All that glitters is litter? Ecological impacts of conventional versus biodegradable glitter in a freshwater habitat

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Abstract

Biodegradable plastics are becoming increasingly popular due to global concerns about plastic pollution. In this study, the impacts of glitter manufactured of conventional, non-biodegradable polyethylene terephthalate (PET) versus glitter of alternative materials (modified regenerated cellulose (MRC), mica or synthetic mica) on the biodiversity and ecosystem functioning of freshwater, lotic habitats were compared using a semi-natural mesocosm experiment. After 36 days, there was no effect of glitter on overall assemblage structure or diversity indices, however there was a two-fold increase in the abundance of New Zealand mud snails (*Potamopyrgus antipodarum*) in response to MRC glitter. In addition, the root length of common duckweed (*Lemna minor*) and phytoplankton biomass (based on chlorophyll content) were significantly reduced by exposure to any type of glitter. On the contrary, the chlorophyll content in the sediment (indicating microphytobenthos biomass) was significantly greater in those exposed to synthetic mica glitter. Organic matter content of sediment did not differ amongst any of the treatments. However initially, on days 8 and 15, NO_3^{2-} concentration in the control treatment were significantly greater than in all glitter treatments, but this observation disappeared over time. Overall, results indicate that both conventional and alternative glitters can cause ecological impacts in aquatic ecosystems.

1. Introduction

Microplastics are the most abundant form of solid waste worldwide (Eriksen et al. 2014) and pose a significant biological and ecological threat to aquatic ecosystems (Galloway et al. 2019). Although still lagging behind relative to marine systems (Blettler et al. 2018), studies on the prevalence and effects of microplastics in freshwater ecosystems have increased in recent years and we now know that microplastics are present in rivers and lakes worldwide (Rios Mendoza and Balcer 2019), and can be ingested by a range of vertebrates (O'Connor et al. 2019) and invertebrates (Windsor et al. 2019). Microplastics can occur in particularly great abundance in

freshwater sediments, for example, reaching >70,000 microplastics kg^{-1} in river sediments in the UK (Hurley et al. 2018) and in China (Wang et al. 2018) and can have biological (Bellasi et al. 2020) and ecological (Redondo-Hasselerharm et al. 2020) impacts on freshwater organisms and communities.

In response to concerns about plastic pollution, biodegradable alternatives are becoming commonplace especially for substitution of conventional plastics in single-use items including primary microplastics, such as microbeads. Indeed, due to the phase-out of plastic microbeads (banned by >10 countries since 2015; Lam et al. (2018); Nelson et al. (2019) and being restricted in European-wide legislation; ECHA, (2019)) replacement with biodegradable alternatives including poly(lactic acid) (Nam and Park 2019), polyhydroxyalkanoate (Govindasamy et al. 2019) and cellulose (O'Brien et al. 2017) is already widespread. As litter in the aquatic environment, however, biodegradable microplastics may persist for years (Narancic et al. 2018) and result in similar negative biological and ecological consequences as conventional microplastics in marine (Green 2016; Green et al. 2016; 2017; 2019) and freshwater (Straub et al. 2017; González-Pleiter et al. 2019) habitats.

Another type of primary microplastic, that has received less attention from the environmental science community is glitter (Tagg and Ivar do Sul 2019; Yurtsever 2019a). Glitters are flat, reflective particles that are precision-cut into uniform shapes and sizes, ranging from 50 to >5000 μm, with the most common being ~200 μm (Blacksedge and Jones 2007) that are widely used as decoration in e.g. clothing, arts and crafts, cosmetics and body paint for humans and pets (Yurtsever 2019b). They have been used in great quantities at protests (a.k.a. "glitter bombing") and celebratory events such as festivals (Yurtsever 2019b). Although we cannot currently estimate emissions, glitter can be released into the aquatic environment directly or indirectly (Tagg and Ivar do Sul 2019). Direct releases could arise from glitter being rinsed off down the drain whilst washing off glitter body paint or make-up or at outdoor events as

described above. Indirectly, even if glitter is retained by waste-water treatment plants in sludge, the application of biosolids to soil can result in almost 100% of the microplastics being transported into aquatic habitats (Crossman et al. 2020). Although the presence of glitter as contamination in aquatic habitats is not routinely reported in studies on microplastics (Tagg and Ivar do Sul 2018), glitter has been found in freshwater sediments (Ballent et al. 2016; Hurley et al. 2018) and is likely to be currently underestimated due to methodological constraints and incorrect categorisation (Yurtsever 2019a). For example, a lack of clear reporting (i.e. being categorised as "films" or "fragments") or extraction methods which dissolve the coating on the surface of glitter leaving them transparent and difficult to detect coupled with density separation with salts such as NaCl which do not float denser polymers such as those used in glitters could all lead to an under-estimation of glitter abundance (Yurtsever 2019a).

