

RESEARCH

Open Access



# Effects of dissolved organic matter on the toxicity of micro- and nanoplastic particles to *Daphnia* - a meta-analysis

Sophia Salomon<sup>1</sup>, Eric Grubmüller<sup>1</sup>, Philipp Kropf<sup>1</sup>, Elisa Nickl<sup>1</sup>, Anna Rühl<sup>1</sup>, Selina Weigel<sup>1</sup>, Felix Becker<sup>1</sup>, Ana Leticia Antonio Vital<sup>2</sup>, Christian Laforsch<sup>3,4</sup>, Matthias Schott<sup>3,4</sup> and Magdalena M. Mair<sup>2,4\*</sup>

## Abstract

Effects of micro- and nanoplastic particles (MNP) on organisms have been increasingly reported in recent years, with a large number of studies conducted on water fleas of the genus *Daphnia*. Most of the available studies used pristine particles that have not been exposed to the environment or to organic substances. In natural environments, however, organic substances like dissolved organic matter (DOM) attach to the MNP, forming an ecocorona on the particles' surface. How the formation of an ecocorona influences MNP toxicity is still uncertain. While some studies suggest that DOM can mitigate the negative effects of MNP on organisms, other studies did not find such associations. In addition, it is unclear whether the DOM attached to the particles' surface attenuates the effects of MNP directly or whether co-exposure with DOM solved in the medium attenuates MNP toxicity indirectly, for instance by increasing *Daphnia*'s resilience to stressors in general. To draw more solid conclusions about the direction and size of the mediating effect of DOM on MNP-associated immobilization in *Daphnia* spp., we synthesized evidence from the published literature and compiled 305 data points from 13 independent studies. The results of our meta-analysis show that the toxic effects of MNP are likely reduced in the presence of certain types of DOM in the exposure media. We found similar mediating effects when MNP were incubated in media containing DOM before the exposure experiments, although to a lesser extent. Future studies designed to disentangle the effects of DOM attached to the MNP from the general effects of DOM in the exposure medium will contribute to a deeper mechanistic understanding of MNP toxicity in nature and enhance the reliability of MNP risk assessment.

**Keywords** Water flea, Microplastic, Nano-plastic, Biofilm, Ecocorona, Mortality, Immobilization, Ecotoxicology

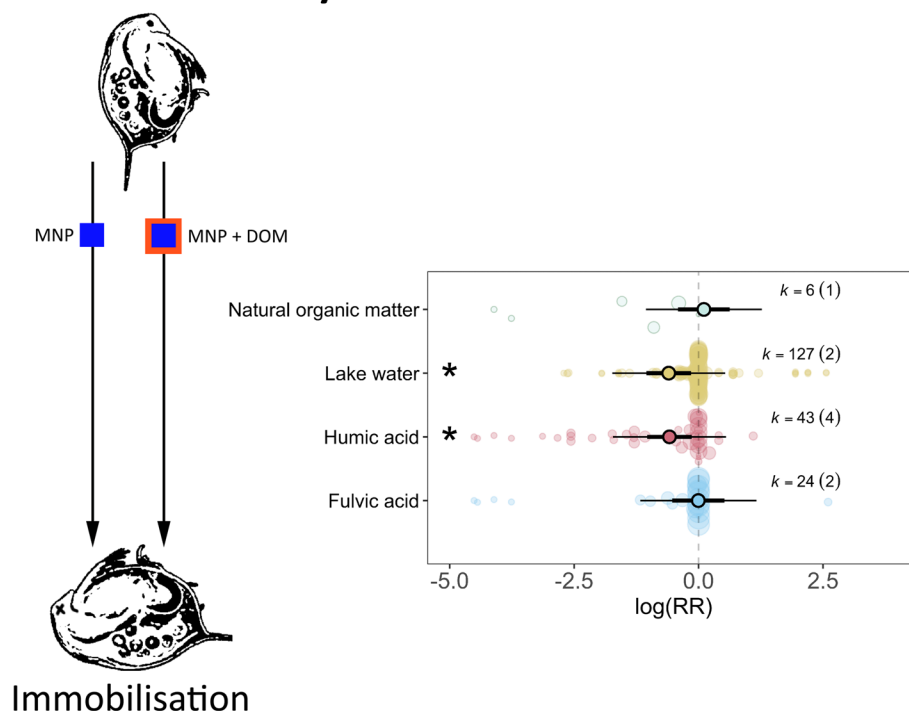
\*Correspondence:

Magdalena M. Mair  
magdalena.mair@uni-bayreuth.de

Full list of author information is available at the end of the article

## Graphical Abstract

## Meta-Analysis



## Introduction

The number of studies investigating the potential harm of micro- and nanoplastic particles (MNP) to organisms has increased substantially over the last years [1]. MNP are ingested by animals and can block their digestive tract, injure gut epithelia and change the gut microbiome [2, 3]. If small enough, particles can translocate into tissues and cells [4–7] and induce inflammatory responses and cellular damage [8,9]. On an organismic level, effects on body growth, reproduction and survival have been reported, among others [10–12]. In addition to effects elicited by the particles themselves, additives such as UV-stabilizers [13] or plasticizers [14] added to the polymers may leach from the particles and induce toxic responses [15, 16]. Pollutants or pathogens may attach to the particle surfaces and induce toxic effects upon ingestion (vector effect, [17, 18], but see also [19, 20]).

Due to their complexity, the effects of MNP cannot be easily generalized. Unlike chemicals which have distinct molecular structures and stable properties and can usually be assigned unique identifiers (e.g., Chemical Abstracts Service (CAS) number), MNP are more diverse. Each single particle possesses its own set of chemical and physical

properties. These properties include simple characteristics such as polymer type, size and shape, as well as more complex properties like mixtures of plastic-associated chemicals, surface structure and charge, and substances attached to the particles' surface including organic substances from the environment (ecocorona; [21]), proteins (protein corona, [22, 23]) and bacteria (biofilm, [24, 25]). In addition, all these properties can change over time. This complexity makes extensive testing necessary if we want to understand how specific MNP properties relate to specific toxicity outcomes and how strong effects are to be expected in natural environments.

Water fleas of the genus *Daphnia* are important standard test organisms for aquatic systems that occur in most stagnant and slowly flowing freshwater habitats. As part of the zooplankton, *Daphnia* spp. play an essential role in the aquatic food web. They form a link from primary producer plankton which contains essential fatty acids to higher trophic levels of the food web and are considered sensitive keystone species. As highly effective filter feeders they are exposed to substances and matter contained in the surrounding water. The non-selective feeding method makes them particularly vulnerable to accidental

ingestion of particulate pollutants. *Daphnia* spp. are easy to maintain in the lab, as they have short generation times and typically reproduce parthenogenetically, if not stressed. Consequently, *Daphnia* spp. are widely used in acute and chronic standard toxicity tests [26, 27]. *Daphnia magna* is the most tested aquatic invertebrate species in studies investigating the ecotoxicological effects of MNP on organisms (see [28] and the Toxicity of Microplastics Explorer (ToMEx) database: [29]).

Several studies have shown effects of MNP on *Daphnia* spp. including a variety of endpoints, concentrations and particle characteristics (for an overview see [28]). For example, Eltemsah & Bøhn [30] observed increased mortality rates, decreased growth, and stimulation of early reproduction at the expense of later reproduction in *Daphnia* exposed to polystyrene microbeads, Zhang et al. [31] observed changes in levels of radical oxygen species (ROS), and Lin et al. [32] observed changes in the daphnids' swimming activity. While earlier studies often tested only one specific type of MNP, later studies more frequently demonstrated that effects are not uniform across all types of MNP, but instead depend on the MNPs' properties. Effects have been shown to depend, among others, on polymer type, particle size and shape, surface charge and the presence of additives [15, 16, 32–36]. Most studies so far have worked with pristine particles [28], i.e., particles that have not had contact with natural environments. Only a few studies have attempted to investigate effects under more realistic circumstances. Whether effects observed in the lab are representative of true effects expected in nature is therefore still under debate [21, 37] (see also [38]).

