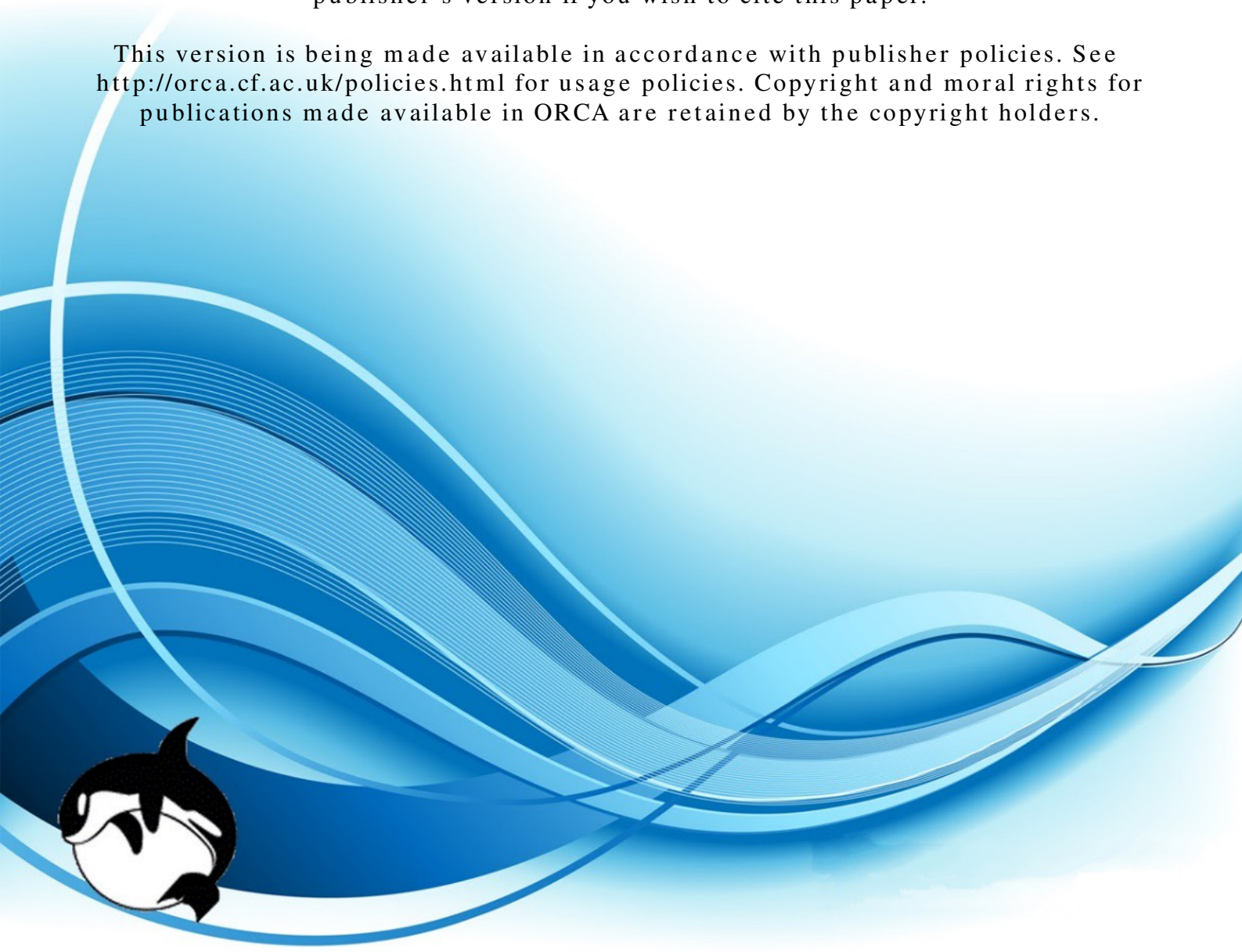

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Polystyrene Microplastics Decrease Accumulation of Essential Fatty Acids in Common Freshwater Algae

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ABSTRACT

Despite growing concern about the occurrence of microplastics in aquatic ecosystems there is only rudimentary understanding of the pathways through which any adverse effects might occur. Here, we assess the effects of polystyrene microplastics (PS-MPs; <70 µm) on a common and widespread algal species, *Chlorella sorokiniana.* We used laboratory exposure to test the hypothesis that lipids and fatty acids (FAs) are important molecules in the response reactions of algae to this pollutant. Cultivation with PS-MPs systematically reduced the concentration of essential linoleic acid (ALA, C18:3n-3) *in C. sorokiniana*, concomitantly increasing oleic acid (C18:1n-9). Among the storage triacylglycerols, palmitoleic and oleic acids increased at the expenses of two essential fatty acids, linoleic (LIN, C18:2n-6) and ALA, while PS-MPs had even

more pronounced effects on the fatty acid and hydrocarbon composition of waxes and steryl esters. The FA composition of two major chloroplast galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), were affected implying changes in the conformational structure of photosynthetic complexes that can impair the photosynthesis. These data reveal how exposure to polystyrene microplastics can modify the concentrations of lipid molecules that are important intrinsically in cell membranes, and hence the lipid bilayers that could form an important barrier between algal cellular compartments and plastics in the aquatic environment. Changes in lipid synthesis and fatty acid composition in algae could also have repercussions for food quality, growth and stressor resistance in primary consumers. We advocate further studies of microplastics effects on the lipid composition of primary producers, and of their potential propagation through aquatic food webs.

Main finding: Polystyrene causes fundamental changes in lipid composition of widespread algae opening a new front in understanding microplastic effects on food webs.

Keywords:

Chlorella, plastic pollution, lipids, primary producers, aquatic ecosystems

1. Introduction

The production of synthetic polymers is increasing exponentially with over 280 million tonnes of plastics now produced every year. Once discarded, there is a large risk that this material will pollute either marine or freshwater ecosystems where it has the potential to affect individuals and populations of a range of organisms as well as ecosystem processes (De Sá et al., 2018). Physical characteristics such as chemical inertness and slow biodegradation rates, coupled with large production, has resulted in an accumulation of plastic debris in benthic sediments so far up to 500,000 fragments m^{-2} and in the water column to over 4000 particles m^{-3} (Yangtze estuary system, East China Sea) (Lusher, 2015). These concentrations reflect contributions either from primary microplastics (e.g., fibres, tyre dust, road paint, cosmetics) or from the breakdown of larger plastic items through mechanical erosion, physical abrasion, solar radiation and/or biological degradation, whereas chemical degradation is very slow (De Sá et al., 2018). Among plastic pollutants in aquatic ecosystems, microplastics (MPs) are defined as plastic particles of 0.1 µm-5 mm in size, while nanoplastics (NPs) are 1-100 nm in size (Akdogan and Guven, 2019).