Glitter is a unique type of microplastic, typically consisting of three layers; a plastic core usually made of a type of stretched polyester PET film known as BoPET (biaxially-oriented polyethylene terephthalate), often coated with aluminium to create a reflective appearance and topped with another thin plastic layer, e.g. styrene acrylate (Yertsever 2019b). Similar to other types of single-use microplastics, there has been a phase-out of PET glitter in favour of biodegradable alternatives. For example, in the United Kingdom alone, >60 festivals have already pledged to switch to using biodegradable glitter instead of PET glitter (Street 2018). In response to this demand for "eco-friendly" glitter there is a rapidly growing market for alternative glitters with many new brands entering the marketplace. Biodegradable glitters predominately use regenerated cellulose or modified regenerated cellulose (MRC) (sourced mainly from *Eucalyptus* trees) as their core and are coated with aluminium and/or mineral pigment for reflectivity and topped with a thin plastic layer (e.g. styrene acrylate). Moreover, natural or synthetic fluorphlogopite mica (Becker et al. 2015) (which is seen as a more ethical

92 alternative to natural mica; Bliss 2017) are also used as alternative glitters in cosmetics as
93 shimmers (Yertsever 2019b). As litter in the environment, the biological or ecological effects
94 of any type of glitter, conventional or biodegradable, have never been tested. Here a mesocosm
95 experiment was used to test whether alternative, cellulose-based and mica glitters have a
96 different effect than traditional PET glitter on the biodiversity and ecosystem functioning of a
97 lotic, sedimentary habitat. It was hypothesised that any type of glitter used (PET or the
98 alternative materials) would have similar negative effects on primary producers and on
99 communities of sediment infauna.

100

101 **2. Materials and methods**

102 *2.1. Experimental design and set-up*

103 The experiment was conducted at Anglia Ruskin University, Cambridge, UK. The experiment
104 consisted of a single factor 'Glitter' with 5 levels including a Control group with no glitter
105 added, and four treatments with glitter composed of either PET (~100 μm diameter), modified
106 regenerated cellulose (~150 μm diameter), mica (40 – 200 μm diameter) or synthetic mica (70
107 - 200 μm diameter) glitter added. All glitter used was silver in colour, to cease colour being a
108 potential extraneous variable. This equalled a total of 5 individual treatments, with all
109 treatments being replicated 7 times, for a total of 35 mesocosms (n = 7, N = 35). The
110 mesocosms were constructed using transparent, polypropylene buckets with a 10 L capacity
111 (height x diameter = 23 cm x 30 cm).

112 Sediment was collected from a static area of the River Glaven, Norfolk, UK from a depth of
113 ~50 cm. Floating plants in this stretch of river consisted mainly of *Lemna minor* (common
114 duckweed, Linnaeus 1753), which was collected with a net before being transferred into
115 buckets containing water from the river. In order to collect phytoplankton, water from the water
116 column (depth ~10 cm) of the river was also collected and stored in 10 L buckets. All material

was transported back to the laboratory and left overnight with bubblers to keep oxygenated. In the morning *Potamopyrgus antipodarum* (New Zealand mud snail, Gray 1843) which were positioned on the sides of the buckets (and were, therefore alive) were collected for later distribution. Sediment was homogenised in a large tub by gently mixing with a trowel and distributed evenly amongst the 35 mesocosms, with an average depth of ~8 cm and weight of ~1150 (± 58) g in each mesocosm. River water was pooled to mix and then distributed by adding 1 L to each of the mesocosms to inoculate them with natural phytoplankton communities. Each mesocosm was then topped up with 7 L of dechlorinated tapwater (using Tetra Aquasafe), giving an overlying water column of 8 L. Adult (diameter ~5 mm) *P. antipodarum* (mud snails) were distributed evenly between the mesocosms with 50 individuals placed into each. Each mesocosm also received 500 individuals of *L. minor*. Bubblers were placed into each mesocosm to supply oxygen and mimic the low energy conditions where the material was collected from. All mesocosms were left to acclimatise for 48 hours, before 500 mg of either PET, MRC, mica or synthetic mica glitter was added by pouring gently into the centre of the water (equal to ~60 mg L^{-1} or ~435 mg kg^{-1} sediment). Glitter was observed to have sunk to the surface of the sediment within 24 h. Although a density of 435 mg kg^{-1} is relatively high, densities of microplastics as high as 1000 mg kg^{-1} (Klein et al. 2015) and >2000 mg kg^{-1} (Toumi et al. 2019) have been found in heavily contaminated freshwater sediments in Germany and Africa respectively. Water was topped up daily with deionised water, to keep the water column at 8 L. A ~20 % water change was done on day 20 of the experiment, where 1.5 L of water was removed and replaced with dechlorinated tapwater by pouring gently at the edge of each mesocosm. The experiment ran for 36 days, from 2nd July to 6th August 2018. Water temperatures ranged between 21.4 °C and 22.9 °C and were on average 21.8 °C. pH did not significantly differ amongst treatments (ANOVA: $F_{4,30} = 1.18$, $P = 0.340$) and averaged (± S.E.) 8.52 ± 0.01.

