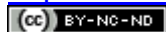Article (refereed) - postprint

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Effects of a microplastic mixture differ across trophic levels and taxa in a freshwater food web: in situ mesocosm experiment.

EFFECTS OF A MICROPLASTIC MIXTURE on different taxa and TROPHIC LEVELS IN A FRESHWATER FOOD WEB: AN OUTDOOR MESOCOSM EXPERIMENT

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***We would reproduce the illustrations in figures in black and white for print upon acceptance.

HIGHLIGHTS

- Impacts of microplastic exposure on the food web were lower than hypothesized.
- Zooplankton microplastic ingestion was low, and mostly by large-bodied *Daphnia*.
- Microplastics were trophically transferred to odonate larvae.
- Exposure to microplastics altered wing morphology in chironomids.
- The first *in-situ* transfer of microplastics to terrestrial ecosystems was recorded.

ABSTRACT

The ubiquitous presence of microplastics (MP) in aquatic ecosystems can affect organisms and communities in multiple ways. While MP research on aquatic organisms has primarily focused on marine ecosystems and laboratory experiments, the community-level effects of MP in freshwaters, especially in lakes, are poorly understood. To examine the impact of MP on freshwater lake ecosystems, we conducted the first *in situ* community-level mesocosm experiment testing the effects of MP on a model food web with zooplankton as main herbivores, odonate larvae as predators, and chironomid larvae as detritivores. For seven weeks, tThe mesocosms were exposed for seven weeks to a mixture of the most abundant MP polymers found in freshwaters, added at two different concentrations in a single pulse to the water surface (polyethylene (PE), polypropylene (PP), 0.007 g m^{-2} and 0.07 g m^{-2}), andwater column (PE, 2 mg L^{-1} and 20 mg L^{-1}), and sediment (polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA) and polyethylene terephthalate (PET), 8 g m^{-2} and 80 g m^{-2}). Water column MP concentrations declined sharply during the first two weeks of the experiment. Contrary to expectations, MP ingestion by zooplankton was low and limited mainly to large-bodied *Daphnia*, causing a decrease in their biomass. Biomass of the other zooplankton taxa did not decrease. Presence of MP in the faecal pellets of odonate larvae that feed on zooplankton was indicative of a trophic transfer of MP. For chironomids, MP had only a low, short-term impact on emergence patterns while their wing morphology was significantly affected. Overall, the impact of MP exposure on the experimental food web and cross-ecosystem biomass transfer was lower than expected, but the experiment provided the first *in situ* observation of MP transfer to terrestrial ecosystems by emerging chironomids.

1| INTRODUCTION

Plastics have become a global anthropogenic problem due to their ubiquitous presence in the environment, especially in aquatic systems (Vince and Stoett, 2018). The annual plastic production rate increased to approximately 370 million metric tons in 2019 (Plastics Europe, 2020). Up to 4.6% of this plastic is transported into marine ecosystems through rivers and lake ecosystems, and runoffs (Drist et al., 2017; Güven et al., 2017). Plastic particles between 1 µm and 5 mm in size, termed "microplastics" (hereinafter, MP), represent a considerable proportion of the plastics found in freshwater ecosystems including lakes, rivers, and reservoirs across the globe, making freshwater MP contamination an issue of global concern (e.g. Li et al., 2020; Rochman, 2018). As a result, research of MP impacts on species, communities and ecosystems is growing exponentially (Sorensen and Jovanović, 2021).

The most abundant plastic polymers found in freshwater ecosystems globally are polyethylene (PE) and polypropylene (PP), followed by polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA) and polyethylene terephthalate (PET) (e.g. Canniff and Hoang, 2018; Koelmans et al., 2019; Martins and Guilhermino, 2018). While PE and PP ~~(density <1 g cm⁻³)~~ are typically buoyant (density <1 g cm^{-3}) and common in surface waters, the denser PS, PA, PVC and PET (density> 1 g cm^{-3}) rapidly sink to the sediment (Bond et al., 2018; Koelmans et al., 2019). The large surface area-to-volume ratio of MP is well suited for the growth of microorganisms, with surface biofilms ~~starting to~~ form~~ing~~ within hours (Rummel et al., 2017; Oberbeckmann et al., 2015), making ~~the~~ MP more palatable for consumers and more likely to sink faster.

Due to their small size and high availability, MP can enter aquatic food webs via direct ingestion (Setälä et al., 2014) or indirectly through prey-predator interactions resulting in transfer to higher trophic levels (Cole et al., 2013; Scherer et al., 2018). Direct ingestion and trophic transfer of MP may have profound toxic and behavioural effects on prey and predators (da Costa Araújo et al., 2020; da Costa Araújo and Malafaia, 2021) and hence can alter trophic interactions, population dynamics and energy transfer in food webs. Trophic transfer of MP occurs in many taxa: from mussels to crabs (Farrell and Nelson, 2013), from cladocerans to *Chaoborus* (Scherer et al., 2018), from copepods to

3

54 macrozooplankton (Cole et al., 2013; Setälä et al., 2018), from large-sized cladocerans to fish (Chae et

55 al., 2018; Wang et al., 2021). MP can also be transferred during ontogeny, e.g. from larvae to adults in

56 mosquitoes (Al-Jaibachi et al., 2018), indicating a possible MP transfer pathway from aquatic to

57 terrestrial environments (Hu et al., 2018).

58   Despite the ubiquitous presence of MP in freshwater environments, most of the research has

59 focused on the marine environment and single-species laboratory experiments, leaving a substantial

60 knowledge gap on the community-level effects of MP in freshwater habitats (Meng et al., 2020).

61 ~~Freshwater s~~Studies focus~~sing~~ed on MP ~~in freshwater systems mostly investigated MP~~ ingestion of one

62 or a few species in laboratory experiments ~~such as~~mostly focus on crustaceans (Cole et al., 2013 and

63 2015; Ogonnelowski et al., 2016; Ziajahromi et al., 2017), macro-invertebrates (Redondo-Hasselerharm

64 et al., 2018), and sediment-dwelling organisms (Nel et al., 2018; Silva et al., 2022). These studies ~~mostly~~

65 often used very high concentrations of a single MP type (Canniff and Hoang, 2018; Greven et al., 2016;

66 Rochman et al., 2013). The reported individual-level adverse effects of MP ingestion include physical

67 injury (Gall and Thompson, 2015), reduction in feeding rate (Cole and Galloway, 2015), reproduction,

68 growth, and survival (Lee et al., 2013; Sussarellu et al., 2016).

69   Most studies on freshwater crustaceans found that MP ingestion reduced their biomass, growth

70 rate, feeding capacity, and fitness (e.g., Bosker et al., 2019; Cole et al., 2013 and 2015; Rehse et al.,

71 2016). MP ingestion rates can vary substantially between species and individuals and can be affected

72 by foraging strategy and MP concentration in the environment (e.g., Canniff and Hoang, 2018; Frydkjær

73 et al., 2017). In particular, generalist filter feeders such as *Daphnia* and *Ceriodaphnia* are expected to

74 ingest MP more readily compared to selective (e.g., calanoid copepods) or raptorial feeders (e.g.,

75 cyclopoid copepods) (Scherer et al., 2018). Nevertheless, copepods can also ingest MP (Cole et al.,

76 2013) despite their ability to discriminate against non-food particles (DeMott, 1986). Non-selective~~ly~~

77 feeding benthic chironomid larvae can also ingest MP (Nel et al., 2018; Scherer et al., 2017), ~~and the~~

78 ~~negative effects of ingested MP can be observed~~and can result in altered morphology (e.g., wing

79 deformation; Silva et al., 2019; Stanković et al., 2020). However, the significance of individual-level

80 responses to MP ingestion for the population-, community- and ecosystem-level responses to MP

81 pollution are not well understood (Sorrentino and Senna, 2021).

82   This study investigated MP impacts on a freshwater food web using an outdoor mesocosm

83   experiment. Besides a control treatment ('no MP'), ~~T~~the two different MP concentrations ~~were~~ tested

84   ~~with 'no MP' control,~~were (a) a low MP dose representing the current environmentally relevant

85   concentration, and (b) a high MP dose representing the 'likely future business-as-usual' scenario

86   concentration (ten times higher than the current scenario). MP were added to the water surface, water

87   column and sediment. The hypotheses included: (1) MP ~~stay~~ will be maintained in the water column

88   long enough ~~time~~ to be readily ingested by zooplankton, particularly large-bodied cladocerans ~~for which~~

89   ~~the MP size would fit within the size range of ingested food particles~~, and ingestion of MP ~~causes~~ will

90   lead to a decrease in zooplankton biomass, especially in the high MP concentration; (2) MP ~~are~~ will be

91   transferred from zooplankton to higher trophic levels; (3) MP ~~are~~ will be ingested by ~~the~~ benthic

92   chironomid larvae ~~and lead to altered~~causing changes in morphology and adult emergence patterns; and

93   (4) emerging insects will transfer MP ingested during the aquatic larval stage ~~from freshwater~~ to the

94   terrestrial environment.

