C/L per day) of algae, to avoid force-feeding of microplastic. Following Ogonowski et al. (2016), this food level, which is intentionally lower compared to standard guidelines, was used to mimic oligotrophic systems containing high concentrations of inorganic particles and microplastics. These test conditions simulate the environmentally relevant "worst case scenario" needed to investigate the hypothesized trade-off mechanism (Ogonowski et al., 2016; Enserink et al., 1995). Note, the concentration of food used was below the incipient limiting concentration (Ogonowski et al., 2016).

Microplastic suspensions were very gently homogenised on a daily basis with a 5 mL tip pipette. Furthermore, continous aeration caused gentle mixing of the suspentions. Daphnids were actively swimming and grazing during exposure therewith compensating for small remaining inhomogeneities, if any. For 14 days, each testing tube was checked for

immobilization and the numbers of mobile organisms were counted. According to the standard operating protocol, individuals that settled on the bottom of the test tube and were non-responsive 15 s after gentle movement were considered as immobilized (OECD, 1984). In an additional experiment, microplastic ingestion was qualitatively visualized by digital microscopy (Nikon Eclipse E200) recording (Fig. S2). Bioassays were performed while adhering to the quality assurance/quality control (QA/QC) criteria for microplastic effect studies for ecological risk assessment by de Ruijter et al., 2020. A complete description of QA/QC methodology and a QA/QC evaluation score is provided as SD (Table S1; Fig. S1; Fig. S2).

2.4. Modelling framework

Population Dynamics. We simulated the biomass density of daphnids as a function of growth (dD/dt) upon (1) ingestion of food, where part of the food is biofilm which grows dynamically on microplastic and gets detached from biofouled microplastic and part is phytoplankton dispersed in the medium, and loss due to (2) respiration, (3) natural mortality, and (4) dose-dependent mortality due to ingestion of microplastic:

$$\frac{dD}{dt} = \varepsilon \times \langle I_{max} \times \left\{ \frac{\left[F_{biofilm,max} \times (1 - e^{-k_{det}t})\right] + F_{phyt}}{k_h + \left[F_{biofilm,max} \times (1 - e^{-k_{det}t})\right] + F_{phyt}} \right\} \rangle - k_{resp} - \mu_{nat} - \frac{1}{t} ln \left[1 + \left(\frac{C_{MP}}{LC_{50,MP}} \right)^{b_{MP}} \right] \quad (1)$$

where D is the biomass of daphnids (mg), ϵ the dimensionless food assimilation efficiency, I_{max} is the maximum ingestion rate (mg-C/mg d^{-1}), F_{phyt} (mg-C/L) is the food carbon *via* algae (phytoplankton) constantly added in the system, $F_{biofilm,max}$ is the maximum food carbon concentration from biofilm (mg-C/L) which is proportional to the microplastic concentration in the system and dependent on biofouling incubation time, k_{det} is a detachment rate coefficient of the biofilm; t is time (d), k_h is the half saturation food carbon concentration (mg-C/L), k_{resp} is the respiration rate (d^{-1}), μ_{nat} is the rate constant for natural mortality (d^{-1}). $LC_{50,\ MP}$ is the concentration which cause 50% mortality in the pristine microplastic toxicity test (mg/L), b_{MP} (>0) is a slope parameter of the concentration−response curve on a linear scale. Basis for this theoretical framework is a previously developed population dynamic model (Kupryianchyk et al., 2012). A detailed motivation and parametrization of the model is provided as SD (Eqs. S1-S11; Figs. S3-S4; Tables S2-S3).

Trade-off calculation. The trade-off between the beneficial effect of biofilm as extra-food source and the direct negative effects of microplastic ingestion on *Daphnia* was calculated using the parametrized and calibrated model at population equilibrium (zero growth, eq. (1); dD/dt = 0):

$$\varepsilon \times \langle I_{max} \times \left\{ \frac{\left[F_{biofilm,max} \times (1 - e^{-k_{det}t})\right] + F_{phyt}}{k_h + \left[F_{biofilm,max} \times (1 - e^{-k_{det}t})\right] + F_{phyt}} \right\} \rangle - k_{resp} - \mu_{nat} - \frac{1}{t} ln \left[1 + \left(\frac{C_{MP}}{LC_{50,MP}} \right)^{b_{MP}} \right] = 0 \quad (2)$$

Finally, the trade-offs were plotted by drawing the line of zero growth (isocline) for each of the experimental conditions. The isoclines separate the zones where the population decreases (dD/dt < 0) from the zones where the population increases (dD/dt > 0). Sensitivity analysis on the isoclines was done varying the model parameters with ±10%.