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143 *2.2. Biomass, root length and abundance of Lemna minor*

144 At the end of the experiment, all *L. minor* was removed from each mesocosm. Upon removal,
145 all individual plants that were whole were counted (i.e. with at least 3 leaves, green in colour
146 and with a root). Root length, which is an optimal toxicity endpoint (Gopalapillai et al. 2014),
147 was measured to the nearest millimetre from 5 haphazardly chosen (by placing all plants onto
148 a tray and selecting each individual with eyes closed) *L. minor* individuals from each mesocosm
149 which were later pooled to give one value per replicate mesocosm. For each separate
150 mesocosm, *L. minor* was then blotted dry on a paper towel and weighed to obtain wet biomass,
151 and a subsample of ~200 mg was removed and stored in 15 mL capped centrifuge tubes in a
152 freezer at -18 °C until needed for chlorophyll analysis. Dry biomass of the remaining sample
153 was then quantified by desiccation at 50 °C until they reached a constant weight to assess
154 moisture content gravimetrically.

155

156 *2.3. Chlorophyll content of L. minor, water column and sediment*

157 Chlorophyll was extracted from 200 mg of frozen *L. minor*, 1 L of filtered water from the water
158 column (using filter paper with a pore size of 1.6µm, chlorophyll was extracted from any algae
159 remaining on the filter) and 1 g of surface (oxic layer) sediment from each mesocosm (collected
160 with sterile spatulas after the mesocosms were drained of water). Each sample was placed
161 inside separate 15 mL capped centrifuge tubes wrapped in aluminium foil to block light and
162 stored in the freezer at -18 °C until needed. Chlorophyll was extracted for 1 hour using 90%
163 acetone, shaking for 30 seconds every 5 minutes in the dark. The samples were centrifuged at
164 2500 rpm for 1-2 minutes to settle any debris. Chlorophyll a & b (for *L. minor*), chlorophyll a,
165 b & c (for phytoplankton in the water column), and chlorophyll a & c (for microphytobenthos
166 in the sediment) were measured from the supernatant using a spectrophotometer (at λ = 630,
167 647 and 664 nm) according to equations by Jeffrey and Humphrey (1975). Final concentrations

were calculated for *L. minor*, the water column and the sediment and were expressed as mg g^{-1} of dry plant biomass, mg L^{-1} water or mg g^{-1} dry sediment respectively.

2.4. Sediment communities

On day 36, all sediment from each mesocosm was sieved through a 500 µm mesh in order to retain macrofauna and sorted by hand. Samples were preserved in 70% ethanol and later quantified and identified to species level where possible. The shells of all bivalves and gastropods were cracked using forceps in order to assess whether or not they were viable at the time of collection. Only viable specimens were used in the analysis.

2.5. Measurements of nitrate, community respiration and organic matter content

Nitrate concentrations was measured on days 8, 15, 22, 29 and 36 of the experiment using a Go Direct® Vernier Nitrate Ion-Selective Electrode probe with amplifyer and a Vernier LabQuest 2 computer interface, The device was calibrated to the manufacturer's instructions prior to each measurement data and tested for drift against a known concentration after every 5 measurements. The probe tip was submerged in the centre of each mesocosm to a depth of 10 cm for around 30 seconds until the readings had stabilised. Between each measurement, probes were rinsed in distilled water.

Community respiration was measured in the late afternoon (16:00) on day 22 of the experiment using a Vernier Optical Dissolved Oxygen Probe and a Vernier LabQuest 2 computer interface. In turn, each mesocosm was wrapped in foil to block out any light, and its bubbler was turned off for the duration of the measurements. A small hole was made in the foil in the centre of each mesocosm to insert the probe tip into the water. The probe tip was submerged for ~90 seconds or until the reading had stabilised. Upon removal of the probe, the hole in the foil was covered. Readings for each mesocosm were repeated 3 times, with each measurement around

193 an hour apart. Community respiration (O_2 mg L^{-1} h^{-1}) was calculated for each mesocosm as the
194 slope of each line over the time.

195 At the end of the experiment, approximately 50 g of sediment was taken from the surface of
196 each mesocosm and oven-dried at 105℃ until a constant weight was achieved. From this 5 g
197 subsamples were combusted at 550℃ for 12 hours in a muffle furnace and reweighed and
198 organic matter content was determined by calculating loss on ignition (LOI).

199

200 *2.6. Statistical Analysis*
201 All statistical analyses were done in R v3.6.2. (R Core Team, 2019). Univariate data (duckweed
202 abundance and biomass, root length, chlorophyll content, densities of individual species,
203 organic matter, nitrate concentrations and community respiration) were screened for
204 heterogeneity of variance and for normality (q-q plots, and Shapiro-Wilk tests) to fulfil
205 assumptions for ANOVA. A one-way ANOVA was calculated with "Glitter" as a single factor
206 and post-hoc pairwise comparisons were computed using Tukey HSD tests when the ANOVA
207 was significant. Statistical significance was assumed at $\alpha = 0.05$ for all analyses. Infaunal
208 assemblages were visualised using a non-metric multidimensional scaling diagram and
209 differences in assemblage structure and composition were tested with a one-way
210 PERMANOVA using *vegan* R package version 2.5-6 (Oksanen et al. 2019) on Bray-Curtis
211 dissimilarities (Bray and Curtis 1957).