95   **2 | MATERIALS AND METHODS**

96   **2.1 | Experimental set-up**

97   The mesocosm experiment ran at the METU Outdoor Experimental System (mesocosm) located

98   at the deepest point of the experimental lake of the Middle East Technical University campus (Ankara,

99   Turkey; 39° 52'13.18 "N, 32° 46'31.92 "E, 998 m above sea level) for seven weeks from June to August

100   2018. This period was required in order to include effects of MPs on trophic interactions (i.e., beyond

101   the timescales of hours to days commonly used in laboratory experiments)~~,~~ and allowed us to investigate

102   aquatic insect emergence patterns~~,~~ and MP transfer to the terrestrial environment by the emerging

103   insects. For example, *Sympetrum* larvae used in the mesocosm experiment need approximately three

104   weeks to complete the last instar under a diet similar to that available in the experimental mesocosms

105   (Dudová et al., 2019). Experimental treatments included a control (no MP), low MP dose

106   (environmentally relevant concentration), and high MP dose (ten times low MP treatment concentration

107   - a 'likely business as usual future scenario') with four replicates of each treatment ($N = 12$). The total

108   MP concentrations added to the low MP mesocosms were 0.007 g m^{-2} for the water surface, 2 g m^{-3} (2

109 mg L^{-1}) for the water column, and 8 g m^{-2} for the sediment, and the respective concentrations were 10

110 times higher in the high MP mesocosms. The concentrations were based on previous studies (for details

111 see supplementary Text S1, Castañeda et al., 2014; Dubaish and Liebezeit, 2013; Lechner et al., 2014;

112 Moore et al., 2011; Zhang et al., 2015). Individual mesocosms were made of fiberglass and measured

113 1.2 m in depth and 1.2 m in width. Mesocosms were submerged in lake water and filled with lake water

114 to obtain a 1m water column depth. The top 20 cm were not filled in order to avoid incursion of lake

115 water into the mesocosms during windy periods (volume 1360 L; for further details see Coppens and

116 Hejzlar, 2016; Ersoy et al., 2020). Each mesocosm contained 10 cm of mixed sediment: 10% of natural

117 sediment mixture from four oligotrophic lakes (Lakes Gölcük, Abant, Çubuk and Poyrazlar) and 90%

118 washed sand (by volume, grain diameter <1 mm) (**Figure 1** and also see Ersoy et al., 2020 for further

119 details). The sediment was equilibrated to the desired experimental total phosphorus (TP) concentration

120 of 20±5 μg L^{-1} in the laboratory prior to the experiment.
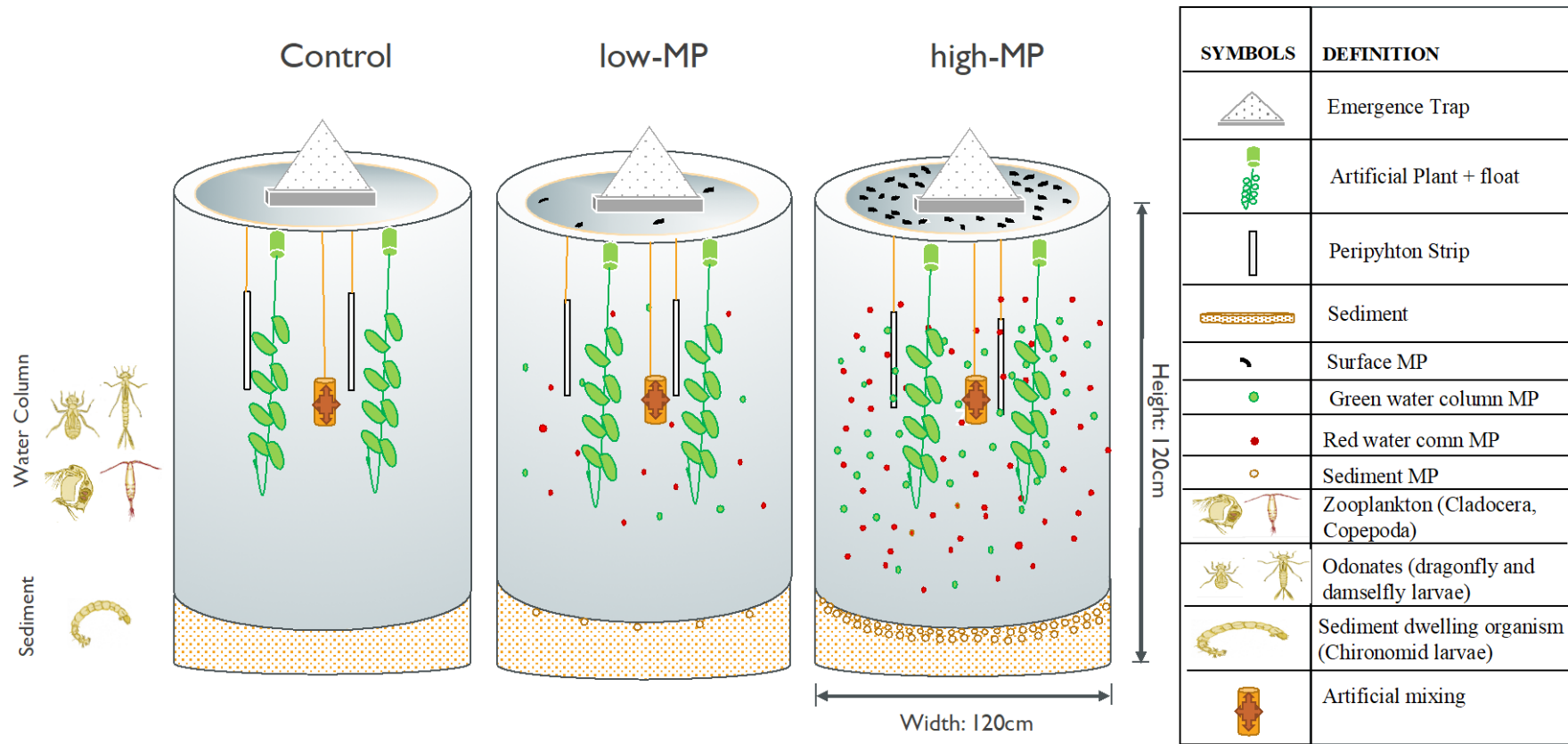
Figure 1 Unscaled schematic view of the mesocosm set-up with inocula and MP treatment.

7

123   Defined MP polymer mixtures were added to the water surface, water column, and the sediment

124   in the low and high MP concentrations. PS (Sigma-Aldrich cat no. 331651), PET (Rynite 530 polyester

125   resin, Sigma-Aldrich cat no. 429252) and PP (viscosity 10 poise, Sigma-Aldrich cat no. 428175) were

126   grounded with a coffee grinder and then filtered through a 500 μm mesh before inoculation. Plastics

127   such as PA (Sigma-Aldrich cat no. 02395), PVC (Sigma-Aldrich cat no. 81387) and PE (Sigma-Aldrich

128   cat no. 434272), and neutrally buoyant fluorescent red and green PE (Cospheric LLC: red cat no.

129   UVPMS-BR; green cat no. UVPMS-BG) were available as microspheres (powder form) (for details see

130   Stanković et al., 2020). To determine the mean size of inoculated MP, 100 randomly chosen particles

131   for each plastic type were measured under a Leica M125 microscope with Leica Application Suite

132   version 4.12.0 (**Table 1**). MP were added and mixed to the top 2 cm of the sediment layer before the

133   mesocosms were filled with filtered lake water (500-μm mesh) ten days prior to the experimental start.

134   To prevent re-suspension and disturbance of the sediment, a wooden disc was placed on top of the

135   sediment in each mesocosm during lake water addition. MP were added to the water surface and column

136   at the start of the experiment (day 0). MP in the water column included the green and red fluorescent

137   PE microspheres (density of 1.00 g cm^{-3} and 0.995 g cm^{-3}, respectively) , hereafter referred to as 'green

138   MP' and 'red MP', ensuring the detection of the MPs within animals . To prevent MP aggregation, water

139   column and surface MP were suspended in 50 mL of water and 5 mL of Tween80 before addition to the

140   mesocosms. Control treatments received the same amount of Tween80 s.

141

Table 1. Inoculated MP types, particle properties and relative volume used for each layer in the mesocosms: Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA) and polyethylene terephthalate (PET). Length and width of MP, measured via a stereomicroscope, are given as mean±SD ($N = 100$ particles for each type of MP).