A range of plastic types can constitute MPs, with European data showing the most common subtypes to be 28% polyethylene, 19% polypropylene and 7% polystyrene (plasticseurope.org). Owing to their small size, as well as differences in shape and density, MPs are distributed among water surfaces, the water column

and sediments. This enables MPs to penetrate aquatic food webs through several trophic levels and entry routes (Windsor et al., 2019). A multitude of MP types with varying physicochemical properties can therefore interact with biota via different mechanisms, including ingestion or external contact (Eerkes-Medrano et al., 2015; De Sá et al., 2018). Moreover, the contamination of plastics with plasticizers and chemical additives can occur during manufacture. In addition, MPs can transport some pollutants sorbed to their surfaces through aquatic and terrestrial environments (Engler, 2012; Diepens et al., 2018; Bradney et al., 2019; Gassel and Rochman, 2019). Despite the potential for adverse effects on organisms, the mechanisms of any MP impacts at the molecular level are poorly known. This is particularly true for primary producers such as algae. In standing waters, suspended algae, or phytoplankton, are critical basal resources that power food webs, oxygen production and biogeochemical cycling, and represent significant biodiversity (Stevenson, 2014). As a result, algae also have a long history of use in ecological monitoring, environmental assessment, and as bioindicators of environmental conditions (Gökçe, 2016). Current understanding of the effects of MPs on algae is limited, especially among freshwater species, despite the fact that freshwater ecosystems sit within terrestrial landscapes that are the source of much plastic pollution (Windsor et al., 2019). Initial data indicate that MPs could affect algal growth, chlorophyll content and photosynthetic activity (Sjollema et. al., 2016; Wu et al., 2019), while the production of reactive

oxygen species induced by MPs might lead to oxidative stress (Bhattacharya et al., 2010; Prata et al., 2019).

Anthropogenic factors can affect lipid metabolism in algae, including the synthesis of polyunsaturated fatty acids (PUFAs) (Guschina and Harwood, 2006; Guschina and Harwood, 2009). These are important and major dietary components for primary consumers as a source of energy and essential nutrients, including polyunsaturated fatty acids (PUFAs) that cannot be synthesised by animals. PUFAs are critical regulators of the survival, reproduction and population growth in invertebrates and fish (Parrish 2009; Muller-Navarra et al., 2004; Kainz et al., 2004). As they are highly retained during transfer through freshwater food webs, any factors affecting the quantity and quality of PUFAs in phytoplankton could have subsequent effects on the growth, reproductive capacities and fitness of aquatic invertebrates and fish. However, we are aware of no studies assessing the effects of MPs on algal lipids, including PUFAs.

Here, we assess the effect of polystyrene microplastics (PS-MPs) on lipid and fatty acid composition of a unicellular, freshwater, green alga *Chlorella sorokiniana* under laboratory conditions. This species has been used extensively in controlled laboratory experiments as a food source for consumers, as well as to study the role of algal lipids in adaptation to various environmental factors. *C. sorokiniana,* like other Chlorophytes, synthesises essential fatty acids (FAs) such as linoleic acid (LIN; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3), the precursors of long-chain PUFAs which plankton and organisms on the higher

trophic levels need for survival (Sargent et al., 1999). We test the hypothesis that the lipids and FAs are important molecules in the response reactions of algae to polystyrene contamination.

2. Material and methods

2.1. Algal Cultivation.

Chlorella sorokiniana (211-31; Sammlung von Algenkulturen, Gottingen University, Germany) was used for the experiments. The alga was grown in 50-ml cultures on a 12:12 h (L:D) cycle (PAR = 35.4 µmol/m^2/s) at 22 °C in Bold's basal medium (Bold, 1949) on a table shaker (125 rpm).

2.2. PS-MPs treatment.

Polystyrene granules (Sigma-Aldrich, Gillingham, UK; product specification 331651; identity and purity shown by infrared techniques, as confirmed by the Merck Company, including the lack of any coating) were ground and the size fraction of <70 µm isolated by sieving.

The PS-MP suspension was prepared in sterile cultivation media at the stock concentration of 240 mg/L, and sonicated prior to use to ensure full dispersion: we followed this step based on other investigators, and no sonication was applied to algal cultures. On the first day of the experimental exposure, the PS-MP suspension (40 mL) was added to 10 mL of algal cultures stocked in the stationary phase. This gave a concentration of PS-MPs of 60 mg/L in the algal media at the

beginning of the experimental cultivation, when algae were in the logarithmic growth phase. After 4-weeks of experimental cultivation, algal cells (once more in their stationary growth phase as a batch culture) were harvested by centrifugation (1,500 rpm) and compared against control cultures grown using the same cultivation methods. This approach was based on our own previous experience (as well on available literature) of green algae culture and lipid composition which shows using growth curves that the majority of green algae enter the stationary phase, after four week of cultivation,. Optical density methodology could not be used here to assess growth patterns in this investigation because the presence of microplastics would have interfered with any optical density measurements. However, the accumulation of large amounts of triacylglycerols (TAGs) in our cultures confirmed that cultured algae were in their stationary stage.

2.3. Lipid Extraction.

Algal cell pellets were washed once with dechlorinated water, and total lipids extracted according to Kates (1986). Briefly, total lipids were pre-extracted from fresh biomass (about 250 mg wet weights) with 2 ml of isopropanol heated at 70 °C during 30 min to inactivate endogenous lipases (twice). The isopropanol extracts were combined, dried under a stream of nitrogen and then redissolved in 3 ml of 2:1 (v/v) chloroform/methanol. Total lipids were further separated by adding 2 ml of the solution of 2 M KCl in 0.5 M phosphate buffer, mixed and

centrifuged at 200 g for 5 min to separate two layers. The lower chloroform fractions were collected, and the solvents were evaporated under a stream of nitrogen. Total lipid extracts were stored in chloroform at -20 °C under nitrogen until further analysis.

2.4. Thin-layer chromatography (TLC).

The major lipid classes, namely total polar lipids (TPL), triacylglycerols (TAG) and steryl esters (SE) were separated using one-dimensional TLC on 10 x 10 cm silica gel G plates (Merck KGaA, Darmstadt, Germany) using 80:20:1 (v/v/v) hexane/diethyl ether/acetic acid. Phospholipids (PL) and glycosylglycerides (GL) were separated using two-dimensional TLC using 65:25:4 (v/v/v) chloroform/methanol/water in the first dimension and then 50:20:10:10:5 (v/v/v/v/v) chloroform/acetone/methanol/acetic acid/water in the second. After drying, the plates were sprayed with a 0.1% solution of 8-anilino-4-naphthosulphonic acid in methanol (w/v) and viewed under UV light to reveal lipids.