212

213 **3. Results**
214 *3.1. Effects of glitter on primary producers*
215 Although there was no significant difference in the biomass, abundance or chlorophyll content
216 of *L. minor* (Table 1), roots were~2 times longer in control mesocosms than in mesocosms
217 dosed with PET, cellulose or synthetic mica glitter (Figure 1). In the water column, the
218 chlorophyll a content did not significantly differ amongst treatments (Figure 2), however,

control mesocosms had ~3 times greater chlorophyll b & c concentrations than any of the mesocosms dosed with any type of glitter (Figure 2). In the sediment, chlorophyll a content also did not significantly differ amongst treatments, but sediment dosed with synthetic mica had ~2 times more chlorophyll c than sediment in the control or mesocosms dosed with mica (Figure 3).

3.2. Effects of glitter on fauna diversity in sediment

A total of 11 different taxa were identified in mesocosms (Table 2) and there were no significant differences in the assemblage structure amongst any of the treatments (Figure 4, PERMANOVA: pseudo-$F_{4,30}$ = 1.57, P = 0.146). Mesocosms with MRC glitter, however, had a greater overall abundance (N) of individuals and this was due to ~2 times greater density of *P. antipodarum* than in Controls or mesocosms dosed with PET glitter (Table 2). There were also more *Physa* sp. snails in mesocosms with synthetic mica, however post-hoc tests could not resolve any significant differences amongst treatments (Table 2).

3.3. Effects of glitter on nutrient cycling

Organic matter content of the sediment averaged at 25.1 (± 0.86) % across all mesocosms and did not significantly differ amongst treatments (Table 1). Similarly, community respiration was 0.18 (± 0.01) mg O_2 L^{-1} h^{-1} on average and did not significantly differ amongst treatments (Table 1). Nitrate concentrations, however, were significantly greater in Control mesocosms than in those with any type of biodegradable glitter (MRC, mica or synthetic mica) at 8 and 15 days, but did not significantly differ amongst treatments after 22, 29 or 36 days (Table 1).

4. Discussion

The present study, which is the first to examine the environmental impacts of glitter, found that alternative biodegradable glitters had several effects similar to those observed for as

conventional PET glitter. Any type of glitter (PET, MRC, mica and synthetic mica) resulted in less chlorophyll b & c in the water column and shorter root lengths of *L. minor* compared with controls. Less chlorophyll b & c suggests less biomass of green microalgae (Wetzel 2001), diatoms and dinoflagellates (Dougherty et al. 1970) which are vital primary producers in freshwater systems. Pure cultures of freshwater microalgae have also been found to decrease in biomass in response to microplastics (Wu et al. 2019) possibly due to the formation of hetero-aggregates between the microalga and the microplastics (Legarde et al. 2016) causing the phytoplankton to become more dense and sink out of the water column. In the current study, however, the glitter rapidly sank and was visible on the sediment of the mesocosms, so the formation of hetero-aggregates in the water column is not a plausible explanation. In addition, the results for *L. minor* are similar to those of Kalčíková et al. (2017) who also found no effect on growth or chlorophyll a but did find shorter roots in response to floating microplastics (polyethylene) attributed to mechanical blocking of the pores. However, the glitter in the current study sank to the bottom of the mesocosms and was not observed adhering to *L. minor*, so it is unlikely to be the same mechanism to explain this reduction in root length. Instead, it is more likely that leachate from the glitters (possibly from the aluminium-based and acrylic coatings) caused the reduction in phytoplankton biomass and the shorter root lengths of *L. minor*. Leachate from plastic has been found to reduce the growth and photosynthesis of marine microalgae (e.g. Tetu et al. 2019) and leachate from conventional and biodegradable plastic bags altered germination and development of sand dune plants (Menicagli et al. 2019). Leachate from the glitters were not measured in the current study but could contain a myriad of chemical compounds as additives (Hahladakis et al. 2018) migrating from the core material (i.e. PET, MRC, mica or synthetic mica), reflective metal (e.g. aluminium, but note that solubility, and therefore bioavailability, are dependent on pH; Gensemer and Playle 1999) and / or additional (e.g. styrene acrylate) coatings. The next logical step in order to gain a

mechanistic understanding of the effects of glitter on primary producers is to conduct experiments comparing the effects of glitter with that of leachate derived from glitter.

In addition, sediment treated with synthetic mica had a greater concentration of chlorophyll c, representing benthic diatoms and dinoflagellates, than sediment treated with natural mica or control sediment. Benthic diatoms are used as indicators of environmental change in freshwater habitats around the world (Stevenson et al. 2010), further studies are required to unravel which types of benthic microalgae have responded to synthetic mica and any ecological consequences this may have.

Most notably, however, glitter made out of modified regenerated cellulose (MRC) resulted in an increase in an invasive species: *P. antipodarum*, a snail native to New Zealand that has been in the UK since the 1880's. *P. antipodarum* is a successful invader due to its' high reproductive capacity, which can lead to explosive population growth (Alonso and Castro-Díez 2008). An increase in reproductive output has occurred in *P. antipodarum* in response to other contaminants and is sometimes associated with endocrine disrupting compounds (Zounkova et al. 2014). They also may be resistant to microparticle contamination, for example there was no effect of a mixture of non-buoyant microplastics which included PET (of approximately same size as used in this study average ~100 µm) on fecundity (number of offspring), growth and development of *P. antipodarum* (Imhof and Laforsch 2016). In this way, *P. antipodarum* could be useful as an indicator species for pollution and indeed there is a trend for them to be found in greater densities in polluted than in pristine habitats (Schreiber et al. 2003; Alonso and Castro-Díez 2008; Zounkova et al. 2014). As an invasive species, they could lead to undesirable ecological impacts through high consumption rates (up to 75% of the primary productivity; Hall et al. 2003), dominance in the community in terms of their biomass and prevention of other species from becoming established during early stage of succession (Alonso and Castro-Díez 2008).