In natural environments, an ecocorona forms on the particles within seconds when organic molecules including dissolved organic matter (DOM) attach to the MNPs' surface (e.g., [39]). This ecocorona alters the particles' surface structure and charge [5, 6, 25, 40, 41], influences their behavior in the water column [42, 43], and modifies their attachment rate to cellular surfaces [5, 6]. These alterations may in consequence also affect outcomes on the organismic level, for instance mediated by changes in uptake and tissue translocation rates [5, 6, 44, 45]. In *Daphnia*, it has been demonstrated that the presence of an ecocorona influences the uptake rates of MNP and their retention time in the gut [37]. In MNP-exposed organisms, the presence of DOM has for instance been shown to alter effects on mobility/survival (e.g., [22, 46, 47]), feeding behavior [37] and molecular effects (e.g., [22]). However, deriving a general pattern for the direction of these mediating effects is still challenging. One reason for this is that results in the published literature are not entirely consistent: while ameliorating effects of

ecocorona formation and presence of DOM on organisms were found for some MNP (e.g., [34, 48, 49]), this was not the case for other MNP (e.g., [50, 51]), and also increased toxicities have been observed [37]. Another factor complicating generalized conclusions is the way experiments are conducted. While some experiments co-exposed organisms to MNP and DOM simultaneously [51], other studies incubated MNP in media containing DOM prior to being transferred to the exposure medium (i.e., no additional DOM in the exposure medium, e.g. [47]). It is thus unclear, whether it was the DOM attached to the particles' surface itself or the DOM in the media that led to the observed differences. Furthermore, the type of DOM used in experiments affects the composition and thickness of the formed ecocorona and may in turn influence observed outcomes [52, 53].

Meta-analyses are a tool for addressing exactly these kinds of questions, where the presence, direction and size of effects are unclear [54]. By aggregating data from several studies in a quantitative way, meta-analyses aim to derive effect size estimates with reduced bias and greater precision (lower uncertainty) than estimates from single studies [55, 56].

We performed a meta-analysis to answer the question of how the presence of DOM alters the effects of MNP on *Daphnia* immobilization rates. Through a systematic literature search of experimental studies, we compared the effects of MNP with DOM to effects of the exact same particles without DOM. Based on the gathered data, we discuss the strength of evidence regarding mediating effects of DOM on MNP toxicity. In addition, we investigated whether the mediating effects depend on the type of DOM and the type of experimental approach used (either co-exposure with DOM or incubation prior to exposure).

## Materials and methods

### Literature search

We conducted a literature search for studies that investigated effects of MNP on immobilization rates (including mortality) in water fleas of the genus *Daphnia*. The aim was to compile data from studies that met all of the following inclusion criteria: (1) experimental research (excluding books and reviews) published in English, (2) investigation of alterations of MNP effects due to DOM (i.e. studies contained at least one MNP treatment with DOM and one treatment with the same particles without DOM), (3) testing of water fleas of the genus *Daphnia*, and (4) absence of additional stressors during exposure (e.g., chemicals). The final search was conducted in December 2022 on Web of Science (WoS) and PubMed using the search string “((micro\* OR nano\*) AND

(plastic\* OR particle\*) OR microplastic OR nanoplastic) AND *Daphnia* AND (eco-corona OR ecocorona OR bio-film OR humic acid OR DOC OR DOM OR fulvic acid OR lake water OR protein corona OR protein-corona OR proteincorona OR incub\*). In parallel, we searched for review articles addressing effects of MNP on *Daphnia* or freshwater organisms as additional sources of literature. After removing duplicates, all titles and abstracts were screened. Studies that clearly did not follow the inclusion criteria were removed. All remaining studies were subjected to full text screening and only those studies that met all selection criteria were kept for data extraction (see full text screening list in the supplementary online material).

### Data extraction

The extracted data consist of immobilization and mortality measurements, information on added DOM, characteristics of the used MNP, information on the test organisms and experimental parameters. As studies frequently did not distinguish between immobilization (absence of movement after agitation; [26]) and mortality (absence of heartbeat in addition to the absence of movement), we will refer to both as immobilization.

We extracted immobilization rates (i.e., the number of immobile/mobile individuals or the proportion of immobile individuals) for both the MNP treatment with DOM and the control treatment without DOM. Whenever possible, we extracted the rates directly from the text, data tables or raw data files provided in the supplementary online material. In cases where the data was instead presented in figures, the rates were extracted from the plots using the R package *metaDigitise* [57]. If daphnids were observed repeatedly over time, immobilization rates were extracted for the latest time point (one of either 24, 48, 72 or 96 hours). If none of these time points were measured, we used the latest reported time point in the study. Immobilization rates for all other time points were neglected. Additionally, we extracted the number of replicates (i.e., the number of independent test vessels) and the number of individuals per replicate (i.e., the number of daphnids per test vessel). These numbers were used to calculate the total number of immobile and mobile individuals if immobilization rates were reported as proportions.

The pairing of treatments using identical experimental setups and particles that differed only in the presence or absence of DOM allowed us to directly control for confounding by other MNP properties, different experimental parameters, and characteristics of the test organisms. As the main explanatory variables, we thus only noted (1) the type of DOM used in experiments

(*DOM\_type*; e.g., humic or fulvic acid, different types of lake water, etc.), (2) whether DOM was added during exposure (*DOM\_conditioned*: no) or whether the particles had instead been incubated in DOM-containing media prior to their use in experiments (i.e. particles were removed from the DOM-containing media prior to transfer to the exposure vessels without DOM; *DOM\_incubated*: yes).

For completeness and to enable extended use of the data in the future, we extracted the following additional information from the studies if available: particle concentration (in mg per ml/l and particles per ml), MNP properties including polymer type, particle size (mean  $\pm$  standard deviation), particle shape (spherical, fragment, fiber), the particles' chemical surface modification (e.g. carboxylation, amination) and surface charge (either positive or negative); characteristics of the test organisms including species, clone and age at the start of exposure; experimental conditions including temperature, pH of the test medium, whether food was provided during exposure and its concentration, and the concentration of DOM added during MNP incubation or during exposure.

### Statistical analysis

As a measure of effect size, we calculated log risk ratios (log (RR)) for immobilization. A multiple mixed meta regression model without intercept was fit to the data including the two factors *DOM\_type* (humic acid, fulvic acid, type of lake water, etc.) and *DOM\_conditioned* (either “yes” for treatments with MNP conditioned in DOM-containing media or “no” for treatments with DOM added during exposure) as moderators and a random intercept for the unique publication identifier *Publication ID* consisting of the first author name and the year of publication:

$$\log(RR) \sim \text{DOC\_type} + \text{DOM\_conditioned} - 1 + (1|\text{PublicationID})$$

Moderator effects, i.e., the effects of the two fixed factors, were investigated by fitting reduced models including only one of the moderators at a time and comparing these reduced models to the full model through likelihood ratio tests. In addition, the variance component attributed to *Publication ID* (sigma squared) was checked using profile likelihood plots to ensure that the component was successfully identified, indicated by the curve peaking at the maximum likelihood estimate. To see whether the two factors in the model adequately accounted for the heterogeneity among data points, a test for residual heterogeneity was conducted and the variance attributable to among-sample heterogeneity rather than sampling variance ( $I^2$ ) was calculated.

Based on the full model, an orchard plot was generated to visualize the average marginal effect estimates for each combination of moderator levels. In contrast to forest plots, orchard plots include individual effect size estimates, 95% confidence intervals (CI) and 95% prediction intervals.

To investigate potential publication bias visually, standard errors were plotted against residuals of the full model in a funnel plot. In general, skewed, asymmetric funnel plots or funnel plots with apparent gaps can indicate potential publication bias (for a more detailed explanation and limitations see [58]). In the funnel plot, we expected that selective publishing would result in a clear shift of data to higher effect sizes in studies with high standard errors, suggesting that studies with low power were preferentially published when observed effects were large.