MP type	Layer Used	Density (g.cm^{-3})	Length (µm)	Width (µm)	Relative Volume (%)	Form and shape
PE	Surface water	0.94	22±31	15±11	50	Powder (mostly spherical)
PP	Surface water	0.90	336±90	254±67	50	Irregular
Green PE	Water column	1.00	56±3 (diameter)		50	Microspheres (perfect spheres)
Red PE	Water column	0.995	28±8 (diameter)		50	Microspheres (perfect spheres)
PS	Sediment	1.06	118±37	82±24	20	Irregular
PET	Sediment	1.68	42±32	21±17	45	Irregular
PVC	Sediment	1.14	111±27	88±21	20	Powder (mostly spherical)
PA	Sediment	1.14	99±37	71±25	15	Powder (mostly spherical)

Mesocosms were inoculated with an additional mixture of phytoplankton and zooplankton communities, collected from five local lakes (Lakes Gölcük, Abant, Çubuk, Poyrazlar and Eymir), five days before the start of the experiment (Landkildehus et al., 2014). Water for phytoplankton inoculum was collected at every meter of the water column of each lake using a Ruttner water sampler (KC Denmark) and filtered through a 55 µm mesh to exclude zooplankton. Zooplankton for initial inoculum was collected via five vertical plankton net hauls (net diameter 25 cm, mesh size 55 µm) of the entire water column in each lake. Samples were thoroughly mixed in barrels before aliquots were added to each mesocosm (2 L per mesocosm). Mesocosms were also inoculated with intermediate to late-instar

odonate larvae collected from a nearby wetland: 25 *Sympetrum* cf. *striolatum* larvae and 47 damselfly larvae, mostly *Ischnura* spp.; hereinafter: *Sympetrum* and Zygoptera. Chironomid larvae were collected from the same wetland area and added three days before the start of the experiment. Each mesocosm received 200 mL of sieved larvae identified by the relevant identification keys (Andersen et al., 2013, Vallenduuk and Pillot 2007, Vallenduuk, 2017, **Text S1**).

One pyramid-shaped floating emergence trap (35 x 35 cm water surface area) with a 250 mL collection bottle containing glycerated ethanol (Cadmus et al., 2016) was installed in each mesocosm on day 0. Two periphyton growth strips (16 cm ~~height~~ length x 2 cm width) were placed in each mesocosm 0.5 m below the water surface. Two artificial broad-leaved plastic plant models resembling a *Potamogeton* sp. with multiple leaves were placed in each mesocosm. The plant models spanned the entire water column from bottom to surface (ca. 1m) ~~were~~ and were attached to a float to provide refuge and perching sites for the odonate larvae (Gingras et al., 2008; Grutters et al., 2015; Tavşanoğlu et al., 2015). All mesocosms were covered with a bird net (3 x 3 cm mesh size) to prevent birds, turtles, and fish from entering the mesocosms. Water in the mesocosms was gently mixed with a pump to mimic natural water movement (e.g., due to wind action). Nitrate and phosphate were added to each mesocosm to keep stable nutrient concentrations (see **Text S1**).

2.2 | Sampling and analyses

For water samples, the entire water column (surface to approximately 5 cm above the sediment) of each mesocosm was sampled at three horizontal locations (at 10, 30, and 60 cm distance to the mesocosm walls) using an integrated tube sampler. Water taken with the tube sampler was pooled in a 10 L bucket for each mesocosm (hereinafter: pooled sample) and subsampled for water chemistry (alkalinity, TN, TP, soluble reactive phosphorus (SRP), ammonium (NH_4-N)), red and green MP counts, chlorophyll-*a* (Chl-a) concentration, and zooplankton community for further analyses in the laboratory (for details see **Text S1**). All water samples were stored in ~~a~~the cool box until arrival to the laboratory. The first three samplings for all parameters were conducted 2, 24, and 48 hours after adding MP to the surface and water column. From then onwards, weekly sampling for chemical parameters and Chl-a were conducted on the same day for seven weeks. Temperature, conductivity, pH, and dissolved oxygen

183   (DO) concentrations were recorded in each mesocosm at 0.5 m depth using a YSI 556 MPS probe

184   (®YSI, USA) during each sampling. Water transparency was assessed using a Secchi disc. Periphyton

185   samples were taken after 35 days of incubation, and the strips were replaced with new ones and sampled

186   again on day 49. Zooplankton were collected on day 0, day 1 and day 2, weekly for the first two weeks,

187   and then fortnightly until the end of the experiment. Odonate larvae were sampled on days 1, 14, 28,

188   and 42 of the experiment for gut MP content. Emerging adult insects and last-instar exuviae of emerged

189   odonate larvae were sampled from the emergence traps five times a week during the first three weeks,

190   followed by two sampling events per week for the rest of the experiment.

191   For MP counts, 100 mL from the pooled sample was filtered through a GF/C Whatman filter

192   (1.2 μm pore size), and the red and green MP spheres on the filter counted under a stereomicroscope at

193   10x magnification. To prevent MP aggregation, 1 mL of TWEEN80 was mixed with water samples

194   before filtration. Ingestion of red and green MP by zooplankton was examined under a stereomicroscope

195   at 10x magnification and an inverted microscope (Leica, DMI4000B) at 63x magnification with a

196   fluorescent filter. Zooplankton were identified to species level whenever possible, and length was

197   measured for dry weight (DW) calculations (see supplementary information for details). Cladocerans

198   were further divided into two size classes; small (<0.5 mm) and large (>0.5 mm). The overall incidence

199   of ingestion (hereinafter: MP-IOI) was calculated for zooplankton, odonates and adult chironomids as

200   the percentage of individuals examined for MP content with MP in their gut (Steer et al., 2017). The

201   degree of MP ingestion (hereinafter: MP-DOI) was also calculated for all groups as the number of

202   ingested MP particles per individual that had at least one MP particle in its gut (Desforges et al., 2015).

203   Other MP types were not counted as only the fluorescent red and green water column MPs were used to

204   investigate the trophic transfer. Therefore, the effect of other MP types was only evaluated indirectly

205   through their effect on population sizes and biomasses.

206   **2.3 | Statistical analyses**

207   Principal response curves accounting for repeated observations over time were used to assess if

208   key physicochemical variables and zooplankton community composition in individual mesocosms

209   differed between the MP scenarios (Szöcs et al., 2015). The physicochemical variables included water

11

temperature, conductivity, pH, DO concentration, water transparency (Secchi disc depth), total alkalinity, TP, SRP, TN, NH_4-N, and Chl-a. Zooplankton community composition was quantified as the biomass of individual taxa and developmental stages (see **Text S1** for details). One mesocosm from the low MP treatment deviated markedly in water transparency and other properties, and a turtle was found in the mesocosm one week after experimental start; we excluded this replicate from the analyses.

Model selection approach was used for all univariate responses defined below, except for the data on total biomass of emerged chironomids on days 7 and 42 and the probability of emergence of the odonate larvae (see **Text S1**). The models always included the MP concentrations either alone or in combination with other predictors in order to quantify the change of response of the MP addition . The most parsimonious (hereafter 'best') model for each response was identified using the corrected Akaike information criterion (AIC) (Burnham and Anderson, 2002), and a likelihood ratio test (LRT) was used to quantify the significance of the treatment effect or its statistical interaction with time where appropriate.

The first set of models tested the relationship between red, green MP and Chl-a concentrations, periphyton biomass, zooplankton abundance, and biomass of the dominant zooplankton groups. Generalized linear mixed models (GLMMs) were used to account for repeated observations in individual mesocosms with appropriate distribution and link functions; generalized linear models (GLMs) were used for responses measured only once or twice (for details see **Text S1**). Mesocosm identity was included as a random intercept in each GLMM analysis. Adult chironomids emerged continuously throughout the experiment; piecewise linear approximation was used to interpolate the MP concentration values on days when MP were not measured, and the uncertainty in the link between current MP concentrations and individual MP content was accounted for by using the day as an additional random intercept in the analysis of MP content in adult chironomids. Note that this approach using model selection (instead of standard hypothesis testing) and GLMMs (instead of, e.g., repeated measures ANOVA) allows comparing multiple models at once and provides insights into the temporal dynamics of the response variables. All analyses were run in R version 4.0.2 (R Core Team, 2020) (for details see **Text S1**).

237   Finally, the software tpsDig2 (Rohlf, 2018) was used to digitize 13 specific wing landmarks in

238   the morphometric analysis of *C. riparius* individuals. Data on both sexes were thus analyzed separately

239   to account for ~~the~~ sexual dimorphism. Landmark positions on the right wings of males and females were

240   determined according to Savić-Zdravković et al. (2018). MorphoJ software (Klingenberg, 2011) was

241   used for further geometric morphometric analysis and the data on wing shape analyzed in Statistica (Stat

242   Soft Inc. version 7.0) as in Savić-Zdravković et al. (2018).

3 | RESULTS

3.1 | Environmental conditions

245   Temperature, conductivity, pH, DO concentration, total alkalinity, TP, SRP, TN, and NH_4-N

246   concentrations fluctuated over the course of the experiment but did not vary significantly among

247   mesocosms (**Table S1**, first PRC axis: $F_{1,56} = 9.82$, $P = 0.77$). Temporal dynamics of the red and~~,~~ green

248   MP and Chl-a concentrations in the water column and periphyton DW significantly differed between

249   low and high MP concentrations, i.e., the best models for all four responses (**Table S2**) contain~~ed~~ing the

250   time-by-treatment interactions (LRT, treatment × time: red MP, $\chi_2^2 = 51.1$, $P < 10^{-4}$; green MP, $\chi_2^2 =$

251   21.3, $P < 10^{-4}$; Chl-a, $\chi_2^2 = 24.5$, $P < 10^{-4}$; periphyton, $\chi_2^2 = 15.4$, $p = 0.0004$; treatment × time^2: red MP,

252     $\chi_2^2 = 19.3$, $P < 10^{-4}$; green MP, $\chi_2^2 = 7.15$, $p = 0.028$; Chl-a, $\chi_2^2 = 0.11$, $p = 0.95$).