2.5. Analysis of fatty acids.

Aliquots of the total lipid extracts (for analysis of the total FAs) or individual lipid classes separated using TLC were used for fatty acid methyl ester (FAME) preparation. FAMEs were prepared by trans-methylation with 2.5% H_2SO_4 (v/v) in 2:1 (v/v) dry methanol/toluene at 70 °C for 2 h. A known amount of nervonic

173 acid, C24:1n-9, was added as an internal standard for quantification. FAMEs
174 were extracted with HPLC grade hexane. A Clarus 500 gas chromatograph with
175 a flame ionizing detector (FID) (Perkin-Elmer 8500, Norwalk, CT, USA) and
176 fitted with a 30 m x 0.25 mm i.d. capillary column (Elite 225, Perkin Elmer) was
177 used for separation and analysis of FAs. The oven temperature was programmed
178 as follows: 170 °C for 3 min, increased to 220 °C at 4 °C/min, and then held at
179 220 °C for 15 min. FAMEs were identified routinely by comparing retention
180 times of peaks with those of G411 FA standards (Nu-Chek Prep. Inc., Elysian,
181 MN, USA). Perkin Elmer Total Chrom Navigator software was used for data
182 acquisition (Fuschino et al., 2011).

183 *2.6. Microplastic size distribution: particle measurements.*

184 To verify the nominal size distribution of plastic particles following sieving,
185 samples of polystyrene microplastics in glass petri dishes were imaged on a Meiji
186 Optem Zoom 125 macro imaging system (Meiji Techno, UK) coupled to a
187 Jenoptik Progres CFscan colour digital camera (Jenoptik, UK) (Fig. 1). Ten
188 randomly selected image fields were taken under transmitted light illumination.
189 Images were calibrated for subsequent measurements using a 1mm/0.01mm stage
190 micrometre. All image data analysis was performed in Fiji
191 (https://imagej.net/Fiji/Downloads) (Schindelin, 2012). To quantify the size of
192 individual particles an automated counting procedure was utilised as follows: 16
193 bit colour images of the particles were converted to 8 bit greyscale images,
194 inverted and thresholded using the maximum entropy algorithm of Fiji's

thresholding tool. The particle analysis tool was then used to identify, trace and calculate the area (μm^2) occupied by each microplastic particle within the thresholded image field. Data were output into Microsoft Excel for further analysis.

2.7. Chlorophyll extraction and analysis.

To assess any effects of plastic exposure, Chlorophylls were extracted with 1 ml of DMSO from 0.06 g of fresh algal biomass for 5 min at 70 °C. The chlorophyll concentrations were determined in DMSO extracts spectrophotometrically using Ultrospec 2000UV/Visible spectrophotometer (Pharmacia Biotech) and quantified according to Solovchenko et at. (2010).

2.*8. Statistics.*

Comparison of the control and PS-MPs treatment means was performed using *t*-test and significant effects were reported at $P < 0.05$ (SPSS 25 Software). Data were expressed as mean ± standard deviations when n=3 replicates for control units and n=4 for PS-MP treatment units.

3. Results

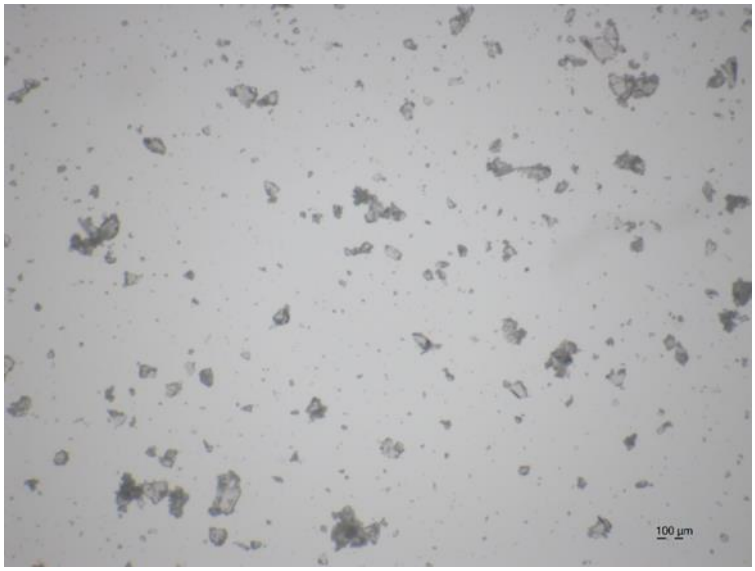


Figure 1.

Transmitted light image of the PS-MPs material following sieving at 70 µm prior to suspension with algae (see MATERIALS AND METHODS). Over 49% of particles were 1-50 µm, but some particle aggregation meant that 25% were in the range 100 – 500 µm. Particles shapes were irregular, fragmented and mostly angular.

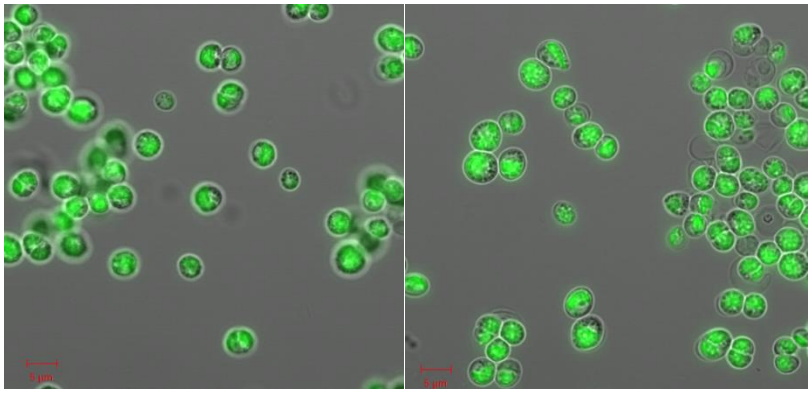


Figure 2. Confocal laser scanning microscopy images of the control samples of *C. sorokiniana* (see MATERIALS AND METHODS).