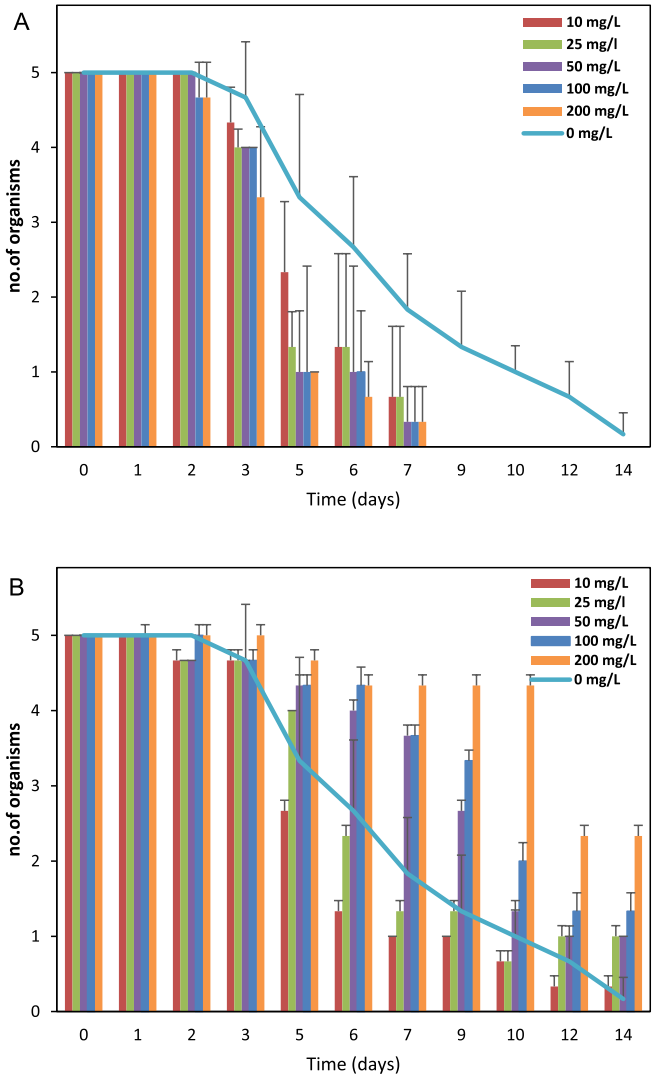

Fig. 1. Effects of PE microplastic on *D. magna* immobilization during 14-day exposure. Number of mobile organisms as a function of microplastic dose and time, for (A) Pristine PE miroplastic (B) Biofouled PE microplastic in WWTP influent (3weeks). Data represent the mean of three replicates (n = 3). Error bars represent the sd. Pristine MP treatments cause mobility to be lower than the zero microplastic control (blue continuous curve), whereas biofouled microplastic treatments cause mobility to be higher than the control. See Tables S4, S5, and S8 for a summary of the statistical analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.5. Statistical analysis

ANOVA analysis coupled with Tukey's HSD (honest significant difference) post-hoc test was performed employing IBM SPSS Statistics software. Statistical significance was considered at $p < 0.05$. Data were checked for normality and homoscedasticity of residuals. The error values of effect thresholds LC_{50} were calculated using IBM SPSS

Statistics software.

3. Results

3.1. Effects of pristine and biofouled microplastics

Pristine microplastic showed significant negative effects ($p < 0.05$, Table S4) with dose and over time on *D. magna* (Fig. 1-A, see Tables S4 and S5 for the summary of the statistical analysis), with LC_{50} ranging from 485 mg/L, after 3 days of exposure, to 1.26 mg/L, after 7 days of expousre (Table S6). PE microplastic had no significant effect on *D. magna* immobilization during the first 2 days of exposure ($p = 1.00$, Table S4), while 100% decrease in the number of mobile organisms was observed from 9 days to the end of exposure ($p < 0.05$, Table S4). LC_{50} values show a wide range (see Fig. S5 for a comparison of present versus literature effect threshold data). As foreseen, control mortality increased over time, in order to be able to tests positive as well as negative effects of the treatments on *D. magna* survival and to get mortality rate parameters for the zero microplastic treatments (Kupryianchyk et al., 2012). Note that this is different for standardized tests used within a regulatory context, where control mortality often needs to be better than 80%.