Interestingly, the alternative biodegradable glitters tested in this experiment elicited stronger effects than the non-biodegradable PET glitter overall. For example, over the first 2 weeks of the experiment mesocosms exposed to any of the three biodegradable glitters had a lower concentration of nitrate compared with control mesocosms and this could be due to the glitter adsorbing these nutrients from the surrounding water or sediment (Prata et al. 2019), or due to microbially-mediated processes in the sediment. Indeed, biodegradable polymers are used in the process of "solid-phase denitrification" providing a carbon source and biofilm carrier for denitrifying microorganisms in order to remove nitrate from a range of applications including drinking water, groundwater and aquaculture wastewater (Boley et al. 2000; Wang and Chu 2016). This effect, however, disappeared halfway through the experiment and there were no other differences in the abiotic responses measured.

Glitters are primary microplastics which are found in wastewater treatment plants and sewage sludge (Murphy et al. 2016; Lusher et al. 2017; Lares et al. 2018; Magni et al. 2019; Sun et al. 2019) and can make their way into freshwater sediments (Ballent et al. 2016; Hurley et al. 2018) where they have the potential to alter primary productivity (current study). Although PET glitters are included in the restrictions proposed on 'intentionally added microplastics' by the European Chemicals Agency (ECHA, 2019), derogations have been made for biodegradable or natural polymers. These derogations occur despite mounting evidence that the persistence of biodegradable microplastics is uncertain (Narancic et al. 2018) and that they can evoke the same biological and ecological impacts as conventional microplastics in terrestrial (Boots et al. 2019), freshwater (González-Pleiter et al. 2019; Straub et al. 2017) and marine (Green 2016; Green et al. 2016; Green et al. 2017) habitats. Moreover, only the core material of glitter (without the reflective coatings and sealants) needs to be tested in order to be certified as "biodegradable", so the biodegradability and ecotoxicity of glitter, in its final form, is not actually assessed.

In conclusion, the current study, which found that alternative biodegradable glitters can cause the same and even stronger effects than non-biodegradable glitter, emphasises that these derogations, and certification conditions for biodegradability, require further consideration.

Author Contributions

DSG: conceptualisation, methodology, visualisation, writing - original draft. MJ: ran the experiment, data collection, writing - review and editing. BB: data analysis, visualisation, writing - review and editing. LS: data collection, writing - review and editing.

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Tables and Figures

Table 1.

Average (±S.E.) abundance, dry weight (g), chlorophyll a and b content (mg g^{-1}), organic matter content (%) of the oxic sediment, community respiration (measured as O_2 consumption mg L^{-1} h^{-1}), NO_3^- concentration (mg L^{-1}) found in mesocosms exposed to either no glitter (Control), PET glitter (PET), Cellulose-based glitter (Cellulose), mica glitter (mica) or synthetic mica glitter (Syn. Mica) after 8, 15, 22, 29 and 36 days. Where significant differences were found F-ratios (F) and P-values (P) are in **bold** and significant differences resolved by post-hoc Tukey tests they are indicated by subscript letters.