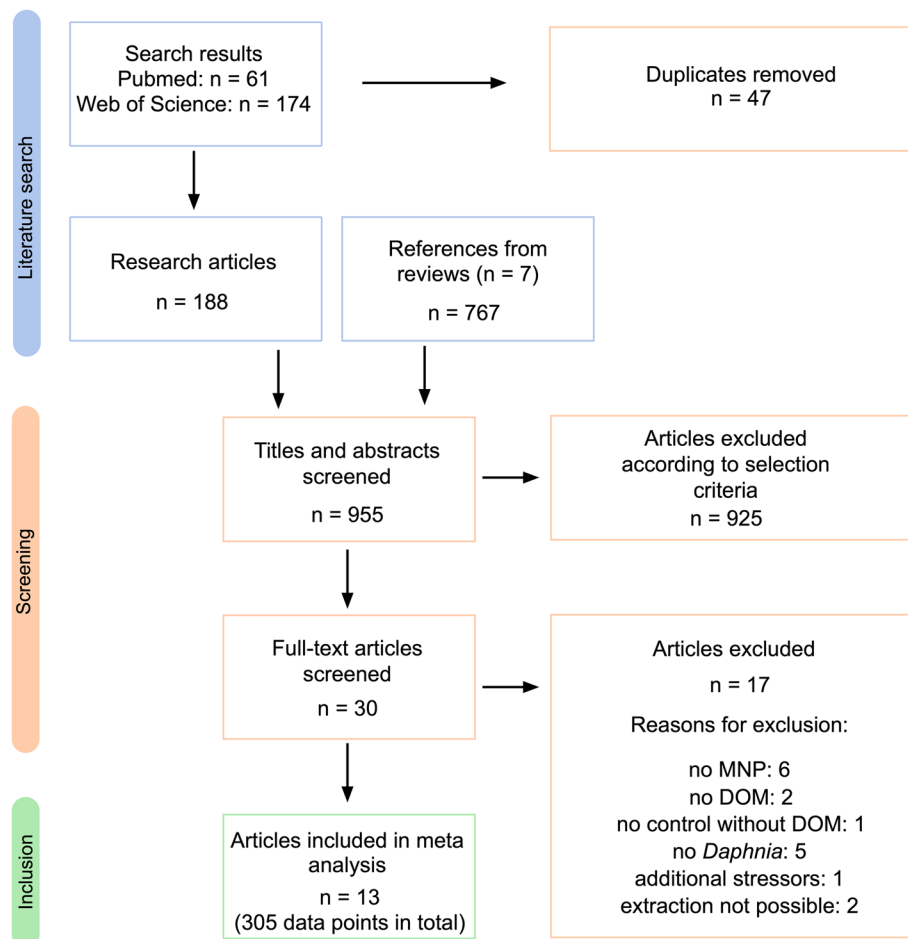
All analyses were done in R version 4.3.1 (R Core Team, 2023). The *metafor* package version 4.2 [59] was used for effect size calculation, fitting meta regression

models and investigating publication bias. The *orchaRd* 2.0 package [58] was used for creating the orchard plot and calculating  $I^2$ .

## Results

### Literature search and data extraction

The literature search resulted in 955 publications, of which 925 failed to meet at least one of the selection criteria. After full text screening of the remaining 30 publications, 17 studies were excluded either because they did not fulfill all the selection criteria or data extraction was not possible. The remaining 13 publications were used for data extraction. In total, we extracted 305 data points (Fig. 1). We grouped the types of DOM used in the studies into seven main categories: metabolites excreted from *Daphnia* (2 studies, 21 data points), humic acid (4 studies, 43 data points), fulvic acid (2 studies, 24 data points), commercially bought natural organic matter (1 study, 6 data points), stream water (3 studies, 34 data points), lake water (3 studies, 128 data points) and wastewater (4



**Fig. 1** PRISMA flow diagram illustrating the results from the systematic literature search and stepwise exclusion of studies not fulfilling defined selection criteria. MNP: micro- and nanoplastic particles. DOM: dissolved organic matter

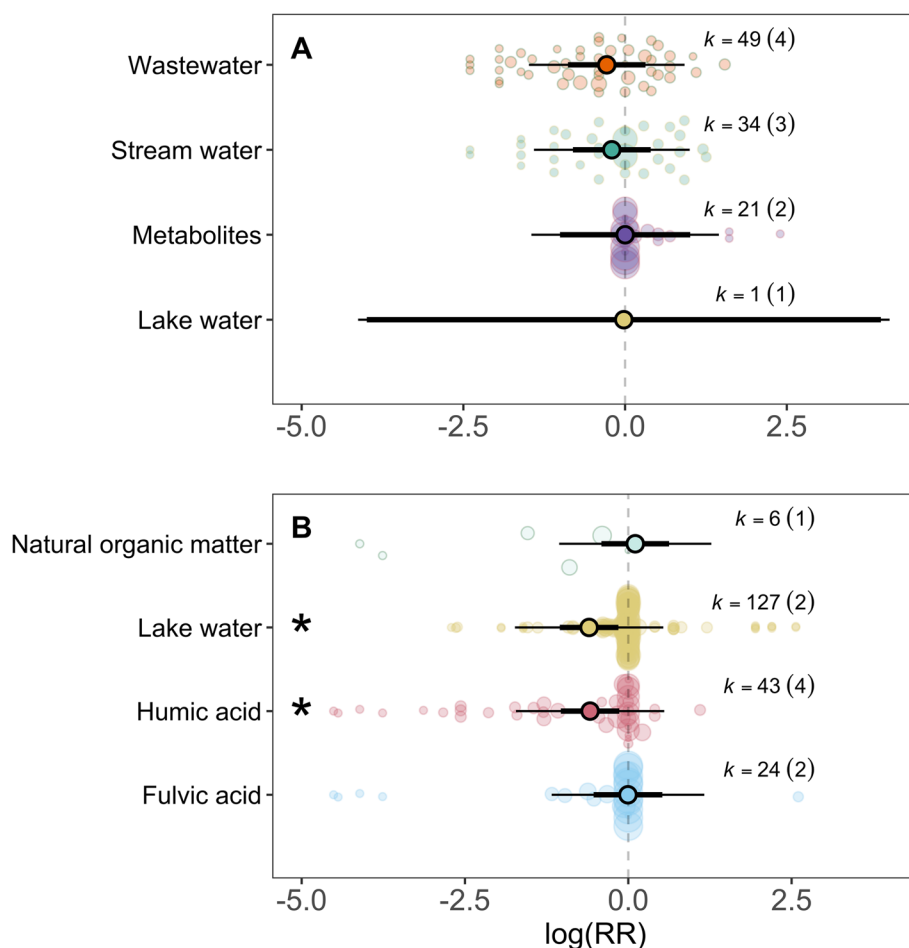


studies, 49 data points). In seven of the studies, the MNP were incubated in the respective DOM type prior to exposure (105 data points). In the other six studies, DOM was added during exposure or exposure was conducted in water sampled from natural environments (200 data points). All included studies tested the species *Daphnia magna* (for a full overview of experimental parameters covered in the studies see raw data in the supplementary online material).

#### Effects of DOM type and conditioning on immobilization rates

In the full model, moderators had a significant effect on immobilization risk (test of moderators:  $Q_M = 17.33$ ,  $df = 8$ ,  $p = 0.023$ ) indicating that at least one of them accounted for differences in effects across samples. A comparison of full and reduced models showed that

DOM type was more important for the model fit (comparison of the full versus the reduced model without factor *DOM\_type*: LR = 12.47,  $p = 0.052$ ) than whether the particles had been conditioned prior to their use in exposure experiments (comparison of the full versus the reduced model without factor *DOM\_conditioned*: LR = 0.08,  $p = 0.78$ ). A reduction in immobilization risk was observed when DOM was added in the form of humic acid (log(RR): -0.58 (CI: -1.03, -0.14), RR: 0.56 (CI: 0.36, 0.87),  $z = -2.56$ ,  $p = 0.01$ ) or lake water during exposure (log(RR): -0.60 (CI: -1.05, -0.15), RR: 0.55 (CI: 0.35, 0.86),  $z = -2.62$ ,  $p = 0.009$ ), and to a lesser degree when MNP were incubated in wastewater (log(RR): -0.28 (CI: -0.88, 0.32), RR: 0.76 (CI: 0.41, 1.37),  $z = -0.93$ ,  $p = 0.35$ ) or stream water (log(RR): -0.21 (CI: -0.81, 0.40), RR: 0.81 (CI: 0.44, 1.49),  $z = -0.67$ ,  $p = 0.50$ ) prior to exposure



**Fig. 2** Mean treatment effects of different dissolved organic matter (DOM) types from the selected studies. A: effects shown for micro- and nanoplastic particles (MNP) conditioned in DOM containing medium; B: effects of MNP with DOM added to the medium during exposure; Metabolites: medium containing metabolites excreted by *Daphnia*; log (RR): log risk ratio; thicker black lines show 95% confidence intervals; narrow lines show prediction intervals; asterisks indicate significant moderation of MNP effects ( $p < 0.05$ ). Point sizes reflect inverse standard errors

(Fig. 2). Changes in immobilization risks due to DOM were below 15% in all other treatments.

In general, we found high variance in the data in all groups. In addition, while DOM type and experimental approach (*DOM\_conditioned*) explained part of the variance in the data, the model left significant residual heterogeneity ( $Q_M = 680.25$ ,  $df = 297$ ,  $p < 0.0001$ ) indicating that other factors not included in our analysis additionally contribute to the differences. In accordance with this, 97% of the variance among data points can be attributed to sample heterogeneity ( $I^2 = 0.97$ ) and only 3% are estimated to result from sampling variance.