In contrast, in water with biofouled microplastic there was a significant positive effect of microplastic dose compared to the control ($p < 0.05$, Table S4 and Table S8), for which the treatment with biofilm and its additional nutritional value is the only plausible explanation (Fig. 1-B). This empirically confirms our hypothesis that microplastics acquire an extra-food source, which can compensate the negative effects of microplastics ingestion. In addition, our results show that the positive effect on immobilization is higher for microplastic biofouled in wastewater influent than for microplastics biofouled in both river (upstream and downstream) or wastewater effluent sources, for each of the incubation times (1, 2 or 3 weeks) (see Figs. S6 and S7 for all biofouled PE microplastic effects on *D. magna*). Moreover, the daphnids in contact with microplastic biofouled in wastewater influent increased in size and were still able to reproduce at high doses of microplastics (Table S7). We did not observe a significant difference in immobilization, size and reproduction between microplastics biofouled in river upstream, river water and wastewater effluent for either incubation time ($p < 0.05$, Table S8). The HSD Tukey test ($p < 0.05$) was used to analyze differences between all tested conditions: concentration, time and microplastic types (see Tables S4, S5, S8 for a summary of the statistical analysis).

These results suggest that the compensatory pattern on daphnids is influenced by the amount of extra food acquired by microplastic. Microplastics were thus analyzed to determine whether there is evident biofilm formation on their surface during the aging process in the freshwater sources, prior to exposure. Biological abundance measurements demonstrated that metabolically active microorganisms and polymeric material were attached to the microplastic surface (Fig. S8). Comparing biofilm density in the four water types, the abundance of bacteria, algae and organic material on microplastics incubated in wastewater influent was higher than in river waters (upstream and downstream) and wastewater effluent (Figs. S8-A, S8-B and S8-C, see Tables S9 and S10 for summary of the statistical analysis). Microplastic incubated in upstream river water resulted in biological material abundance values that were similar to those of downstream river water samples. TOC measurements allowed the quantification of the biofilm content in terms of total carbon per mass of microplastic (Fig. S8-D). Microplastic incubated with wastewater influent acquired the highest amount of biofilm compared to both river water (upstream and downstream) and wastewater effluent. In addition, biofilm abundance varied with incubation period, with 3 weeks generating the highest biofilm loading for all water sources. We also inspected the surface of the microplastic using CLSM. The CLSM micrographs revealed the attachment of green-marked bacteria on microplastic after the first week (Fig. S9), and the self-production of EPS stained in red within 3 weeks (Fig. S10). The most prominent bio cover occurred in the case of microplastic incubated with wastewater influent, which exhibited mature biofilms with their characteristic pronounced EPS, high bacterial colonization, and aggregation tendency. Microplastic incubated in river waters and wastewater effluent were totally covered by bacteria but not by EPS. The surface of microplastic maintained in DW, i.e. pristine microplastic, remained clean. The presence of biofilm on microplastics was confirmed also *via* visual inspection (Fig. S11). Considerable differences were observed in appearance and water column distribution between pristine and aged microplastics. Again, these varied with water sources type. White pristine microplastic (MP-DW) were maintained close to the surface of the water. Microplastic aged in river and WWTP effluent water were distributed vertically throughout the water column, despite early patchy discoloration; whereas microplastic incubated in WWTP influent had sunk to the bottom of the beaker. This distribution likely corresponds to the density and amount of biofilm attached to the microplastics (Fig. S8). The highest amount of biofilm formed was in the WWTP influent water. In addition, the vertical distribution in the water column varied with the incubation period, 3 weeks showing the highest change on sinking, when the microplastics were abundantly biofouled. The differences in the biofilm abundance for wastewater influent versus river waters or wastewater effluent sources are consistent with the combination of both basal largest planktonic density and the higher TOC (Fig. S12). Full data of initial abiotic and biotic characteristics for the employed water matrices are presented in Fig. S12. The remaining water fraction after removing biofouled microplastics from the aging experiment did not show a significant variability in abiotic characteristics among sampling dates (Fig. S12-A, S12-B). Turbidity, TOC, bacterial and algal abundance (Fig. S12-C, D, E and F) decreased at all incubation times and for all waters matrices, confirming biofouling, with WWTP influent being most pronounced, with river and waste water effluent being a temporary exception for the first week.