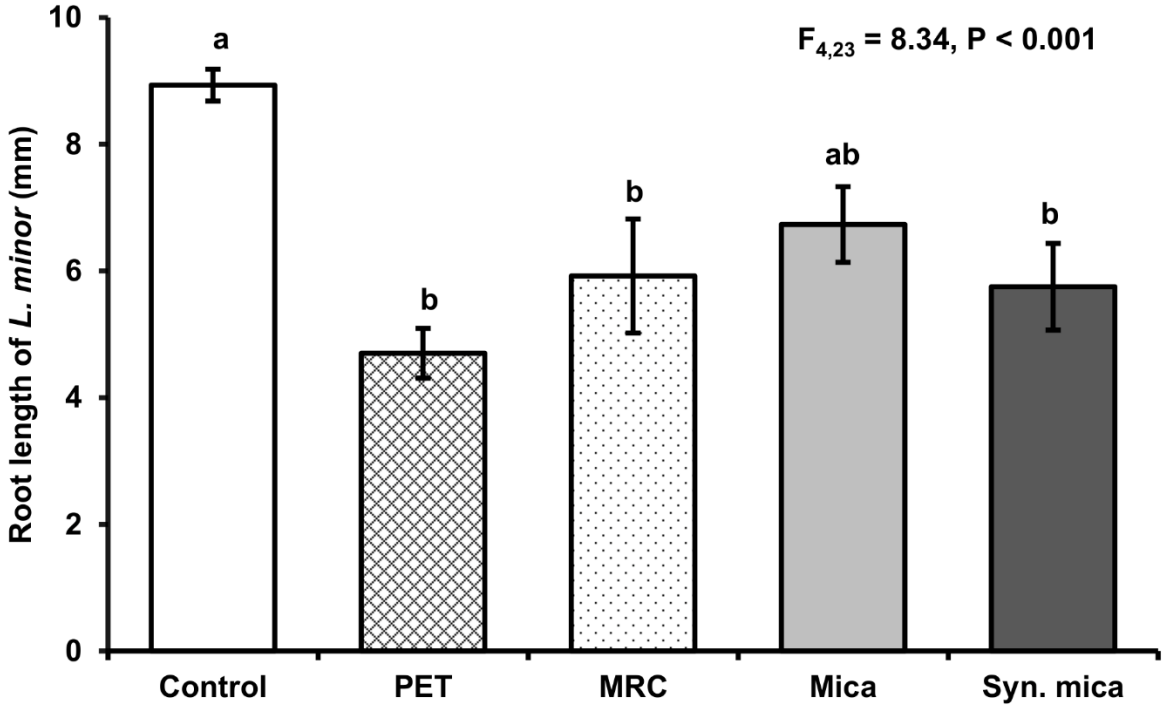
Response / Treatment	Control	PET	Cellulose	Mica	Syn. Mica	F, P
L. minor abundance	211 ± 58	283 ± 70	204 ± 59	240 ± 53	149 ± 69	0.63, 0.644
L. minor dry weight	0.22 ± 0.05	0.29 ± 0.05	0.21 ± 0.07	0.27 ± 0.07	0.18 ± 0.08	0.50, 0.733
L. minor chl a	3.16 ± 0.56	3.51 ± 0.67	2.63 ± 0.92	3.46 ± 0.62	2.22 ± 0.81	0.58, 0.678
L. minor chl b	2.13 ± 0.38	2.34 ± 0.44	1.74 ± 0.63	2.26 ± 0.41	1.42 ± 0.51	0.64, 0.641
Sediment OM	27.8 ± 3.04	26.1 ± 0.51	24.4 ± 0.95	21.8 ± 2.64	25.6 ± 0.49	1.38, 0.265
Community respiration	0.20 ± 0.02	0.23 ± 0.04	0.18 ± 0.02	0.16 ± 0.02	0.14 ± 0.01	1.89, 0.138
NO_3^- conc. day 8	**3.68 ± 0.49$_a$**	**2.85 ± 0.15$_{ab}$**	**2.20 ± 0.23$_b$**	**2.21 ± 0.21$_b$**	**2.32 ± 0.18$_b$**	**5.12, 0.003**
NO_3^- conc. day 15	**1.97 ± 0.25$_a$**	**1.46 ± 0.06$_{ab}$**	**1.10 ± 0.10$_b$**	**1.33 ± 0.08$_b$**	**1.37 ± 0.12$_b$**	**5.40, 0.002**
NO_3^- conc. day 22	1.09 ± 0.07	1.01 ± 0.04	0.97 ± 0.03	1.00 ± 0.03	1.00 ± 0.04	0.98, 0.430
NO_3^- conc. day 29	0.94 ± 0.06	0.86 ± 0.07	0.91 ± 0.05	0.90 ± 0.06	0.93 ± 0.05	0.31, 0.870
NO_3^- conc. day 36	0.93 ± 0.07	0.94 ± 0.06	1.14 ± 0.09	1.20 ± 0.10	1.03 ± 0.11	1.82, 0.150

Table 2.

Average (±S.E.) species richness (SR), numbers of animals (N), Shannon-Wiener index (H') and densities of viable taxa found in mesocosms exposed to either no glitter (Control), PET glitter (PET), Cellulose-based glitter (Cellulose), mica glitter (mica) or synthetic mica glitter (Syn. Mica) for 36 days. F-ratios (F) and P-values (P) from ANOVA are included and values are highlighted in **bold** when significant differences were found ($\alpha < 0.05$). Subscript letters are used to indicate where these differences could be resolved by post-hoc Tukey tests.

Response / Treatment	Control	PET	Cellulose	Mica	Syn. Mica	F, P
SR	1.86 ± 0.26	2.29 ± 0.56	2.42 ± 0.43	2.29 ± 0.36	3.43 ± 0.48	1.83, 0.149
N	**37.7 ± 6.81$_a$**	**40.7 ± 4.37$_a$**	**90.1 ± 22.6$_b$**	**47.4 ± 11.6$_{ab}$**	**45.7 ± 9.3$_{ab}$**	**2.90, 0.039**
H'	0.14 ± 0.04	0.22 ± 0.10	0.13 ± 0.05	0.24 ± 0.06	0.39 ± 0.09	2.22, 0.091
P. antipodarum	**36.4 ± 6.62$_a$**	**38.6 ± 4.56$_a$**	**87.3 ± 22.3$_b$**	**44.9 ± 11.3$_{ab}$**	**41.0 ± 8.4$_{ab}$**	**2.97, 0.035**
Lymnaea stagnalis	-	-	-	-	0.14 ± 0.14	1.00, 0.423
***Physa* sp.**	**0.14 ± 0.14$_a$**	**-$_a$**	**-$_a$**	**-$_a$**	**0.43 ± 0.20$_a$**	**2.83, 0.042**
Planorbis sp.	-	0.14 ± 0.14	0.43 ± 0.20	0.29 ± 0.29	0.86 ± 0.55	1.21, 0.329
Valvata sp.	-	-	-	-	0.57 ± 0.57	1.00, 0.423
Bithynia sp.	-	-	-	-	0.29 ± 0.18	2.40, 0.072
Sphaerium sp.	0.43 ± 0.30	1.00 ± 0.44	2.14 ± 1.20	2.00 ± 0.58	1.86 ± 0.88	0.96, 0.443
Chironomidae	0.71 ± 0.36	0.71 ± 0.57	0.29 ± 0.18	0.14 ± 0.14	0.43 ± 0.30	0.55, 0.699
Sialidae	-	0.14 ± 0.14	-	0.14 ± 0.14	-	0.75, 0.566
Asellus aquaticus	-	0.14 ± 0.14	-	-	-	1.00, 0.423
Limnephilidae	-	-	-	-	0.14 ± 0.14	1.00, 0.423