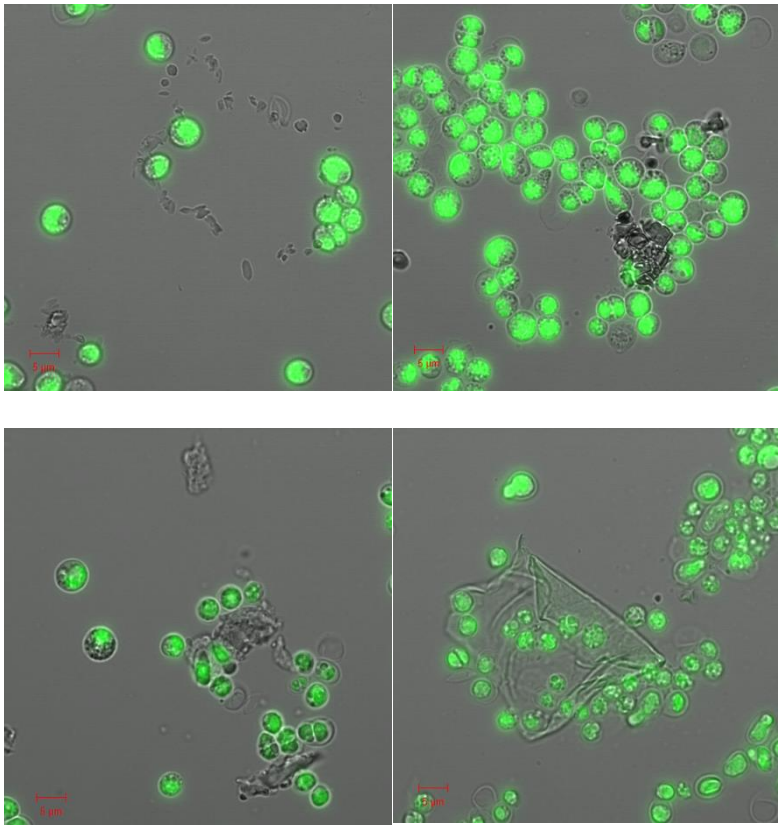


Figure 3. Confocal laser scanning microscopy images of the PS-MP treated samples of *C. sorokiniana.* The images illustrate the variations in the size and shape of PS-MP particles as well as their varying attachments to the algal cells (see MATERIALS AND METHODS).

Algal cell size (area) was reduced significantly following microplastic exposure by around 11% from 13.7 μm^2 (SD 3.6 μm^2) to 12.2 μm^2 (SD = 4.3 μm^2; $t = 112.2$, $P < 0.001$, $df = 5,136$). The chlorophyll *a* concentration increased from 8.33 ± 0.11 in control samples to 10.10 ± 0.04 µg/mLin PS-MP treated sample ($t = 27.05$, $P < 0.001$, $df = 4$), while chlorophyll *b* increased from 5.15 ± 0.04 to 5.77 ± 0.03 µg/mL in the PS-MP treated algae ($t = 23.62$, $P < 0.001$), increases respectively of 21% and 12% (Figs. 2 and 3).

237

238 *3.1 Lipid accumulation and major lipid classes*

239 Incubation with PS-MPs increased the total lipid accumulation in *C. sorokiniana*
240 from 486.7 ± 58.5 µg of FAs per 100 mg fresh weight (FW) in controls to 652.6
241 ± 126.64 µg of FAs in PS-MP treated samples.

242 Among the major lipid classes which include total polar lipids (TPL),
243 triacylglycerols (TAG) and the combined fraction of waxes and steryl esters (Fig.
244 4), storage TAG accounted for up to 80% of total lipids, followed by membrane
245 polar lipids, TPL (up to 18%) and the fraction of waxes and steryl esters (up to
246 3%; Fig. 4). The latter was a minor class, but decreased in *C. sorokiniana* after
247 30 day- incubation with PS-MPs, whereas TAG and TPL were unchanged (Fig.
248 4).

249 In keeping with widespread practice in lipid analysis, individual lipids were
250 assessed from the relative (%) distribution of individual lipid classes as this was
251 considered to give a more appropriate indication of lipid re-arrangement in the
252 cells under MP treatment. The percentages reveal the re-arrangement of lipid
253 membrane compounds which reflects the interdependence of the metabolic
254 pathways involved (Fuschino et al., 2011).

255 The fatty acid profile in total lipids of *C. sorokiniana* was typical of green algae
256 with domination of palmitic acid (C16:0), oleic acid (C18:1n-9), essential LIN
257 and ALA as well as C16 PUFA, namely C16:3n-3 and C16:4n-3 (Fig. 5).

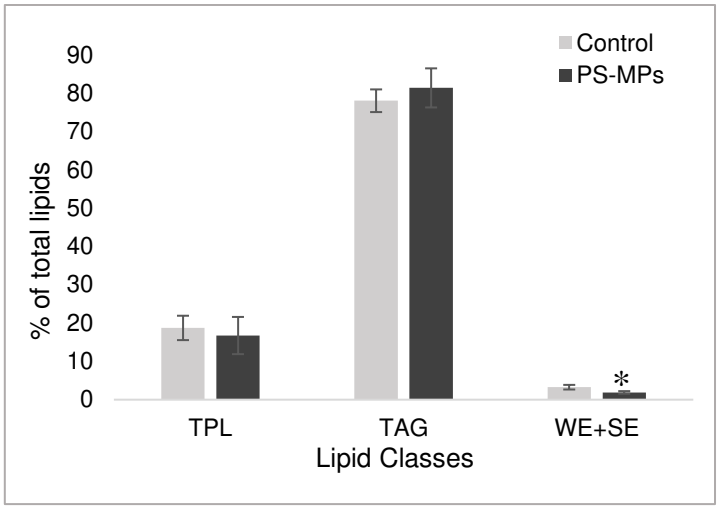


Fig. 4. PS-MP effect on distribution of major lipid classes (% of total), total polar lipids (TPL), triacylglycerols (TAGs) and the fraction of waxes and steryl esters (WE+SE), in *C. sorokiniana.* The asterisk (*) indicates a significant effect of PS-MPs when compared to control samples ($p < 0.05$, n=3-4).

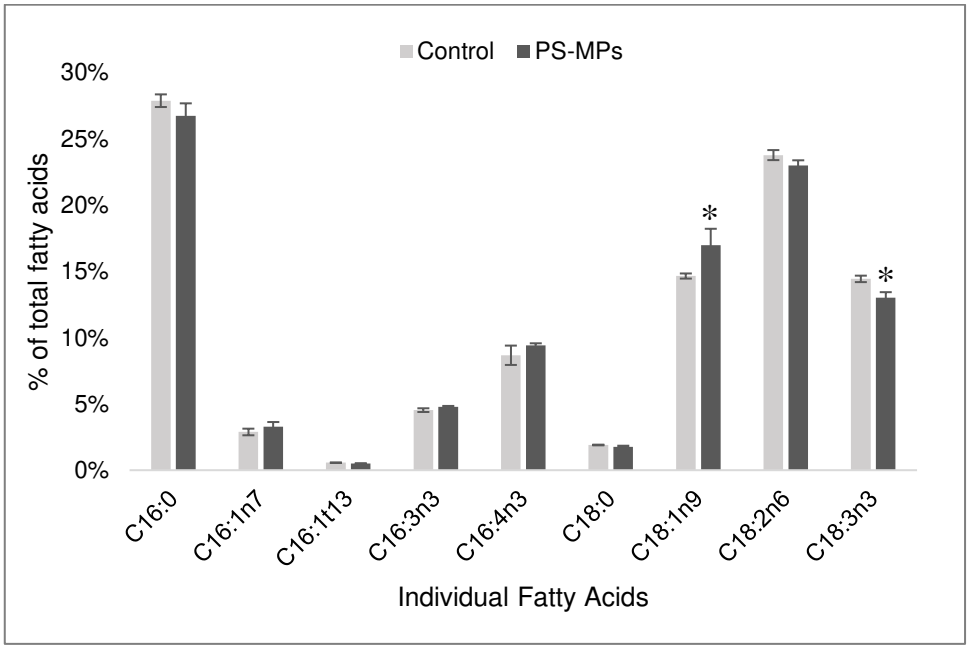


Fig. 5. PS-MP effect on distribution of fatty acids (% of total FA) in total lipids of *C. sorokiniana.* FAs are indicated with the number before colon showing the number of carbon atoms, the figure afterwards denoting the number of double

bonds. The position of the first double bond is shown after "n". Values are means ± SD . The asterisk (*) indicates a significant effect of PS-MPs when compared to control samples ($p < 0.05$, n=3-4).