3.2. Modelling the trade-off between microplastic food dilution and microplastic biofilm feeding

Our next step was to quantify the trade-off between negative effects of microplastic ingestion and beneficial effects of biofilm as food source for *D. magna*. We first evaluated a new model which simulates biomass density (D) of daphnids as a function of time (dD/dt) upon (a) ingestion of food, where part of the food is biofilm which grows dynamically on microplastic and gets detached from biofouled microplastic and part is phytoplankton, and loss due to (b) respiration, (c) natural mortality, and (d) microplastic dose-dependent mortality due to 'food dilution' triggered by their ingestion. Basis for this theoretical tool is a logistic population growth model combined with dose-effect relationships for microplastic and ingestion of food (see Materials and Methods and Supporting Data). The main concepts and initial parameters were in part independently taken from literature, or estimated from the separate experiments with pristine microplastic (detailed in Tables S2 and S3). For the full trade-off model, all parameters were used as such, and not further optimized.

Whereas in the previous section we evaluated the microplastic effects using statistical testing, here we evaluate the results by modelling the population dynamics. The results of the whole 14-day toxicity tests with *D. magna* in contact with pristine microplastic and biofouled microplastic as well as data on biofilm abundance, were used as input to model the population development of *D. magna*.

The agreement between measured and modelled data of *D. magna* biomass in the 14 day-bioassays for pristine microplastic is very good as it explains 99.1% of the variance in the data, with a slope close to 1 and intercept close to zero ($y = (0.956 \pm 0.011)\ x + (0.025 \pm 0.004)$, $R^2 = 0.991$, Fig. S13, panel A). Similarly, for the bioassays with biofouled microplastic the agreement between measured and modelled data was very good with slopes between 0.934 and 0.973, and R^2 from 0.868 to