253   Best models for red and green MP concentrations showed that their water column concentrations

254   declined sharply over time, especially within the first ten days, until ca. day 20 to day 30 and then

255   remained constant or slightly increased depending on the concentration. The final MP concentrations in

256   the water column were at least ten times lower in the low MP concentration and 100 times lower in the

257   high MP concentration compared to the initial concentrations (**Figure 2**). Small MP quantities observed

258   in controls were probably caused by cross-contamination despite rinsing all equipment between

259   treatments (day 0, red MP, controls: $(2.0 \pm 0.64) \times 10^2$ particles L^{-1}, low MP: $(7.5 \pm 5.2) \times 10^3$ particles

260   L^{-1}, high MP: $(5.6 \pm 2.1) \times 10^4$ particles L^{-1}; green MP, controls: $(0.45 \pm 0.77) \times 10^2$ particles L^{-1}, low

261   MP: $(3.9 \pm 2.2) \times 10^3$ particles L^{-1}, high MP: $(1.9 \pm 0.7) \times 10^4$ particles L^{-1}; **Figure 2** and **S1**).

262   Chl-a concentrations were very low throughout the experiment and first declined and then

263   remained approximately stable in the controls while it increased in the low and high MP concentrations

264 (**Figure 2** and **S2a**). Finally, periphyton DW remained similar among MP treatments although it was

265 lower during the second sampling in the high MP treatments (**Figure 2** and **S2b).**
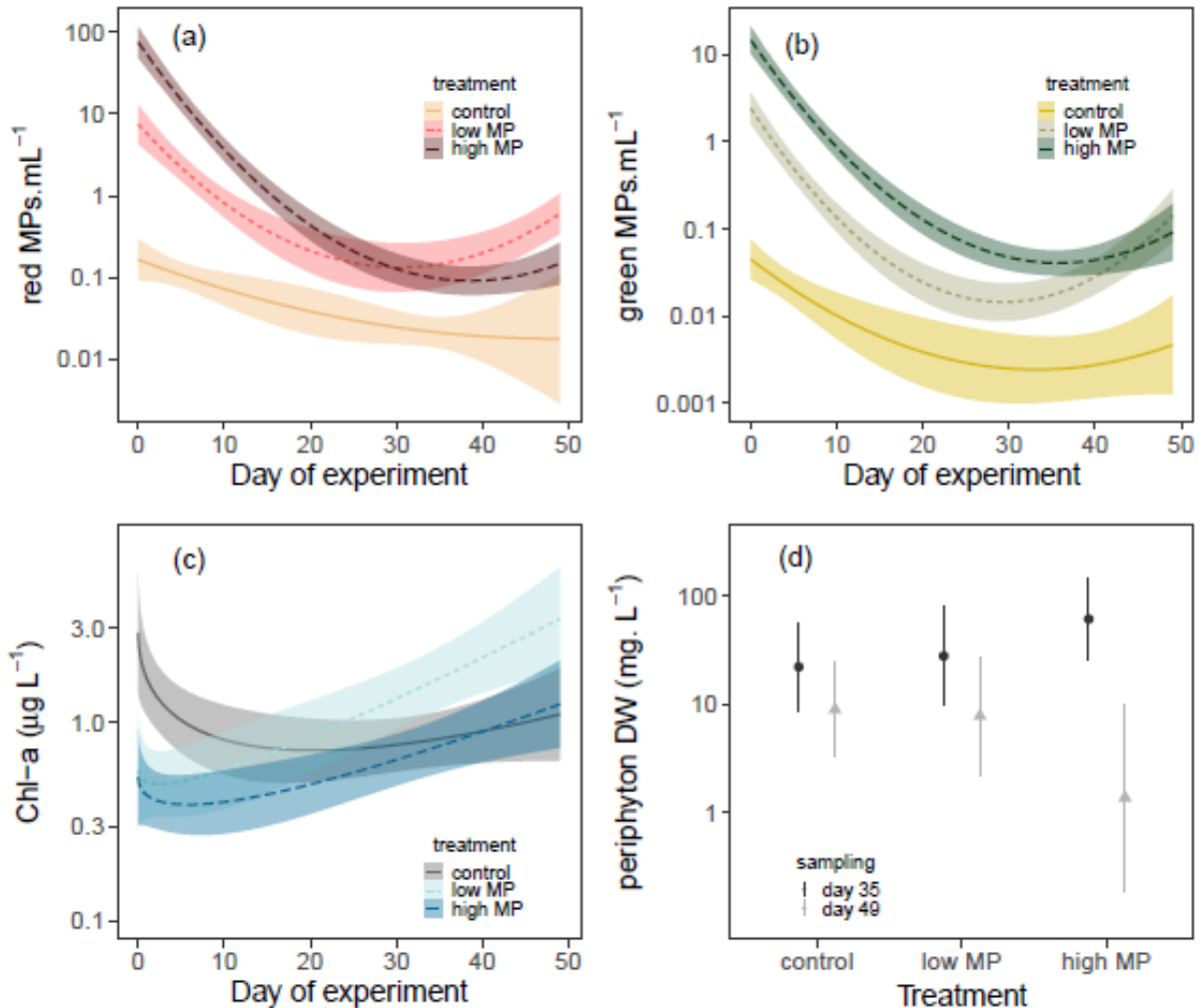
266 **Figure 2** Best models of the treatment-specific dynamics of (a) red MP concentration, (b) green MP
267 concentration, ~~and~~ (c) Chl-a concentration in the water column, and (d) periphyton dry weight (DW) in
268 the experiment. Model estimates are shown as mean values with 95% confidence intervals based only
269 on fixed effects.

270

271 **3.2 | Zooplankton dynamics**

272 Biomass-based composition of the zooplankton community did not differ significantly among the

273 concentrations of MP (first PRC axis: $F_{1,56} = 5.26$, $p = 0.96$). The best models on the temporal dynamics

274 of total zooplankton abundance and total biomass of each major group, except total copepods, calanoid

275 copepods and *Daphnia* (**Table 2, S3 and S6**, **Figure 3)**, were consistent with a small initial increase

276 followed by a stronger decrease. There were no differences between concentrations (LRT, treatment:

14

277 total abundance, $\chi_2^2 = 0.09$, $p = 0.96$; total biomass, $\chi_2^2 = 0.41$, $p = 0.81$; total copepods, $\chi_2^2 = 4.51$, $p =$

278 0.11; calanoid copepods, $\chi_2^2 = 3.54$, $p = 0.17$; cyclopoid copepods, $\chi_2^2 = 0.75$, $p = 0.69$; all cladocerans,

279 $\chi_2^2 = 2.08$, $p = 0.35$; large cladocerans, $\chi_2^2 = 1.47$, $p = 0.48$; small cladocerans, $\chi_2^2 = 1.67$, $p = 0.43$).

280 Zooplankton biomass peaked at different times for different zooplankton groups (**Figure 3**). While large

281 cladocerans (body size > 0.5 mm, such as *Daphnia magna* and *D. ~~aphnia~~ pulex*, the main contributors to

282 the total biomass) and small cladocerans (body size < 0.5 mm) peaked early (during first week),

283 cyclopoid copepods peaked a few days later. MP concentrations did not show any significant effects on

284 total zooplankton biomass (**Table S3**). Although calanoid copepod biomass declined slightly over time

285 in all treatments, it largely replaced cladoceran biomass towards the end of the experiment (**Figures S3–**

286 **S5**). Only *Daphnia* (*D. pulex*, *D. magna*, and *D. longispina*) dynamics differed significantly between

287 concentrations: their biomass declined over time and the decline in the second half of the experiment

288 was steeper in the high MP treatment, leading to a more than 10 times lower final biomass compared to

289 low MP and control treatments (LRT, treatment × time: $\chi_2^2 = 14.0$, $p = 0.0009$; treatment × time^2: $\chi_2^2 =$

290 9.11, $p = 0.011$; **Table 2**, **Figure 3**).

291 **Table 2** Parameters of the best GLMM models describing the temporal dynamics of MP load and key
292 phytoplankton and zooplankton community characteristics in the experiment. Intercept corresponds to
293 controls on day 0 (day 35 for periphyton biomass); *time* and *time*^{2} describe time dependence in controls
294 (time scaling reported in the heading of each model); *low MP* and *high MP* describe differences between
295 the given treatment and controls. Fixed effect parameters are given as mean estimate with 95%
296 confidence interval in parentheses. Model structure: NB = negative binomial, Gamma = Gamma with
297 log-link function, Log-normal = Gaussian with log-link function. Time scaling: difference between day
298 49 and day 35 (periphyton biomass) or scaling used for time variable (all other models). Random effects:
299 σ^2 = residual variance; τ_{00} = random effect variance; $N_{mesocosm}$ = number of random effect levels; N_{tot} =
300 number of observations. R_m^2 = marginal R^2, R_c^2 = conditional R^2. See methods for details.