631



632

633 *Figure 1.*

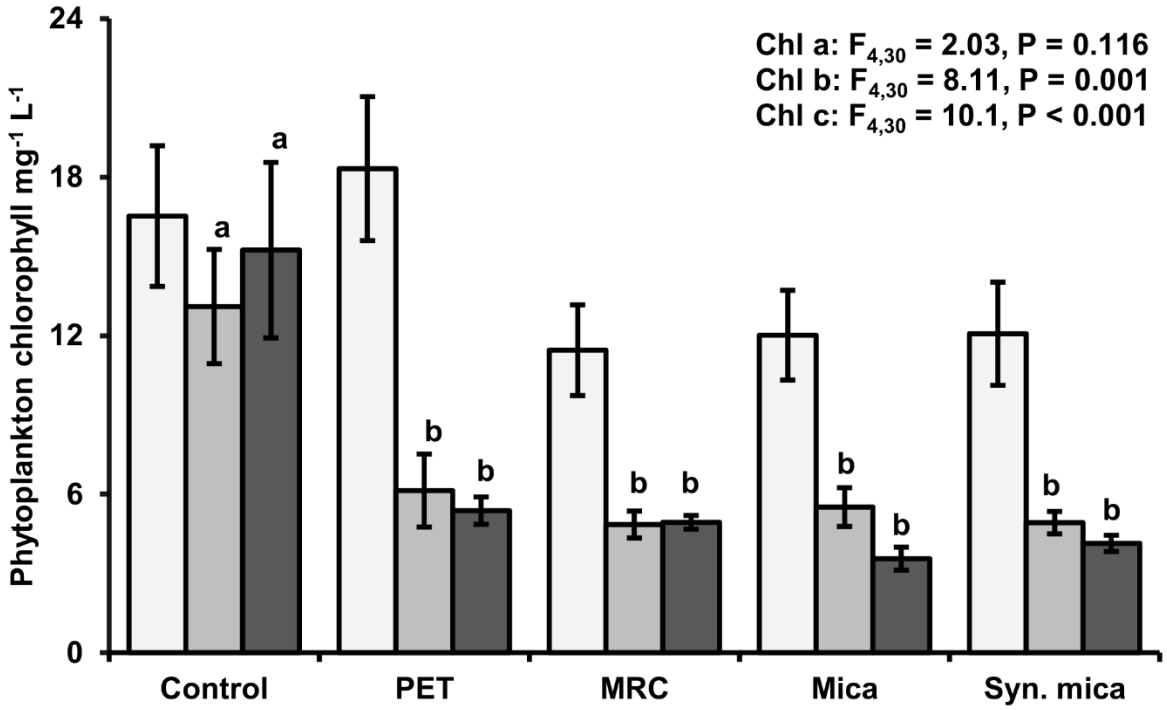
634 Average (± S.E) root length (mm) of *Lemna minor* from mesocosms with no glitter (Control)

635 or with non-biodegradable (PET) or biodegradable modified regenerated cellulose (MRC),

636 mica or synthetic mica) glitter. Included are ANOVA results with F-ratios and P values, letters

637 indicate significant differences determined by post-hoc tests.