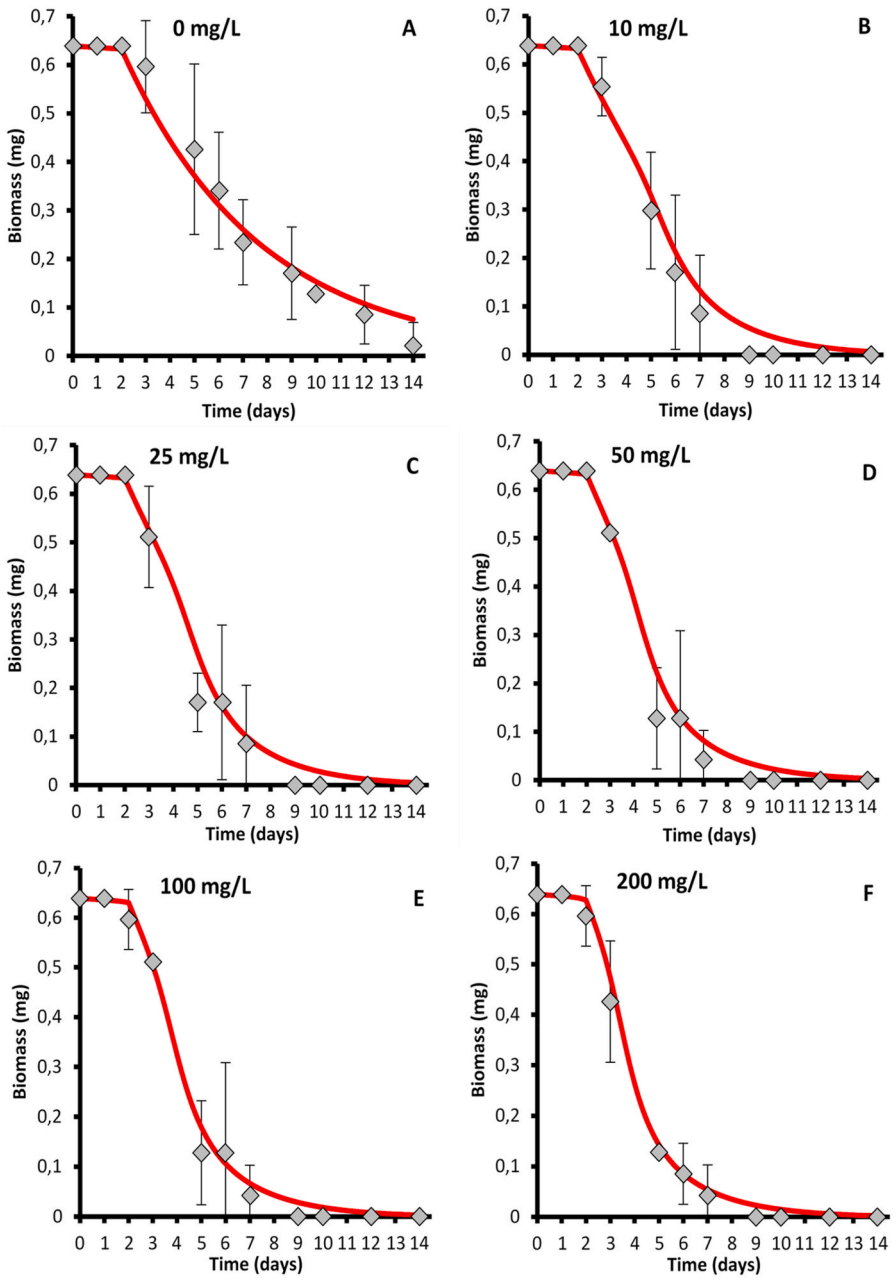

Fig. 2. Population dynamics of *D. magna* in contact with pristine microplastic at 0 (A), 10 (B), 25 (C), 50 (D), 100 (E), and 200 (F) mg/L. The markers correspond to biomass (±sd) measured during 14-day toxicity test, whereas the red curve represents model output. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

0.966 (for all regression curves see Fig. S13).

The effect of pristine microplastic on *D. magna* is acute and leads to a rapid decline of the population size within 9 days of exposure. Furthermore, at higher microplastic dose, an exponential decrease of the population is observed (Fig. 2B–F). Addition of extra-food through ingestion of biofouled microplastic improves survival over the 14 days of exposure (Fig. 3 and Figs. S14-S24). However, exposure to microplastics biofouled in river and wastewater effluent sources (Figs. S14-S22), still results in a predicted extinction of the population, even at the highest doses of biofouled microplastics. At these high microplastic concentrations, the negative effects of food dilution due to microplastic ingestion are still bigger than the positive effects of the presence of biofilm. Only microplastics with highest biofilm abundance, which were biofouled in wastewater influent for either incubation time (1, 2 or 3 weeks), completely compensate for the adverse effects of microplastic ingestion and result in a predicted growth of the population even larger than the control (Fig. 3 and Figs. S23 and S24).

Finally, we calculated the trade-off between the beneficial effect of biofilm as extra-food source and the direct negative effects of microplastic ingestion on *Daphnia*, using the model outputs for the population equilibria (zero growth of population density (D); $dD/dt = 0$) (see Materials and Methods) and visualized where a state of population growth tips into a state of population decline by drawing the curves of zero growth (isoclines) for each of the experimental conditions (Fig. 4 and Figs. S25-S37). The isoclines separate the zones where the population decreases ($dD/dt < 0$) from the zones where the population increases ($dD/dt > 0$).

For pristine microplastic, the population equilibrium is observed at food carbon concentration cfood = 0.054 mg-C/L where the isocline intersects with the x-axis at 0 mg/L microplastic (Fig. 4 A1-4). This