### Publication bias

A visual inspection of the funnel plot did not show severe deviations from symmetry, but showed a small gap in data points on the right for medium powered studies (see gap on the right at medium standard errors in Fig. 3).

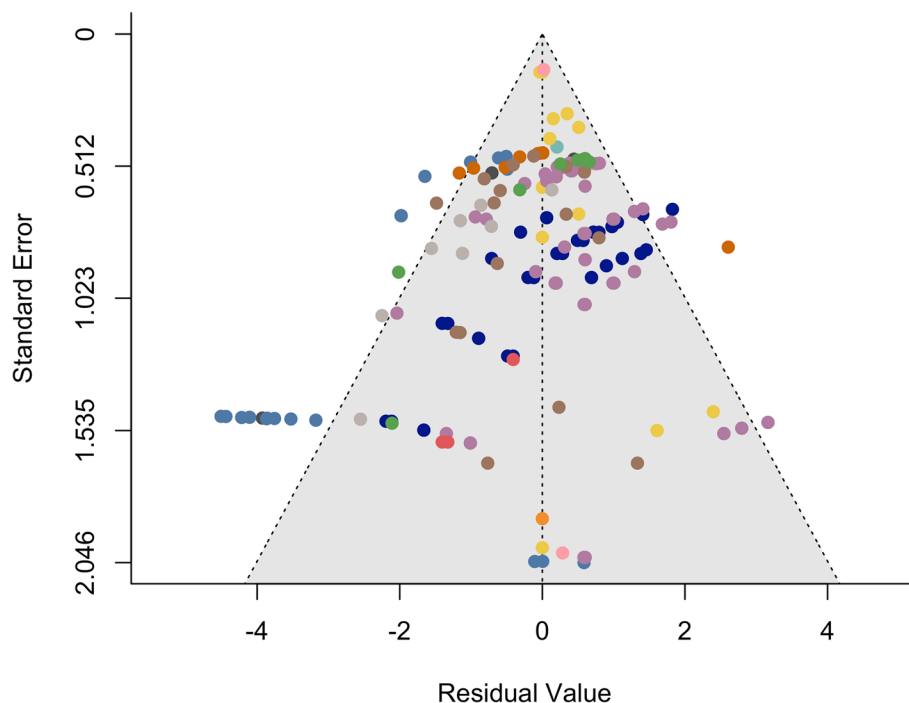
### Discussion

Our meta-analysis shows that immobilization of *Daphnia* by MNP can be alleviated by DOM. While it has been argued that the reduction in negative effects in the presence of DOM might be attributed to the formation of an ecocorona on the MNP surface alone (e.g., [22, 60]), our data indicate that DOM present in the media during exposure may contribute additionally

to the observed mitigating effects. Furthermore, the compiled data suggest that moderating effects of DOM depend on the type of DOM used.

DOM is known to alleviate effects of various pollutants in *Daphnia* spp. For example, humic acid has been shown to attenuate negative effects of soluble substances (e.g., [31, 61, 62]) including pesticides (e.g., [63]), and various particulate pollutants (e.g., [64]). For example, natural organic matter has been shown to reduce the toxicity of perfluorooctane sulfonate (PFOS, [65]) and heavy metals (e.g., [66, 67]), among others. In addition, beneficial impacts of DOM on effects imposed by MNP and other pollutants have also been demonstrated in several other aquatic organisms. Saavedra et al. [34] for example tested the toxicity of MNP to the rotifer *Brachionus calyciflorus* and larvae of *Themnocephalus platyurus* and found lowered effects for ecocorona-coated compared to pristine MNP. Similarly, ameliorating effects of DOM have, among others, been found for MNP-induced oxidative stress responses (ROS production) in algae and fish [40, 68], copper-induced mortality in freshwater mussels [33], and for pesticide-induced mortality in the freshwater mysid shrimp *Americamysis bahia* [69].

One mechanism by which DOM can reduce toxic effects is its ability to bind pollutants [70]. For instance, humic acid and, to a lesser extent, fulvic acid bind



**Fig. 3** Standard errors of data points from the studies included in the meta-analysis plotted against residual log risk ratios. Different colors represent data points extracted from different publications; the grey shaded area represents the 95 % pseudo confidence interval

hydrophobic organic pollutants including pesticides [71–73], and DOM binds pesticides and metal ions [74–76]. In contact with particulate pollutants, DOM leads to ecocorona formation (e.g., [39, 42]) altering the particles' surface characteristics [25, 40, 41] and changing interactions with tissues and cells [5, 6]. Changes in physico-chemical properties also lead to altered colloidal interactions [41] and altered aggregation in the test media [74, 77, 78]. Particle aggregation in turn affects the particles' transport behavior, can lead to increased sinking velocities and consequently lower the availability of particulate pollutants in the water column [42, 78–80]. In consequence, particle aggregation can alter MNP toxicity, for instance by altering uptake rates [49, 77, 81]. Furthermore, DOM attached to the particles may add nutritional value to the particles and thus partly reduce food dilution effects [82]. Nevertheless, all these effects of DOM on particle behavior and properties cannot explain sufficiently why moderating effects in our dataset were stronger in co-exposure as compared to MNP incubation setups, as these effects should show up in both setups similarly.

A potential alternative explanation for the strong attenuating effects of humic acid and lake water in co-exposure experiments may be that DOM in the media generally contributes to the well-being of the daphnids, thus making them more resilient to stressors. In general, stressed *Daphnia* are more sensitive towards additional stressors [83, 84] while beneficial environments help daphnids become more resilient [85, 86] (for a conceptual discussion of stress addition, see [87]). Supplementary DOM for instance may serve as a nutrient source for phytoplankton, indirectly leading to better food supply for the daphnids [88]. In addition, DOM can increase food supply through the microbial loop in particular when algal food becomes limited (e.g., [89, 90]). Second, similar to other organisms, daphnids may benefit from the direct uptake of DOM leading to improved intestinal health, increased reproduction or increased growth induced by mild chemical stress responses [91, 92]. In contrast to these findings however, other studies have demonstrated adverse effects of DOM on *Daphnia* [93–95] and on other invertebrate freshwater species (e.g., [96]), indicating that the processes and mechanisms in natural waters are likely more complex and not understood well enough yet.

Among the types of DOM used in the screened literature, humic acid and lake water added during exposure had the strongest mitigating effects, decreasing the risk of immobilization caused by MNP by almost 50% (RR = 0.56 and 0.55, respectively). Whether these effect sizes are comparable to effects in natural environments depends, among other factors, on how realistic the

concentrations of DOM applied in experiments were. In the studies included in our meta-analysis that reported DOM concentrations, test concentrations ranged from 1 to 50 mg l<sup>-1</sup> (see raw data in the supplementary online material). Similar ranges have been reported for natural aquatic systems, spanning for instance from 0.1 to 322 mg l<sup>-1</sup> in a dataset of measurements of dissolved organic carbon (DOC) from 7,500 lakes [97] (see also [88]). It is therefore likely that attenuating effects of DOM on MNP toxicity can occur in a similar way in natural habitats.

Although the compiled dataset indicates that the mere conditioning of particles in DOM-containing media has lower moderating effects on immobilization risks than co-exposure with DOM, and that effect sizes differ between different DOM types, the dataset also has some important limitations. The types of DOM tested in conditioning experiments were different from the types tested in co-exposure experiments (except for one data point from an experiment using lake water-conditioned MNP). Due to this limited overlap, it is difficult to disentangle the effects of DOM type and experimental approach at this point. However, the observed patterns can serve as valuable hypotheses that can be easily validated (or disproved) in experiments or when more studies become available in the future.

Publication bias can lead to wrong effect size estimates derived from meta-analyses. Bias arises when some effect sizes are published selectively, e.g., when only significant outcomes are published or when confirmatory results are preferentially published [98]. A second factor that can lead to wrong effect size estimates is study (or sample) heterogeneity [99, 100]. Significant heterogeneity indicates that the variance among data points cannot be sufficiently explained by sampling variance, but instead likely results from measured effects not being derived from a true common effect. The funnel plot from our meta-analysis shows a slight gap of data points on the side of increased immobilization risk in the presence of DOM and the full model showed significant residual heterogeneity. A potential reason for the gap of data points could be the preferential publication of low-powered studies where MNP effects are mitigated in the presence of DOM, while low-powered studies where the presence of DOM increased negative effects of MNP were published less often. In combination with the high residual heterogeneity, another likely reason for these patterns is however that the effect sizes in our dataset are moderated by additional factors not accounted for in our analysis [101]. For example, it is possible that the effect of DOM on immobilization caused by MNP is further moderated by experimental temperature, food availability during exposure, different MNP concentrations, different concentrations of DOM or other experimental parameters



and MNP properties. Although we accounted for confounding by pairing measurements from treatments that differed solely in DOM presence/absence while keeping all other parameters the same, we cannot rule out interaction effects. For example, it might be possible that the strength of the mediating effect of DOM on MNP toxicity differs for different polymer types, experimental temperatures, or any other parameter.