3.2 Essential fatty acids

Cultivation with PS-MPs significantly decreased the concentration of essential linoleic acid (C18:3n-3) with a concomitant increase in oleic acid (C18:1n-9) (Fig. 5). Analysis of fatty acids in the storage TAGs revealed some subtle but statistically significant increase in palmitoleic and oleic acids at the expenses of two essential fatty acids, LIN and ALA (Fig. 6A). The effect of PS-MPs on fatty acid and hydrocarbon (nC in Fig. 6B) composition of waxes and steryl esters was more pronounced (Fig. 6B). Exposure led to a substantial reduction in the relative amounts of LIN (from 14.3% to 11.7%) and ALA (from 22.4% to 18.8%) alongside elevation in the levels of saturated myristic (C14:0) and palmitic acids. The principal hydrocarbon in this lipid fraction was nC17:0, which declined reduced from 14.3% in control culture to 11.7% in PS-MP treated samples (Fig. 6B).

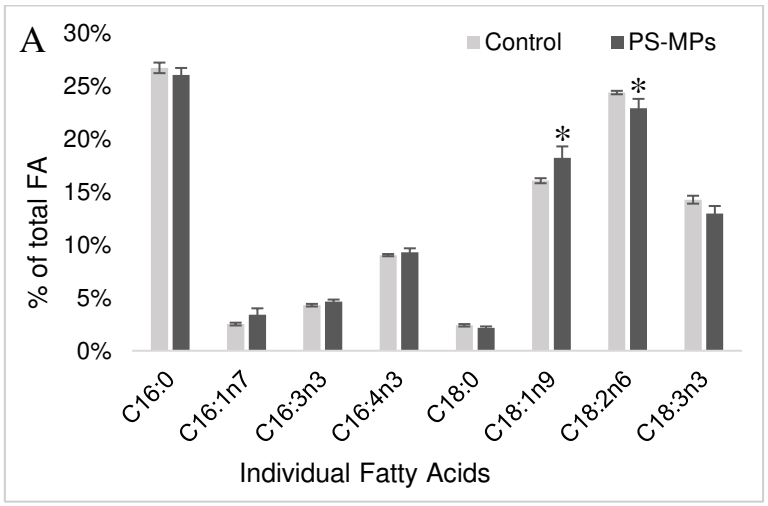
3.3 Polar lipids

Polar lipids were of particular interest in analysis. The fraction of total polar lipids consists of two groups of glycerolipids, glycosylglycerolipids (or glycolipids) and phosphoglycerides (or phospholipids). In algae (as in higher plants and cyanobacteria), glycolipids, namely monogalactosyldiacylglycerol

(MGDG) and digalactosyldiacylglycerol (DGDG) are located mainly in photosynthetic membranes. Another class of glycosylglycerolipids of photosynthetic membranes in green algae is the plant sulfolipid, sulfoquinovosyldiacylglycerol (SQDG). A unique feature of plastid galactolipids is their very high amounts of PUFAs with both C16 and C18 chains.

Phospholipids are located in the extra-chloroplast membrane except phosphatidylglycerol (PG) which is the only phospholipid present in the thylakoid membranes in appreciated amounts. A unique feature of PG is Δ3-trans-hexadecenoic acid (C16:1*t*13) esterified sn-2 position of this phospholipid. In addition to PG, phosphatidylcholine (PC) and phosphatidylinositol (PI) are important phospholipids identified in *C. sorokiniana*. A betaine lipid, diacylglyceryltrimethylhomoserine (DGTS), is a common lipid of many lower plants including algae. In membranes, DGTS plays a similar role that PC does in higher plants and animals (Guschina and Harwood, 2006; Guschina and Harwood, 2009). There is no phosphorus or carbohydrate in this lipid. MGDG and DGDG are uncharged, whereas SQDG, PI and PG carry negative charge, and PC and DGTS are twitterionic molecules. These chemical features of membrane lipids are essential for the binding capacity of the lipid bilayer to pollutants. The polar lipid composition of *C. sorokiniana* (Fig. 7) was typical of common green algae with phosphatidylcholine (PC) and a betaine lipid, diacylglycerylthrimethylhomoserine (DGTS) as the major lipids, followed by the chloroplast lipids, phospholipid phosphatidylglycerol (PG) and three

galactolipids (MGDG, DGDG and a sulfolipid, SQDG). A small amount (about 5% of the total polar lipids) of PI was also detected in *C. sorokiniana* (Fig. 7).