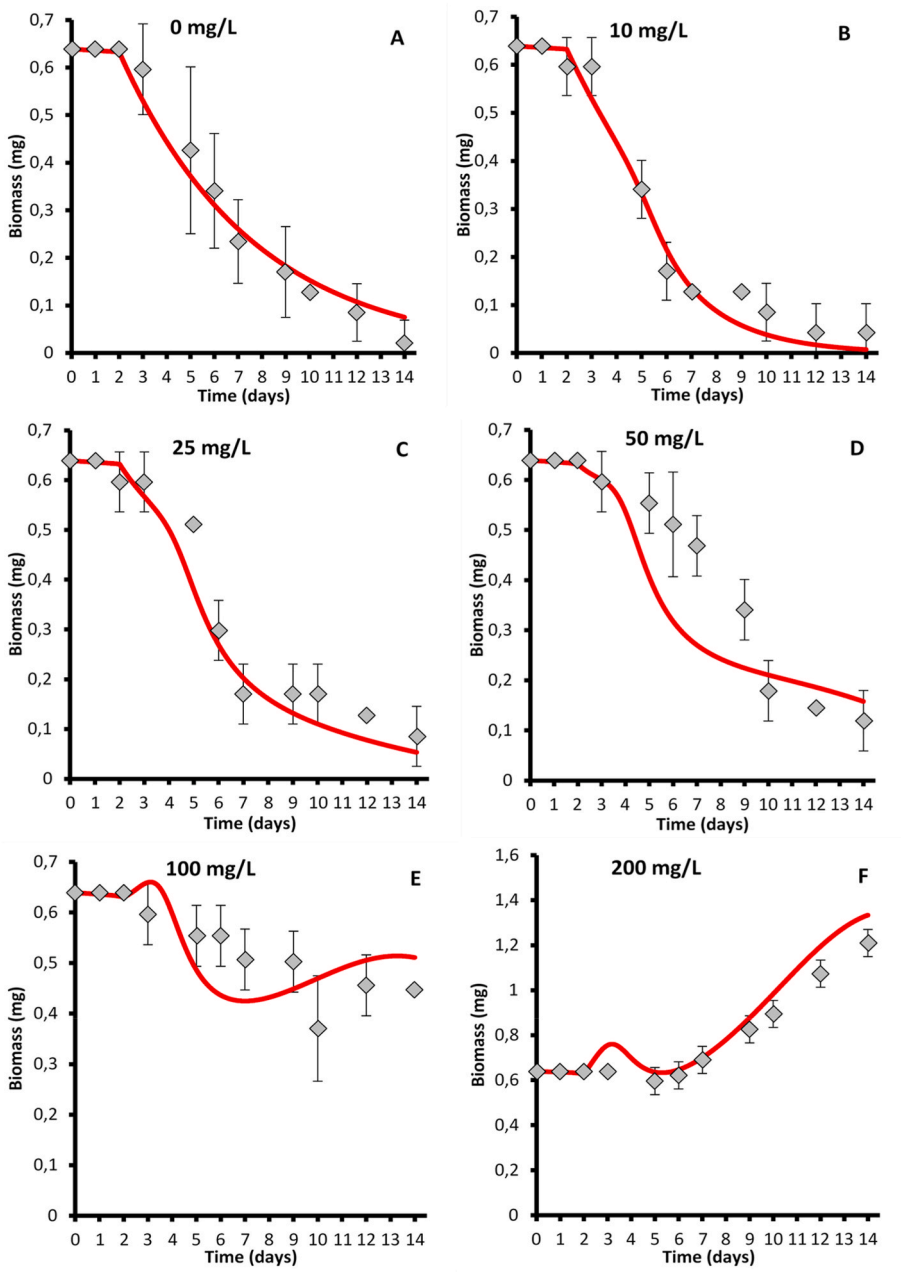

Fig. 3. Population dynamics of *D. magna* in contact with biofouled microplastic in wastewater influent for 3 weeks at 0 (A), 10 (B), 25 (C), 50 (D), 100 (E), and 200 (F) mg/L. The markers correspond to biomass (±sd) during 14-day exposure to MP, whereas the red curve represents model output. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

means that the population grows if the food concentration is higher than this value. An increase in microplastic dose increases the microplastic ingestion-related mortality and therefore requires an increased concentration of food to keep the population at equilibrium. Increasing the microplastic concentration from 0 to the highest dose of 200 mg/L requires a food dose of 0.054 mg-C/L increasing to 0.079, 0.316, 0.396, and 0.493 mg-C/L at 3, 7, 10, and 14 days of exposure, respectively (Fig. 4 A1-4). Because the mortality of daphnids due to microplastic is dose and time dependent, a theoretical increase in the microplastic concentration would become lethal and cause decline of the population. As the isocline is constrained mainly by the toxicity of the microplastic ingestion, no limit to the tolerance (no upper bound) for pristine microplastic can be calculated (Fig. 4 A1-4).

When the dose of biofouled microplastic (river upstream, river downstream, wastewater effluent, and wastewater influent) gradually varies from 0 to 200 mg/L, the tolerance to microplastic decreases to 161.7, 152.8, and 148 mg/L at 7, 10, and 14 days of chronic exposure, respectively (panels B of Fig. 4, and Figs. S25-S37). At these concentrations of biofouled microplastic, the positive effects of biofilm-food supply outweigh direct negative effects of microplastic food dilution. The maximum tolerance for microplastic is reached at a biofouled microplastic concentration of 148 mg/L. This implies a limit to the tolerance for microplastic when biofilms supply extra food to the system, which in this case is calculated as cfood, biofilm ≈0.481 mg-C/L.