Although *Daphnia* is among the most frequently used organisms in ecotoxicological research on MNP effects [28, 29]), we found only 13 studies (published until December 2022) that met our selection criteria and allowed for the extraction of effect size data. For the successful inclusion in future meta-analyses, studies investigating the moderating effects of DOM on MNP toxicity need to: (1) include an appropriate control treatment which differs only in the absence of DOM while all other parameters are kept the same as in the DOM treatment, (2) either report measurement results as means together with an uncertainty measure (standard deviation, standard error or confidence interval) or provide the complete raw data in the supplementary online material or elsewhere and (3) clearly report the number of replicates (i.e., number of independent test vials) and the number of individuals tested per replicates for all treatments.

In general, we think that further research is needed to disentangle the effects of DOM attached to the particles and effects of DOM present in the exposure media on MNP toxicity in *Daphnia*. This includes addressing in particular the mechanisms that lead to observed differences among different approaches (MNP incubation versus co-exposure) and DOM types. Including a clear characterization and validation of the ecocorona formed by different DOM types and including DOM treatments in the absence of MNP in the experimental setup can further help deepen our understanding of the processes and effects that are to be expected in natural environments and thus increase the reliability of MNP risk assessments in the future.

## Conclusions

In the present meta-analysis, we synthesized data from 13 studies that investigated the effects of ecocorona formation and DOM on MNP-induced immobilization risk in *Daphnia* spp. We showed that the mere conditioning of particles in DOM-containing media can moderate MNP toxicity, but the presence of DOM in the test media during exposure appears to be another important predictor for the observed attenuation of negative outcomes. Based on our results and evidence from the literature on other stressors, we hypothesize that DOM and in particular humic acid mitigates negative effects of MNP by either (1) reducing bioavailability

or (2) making daphnids more resilient to stressors in general. Additional experiments are needed to challenge these hypotheses and disentangle the effects of ecocorona formation and the presence of DOM in the media, and to understand how effects of DOM on particle behavior in the medium translate into reduced effects on an organismic level. Such experiments could for example directly compare the impact of DOM-conditioning with the impact of adding the same type of DOM to the media during MNP exposure, or investigate attenuating effects of DOM on negative effects imposed by other stressors such as chemical or particulate pollutants or heat stress.

## Abbreviations

CAS	Chemical Abstracts Service
CI	Confidence interval
DOM	Dissolved organic matter
log(RR)	log risk ratio
MNP	Micro- and nanoplastic particle
ROS	Radical oxygen species
WoS	Web of Science

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43591-024-00088-4>.

Supplementary Material 1.  
Supplementary Material 2.  
Supplementary Material 3.  
Supplementary Material 4.

## Acknowledgements

We want to thank Konstantinos Grintzalis for kindly providing the raw data pertaining to Grintzalis et al. [102].

## Authors' contributions

MMM and CL acquired funding. MMM conceptualized the study. MMM and MS supervised the study. The literature search and data extraction were done by MMM, SS, EG, PK, EN, AR, SW and FB. ALAV validated and corrected the extracted data. Statistical analysis was done by PK, ALAV and MMM. EG, ALAV, MS and MMM visualized the results. SS, EG, PK, EN, AR, SW, FB wrote an initial draft of the manuscript. SS, EG, EN, AR, SW, ALAV, CL, MS and MMM reviewed and improved the manuscript. All authors read and approved the final version of the manuscript.

## Funding

Open Access funding enabled and organized by Projekt DEAL. This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – SFB 1357 – 391977956.

## Availability of data and materials

All raw data are provided in the supplemental material. All data and code are also available on github (<https://github.com/StatEcotox/Salomon-et-al-2024>, git) and Zenodo (<https://doi.org/10.5281/zenodo.11477725>).

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All authors agree to the publication.

## Competing interests

The authors declare no competing interests.

## Author details

<sup>1</sup>University of Bayreuth, Bayreuth, Germany. <sup>2</sup>Statistical Ecotoxicology, University of Bayreuth, Bayreuth, Germany. <sup>3</sup>Animal Ecology I, University of Bayreuth, Bayreuth, Germany. <sup>4</sup>Bayreuth Center for Ecology and Environmental Research (BayCEER), Bayreuth, Germany.

