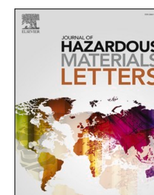
Journal of Hazardous Materials Letters 2 (2021) 100038

Contents lists available at ScienceDirect

Journal of Hazardous Materials Letters

journal homepage: www.sciencedirect.com/journal/journal-of-hazardous-materials-letters



Plastic habitats: Algal biofilms on photic and aphotic plastics

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ARTICLE INFO

Keywords:
Plastic pollution
Biofilms
Freshwater
Algae and diatoms
Microplastic

ABSTRACT

Plastic pollution is abundant in aquatic environments worldwide and many of its detrimental impacts are well documented, but it also represents a novel substrate available to a diversity of organisms. Biofilms – assemblages of bacteria, algae, and fungi – colonise hard surfaces in aquatic environments. They are key agents in biogeochemical cycling and are a food source for grazing organisms, representing a keystone aquatic community, and are known to influence the fate of plastic pollution in aquatic environments. In one of the most temporally thorough assessments of biofilm development on freshwater plastics, here we report on the evolution of algal biofilm assemblages on three plastic polymers (Low Density Polyethylene, Polypropylene, and Polyethylene Terephthalate) over six weeks in the photic and aphotic zones of a freshwater reservoir in Staffordshire, UK. Significant differences were found between diatom assemblages on plastics in the photic and aphotic zones, and between diatom assemblages quantified on weeks 2, 4 and 6 of the study, but total algal photosynthetic pigment concentrations did not vary significantly between polymers in either zone. Scanning Electron Microscopy indicates that degradation of polymer surfaces occurs within six weeks in the aphotic zone, with potential implications for plastic fragmentation and microplastic generation.

1. Introduction

Plastics are a heterogeneous group of polymers predominantly manufactured from fossil fuels that can be chemically tailored to provide a variety of useful functions. Durable, lightweight, and cheap, plastic materials are integral to much of modern-day life and have been increasingly so since the onset of their mass production in the mid-twentieth century. However, at the end of their life plastic products that are not disposed of responsibly may enter aquatic environments. The negative impacts of this plastic debris in the environment are well documented, including ingestion and entanglement (Gregory, 2009), and vary with product type, form and polymer. The majority of plastic pollution research is concerned with the marine environment (Xu et al., 2021), but freshwater catchments represent major pathways for the transport of pollution and are a primary source of marine plastic debris.

Some biota utilise the plastic waste they encounter in the natural environment, where it represents a novel environmental substrate. In urban rivers anthropogenic litter including plastic provides habitats for invertebrate communities where natural substrates have been removed (Wilson et al., 2021); case-building caddisfly are known to utilise microplastic particles in the production of their cases (Ehlers et al., 2019); and hermit crabs have been recorded using plastic materials in lieu of shells (Lavers et al., 2020). But, however resourceful these interactions between organisms and anthropogenic debris are, they are also associated with entrapment (Lavers et al., 2020) and increased susceptibility to predation and exposure to chemical pollutants (Ehlers et al., 2019).

Interactions between biota and plastic debris are not limited to animals. Biofilms – assemblages of bacteria, algae, and fungi – also colonise plastic material that enters aquatic environments (Carson et al., 2013; Oberbeckmann et al., 2015; Kettner et al., 2017). Biofilm formation on plastic material can degrade plastic surfaces (Webb et al., 2009; Zettler et al., 2013; Reisser et al., 2014), and can overcome the buoyancy of plastic polymers, causing plastic to sink to aphotic environments (Andrady, 2011). Biofilms have also been found to change the physiochemical properties of plastic (Keswani et al., 2016) and can impact processes of plastic degradation (Artham et al., 2009; Balasubramanian et al., 2010; Zettler et al., 2013), shielding plastics from the ultraviolet radiation (UVR) that is known to initiate its degradation (Andrady, 2011).

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https://doi.org/10.1016/j.hazl.2021.100038
Received 29 March 2021; Received in revised form 29 July 2021; Accepted 18 August 2021
Available online 19 August 2021
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Algae play an important role in primary productivity (Ascon and Lebault, 1999), nutrient cycling (Boelee et al., 2012) and carbon fixation (Raven and Giordano, 2014) in aquatic environments, with diatoms and other microscopic unicellular algae, playing a particularly significant role in this (Smith, 1856). Factors that influence biofilm composition and species diversity include substrate type (Azim and Wahab, 2002; Murdock and Dodds, 2007); submersion time (Azim and Adaeda, 2005); water chemistry (Chessman et al., 1999); nutrient availability (Biggs and Close, 1989); quality and intensity of light (Goldsborough et al., 2005); temperature (Sanches et al., 2011); and physical parameters such as topography, geology, land use and vegetation type (Biggs, 1996). Despite the ubiquity of plastic in the environment, little is known of the influence of plastic polymers on biofilm formation and composition, and the implications of this functioning on lake ecosystems. There is therefore an urgent need to better understand the relationship between plastics and the biofilms that colonise them (Rummel et al., 2017).