	Red MP	Green MP	Chl-a	Periphyton biomass	Zooplankton biomass	Cladoceran biomass	Copepod biomass	*Daphnia* biomass
Model structure	*NB*	*NB*	*Gamma*	*Log-normal*	*Gamma*	*Gamma*	*Gamma*	*Gamma*
Time scaling	*day*	*day*	$(day)^{0.5}$	*day 49 vs day 35*	$(day)^{0.25}$	$(day)^{0.5}$	*day*	*day*
(Intercept)	1.61 (1.32 – 1.90)	-0.20 (-0.67 – 0.27)	0.08 (-0.17 – 0.32)	1.93 (0.98 – 2.87)	3.78 (3.40 – 4.16)	3.16 (2.63 – 3.69)	2.23 (1.76 – 2.70)	3.17 (2.51 – 3.84)
low MP	2.70 (2.29 – 3.10)	2.79 (2.24 – 3.35)	-0.11 (-0.48 – 0.26)	0.23 (-1.18 – 1.64)	-0.19 (-0.76 – 0.39)	-0.48 (-1.29 – 0.32)	0.71 (0.00 – 1.42)	-1.36 (-2.37 to -0.36)
high MP	3,45 (3.07 – 3.83)	4,06 (3.54 – 4.58)	-0.62 (-0.97 to -0.28)	1,02 (-0.27 – 2.30)	-0.04 (-0.58 – 0.50)	-0.53 (-1.27 – 0.20)	0.62 (-0.04 – 1.28)	-2.17 (-3.10 to -1.24)
time	-8.66 (-11.8 to -5.51)	-9.76 (-14.4 to -5.11)	-3.15 (-5.79 to -0.51)	-0.21 (-0.59 – 0.17)	-5.15 (-7.01 to -3.28)	-13.6 (-15.6 to -11.5)	-3.04 (-5.68 to -0.41)	-16.5 (-22.3 to -10.6)
$time^2$	2.19 (-0.67 – 5.05)	6.09 (1.30 – 10.9)	3.01 (0.40 – 5.61)	-	-7.11 (-8.95 to -5.27)	-7.78 (-9.82 to -5.74)	-	-3.49 (-9.81 – 2.83)
time x low MP	-3.06 (-7.38 – 1.25)	-4.04 (-9.43 – 1.35)	9.44 (5.77 – 13.12)	-0.37 (-1.15 – 0.41)	-	-	-	-8.19 (-18.7 – 2.31)
time x high MP	-16.7 (-20.7 to -12.6)	-11.4 (-16.6 to -6.19)	6.24 (2.71 – 9.78)	-2.89 (-4.70 to -1.08)	-	-	-	-23.1 (-31.4 to -14.7)
$time^2$ *x low MP*	7.93 (3.74 – 12.1)	7.61 (2.01 – 13.2)	-0.10 (-4.03 – 3.84)	-	-	-	-	13.40 (1.35 – 25.5)
$time^2$ *x high MP*	8.22 (4.37 – 12.1)	4.56 (-0.75 – 9.88)	-0.53 (-3.99 – 2.93)	-	-	-	-	-7.60 (-16.6 – 1.37)
Random effects								
σ^2	0.47	0.38	0.47	40.1	0.86	0.94	1.54	3.11
τ_{00}	0.00	0.00	0.01	0.77	0.02	0.13	0.00	0.00
$N_{mesocosm}$	11	11	11	11	11	11	11	11
N_{tot}	109	109	110	22	76	76	76	76
R_m^2 / R_c^2	0.926 / -	0.946 / -	0.394 / 0.411	0.024 / 0.042	0.540 / 0.550	0.755 / 0.785	0.129 / -	0.807 / -

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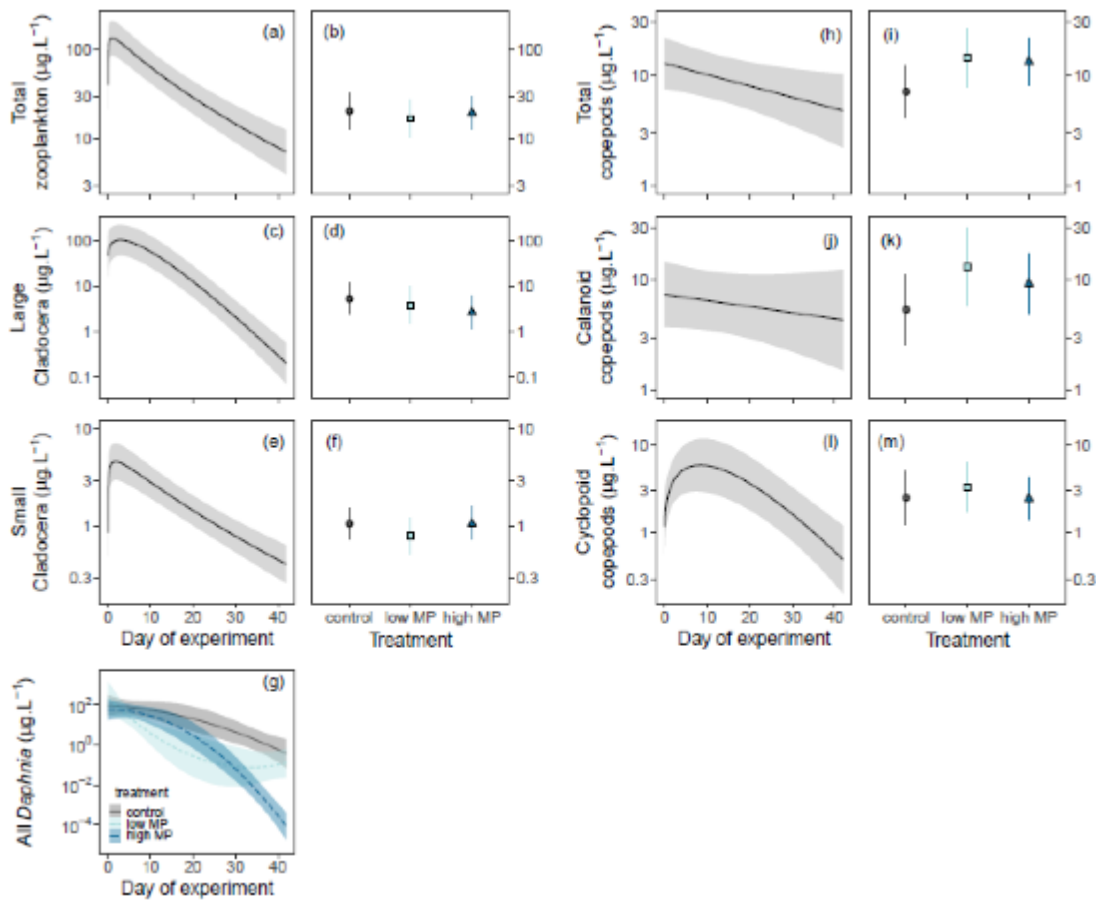
Figure 3 Best models of the treatment-specific dynamics of the biomass of (a, b) total zooplankton and the main zooplankton groups including (c, d) large cladocerans with body length > 0.5 mm, (e, f) small cladocerans with body length ≤ 0.5 mm, (g) all *Daphnia* species (h, i) total copepods, (j, k) calanoid copepods, and (l, m) cyclopoid copepods. Panels (a), (c), (e), (h), (j), (l): average temporal dynamics; panels (b), (d), (f), (i), (k), (m): treatment-specific biomass on day 25. Model estimates shown as mean values with 95% confidence intervals based only on fixed effects. Note that only the model in panel (g) includes a time-dependent treatment effect on all *Daphnia* species.

3.3 | MP ingestion and trophic transfer by zooplankton and insect larvae

Both main zooplankton groups (cladocerans and copepods; total number of examined individuals $N = 3351$) as well as odonate larvae (*Sympetrum*, $N = 7$; Zygoptera, $N = 45$) and chironomid larvae ($N = 497$) ingested MP. MP-IOI and MP-DOI differed greatly between and within groups and between the MP types. In zooplankton, MP were detected only in large-sized individuals (cladocerans, mostly large *Daphnia*, with body length> 0.5~~34~~ mm~~, mostly large *Daphnia*,~~ and copepods> 1 mm). The highest MP-IOI in the zooplankton community occurred during the first week of the experiment. Overall MP-IOI of all zooplankters was only 2.2% and 0.1% for red and green MP, respectively, and no zooplankter from the control was found with ingested MP (**Table S7**). Mean MP-IOI for all zooplankters

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in both MP treatments was low, especially for green MP (red MP: 2.5% in low MP, 4.1% in high MP; green MP: 0.1% in low MP, 0.2% in high MP; **Table S7**). Cladoceran MP-IOI was about 5 times higher than copepod MP-IOI and highest in *Daphnia* (*Daphnia:* red MP: 6.8 % in low MP, 13.1% in high MP; green MP: 0.3% in low MP, 0.7% in high MP; for cladocerans see **Table S7**).

MP-DOI increased substantially with MP treatment for the largest daphniids, *D. magna* and *D. pulex*. Red MP-DOI differ~~eds~~ more in cladocerans than in calanoid copepods throughout the experiment, with mean±SD across all replicates on a given sampling date varying between 1.0±0.0 and 5.4±7.4 particles per individual *Daphnia*, 1.0±0.0 to 11.5±13.4 particles per *Ceriodaphnia,* and 1.0±0.0 to 2.0±0.0 particles per calanoid copepods (**Table S8**), and no red MP were found in most other cladoceran taxa (**Figure S6**). MP-IOI across all chironomid adults was low (red MP: 1.4%; green MP: 0.4%) and water column MP were only found in the largest morphospecies (Chironomid A, red MP: 4.1%; green MP: 1.2%; **Figure S7a** and **Table S7**) except for one individual of the Chironomid B morphospecies. All these findings contrasted with high MP-IOI of both red and green MP in the faeces of odonate larvae (red MP: 63.6%; green MP: 56.4%; **Figure S7bc** and **Table S7**). Odonate faeces also contained substantially higher numbers of water column MP than ingested by zooplankton (**Figure 4**).