Received: 22 December 2023 Accepted: 25 May 2024

Published online: 20 June 2024

## References

- Barbosa F, Adeyemi JA, Bocato MZ, Comas A, Campiglia A. A critical viewpoint on current issues, limitations, and future research needs on micro- and nanoplastic studies: From the detection to the toxicological assessment. *Environ Res.* 2020;182:109089. <https://doi.org/10.1016/j.envres.2019.109089>.
- Qiao R, Deng Y, Zhang S, Wolosker MB, Zhu Q, Ren H, Zhang Y. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere.* 2019;236:124334. <https://doi.org/10.1016/j.chemosphere.2019.07.065>.
- Susanti NKY, Mardiatuti A, Wardiatno Y. Microplastics and the impact of plastic on wildlife: a literature review. *IOP C Ser Earth Env.* 2020;528(1); <https://doi.org/10.1088/1755-1315/528/1/012013>.
- Hara J, Frias J, Nash R. Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters. *Mar Pollut Bull.* 2020;152(January):110905. <https://doi.org/10.1016/j.marpolbul.2020.110905>.
- Ramsperger AFRM, Narayana VKB, Gross W, Mohanraj J, Thelakkt M, Greiner A, Schmalz H, Kress H, Laforsch C. Environmental exposure enhances the internalization of microplastic particles into cells. *Sci Adv.* 2020;6(50):1–10. <https://doi.org/10.1126/sciadv.abd1211>.
- Ramsperger AFRM, Stellwag AC, Caspari A, Fery A, Lueders T, Kress H, Löder MGJ, Laforsch C. Structural diversity in early-stage biofilm formation on microplastics depends on environmental medium and polymer properties. *Water.* 2020;12(11):3216. <https://doi.org/10.3390/w12113216>.
- Von Moos N, Burkhardt-Holm P, Köhler A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ Sci Technol.* 2012;46(20):11327–11335. <https://doi.org/10.1021/es302332w>.
- Solomando A, Capó X, Alomar C, Álvarez E, Compa M, Valencia JM, et al. Long-term exposure to microplastics induces oxidative stress and a pro-inflammatory response in the gut of *Sparus aurata* Linnaeus, 1758. *Environ Pollut.* 2020;266:115295. <https://doi.org/10.1016/j.envpol.2020.115295>.
- Zhang M, Shi J, Huang Q, Xie Y, Wu R, Zhong J, Deng H. Multi-omics analysis reveals size-dependent toxicity and vascular endothelial cell injury induced by microplastic exposure: In vivo and in vitro. *Environ Sci Nano.* 2022;9(2):663–83. <https://doi.org/10.1039/d1en01067k>.
- Lahive E, Walton A, Horton AA, Spurgeon DJ, Svendsen C. Microplastic particles reduce reproduction in the terrestrial worm *Enchytraeus crypticus* in a soil exposure. *Environ Pollut.* 2019;255:113174. <https://doi.org/10.1016/j.envpol.2019.113174>.
- Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet MEJ, et al. Oyster reproduction is affected by exposure to polystyrene microplastics. *PNAS.* 2016;113(9):2430–5. <https://doi.org/10.1073/pnas.1519019113>.
- Ziajahromi S, Kumar A, Neale PA, Leusch FDL. Impact of microplastic beads and fibers on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: implications of single and mixture exposures. *Environ Sci Technol.* 2017;51(22):13397–406. <https://doi.org/10.1021/acs.est.7b03574>.
- Song J, Na J, An D, Jung J. Role of benzophenone-3 additive in chronic toxicity of polyethylene microplastic fragments to *Daphnia magna*. *Sci Total Environ.* 2021;800:149638. <https://doi.org/10.1016/j.scitotenv.2021.149638>.
- Yan Y, Zhu F, Zhu C, Chen Z, Liu S, Wang C, Gu C. Dibutyl phthalate release from polyvinyl chloride microplastics: Influence of plastic properties and environmental factors. *Water Res.* 2021;204:117597. <https://doi.org/10.1016/j.watres.2021.117597>.
- Brehm J, Wilde MV, Reiche L, Leitner L-C, Petran B, Meinhardt M, Wieland S, Ritschar S, Schott M, Boos J-P, Frei S, Kress H, Senker J, Greiner A, Fröhlich T, Laforsch C. In-depth characterization revealed polymer type and chemical content specific effects of microplastic on *Dreissena bugensis*. *J Hazard Mater.* 2022;437:129351. <https://doi.org/10.1016/j.jhazmat.2022.129351>.
- Zimmermann L, Göttlich S, Oehlmann J, Wagner M, Völker C. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. *Environ Pollut.* 2020;267:115392. <https://doi.org/10.1016/j.envpol.2020.115392>.
- Gkoutselis G, Rohrbach S, Harjes J, Obst M, Brachmann A, Horn MA, Rambold G. Microplastics accumulate fungal pathogens in terrestrial ecosystems. *Sci Rep.* 2021;11(1):13214. <https://doi.org/10.1038/s41598-021-92405-7>.
- Rochman CM, Hoh E, Kurobe T, Teh SJ. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci Rep.* 2013;3:1–7. <https://doi.org/10.1038/srep03263>.
- do Prado Leite I, Menegotto A, da Cunha Lana P, Júnior LLM. A new look at the potential role of marine plastic debris as a global vector of toxic benthic algae. *Sci Total Environ.* 2022;838: 156262. <https://doi.org/10.1016/j.scitotenv.2022.156262>.
- Koelmans AA, Bakir A, Burton GA, Janssen CR. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environ Sci Technol.* 2016;50(7):3315–26. <https://doi.org/10.1021/acs.est.5b06069>.
- Nasser F, Constantinou J, Lynch I. Nanomaterials in the environment acquire an “eco-corona” impacting their toxicity to *Daphnia magna*—a call for updating toxicity testing policies. *Proteomics.* 2020;20(9):1–15. <https://doi.org/10.1002/pmic.201800412>.
- Fadare OO, Wan B, Liu K, Yang Y, Zhao L, Guo LH. Eco-corona vs protein corona: effects of humic substances on corona formation and nanoplastic particle toxicity in *Daphnia magna*. *Environ Sci Technol.* 2020;54(13):8001–9. <https://doi.org/10.1021/acs.est.0c00615>.
- Kihara S, Ghosh S, McDougall DR, Whitten AE, Mata JP, Köper I, McGilivray DJ. Structure of soft and hard protein corona around polystyrene nanoparticles—Particle size and protein types. *Biointerphases.* 2020;15(5):051002. <https://doi.org/10.1116/6.0000404>.
- Barros CHN, Fulaz S, Vitale S, Casey E, Quinn L. Interactions between functionalised silica nanoparticles and *Pseudomonas fluorescens* biofilm matrix: A focus on the protein corona. *PLoS ONE.* 2020;15(7):e0236441. <https://doi.org/10.1371/journal.pone.0236441>.
- Shi X, Chen Z, Wei W, Chen J, Ni B-J. Toxicity of micro/nanoplastics in the environment: Roles of plastisphere and eco-corona. *Soil Environ Health.* 2023;1(1):100002. <https://doi.org/10.1016/j.seh.2023.100002>.
- OECD. Test No. 202: *Daphnia* sp. acute immobilisation test. OECD guideline for the testing of chemicals, section 2, OECD Publishing, Paris. 2004. <https://doi.org/10.1787/9789264069947-en>.
- OECD. (2012). Test No. 211: *Daphnia magna* reproduction test; OECD guideline for the testing of chemicals, section 2, OECD Publishing, Paris. 2012. <https://doi.org/10.1787/9789264185203-en>.
- Brehm J, Ritschar S, Laforsch C, Mair MM. The complexity of micro- and nanoplastic research in the genus *Daphnia* – A systematic review of study variability and a meta-analysis of immobilization rates. *J Hazard Mater.* 2023;458(March):131839. <https://doi.org/10.1016/j.jhazmat.2023.131839>.
- Thronton Hampton LM, Lowman H, Coffin S, Darin E, De Frond H, Hermabessiere L, Miller E, de Ruijter VN, Faltynkova A, Kotar S, Monclús L, Siddiqui S, Völker J, Brander S, Koelmans AA, Rochman CM, Wagner M, Mehinto AC. A living tool for the continued exploration of microplastic toxicity. *Microplast Nanoplast.* 2022. <https://doi.org/10.1186/s43591-022-00032-4>.
- Eltensah YS, Bøhn T. Acute and chronic effects of polystyrene microplastics on juvenile and adult *Daphnia magna*. *Environ Pollut.* 2019;254:112919. <https://doi.org/10.1016/j.envpol.2019.07.087>.
- Zhang P, Yan Z, Lu G, Ji Y. Single and combined effects of microplastics and roxithromycin on *Daphnia magna*. *Environ Sci Pollut R.* 2019;26(17):17010–20. <https://doi.org/10.1007/s11356-019-05031-2>.