638

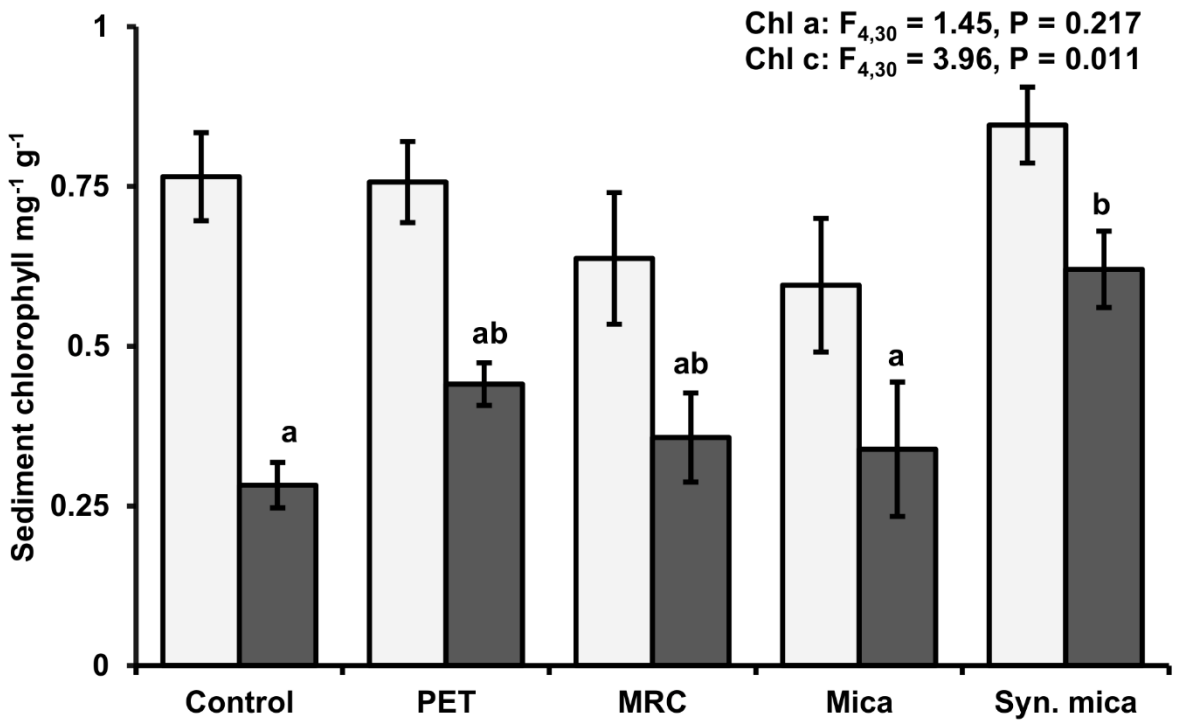


639

640 *Figure 2.*

641 Concentration (mg L^{-1}) of chlorophyll a (white), chlorophyll b (light grey), and chlorophyll c
642 (dark grey) in the water column from mesocosms with no glitter (Control) or with non-
643 biodegradable (PET) or biodegradable modified regenerated cellulose (MRC), mica or
644 synthetic mica) glitter. Letters indicate significant differences between treatments for each type
645 of chlorophyll. Included are ANOVA results with F-ratios and P values.

646



647

648 *Figure 3.*

649 Concentration (mg g^{-1} dw) of chlorophyll a (white) and chlorophyll c (dark grey) in oxic

650 sediment in from mesocosms with no glitter (Control) or with non-biodegradable (PET) or

651 biodegradable modified regenerated cellulose (MRC), mica or synthetic mica) glitter. Letters

652 indicate significant differences between treatments determined by post-hoc tests.

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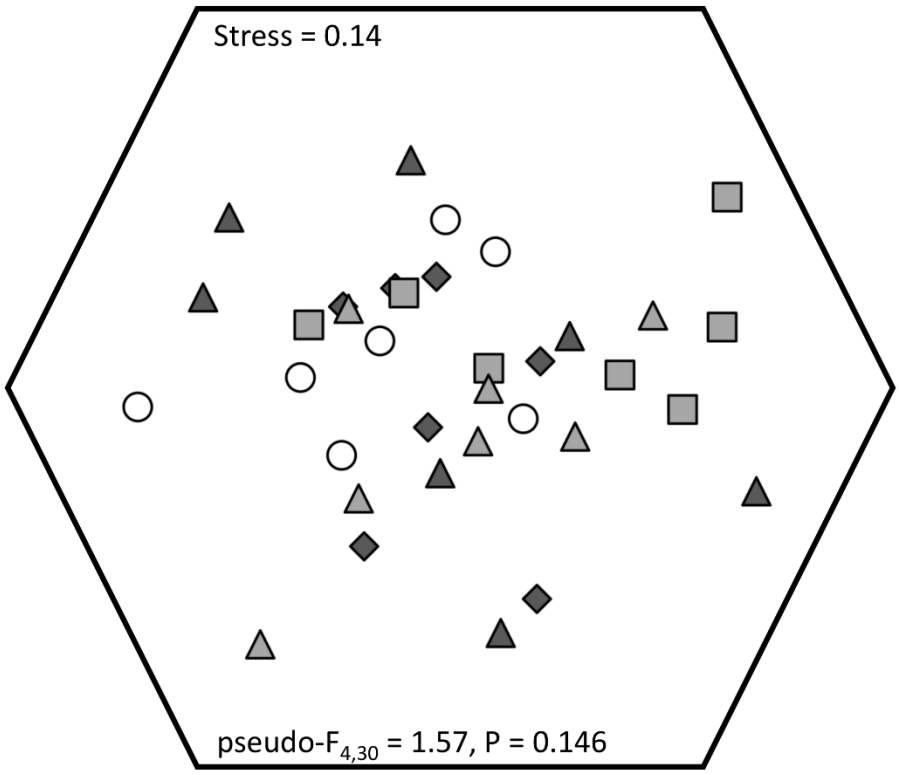

Figure 4.

Non-metric multidimensional scaling ordination of square root transformed data of fauna assemblages in sediment with either no added glitter (○), or glitter made of PET (◆), modified regenerated cellulose (□), mica (△) or synthetic mica (▲) after 36 days of exposure. Included are the results of the multivariate ANOVA.