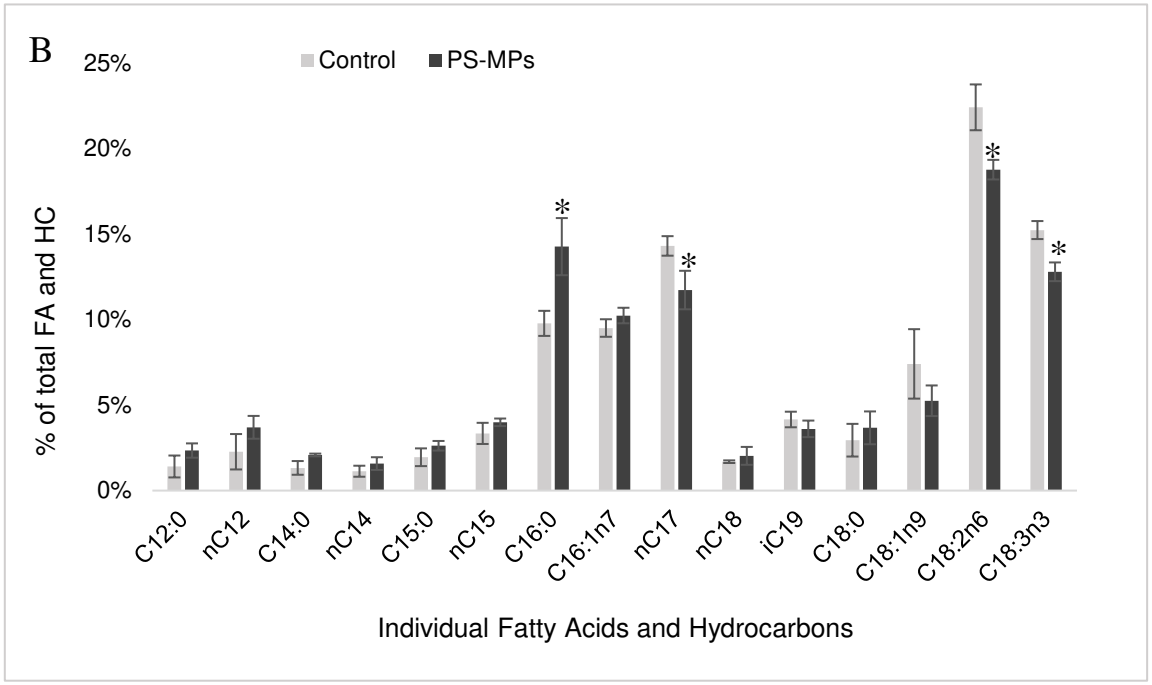


Fig. 6. PS-MP effect on distribution of fatty acids (% of total FA) in triacylglycerols (A) and in the fraction of waxes and steryl esters (B) of *C. sorokiniana.* FAs are indicated with the number before colon showing the number of carbon atoms, the figure afterwards denoting the number of double bonds; iC19 – isoC19. The position of the first double bond in FAs is shown after "n". Hydrocarbons (nC) are indicated with the number "n" as the number of carbon atoms. Values are means ± SD. The asterisk (*) indicates a significant effect of PS-MPs when compared to control samples ($p < 0.05$, n=3-4).

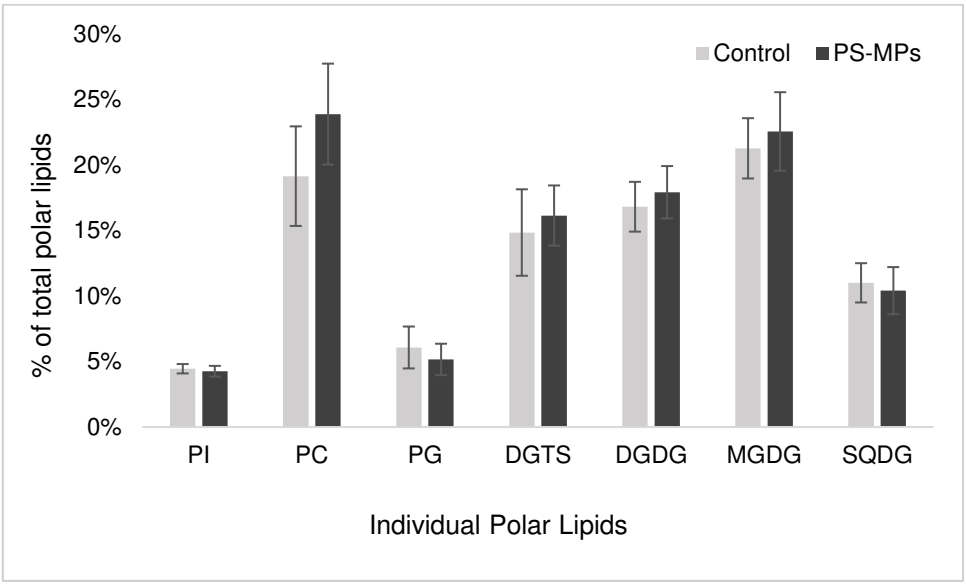
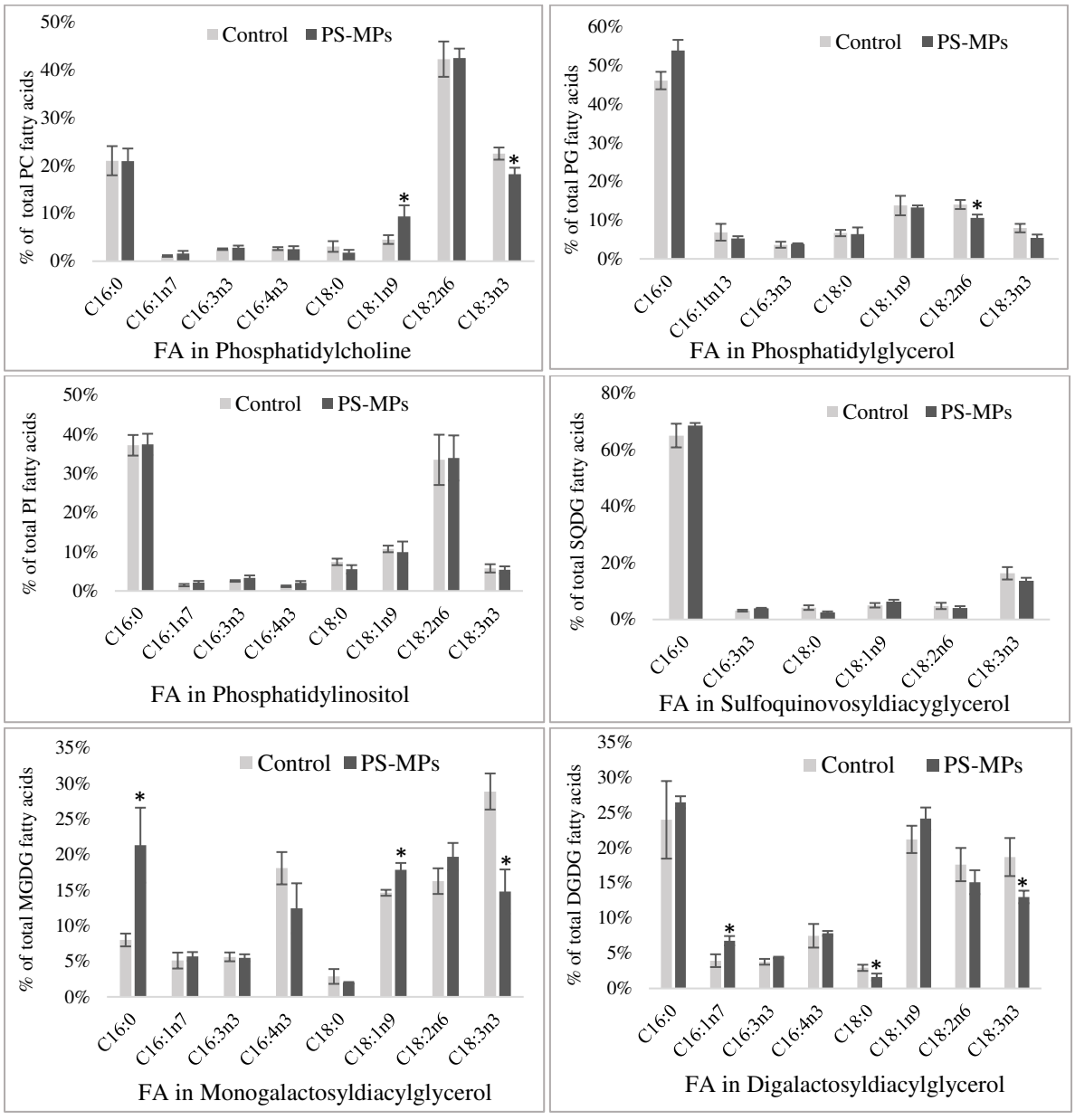


Fig. 7. PS-MP effect on distribution of individual polar lipids (% of total polar lipids) in *C. sorokiniana.* Values are means ± SD (n=3-4). Abbreviations: phosphatidylinositol (PI); phosphatidylcholine (PC); phosphatidylglycerol (PG); diacylglyceryltrimethylhomoserine (DGTS); digalactosyldiacylglycerol (DGDG); monogalactosyldiacylglycerol (MGDG); sulfoquinovosyldiacylglycerol (SQDG).