32. Lin W, Jiang R, Hu S, Xiao X, Wu J, Wei S, Xiong Y, Ouyang G. Investigating the toxicities of different functionalized polystyrene nanoplastics on *Daphnia magna*. *Ecotox Environ Safe*. 2019;180:509–16. <https://doi.org/10.1016/j.ecoenv.2019.05.036>.
33. Gillis PL, Mitchell RJ, Schwalb AN, McNichols KA, Mackie GL, Wood CM, Ackerman JD. Sensitivity of the glochidia (larvae) of freshwater mussels to copper: assessing the effect of water hardness and dissolved organic carbon on the sensitivity of endangered species. *Aquat Toxicol*. 2008;88(2):137–45. <https://doi.org/10.1016/j.aquatox.2008.04.003>.
34. Saavedra J, Stoll S, Slaveykova VI. Influence of nanoplastic surface charge on eco-corona formation, aggregation and toxicity to freshwater zooplankton. *Environ Pollut*. 2019;252:715–22. <https://doi.org/10.1016/j.envpol.2019.05.135>.
35. Schrank I, Trotter B, Dummert J, Scholz-Böttcher BM, Löder MGJ, Laforsch C. Effects of microplastic particles and leaching additive on the life history and morphology of *Daphnia magna*. *Environ Pollut*. 2019;255:113233. <https://doi.org/10.1016/j.envpol.2019.113233>.
36. Schwarzer M, Brehm J, Vollmer M, Jasinski J, Xu C, Zainuddin S, Fröhlich T, Schott M, Greiner A, Scheibel T, Laforsch C. Shape, size, and polymer dependent effects of microplastics on *Daphnia magna*. *J Hazard Mater*. 2022;426:128136. <https://doi.org/10.1016/j.jhazmat.2021.128136>.
37. Nasser F, Lynch I. Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on *Daphnia magna*. *J Proteomics*. 2016;137:45–51. <https://doi.org/10.1016/j.jpro.2015.09.005>.
38. Petersen EJ, Barrios AC, Henry TB, Johnson ME, Koelmans AA, Montoro Bustos AR, et al. Potential artifacts and control experiments in toxicity tests of nanoplastic and microplastic particles. *Environ Sci Technol*. 2022;56(22):15192–206. <https://doi.org/10.1021/acs.est.2c04929>.
39. Rummel CD, Jahnke A, Gorokhova E, Kühnel D, Schmitt-Jansen M. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environ Sci Technol Letters*. 2017;4(7):258–67. <https://doi.org/10.1021/acs.estlett.7b00164>.
40. Natarajan L, Jenifer MA, Mukherjee A. Eco-corona formation on the nanomaterials in the aquatic systems lessens their toxic impact: a comprehensive review. *Environ Res*. 2021;194:110669. <https://doi.org/10.1016/j.envres.2020.110669>.
41. Witzmann T, Ramsperger AFRM, Wieland S, Laforsch C, Kress H, Fery A, Auernhammer GK. Repulsive interactions of eco-corona-covered microplastic particles quantitatively follow modeling of polymer brushes. *Langmuir*. 2022;38(29):8748–56. <https://doi.org/10.1021/acs.langmuir.1c03204>.
42. Elagami H, Ahmadi P, Fleckenstein JH, Frei S, Obst M, Agarwal S, Gilfedder BS. Measurement of microplastic settling velocities and implications for residence times in thermally stratified lakes. *Limnol Oceanogr*. 2022;67(4):934–45. <https://doi.org/10.1002/lno.12046>.
43. Fischer R, Lobelle D, Kooi M, Koelmans A, Onink V, Laufkötter C, Amaral-Zettler L, Yool A, van Sebille E. Modelling submerged biofouled microplastics and their vertical trajectories. *Biogeosciences*. 2022;19(8). <https://doi.org/10.5194/bg-19-2211-2022>.
44. Raftis JB, Miller MR. Nanoparticle translocation and multi-organ toxicity: a particularly small problem. *Nano Today*. 2019;26:8–12. <https://doi.org/10.1016/j.nantod.2019.03.010>.
45. Triebeskorn R, Braunbeck T, Grummt T, Hanslik L, Huppertsberg S, Jekel M, et al. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. *TRAC Trend Anal Chem*. 2019;110:375–92. <https://doi.org/10.1016/j.trac.2018.11.023>.
46. Fadare OO, Wan B, Guo LH, Xin Y, Qin W, Yang Y. Humic acid alleviates the toxicity of polystyrene nanoplastic particles to *Daphnia magna*. *Environ Sci Nano*. 2019;6(5). <https://doi.org/10.1039/c8en01457d>.
47. Schür C, Weil C, Baum M, Wallraff J, Schreier M, Oehlmann J, Wagner M. Incubation in wastewater reduces the multigenerational effects of microplastics in *Daphnia magna*. *Environ Sci Technol*. 2021;55(4):2491–9. <https://doi.org/10.1021/acs.est.0c07911>.
48. Amariei G, Rosal R, Fernández-Piñas F, Koelmans AA. Negative food dilution and positive biofilm carrier effects of microplastic ingestion by *D. magna* cause tipping points at the population level. *Environ Pollut*. 2022;294 (September 2021). <https://doi.org/10.1016/j.envpol.2021.118622>.
49. Wu J, Jiang R, Lin W, Ouyang G. Effect of salinity and humic acid on the aggregation and toxicity of polystyrene nanoplastics with different functional groups and charges. *Environ Pollut*. 2019;245:836–43. <https://doi.org/10.1016/j.envpol.2018.11.055>.
50. Pochelon A, Stoll S, Slaveykova VI. Polystyrene nanoplastic behavior and toxicity on crustacean *Daphnia magna*: media composition, size, and surface charge effects. *Environments*. 2021;8(10):101. <https://doi.org/10.3390/environments8100101>.
51. Zhang F, Wang Z, Wang S, Fang H, Wang D. Aquatic behavior and toxicity of polystyrene nanoplastic particles with different functional groups: complex roles of pH, dissolved organic carbon and divalent cations. *Chemosphere*. 2019;228:195–203. <https://doi.org/10.1016/j.chemosphere.2019.04.115>.
52. Reilly K, Davoudi H, Guo Z, Lynch I. The Composition of the Eco-corona Acquired by Micro- and nanoscale plastics impacts on their ecotoxicity and interactions with co-pollutants. In: Szpunar J, Jiménez-Lamana J, editors. *Environmental Nanopollutants: Sources, Occurrence, Analysis and Fate*. Cambridge, UK: The Royal Society of Chemistry; 2022. <https://doi.org/10.1039/9781839166570-00132>.
53. Schefer RB, Armanious A, Mitrano DM. Eco-corona formation on plastics: adsorption of dissolved organic matter to pristine and photochemically weathered polymer surfaces. *Environ Sci Technol*. 2023. <https://doi.org/10.1021/acs.est.3c04180>.
54. Field AP, Gillett R. How to do a meta-analysis. *Brit J Math Stat Psy*. 2010;63(3):665–94. <https://doi.org/10.1348/000711010X502733>.
55. Gurevitch J, Curtis PS, Jones MH. Meta-analysis in ecology. *Adv Ecol Res*. 2001;32. [https://doi.org/10.1016/s0065-2504\(01\)32013-5](https://doi.org/10.1016/s0065-2504(01)32013-5).
56. Harrison F. Getting started with meta-analysis. *Methods Ecol Evol*. 2011;2(1):1. <https://doi.org/10.1111/j.2041-210X.2010.00056.x>.
57. Pick JL, Nakagawa S, Noble DWA. Reproducible, flexible and high-throughput data extraction from primary literature: the metaDigitiser package. *Methods Ecol Evol*. 2019;10(3):426–31. <https://doi.org/10.1111/2041-210X.13118>.
58. Nakagawa S, Lagisz M, O'Dea RE, Pottier P, Rutkowska J, Senior AM, Yang Y, Noble DWA. orchard 2.0: An R package for visualising meta-analyses with orchard plots. *Methods Ecol Evol*. 2023. <https://doi.org/10.1111/2041-210X.14152>.
59. Viechtbauer W. Conducting meta-analysis in R with metafor package. *J Stat Softw*. 2010;36(3):1–48. <https://doi.org/10.18637/jss.v036.i03>.
60. Junaid M, Wang J. Interaction of Nanoplastics with Extracellular Polymeric Substances (EPS) in the Aquatic Environment: A Special Reference to Eco-Corona Formation and Associated Impacts. *Water Res*. 2021;201:17319. <https://doi.org/10.1016/j.watres.2021.117319>.
61. Oris JT, Hall AT, Tylka JD. Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia. *Environ Toxicol Chem*. 1990;9(5):575–83. <https://doi.org/10.1002/etc.5620090506>.
62. Paulauskis JD, Winner RW. Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna* Straus. *Aquat Toxicol*. 1988;12(3):273–90. [https://doi.org/10.1016/0166-445X\(88\)90027-6](https://doi.org/10.1016/0166-445X(88)90027-6).
63. Day KE. Effects of dissolved organic carbon on accumulation and acute toxicity of fenvalerate, deltamethrin and cyhalothrin to *Daphnia magna* (straus). *Environ Toxicol Chem*. 1991;10(1). <https://doi.org/10.1002/etc.5620100111>.
64. Zhang Y, Meng T, Shi L, Guo X, Si X, Yang R, Quan X. The effects of humic acid on the toxicity of graphene oxide to *Scenedesmus obliquus* and *Daphnia magna*. *Sci Total Environ*. 2019;649:163–71. <https://doi.org/10.1016/j.scitotenv.2018.08.280>.
65. Kovacevic V, Simpson AJ, Simpson MJ. The concentration of dissolved organic matter impacts the metabolic response in *Daphnia magna* exposed to 17 $\alpha$ -ethynylestradiol and perfluorooctane sulfonate. *Ecotox Environ Safe*. 2019;170:468–78. <https://doi.org/10.1016/j.ecoenv.2018.12.008>.
66. De Schampelaere KAC, Vasconcelos FM, Tack FMG, Allen HE, Janssen CR. Effect of dissolved organic matter source on acute copper toxicity to *Daphnia magna*. *Environ Toxicol Chem*. 2004;23(5). <https://doi.org/10.1897/03-184>.
67. Penttinen S, Kostamo A, Kukkonen JVK. Combined effects of dissolved organic material and water hardness on toxicity of cadmium to *Daphnia magna*. *Environ Toxicol Chem*. 1998;17(12):2498–503. <https://doi.org/10.1002/etc.5620171217>.
68. Liu Y, Wang Z, Wang S, Fang H, Ye N, Wang D. Ecotoxicological effects on *Scenedesmus obliquus* and *Danio rerio* co-exposed to polystyrene