After calculating the isocline of zero growth, it can be predicted which range of biofilm additions can be considered safe for *D. magna*, where safe is defined as sustaining a habitat quality sufficient for population growth (Kupryianchyk et al., 2012). For instance, for a medium with cfood = 0.481 mg-C/L, population growth is predicted to occur with microplastic concentrations up to 200 mg/L (Fig. 4).

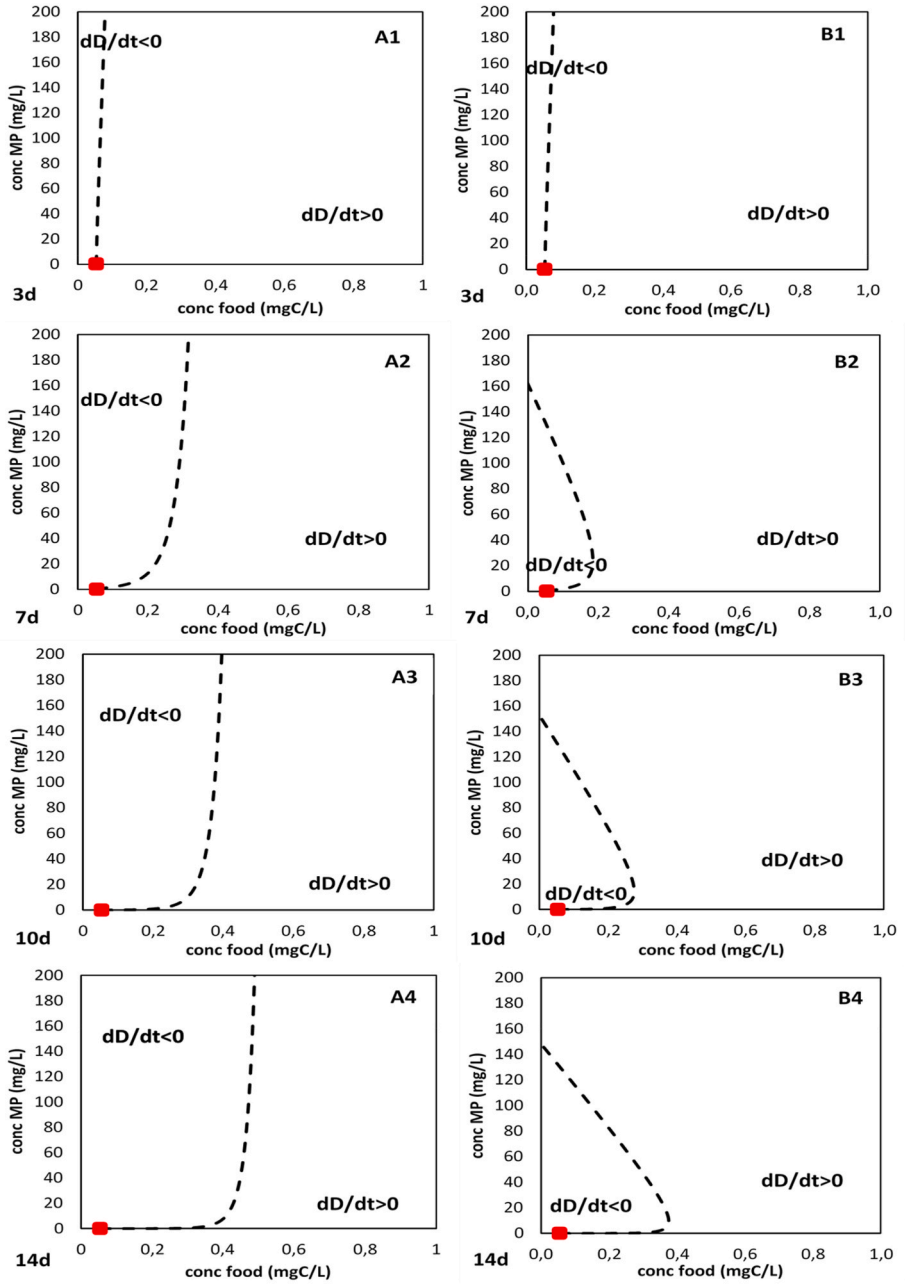

Fig. 4. Isoclines (lines of zero growth of *D. magna*) for pristine microplastic (A) and biofouled microplastic in WWTP influent-3weeks (B) at 3 (A-B.1), 7 (A-B.2), 10 (A-B.3) and 14 (A-B.4) days. Isoclines separates the part of the state space where the populations decrease (dD/dt < 0) from the state space where the populations increase (dD/dt > 0). The red marker defines the point in the state space where food but no microplastic is present. Panels B indicate how biofouling increases the safe operating space for *D. magna* under microplastic stress, leading to full microplastic tolerance at MP doses >148 mg/L. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Sensitivity analysis shows how isoclines change upon a default 10% change of parameter values, a change that appears to be marginal (Fig. S38). Variability of parameter values thus would affect the exact tipping points, but not the occurrence of the trade-off as such.