The best models revealed taxon-specific positive relationships between the number of ingested MP and their concentrations in water in all three major groups (i.e., zooplankton, chironomid adults, and odonate larvae) and taxon-specific size allometry in zooplankton (GLMM; **Table S9** and **S4**). That is, the red MP-DOI increased with body size in zooplankton, and this increase was steeper in copepods than in cladocerans (**Figure 4ab**). Red MP-DOI also increased strongly with MP concentration in cladocerans and adult chironomids, and in the faeces of Zygoptera larvae, while the increase was much weaker to negligible in copepods and in the faeces of *Sympetrum* larvae (**Figure 4cd**). For example, chironomid adults were likely to contain red MP only when their concentration in the water column exceeded ca. 3 particles m L^{-1} (**Figure 4e**), while the faeces of odonate larvae contained red MP even at concentrations of ~0.1 particles m L^{-1} (**Figure 4cd** and **S7bc**). The number of green MP in odonate faeces also tended to increase with MP concentration, with no distinction between *Sympetrum* and Zygoptera larvae according to the best model (**Figure 4cd** and **S7bc**).
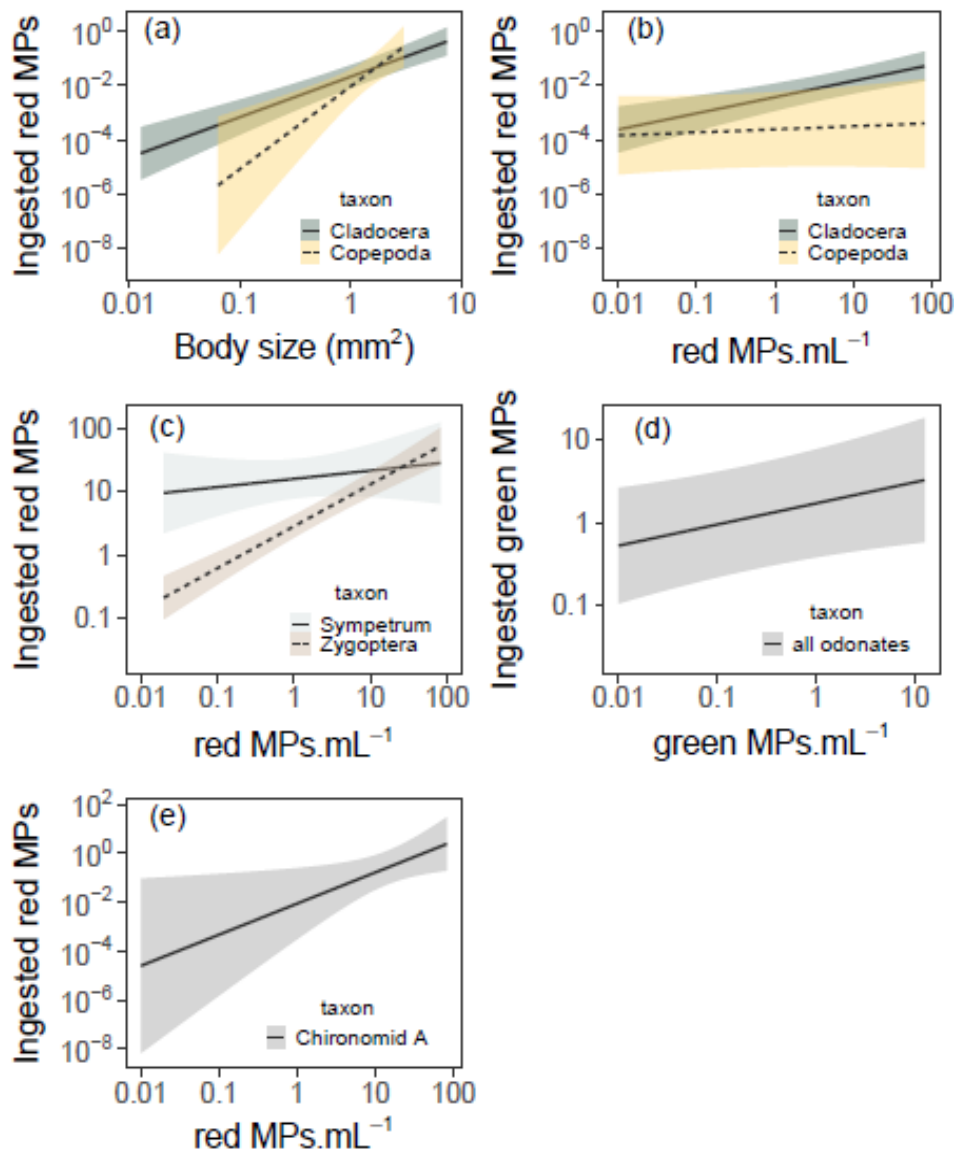
347 **Figure 4** Best models of the number of ingested red MP (a-c, e) and green MP (d) as a function of (a)
348 body size and (b-e) concentration of the same MP type in water for (a, b) zooplankton, (c, d) odonate
349 larvae (*Sympetrum* and Zygoptera; the predicted numbers of green MP are the same for both taxa in
350 panel d), and (e) emerging adults of Chironomid A morphospecies in the experiment. Body size is
351 expressed as body length squared in panel (a). Red MP concentration is fixed at the median value (0.87
352 particles mL^{-1}) in panel (a) and body size fixed at the median value of l^2 (0.295 mm^2) in panel (b). Model
353 estimates shown as mean values with 95% confidence intervals based only on fixed effects.

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3.4 | Effect of MP on insect emergence

Temporal dynamics of the chironomid emergence differed initially between treatments, i.e. the best model of biomass-based emergence rates contained the treatment x time interaction (LRT, treatment × time: $\chi_2^2 = 6.30$, $p = 0.043$; treatment × time^2: $\chi_2^2 = 4.13$, $p = 0.13$; **Tables S5 and S10**). Emergence rates were higher in the controls than in both MP concentrations before ca. day 5; the rates were similar afterwards and declined more or less rapidly after day 15–20 in all treatments (**Figure 5** and **S8**). As a result, these early differences between treatments disappeared quickly and the cumulative chironomid biomass emerged by day 7 and day 42 did not differ significantly between treatments (LRT, treatment: day 7, $\chi_2^2 = 3.61$, $p = 0.16$; day 42, $\chi_2^2 = 2.24$, $p = 0.33$; **Table S10**). Similarly, the emergence probability of *Sympetrum* and Zygoptera larvae did not differ significantly between treatments (LRT, treatment: *Sympetrum*, $\chi_2^2 = 3.27$, $p = 0.20$; Zygoptera, $\chi_2^2 = 1.39$, $p = 0.50$; **Figure 5** and **Table S10**).
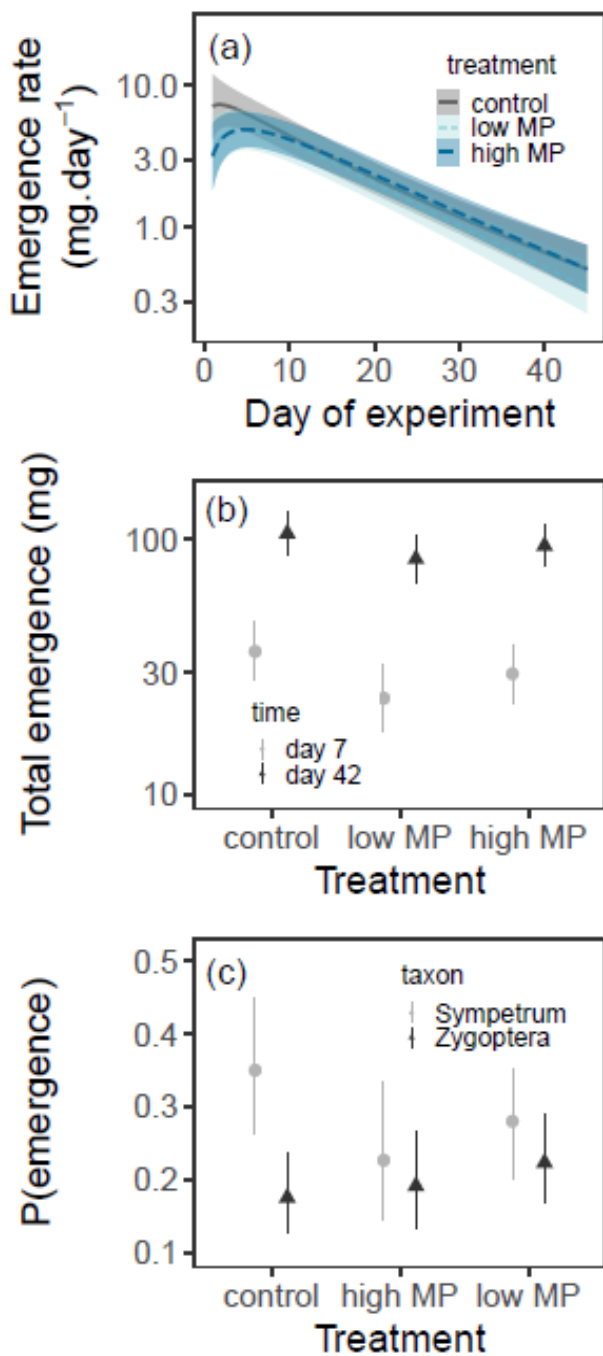
Figure 5 Best models of treatment-specific emergence patterns of aquatic insects in the experiment: (a) temporal changes in the chironomid emergence rate, expressed as total biomass, (b) total chironomid biomass emerged by day 7 and day 42, and (c) emergence probability of odonate larvae. Model estimates shown as mean values with 95% confidence intervals based only on fixed effects.