In one of the most temporally high-resolution studies of biofilm evolution on plastic litter, here we report on the community structure and evolution of biofilm formation on three different plastic polymers (Polyethylene Terephthalate (PET), Low Density Polyethylene (LDPE), and Polypropylene (PP)) over the course of six weeks submersion in a freshwater reservoir. We consider the implications of biofilm colonisation on plastic fate, and explore the influence of plastic litter on the ecosystem functions of algal biofilms.

2. Methods

2.1. Study site

This study was carried out at Knypersley Reservoir, near the source of the River Trent, UK (Fig. 1). The reservoir holds approximately 26 300 m^3 of water within its 142,000 m^2 surface area, and has an average depth of 6.5 m and maximum depth of 12.2 m. The reservoir receives agricultural runoff from farmland that lies upstream of it.

2.2. Sampling design

Six sets of sampling apparatus were deployed in the reservoir, suspended from an inflated bicycle innertube, for analysis of biofilm algal assemblage (pigments), diatom assemblage, and surface degradation. Each set of apparatus suspended 27 pieces of plastic in the photic zone and 27 pieces of plastic in the aphotic zone (Graphical Abstract). Of each set of 27 pieces of plastic, nine were PET, nine were LDPE, and nine were PP (Fig. S1). These polymers were chosen due to their production, representing approximately 44.5 % of European polymer demand (PlasticsEurope, 2019). Pieces of LDPE were taken from a clean carrier bag, pieces of PET were taken from a clean plastic bottle, and pieces of PP were taken from a clean food container. A 10 × 10 cm square was drawn onto each plastic pieces, providing a standardised sampling area across each piece of plastic for all polymers. A hole was punched outside of this square in the plastic and they were attached to the sampling

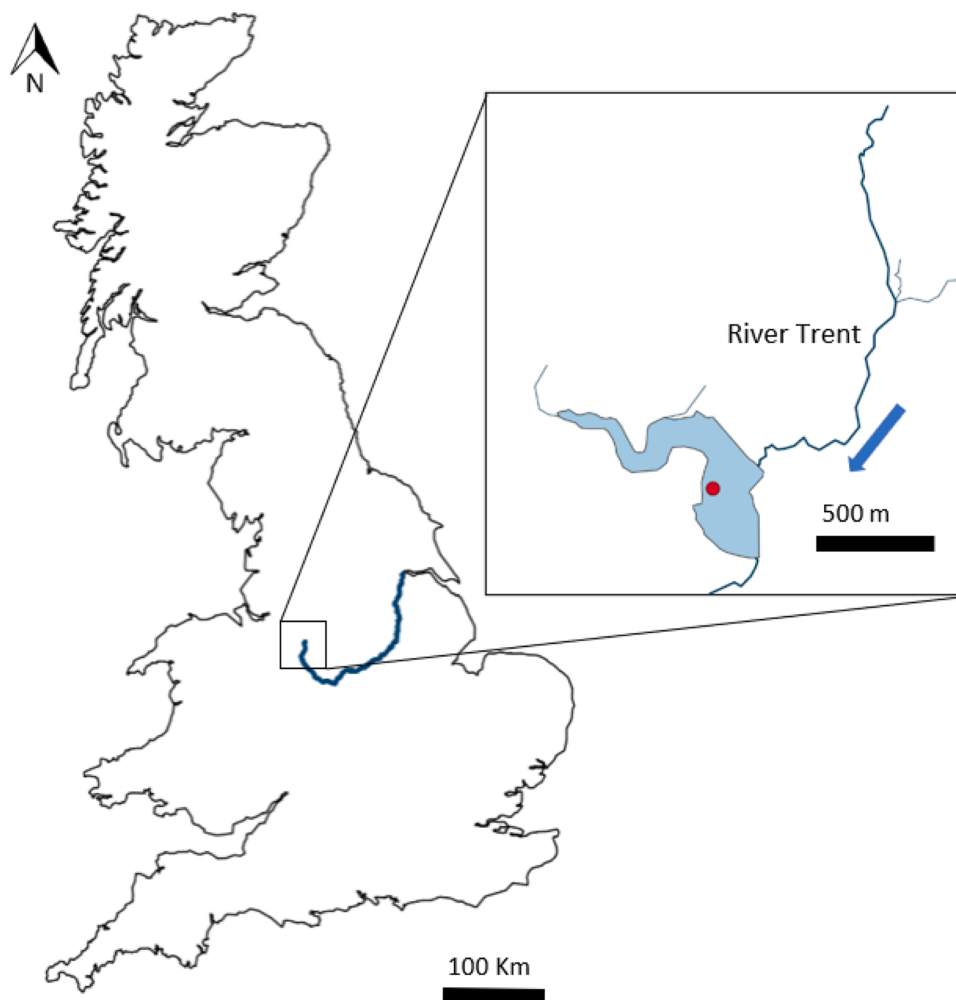


Fig. 1. Location of Knypersley reservoir (Latitude: 53.093251, Longitude: -2.158257) in the Trent catchment. The red circle represents the location within the reservoir that the sampling apparatus was deployed. The source of the River Trent is shown in the top right of the figure inset.

apparatus with rope (Fig. S2).