4. Discussion

So far, microplastic effects have mostly been studied as a function as microplastic dose and characteristics. The nutritional relationships observed here may represent a hitherto overlooked aspect leading to an overhaul of the prevailing paradigm (de Ruijter et al., 2020). This highlights the need for a stress ecological view on microplastic effects in the natural environment (Kong and Koelmans, 2019). Our results show that basic water quality characteristics that change over time cause biofilms to affect microplastic dose-response relationships already within a month of exposure. Since there is no data on time and dose dependency of microplastic effects that include the influence of biofouling, our model can provide the best estimate of the trade-off between microplastic adverse effects and microplastic biofilm nutrition, which can be useful for future eco-toxicological effect studies as well as integrated food web models (Kong and Koelmans, 2019). The present model construct can be implemented in food web models and for more species, to acquire a more realistic tool to evaluate the implications of microplastic in surface waters with different nutrient status.

We used environmentally relevant experimental conditions, thus avoiding concerns regarding the relationship between laboratory-based observations of effects and ecological risks that would occur in nature (de Ruijter et al., 2020; Phuong et al., 2016; Koelmans et al., 2020). PE is one of the most detected polymeric debris in both freshwater and marine compartments and an adequate proxy to study microplastic food dilution effects (de Ruijter et al., 2020; Xu et al., 2020; Koelmans et al., 2019). Irregularly shaped microplastic like the ones we used, with size ranging from 10 to 50 μm, were reported to be an important bioavailable fraction of environmental microplastic in freshwater systems (Koelmans

et al., 2019). Our range of microplastic doses tested, as well as LC_{50} values determined (8.54×10^5–1.71×10^7 #/L, and 1.1×10^5–4.14×10^7, respectively, where 85.38×10^6 #/g is the conversion factor, see table S10) are environmentally relevant for exposure to crustaceans, given microplastic number concentrations up to 10^8 #/L reported for freshwater systems (Koelmans et al., 2019). The ratio of daphnids size (2.7 mm) and maximum ingestible microplastic size tested (10–50 μm) was in the range of 40–50, corresponding well with literature values (Koelmans et al., 2020; Jams et al., 2020). The carbon nutrient concentrations in our systems were similar to the carbon concentrations measured in the environment. Due to natural or anthropogenic factors, dissolved or colloidal carbon concentrations can have ranges of 0.18 up to 30 mg/L in different surface water types. (Al-Said et al., 2018; Shah et al., 2018; Imtiazy et al., 2020; Song et al., 2018; Rodriguez-Murillo and Filella, 2015, Cotovicz et al., 2017), especially in eutrophic waters (Cotovicz et al., 2017; Yang et al., 2008). Only a limited number of studies included the influence of biofouling on microplastic effects (de Ruijter et al., 2020). To our best knowledge this work is the first quantitative assessment of this interaction.

Future research could explore this mechanism further by examining other species, plastic and water types. Furthermore, it is relevant to take a closer look at the microbial composition and food quality of the biofilm.

5. Conclusion

The present work demonstrates that effects of microplastic as observed in laboratory tests are highly conditional, in this case conditional to the level of food abundance and quality used in the test. This challenges the value of the many tests performed thus far that did not include food or that only used one food concentration level. Our findings imply that tests should be performed with different levels of food and with natural biofilms being present on the tested microplastics' surface. We provide a quantitative framework to predict the interactive effects of both varying microplastic and food carbon levels, a framework that may prove to be handy when interpreting and designing effects tests as well as in prospective risk assessment frameworks that aim to include the nutrient status of natural water.

Authors contribution

GA: conceptualization and design; experimental work (GA performed all experimental work); model development; writing. **RR:** conceptualization and design; writing; acquisition and supervision. **FFP:** conceptualization and design; acquisition. **AK:** conceptualization and design; model development; writing; acquisition and supervision. All authors agreed to the final manuscript version.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118622.

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