3.5 | Individual-level effects of MP on adult chironomids

Wing size (ANOVA, $F_5 = 3.22$, $p = 0.01$) and wing shape (MANOVA, Wilks' $\lambda = 0.004$; $F_{110} = 4.89$; $p = 0.001$) differed between male and female *C. riparius*. Regression analysis showed no significant effect

of allometry in both female (only 4.9% of changes in wing shape were influenced by wing size, $p = 0.073$) and male individuals (only 2.4 %, $p = 0.44$). The wing size and shape of female *C. riparius* and wing shape of male *C. riparius* did not differ significantly between treatments (ANOVA females, $F_2 = 0.44$, $p = 0.65$; MANOVA females, Wilks' $\lambda = 0.190$; $F_{110} = 1.05$; $p = 0.434$; MANOVA males, Wilks' $\lambda = 0.131$; $F_{110} = 1.52$; $p = 0.095$), while the size of male wings did (wing size: ANOVA, $F_2 = 6.66$, $p = 0.03$). As outlined by the canonical variate analysis, individuals from the control, and the low MP and high MP treatments were clearly separated along the first two CV axes (accounting for 65.5% and 34.5% of the total variability, respectively; **Figure 6**). Wireframe graphs revealed the tendency of male wings to widen with MP treatments, with the most prominent changes in landmarks 1, 4, and 10. Mahalanobis distances differed significantly between all groups, with the largest distance between those from controls and high MP concentrations (**Table S11**).
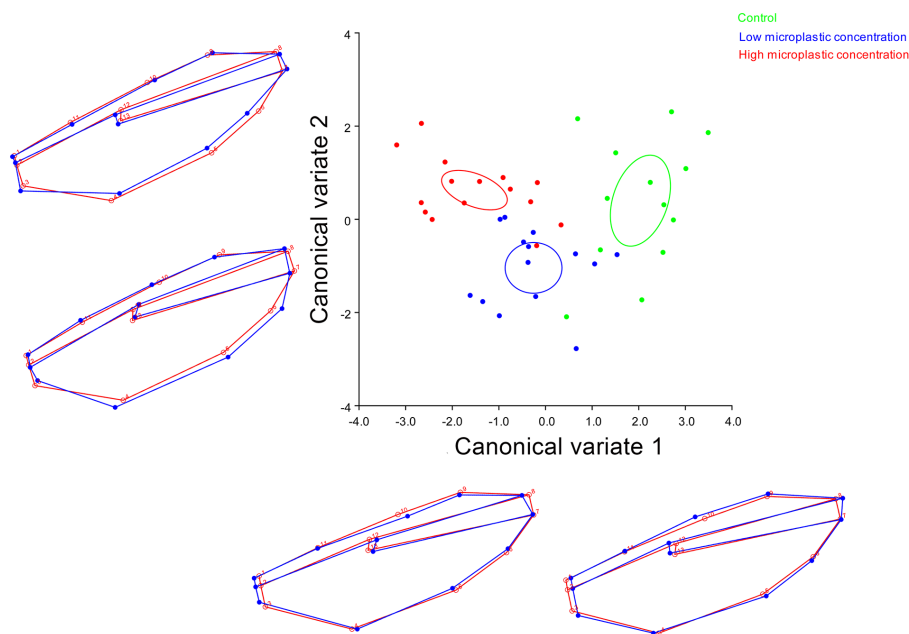
Figure 6 Canonical variate analysis (CVA) of right wings in male *C. riparius*. Diagrams along both axes illustrate changes of wing from the common baseline shape (blue lines) to the shape characteristic for the given quadrant in the morphospace (red lines). Digitized landmarks (numbers 1–13, points on wing contours) describe the wing contour. Ellipses represent 90% confidence intervals for each treatment.

4 | DISCUSSION

MP can affect the structure and functioning of aquatic ecosystems (Bucci et al., 2020; Foley et al., 2018). The current experiment showed that neither environmentally relevant nor 10 times higher MP concentrations substantially alter community structure and dynamics despite the ability of MP to enter multiple trophic levels directly or indirectly in an *in situ* experimentally assembled community. Overall, MP in the water column were not readily ingested by zooplankton (Hypothesis 1), likely due to the sharp decline of~~as~~ water column MP concentrations during the first two weeks of the experiment. MP-IOI of the fluorescent red MP was highest in large-bodied *Daphnia* at the beginning of the experiment. Other zooplankton taxa rarely ingested MP particles and exposure to MP reduced only *Daphnia* biomass (Hypothesis 1). However, MP effects in the experimentally assembled food web reached beyond zooplankton (Hypothesis 2). Ingestion of MP by odonate larvae was probably driven by a combination of trophic transfer from zooplankton and other direct or indirect trophic pathways. An early effect of MP presence on the insect emergence patterns disappeared over the course of the experiment, indicating that population-level MP effects can be low in natural ecosystems (Hypothesis 3). Observed changes in the wing size of male *C. riparius* suggest that MP can measurably affect individual benthic organisms (Hypothesis 3). Finally, this study provided the first evidence of MP transfer to terrestrial ecosystems by emerging aquatic insects *in situ* (Hypothesis 4). These main findings are discussed in more detail below.

4.1 | Fate of MP in the water column

Red and green MP in both MP treatments declined over time, especially within the first 10 days of the experiment. Ingestion by zooplankton was too low to explain the observed declines in water column MP. Similar MP reductions were attributed to settlement of MP to the sediment in another mesocosm experiment (Al-Jaibachi et al., 2020) and in natural freshwater ecosystems (Su et al., 2016). Although direct observational data were not available in the present study, MP settlement appears to drive the temporal patterns of red and green MP concentrations despite the artificial mixing of the water column to prevent stratification. Biofilm growth on MP particles could have increased their density (Rummel et al., 2017), causing them to sink faster and increase the potential for ingestion by benthic fauna (Syberg

23

420 et al., 2015). Additionally, MP might have become attached to the artificial plants and mesocosm walls

421 including the periphyton layer.

4.2 | MP ingestion and trophic transfer

423 Red and green MP were found in the guts of both zooplankton and odonate larvae, revealing

424 trophic transfer of MP (Hypothesis 2) from herbivores to predators (Setälä et al., 2014; Scherer et al.,

425 2018). However, MP ingestion rates by zooplankton were low, especially after MP concentrations

426 declined in the mesocosms. The amount of MP ingested by zooplankton and odonate larvae scaled

427 allometrically with MP concentrations in the water column, as seen in other studies with different

428 organisms (e.g. Catarino et al., 2018; Narmatha Sathish et al., 2020).

429 MP ingestion among zooplankton occurred mainly in large cladocerans (*Daphnia*) in this study.

430 The higher MP-IOI found in cladocerans compared to copepods can be due to their different feeding

431 strategies. While cladocerans are non-selective filter feeders, copepods feed selectively (Demott, 1988;

432 Kiørboe, 2011; Strickler, 1982) and can respond to the presence of MP by a significant decrease in

433 herbivory (Cole et al., 2013; Cole et al., 2015) and ~~by~~ increased selectivity (Cole et al., 2019; Coppock

434 et al., 2019). The observed overall MP-IOI by *Daphnia* was nevertheless low in comparison to results

435 from laboratory studies (Caniff and Hoang, 2018; Frydkjær et al., 2017; Rist et al., 2017). MP

436 concentrations used in those laboratory experiments were mostly above environmentally realistic levels

437 (Bucci et al., 2020), but even comparable concentrations yielded higher MP-DOI estimates. For

438 example, Caniff and Hoang (2018) reported 3.8 ingested particles per individual *Daphnia* using MP that

439 resembled the type, size, and concentration of green MP in the high MP treatment in the present

440 mesocosm experiment. However, we found the MP-DOI to be negligible in *Daphnia*, with a maximum

441 of one ingested MP per individual. Another study with similar conditions to our experiment found 30–

442 50 MP particles ingested per individual *D. magna* (Frydkjær et al., 2017), ca. 10 times the maximum

443 MP-DOI observed in the present study (5.3±5.0 red MP-DOI per individual *Daphnia* on day 0). These

444 results indicate that laboratory experiments may overestimate ingestion rates compared to natural

445 ecosystems, possibly due to the increased exposure to MP in laboratory set-ups lacking the complexity

446 of natural habitats and neglecting other environmental factors and biotic interactions (Widdicombe et

447 al., 2010).