The relative distribution of polar lipids in *C. sorokiniana* did not vary with PS-MP treatment (Fig. 7). In contrast, the FA profiles of individual polar lipids revealed a range of effects (Fig. 8).



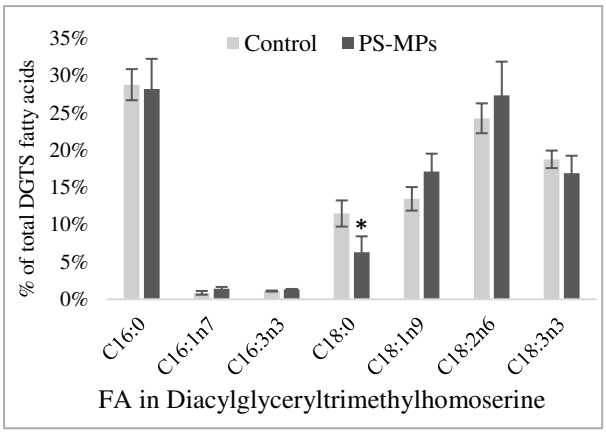


Figure 8. PS-MP effect on fatty acid distribution (% of total FAs) in individual polar lipids of *C. sorokiniana.* Values are means ± SD. The asterisk (*) indicates a significant effect of PS-MPs when compared to control samples ($p < 0.05$, n=3-4).

Among the all-membrane lipids, only fatty acids of PI were not affected by PS-MPs, while FAs of other polar lipids were altered, although in varying proportions, after exposure to PS-MPs. For example, there were only small decreases in stearic acid in SQDG and LIN in PG. In a betaine lipid, DGTS, the level of stearic acid reduced from 11.5% to 6.3% as a result of PS-MPs treatment (Fig. 8). The level of essential ALA was reduced in PC and, to a larger extent, in DGDG and MGDG. In the latter, the level of this important omega-3 fatty acid decreased from 28.9% in control samples to 14.8% in the cultures incubated with PS-MPs (Fig. 8). In this galactolipid, this reduction was accompanied by moderate or significant (from 8.0% to 21.3%) increases in palmitic and oleic acids, respectively, whereas in phospholipid PC, a decrease in ALA co-occurred

366 with an equal increase in oleic acid. In DGDG, the level of stearic acid was also
367 reduced (Fig. 8).
368
369 **4. Discussion**
370 With evidence now clear that microplastics are abundant and widespread
371 pollutants in freshwater ecosystems as well as marine environments, there is
372 increasing recognition of the need to identify and understand any adverse effects
373 on individuals, populations and ecosystem processes (Windsor et al., 2019).
374 Making such assessments in complex environments is challenging, however, not
375 least because organisms at all trophic levels are affected by a wide range of other
376 confounding stressors simultaneously. In this study, we therefore used a
377 controlled experiment to test the hypothesis that lipids and fatty acids (FAs) are
378 important molecules in the response reactions of a common and widespread
379 primary producer to plastic contamination. While our work is so far confined to
380 just one type of plastic – particulate polystyrene – the results supported the
381 hypothesis unequivocally: although effect sizes were variable, exposure to PS-
382 MPs significantly affected a range of lipid molecules. This implies that lipid and
383 FA biosynthesis could be involved in the responses of algae to microplastic
384 pollution in real ecosystems. We now review our observations, draw attention to
385 some possible mechanisms and outline some potential implications.
386 Although our experiment involved treating algal cells with PS-MP at just one
387 concentration (60 mg/L), this represented known environmental conditions (Mao

388 et al., 2018; Li et al., 2020). Moreover, at this one concentration effects on the
389 growth and photosynthesis of *Chlorella pyrenoidosa* were clear. PS-MP particles
390 were also of a size (70 μm) typically found in nature. Although there was some
391 variation in the exact size of plastic particles in the experiments compared with
392 the nominal target (see Fig. 1), this is likely to represent real environments in
393 which microplastic size distributions will also be highly variable both in size and
394 shape. Although previous work has shown that only nanoparticles of 4-5 nm or
395 smaller can penetrate algal cell walls or lipid membranes, MPs of the size range
396 we used can attach to the cell surface (see Fig 3) or be incorporated into the lipid
397 bilayer (Ha et al., 2015; Lagarde et al., 2016). In this lipid bilayer, MPs can attach
398 to the headgroups of membrane lipids and be translocated to fatty acid residues
399 depending on their charge and affinity for the particular molecules involved. As
400 an example, fullerene nanoparticles in water have a higher affinity for unsaturated
401 cationic lipid membrane and membranes containing raft domains (Ha et al.,
402 2015). It is interesting that in other studies, polystyrene MPs caused some
403 morphological changes inside algal cells, as demonstrated for pyrenoid and
404 thylakoid membrane structures in *C. pyrenoidosa,* presumably by affecting cell
405 division or interactions with mixotrophic organisms (Lagarde et al., 2016).

406 Extending these previous observations, our results showed that PS-MPs affect
407 two major compounds of the cell wall, waxes and steryl esters, reducing their
408 relative concentration (Fig. 4) and significantly changing their FA and HC
409 profiles (Fig. 4). A range of consequences are possible, and for example an

410 increase in the level of saturated C16:0 FA with a concomitant decrease in
411 PUFAs, LIN (C18:2n-6) and ALA (C18:3n-3), is likely to decrease the
412 extracellular membrane fluidity while also changing permeability. On this basis,
413 we suggest that PS-MPs could be absorbed by the cells of the algal species we
414 studied and, to some degree, may be incorporated into the cell wall. Once
415 captured in this way, there is a clear possibility of PS-MP biomagnification
416 through trophic transfer from algae to consumers, and we suggest this is an
417 important area for investigation.

418 In contrast to these effects at the cell wall, unaltered levels of TPL and
419 individual polar lipids in our experiment indicate that their structural roles in algal
420 intracellular membranes were unaffected by PS-MPs. This was predictable,
421 because, as discussed above, the size of particles used would be unlikely to allow
422 penetration through the cell membranes. Nevertheless, FA changes were
423 demonstrated among individual polar lipid classes, suggesting some potential
424 changes in both cell membranes and intracellular membranes (Fig. 8).