- nano-plastic particles and natural acidic organic polymer. *Environ Toxicol Phar.* 2019;67:21–8. <https://doi.org/10.1016/j.etap.2019.01.007>.
69. Mézin LC, Hale RC. Effect of humic acids on toxicity of DDT and chlorpyrifos to freshwater and estuarine invertebrates. *Environ Toxicol Chem.* 2004;23(3): <https://doi.org/10.1897/02-431>.
70. Kukkonen J, Oikari A. Bioavailability of organic pollutants in boreal waters with varying levels of dissolved organic material. *Water Res.* 1991;25(4):455–63. [https://doi.org/10.1016/0043-1354\(91\)90082-2](https://doi.org/10.1016/0043-1354(91)90082-2).
71. Chianese S, Fenti A, Iovino P, Musmarra D, Salvestrini S. Sorption of organic pollutants by humic acids: A review. *Molecules.* 2020;25(4):918. <https://doi.org/10.3390/molecules25040918>.
72. De Paolis F, Kukkonen J. Binding of organic pollutants to humic and fulvic acids: Influence of pH and the structure of humic material. *Chemosphere.* 1997;34(8): [https://doi.org/10.1016/S0045-6535\(97\)00026-X](https://doi.org/10.1016/S0045-6535(97)00026-X).
73. Landrum PF, Reinhold MD, Nihart SR, Eadie BJ. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. *Environ Toxicol Chem.* 1985;4(4):459–67. <https://doi.org/10.1002/etc.5620040406>.
74. Aiken GR, Hsu-Kim H, Ryan JN. Influence of dissolved organic matter on the environmental fate of metals, nanoparticles, and colloids. *Environ Sci Technol.* 2011;45(8):3196–201. <https://doi.org/10.1021/es103992s>.
75. He XS, Zhang YL, Liu ZH, Wei D, Liang G, Liu HT, Xi BD, Huang ZB, Ma Y, Xing BS. Interaction and coexistence characteristics of dissolved organic matter with toxic metals and pesticides in shallow groundwater. *Environ Pollut.* 2020;258:113736. <https://doi.org/10.1016/j.envpol.2019.113736>.
76. Reuter JH, Perdue EM. Importance of heavy metal-organic matter interactions in natural waters. *Geochim Cosmochim Acta.* 1977;41(2):325–34. [https://doi.org/10.1016/0016-7037\(77\)90240-X](https://doi.org/10.1016/0016-7037(77)90240-X).
77. Meng Z, Recoura-Massaquant R, Chaumot A, Stoll S, Liu W. Acute toxicity of nanoplastics on *Daphnia* and *Gammarus* neonates: Effects of surface charge, heteroaggregation, and water properties. *Sci Total Environ.* 2023;854:158763. <https://doi.org/10.1016/j.scitotenv.2022.158763>.
78. Yan M, Wang L, Dai Y, Sun H, Liu C. Behavior of microplastics in inland waters: aggregation, settlement, and transport. *B Environ Contam Tox.* 2021;107(4):700–9. <https://doi.org/10.1007/s00128-020-03087-2>.
79. Karakaş G, Nowald N, Schäfer-Neth C, Iversen M, Barkmann W, Fischer G, Marchesiello P, Schlitzer R. Impact of particle aggregation on vertical fluxes of organic matter. *Prog Oceanogr.* 2009;83:1–4. <https://doi.org/10.1016/j.pcean.2009.07.047>.
80. Petosa AR, Jaisi DP, Quevedo IR, Elimelech M, Tufenkji N. Aggregation and deposition of engineered nanomaterials in aquatic environments: Role of physicochemical interactions. *Environ Sci Technol.* 2010;44(17):6532–49. <https://doi.org/10.1021/es100598h>.
81. Albanese A, Chan WCW. Effect of gold nanoparticle aggregation on cell uptake and toxicity. *ACS Nano.* 2011;5(7):5478–89. <https://doi.org/10.1021/nn2007496>.
82. Arruda JA, Marzolf GR, Faulk RT. The role of suspended sediments in the nutrition of zooplankton in turbid reservoirs. *Ecology.* 1983;64(5):1225–35. <https://doi.org/10.2307/1937831>.
83. Serra T, Barcelona A, Pous N, Salvadó V, Colomer J. Synergistic effects of water temperature, microplastics and ammonium as second and third order stressors on *Daphnia magna*. *Environ Pollut.* 2020;267:115439. <https://doi.org/10.1016/j.envpol.2020.115439>.
84. Yin M, Laforch C, Lohr JN, Wolinska J. Predator-induced defense makes *Daphnia* more vulnerable to parasites. *Evolution.* 2011;65(5):1482–8. <https://doi.org/10.1111/j.1558-5646.2011.01240.x>.
85. Lye Koh H, Hallam TG, Ling Lee H. Combined effects of environmental and chemical stressors on a model *Daphnia* population. *Ecol Model.* 1997;103(1):19–32. [https://doi.org/10.1016/S0304-3800\(97\)00073-2](https://doi.org/10.1016/S0304-3800(97)00073-2).
86. Vandenbrouck T, Dom N, Novais S, Soetaert A, Ferreira ALG, Loureiro S, et al. Nickel response in function of temperature differences: Effects at different levels of biological organization in *Daphnia magna*. *Comp Biochem Phys D.* 2011;6(3):271–81. <https://doi.org/10.1016/j.cbd.2011.06.001>.
87. Liess M, Folt K, Knillmann S, Schäfer RB, Liess HD. Predicting the synergy of multiple stress effects. *Sci Rep.* 2016;6:32965. <https://doi.org/10.1038/srep32965>.
88. Thomas JD. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biol.* 1997;38(1):1–36. <https://doi.org/10.1046/j.1365-2427.1997.00206.x>.
89. Hiltunen M, Honkanen M, Taipale S, Strandberg U, Kankaala P. Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to *Daphnia*. *J Plankton Res.* 2017;39(6):861–9. <https://doi.org/10.1093/plankt/fbx050>.
90. McMeans BC, Koussoroplis AM, Arts M, Kainz MJ. Terrestrial dissolved organic matter supports growth and reproduction of *Daphnia magna* when algae are limiting. *J Plankton Res.* 2015;37(6):1201–9. <https://doi.org/10.1093/plankt/fbv083>.
91. Gao Y, He J, He Z, Li Z, Zhao B, Mu Y, Lee JY, Chu Z. Effects of fulvic acid on growth performance and intestinal health of juvenile loach *Parasemigurnus dabryanus* (Sauvage). *Fish Shellfish Immunol.* 2017;62:47–56. <https://doi.org/10.1016/j.fsi.2017.01.008>.
92. Steinberg CEW, Timofeyev MA, Menzel R. Dissolved humic substances: interactions with organisms. In: Likens GE, editor. *Encyclopedia of Inland Waters.* 2009. <https://doi.org/10.1016/B978-012370626-3.00116-2>.
93. Euent S, Menzel R, Steinberg CEW. Gender-specific lifespan modulation in *Daphnia magna* by a dissolved humic substances preparation. *Annals Environ Sci.* 2008;2.
94. Saebelfeld M, Minguez L, Griebel J, Gessner MO, Wolinska J. Humic dissolved organic carbon drives oxidative stress and severe fitness impairments in *Daphnia*. *Aquat Toxicol.* 2017;182:31–8. <https://doi.org/10.1016/j.aquatox.2016.11.006>.
95. Wenzel A, Vrede T, Jansson M, Bergström AK. *Daphnia* performance on diets containing different combinations of high-quality algae, heterotrophic bacteria, and allochthonous particulate organic matter. *Freshwater Biol.* 2021;66(1):157–68. <https://doi.org/10.1111/fwb.13626>.
96. Timofeyev MA, Shatilina ZM, Kolesnichenko AV, Bedulina DS, Kolesnichenko VV, Pflugmacher S, Steinberg CEW. Natural organic matter (NOM) induces oxidative stress in freshwater amphipods *Gammarus lacustris* Sars and *Gammarus tigrinus* (Sexton). *Sci Total Environ.* 2006;366(2–3): <https://doi.org/10.1016/j.scitotenv.2006.02.003>.
97. Sobek S, Tranvik LJ, Prairie YT, Kortelainen P, Cole JJ. Patterns and regulation of dissolved organic carbon: an analysis of 7,500 widely distributed lakes. *Limnol Oceanogr.* 2007;52(3):1208–19. <https://doi.org/10.4319/lo.2007.52.3.1208>.
98. Sterling TD. Publication decisions and their possible effects on inferences drawn from tests of significance—or vice versa. *J Am Stat Assoc.* 1959;54(285):30–4. <https://doi.org/10.1080/01621459.1959.10501497>.
99. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002;21(11):1539–58. <https://doi.org/10.1002/sim.1186>.
100. Kenny DA, Judd CM. The unappreciated heterogeneity of effect sizes: implications for power, precision, planning of research, and replication. *Psychol Methods.* 2019. <https://doi.org/10.1037/met0000209>.
101. Ioannidis JPA, Trikalinos TA. The appropriateness of asymmetry tests for publication bias in meta-analyses: A large survey. *Can Med Assoc J.* 2007;176(8):1091–6. <https://doi.org/10.1503/cmaj.060410>.
102. Grintzalis K, Lawson TN, Nasser F, Lynch I, Viant MR. Metabolomic method to detect a metabolite corona on amino-functionalized polystyrene nanoparticles. *Nanotoxicology.* 2019;13(6):783–94. <https://doi.org/10.1080/17435390.2019.1577510>.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.