448   MP-IOI increased with body size in the present study, suggesting that larger cladocerans tend

449 to ingest more MP (Alomar et al., 2017; McMahon, 1965; Scherer et al., 2017). Moreover, no cyclopoid

450 copepods with ingested MP were observed. This indicates that ambush and selective feeders may not

451 detect non-motile MP or reject them as food particles, emphasizing the importance of funcical feeding

452 traits such as body size and feeding mode in MP trophic transfer (Yu et al., 2020).

453   MP treatments had a minor effect on the total zooplankton biomass and on the biomass of each

454 major group, except *Daphnia,* in this study. The lower *Daphnia* biomass in the high MP treatment can

455 be attributed to higher MP ingestion by *Daphnia* compared to other zooplankton taxa, even if it was

456 very low compared to other studies (see above). Such adverse effects of MP on *Daphnia* biomass could

457 result from mechanical changes to the gut during the evacuation of MP particles (e.g., Bosker et al.,

458 2019; Rist et al., 2017), leading to reduced energy uptake by individual *Daphnia*.

459   Odonate larvae are key predators of zooplankton, especially of large-bodied daphniids, in the

460 littoral zone (Burks et al., 2001; Hirvonen,1999; Thompson and Pickup, 1984). This link can transfer

461 MP to higher trophic levels (Cole et al., 2013; Foley et al., 2018). The presence of relatively high

462 amounts of red MP (including control mesocosms) and some green MP in odonate faecal pellets in this

463 study suggests that the predators consumed multiple large-sized daphniid preys containing MP in their

464 gut but also acquired MP via other pathways. MP attachment to prey body surfaces or mesocosm walls

465 may contribute to MP transfer to higher trophic levels (Cole et al., 2013; Jemec et al., 2016), and some

466 MP found in the faecal pellets—at least the green ones—might have originated from alternative food

467 sources such as mayfly larvae.

468   Chironomid larvae can easily ingest MP since they are non-selective feeders with potentially

469 high MP ingestion rates (> 200 MP particles hr^{-1}, Scherer et al., 2017; Silva et al., 2019). The larvae can

470 ingest particles up to 200 μm in size (Armitage et al., 1995; Henriques-Oliveira et al., 2003), but this

471 size limit likely scales with body size. Thus, except for one adult of the Chironomid B morphospecies,

472 MP were detected only in the largest morphospecies (Chironomid A). Although different types and sizes

473 of MP were added in the sediment, water column and water surface in the current experiment, only the

fluorescent red and green MP originally added to the water column could be examined; chironomid larvae likely ingested them after they settled on and into the sediment. It is most likely that the larvae also ingested the non-fluorescent MP added directly to the sediment that could not be tracked and quantified with the selected methodology of the current experiment, and that these particles were also transferred to the adults.

Overall, the size range of water column MP used in this experiment was comparable to previous studies (Canniff and Hoang, 2018, Stanković et al., 2020) and suitable for direct and indirect ingestion by Daphniids, chironomids and odonates. The results indicate that increasing environmental MP concentrations lead to more frequent ingestion of MP by *Daphnia* (though it was still very low compared to previous laboratory experiments; Rosenkranz et al., 2009) and chironomid larvae, and more frequent trophic transfer to higher trophic levels (zygopteran larvae). Moreover, the findings of the present study emphasized that functional traits such as feeding preference and body size might underpin MP uptake rates, making larger or non-selective feeders more prone to MP exposure in freshwater ecosystems (Scherer et al., 2018). This suggests that MP ingestion by zooplankton may be more limited in habitats with fish, especially in warm or nutrient rich lakes, because their zooplankton communities are typically dominated by small-bodied species (Brooks and Dodson, 1965; Jeppesen et al., 2000).

4.3 | Individual-level effects of MP on chironomids

MP can be ingested by, and affect the morphology of, benthic organisms including chironomids (Silva et al., 2019; Stanković et al., 2020). Changes in chironomid wing size and shape can provide an indirect assessment of the habitat quality, as the wings are crucial for dispersal (McLachlan, 1985; Vepsäläinen, 1986). In the present study, the wing shape of *C. riparius* males differed among the MP concentrations as suggested bywith Hypothesis 3. A similar effect was reported for *C. riparius* females in a controlled laboratory setting with the same MP mixture and concentrations as in the mesocosms (Stanković et al., 2020). The sex specificity might have arisen from temperature conditions; development time differs between the sexes (Stevens et al., 1998) and is temperature-dependent (Frouz et al., 2009). This could have modulated the individual-level response of each sex to MP presence as the temperature in the mesocosms was considerably higher than the 20ºC used in the laboratory experiment (Stanković et al.,

501 2020). Sex differences aside, these results can be interpreted as an indirect evidence of the cumulative

502 effect of all water column and sediment MP on individual chironomids, including the non-fluorescent

503 MP that could not be reliably traced in the present study.

504 **4.4 | Chironomid emergence and MP transfer to terrestrial habitats**

505 Emerging insects may transfer substantial amounts of MP from aquatic to terrestrial habitats due to their

506 often ~~and~~ high biomass flux (D'Souza et al., 2020; Gratton et al., 2008; Likens, 1985). Results from the

507 current experiment provided the first *in situ* evidence for this pathway. The red MP detected in adult

508 chironomids, which were the dominant emerging macroinvertebrate group, were likely ingested in the

509 larval stage and retained through metamorphosis into adults, as found in *Culex* mosquitoes (Al-Jaibachi

510 et al., 2018), and then transferred to the terrestrial habitats.

511   The addition of MP also significantly reduced the emergence rates of chironomids during the

512 first week of the mesocosm experiment as expected, most likely due to the exposure of larvae to MP

513 (Arambourou et al., 2019; Scherer et al., 2017; Ziajahromi et al., 2018). In a laboratory experiment, the

514 same MP mixture and concentrations as in the mesocosms led to prolonged development of the

515 chironomid larvae (Stanković et al., 2020). However, this initial effect on chironomid emergence

516 dynamics quickly disappeared. Similarly, the cumulative emergence probability of odonate larvae did

517 not differ significantly between treatments, implying a negligible effect of environmentally relevant MP

518 concentrations on long-term emergence patterns of aquatic insects.

519

520 **5 | CONCLUSION**

521 The ubiquitous presence and high bioavailability of MP in aquatic habitats may lead to alteration of the

522 structure and functioning of local communities, but relevant experimental data are only beginning to

523 emerge. This mesocosm experiment showed that elevated MP concentrations may only have a~~only~~ low

524 impact on the population dynamics of most taxa in freshwater food webs, despite the propensity of MP

525 to be directly or indirectly transferred to higher trophic levels. In particular, MP were readily ingested

526 by zooplankton at the beginning of the experiment, but overall MP-IOI and MP-DOI were much lower

527 than reported by most laboratory experiments. This was likely driven by the rapid decline of MP

528 concentrations in the water column, presumably due to MP settlement and attachment to different

529 surfaces. On the other hand, wing morphology of adult chironomids was significantly affected by

530 exposure to MP in the larval stage, displaying the same change in pattern previously identified in a

531 laboratory experiment. These observations imply that at least some laboratory experiments may

532 overestimate the presumed population-level effects of MP on freshwater biota despite their ability to

533 reveal important individual-level consequences of MP exposure. The analyses also demonstrated that

534 MP ingestion varies ~~predictably~~ with MP size and concentration and body size and taxonomic identity

535 of the organism, which can help predict the rates of transfer and further effects of MP on freshwater

536 food webs. Moreover, this study confirmed for the first time that *in situ* ~~that~~ MP transfer by emerging

537 aquatic insects could lead to secondary pollution of terrestrial habitats. In sum, this study corroborates

538 some previous results on the impacts of MP on aquatic biota but challenges others. Further long-term,

539 community-level experiments will be required to fully understand potential threats of MP to aquatic

540 biodiversity and ecosystem functioning.

541

542 **Declaration of competing interest**

543 The authors declare that they have no known competing financial interests or personal relationships

544 that could have appeared to influence the work reported in this paper.

545

546 **Authorship contribution statement**

547 **Dilvin Yıldız:** Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft,

548 Visualization, Project administration **Gülce Yalçın:** Formal analysis, Investigation, Resources, Data

549 Curation, Writing - Original Draft, Visualization, Project administration **Boris Jovanović:** Software,

550 Formal analysis, Investigation, Writing - Review and Editing, Visualization, Supervision **David S.**

551 **Boukal:** Software, Formal analysis, Investigation, Writing - Review and Editing, Visualization,

552 Supervision **Lucie Vebrová:** Formal analysis, Investigation **Derya Riha:** Formal analysis, Investigation

553 **Jelena Stanković:** Formal analysis, Investigation , Visualization **Dimitrija Savić-zdraković:** Formal

554 analysis, Investigation , Visualization, Writing - Review & Editing **Melisa Metin:** Investigation

555 **Yasmin Naz Akyürek:** Investigation **Gamze D. Balkanli:** Investigation **Nur Filiz:** Investigation,

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558 **Richardson:** Investigation, Writing - Review & Editing **Meryem Beklioğlu:** Conceptualization,

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