425 As major compound of intracellular lipid droplets, TAG are important storage
426 lipids that provide the majority of energy to algal consumers. Unchanged levels
427 among this lipid group following exposure therefore suggest that the general
428 value of *C. sorokiniana* as an energy source is not affected by PS-MP treatment.
429 Qualitative changes are, nonetheless, possible, shown by a decrease following
430 PS-MP exposure in the level of an essential LA in TAG which account for around
431 80% of the total lipids in *C. sorokiniana* cells (Fig. 4 and 6A).

Two major chloroplast galactolipids, MGDG and DGDG, provided some of the clearest modifications to their FA compositions following PS-MP exposure, namely a reduction of two essential fatty acids, LIN and ALA. MGDG and DGDG are the most abundant lipids of chloroplasts, constituting approximately 50% and 20%, respectively, of total glycerolipids (Dörmann, 2013). In chloroplasts, they occur not only in the lipid bilayer, but also they are a part of the photosynthetic complexes. This includes light-harvesting complex II (LHCII) that harbour the largest fraction of chlorophyll in thylakoid membranes as well as the cytochrome b6f complex involved in electron transfer from photosystem II to plastocyanin. Additionally, the trimeric form of LHCII is supported by glycolipids with high levels of LIN and ALA, thus, their role in photosynthesis is well-established.

Despite detecting some effects of PS-MP on algae using an experimental approach, we cannot yet identify the mechanisms involved. Toxic or physical effects are both possible either alone or in combination. For example, there is some evidence that polystyrene over a range of sizes might be toxic to organisms as diverse as nematodes and fishes, but studies of any toxicity to algae are scarce (Lu et al., 2016; Miao et al., 2019; Mueller et al., 2020). Alternatively, since the biosynthesis of some affected lipids in our work is highly dependent on light conditions, one possible mechanistic explanation for the changes we observed is altered irradiation as a result of algal-microplastics interactions either at the cell wall or through altered light transmission through the medium. The increased

454 level of chlorophylls and reduced size in the algal cells under PS-MP treatment
455 in our experiment indicated photosynthetic reactions in PS-MP treated algae that
456 would be consistent with altered illumination. Illumination effects would also be
457 consistent with previous observations in which shading sufficient to reduce the
458 photosynthetic activity of several algae during hetero-and homoaggregation
459 occurred as a result of MP exposure. The production of exopolymeric substances
460 in these cases were proposed as a possible cause (Prata et al., 2019; Lagarde et
461 al., 2016). Any accumulation of such MPs in exopolymeric substances produced
462 by algae might reduce oxygen, carbon and nutrient availability, and also change
463 microbial communities (Lagarde et al., 2016; Long et al., 2017; Khoironi et al.,
464 2019).

465 Irrespective of the mechanisms, our results reveal some effects of PS-MP
466 microplastics on the lipid and fatty acid composition of algae. We consider this
467 area worthy of further investigation not just with respect to algal productivity, but
468 also the transfer through food webs of important lipid compounds.

469 As well as their links to photosynthesis, LIN and ALA are among the most
470 important molecules transferred across the plant-animal interface. ALA is
471 synthesised in plastids via desaturation from LIN, and this reaction is catalysed
472 by delta-15 desaturase. LIN and ALA are somatic growth limiting compounds
473 for herbivorous zooplankton, and beyond that are critical for the growth, disease
474 resistance of juvenile fish and, ultimately, for human health (Muller-Navarra et
475 al., 2004). These essential FAs are synthesised by delta-12 and delta-15

476 desaturases, two enzymes which are absent in animals. Consumers can perform
477 some further elongation and desaturation of 18:2n-6 and 18:3n-3 with various
478 efficiency, producing other common polyunsaturated FAs (PUFAs) including
479 arachidonic (ARA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3), and
480 docosahexaenoic (DHA, 22:6n-3) acids. Since the involvement of these long
481 chain PUFAs (LCPUFAs) for invertebrate and fish survival, growth,
482 development and reproduction, LCPUFAs are also considered essential to food
483 quality (Muller-Navarra et al., 2004). Any propagation of the effects we observed
484 in *C. sorokiniana* through foodwebs could thus have substantial ramifications.
485 The mechanisms of effects by PS-MP on LIN and ALA, as well as their transfer
486 through food webs, warrant further attention.
487 Overall, we believe our study to be one of very few to have assessed the response
488 of algae to PS-MPs at the molecular level. Our results are particularly significant,
489 therefore in demonstrating PS-MP effects on lipids and FAs in organisms that are
490 the primary biomass producers at the base of freshwater food webs. The algal
491 species we used, *C. sorokiniana*, is widely distributed in freshwater ecosystems
492 as an important part of many phytoplankton communities. The species is also
493 used widely in monitoring research, in experiments that require the culture of
494 model species and in a wide range of biotechnological applications such as
495 biofuel production and bioremediation (Parmar et al., 2016; Olasehinde et al.,
496 2017; Khan et al., 2018). We advocate three key areas from which to extend our
497 work as follows. Firstly, the cell wall compounds on which effects were

498 demonstrated are important together with the extracellular membranes at the
499 interface between the environment and the cell/cytoplasm compartments. They
500 act as the first defence system against a range of pollutants including plastics,
501 where interactions such as binding or absorption at the algal cell surface and in
502 the membrane transport mechanisms of MPs into the cytoplasm of the cell.
503 Second, MP contamination could reduce the tolerance of *C. sorokiniana* to
504 natural stressors, such as changing temperatures, since the level of PUFA
505 determines the fluidity of the cell membranes and adaptation to environment.
506 Third, the transfer through foodwebs of effects on algal quality – particularly
507 involving key lipid groups – could have far-reaching implications and are a
508 priority for further work.
509

510 **5. Conclusions**

511 Despite growing global concern about the occurrence of nano- and micro-
512 plastics (NPs, MPs) in aquatic ecosystems, there is only rudimentary
513 understanding of the pathways through which any adverse effects might occur.
514 Suggestions have included physical impact (eg abrasion, obstruction, surface
515 coating), direct physiological toxicity or toxicity through vectored co-
516 contaminants, but evidence is limited. Prior to this study, however, investigations
517 of effects on primary producers have been rare, particularly for algae and
518 particularly involving consequences for their lipid composition.

519 Our evidence, therefore, extends current understanding by illustrating how
520 exposure to polystyrene microplastics at environmentally relevant concentrations
521 and size distribution significantly affected a range of lipid molecules in a
522 widespread algal species. The lipids affected included essential fatty acids, major
523 structural compounds in algal cell membranes and chloroplast galactolipids with
524 important functions in photosynthesis. In total, these effects hint at potential
525 consequences for the quality of crucial resources at the base of aquatic food webs,
526 and we suggest our data open a new front in understanding the effects of plastics
527 on organisms and ecosystems.

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529

530 **Declarations of interest**

531 None

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535

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