The depth of submersion was measured using an echo sounder and Secchi disk in the field to determine the depths of the photic and aphotic zone as per Vincent (2018). Samples were submerged at around 1 m depth in the photic zone and approximately 6 m depth in the aphotic zone. The movement of water and changes in buoyancy throughout the deployment of sampling devices mean that depths at which samples were suspended can only be approximated. The depth of the water where samples were deployed did not exceed 8 m.

All sets of sampling apparatus were deployed on 12/06/2019, and one piece of apparatus was removed each week for the following 6 weeks. On each sampling occasion the aphotic and photic samples were separated at the lake shore, placed in separate new plastic sample bags, and transported to the laboratory in an icebox. In the laboratory each individual piece of plastic was placed in its own new sample bag before being stored in a freezer.

In addition to removing one sampling device per week, water samples were collected at the reservoir surface and at a depth of 1 m in triplicate during each sampling occasion and analysed for Nitrates, Sulphate, Chloride (Thermo Scientific, Dionex ICS 1000) (Table S1). Results were averaged across these depths.

2.3. Photosynthetic pigment preparation and analysis

To remove biofilms from the piece of plastic analysed for photosynthetic pigments, the marked 10 × 10 cm area (Fig. S1) on each piece of plastic was gently scrubbed for 60 s on each side using a toothbrush and distilled water as per Patil and Anil (2005). Following methods outlined in Leavitt and Hodgson (2001), the biofilm solution was filtered through a Whatman 1.2 μm GF/C glass-fibre filter paper. Pigments were extracted under subdued lighting by submerging filter papers in extraction solvent (80 % acetone, 15 % methanol and 5% deionised water) before being frozen for at least 12 h at −10 °C. Extracts were filtered through a 0.22 μm PTFE filter and air dried under N_2 gas, then re-dissolved in an acetone, an ion pairing agent (0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 ml water) and methanol mixture (75:25:5). The extracted pigments were then analysed by High Performance Liquid Chromatography (HPLC) using an Agilent 1200 HPLC unit.

Pigments were identified based on the absorbance spectra and retention times as described in Chen et al. (2001). Photosynthetic pigment concentrations (PPC) were calculated by calibration against commercial standards and presented as (pmol cm^{-2}). Zeaxanthin (cyanobacteria) and lutein (chlorophyta) did not separate during the HPLC analysis and are therefore reported and analysed together. For every sample taken, two replicates were processed. Replicates were averaged prior to all further analysis.

2.4. Diatom preparation and analysis

As with the photosynthetic pigment samples, for diatom analysis the 10 cm^2 sample area was gently scrubbed for 60 s on each side using a toothbrush and distilled water to remove the algal biofilm that had colonised the plastic. Removed biofilm was collected in individual beakers to which 30 % hydrogen peroxide (H_2O_2) was added dropwise until the contents stopped fizzing. Samples were then heated to 80 °C either using a hotplate or in a water bath and left until the H_2O_2 had digested the organic material.

Samples were centrifuged at 1200 revolutions per minute for 4 min to remove H_2O_2. The supernatant was then decanted and the precipitate resuspended in 30 mL of distilled water. This washing process was repeated 4 times. Following the decanting of the supernatant from the fourth wash, 500 μL of diatom suspension was pipetted onto a cover slip and left to dry overnight before being mounted onto a microscope slide using Naphrax on a hotplate. Diatoms were identified using a Leica DMRA light microscope at 1000X magnification. 300 diatoms in the size range 10−200 μm were identified in each sample as per Battarbee (1986) and Prygiel et al. (2002) and identified using the flora in Krammer and Lange-Bertalot (1986-1991) at the genus level. Species representing ≤1% of the diatom community were excluded from data analysis.

2.5. Scanning Electron Microscopy (SEM) of samples

By following and adapting the methodology of Eich et al. (2015), samples for SEM analysis were cut into 1 × 1 cm squares and gently scrubbed for 30 s on each side and rinsed with deionised water to remove the biofilm and any lose materials attached to the biofilm without damaging the plastic's surface, before being left to dry. Time restraints limited this process to samples from weeks 2, 4 and 6 only. Each 1cm^2 square was fixed onto aluminium stubs using double coated carbon conductive tabs. Samples were imaged using a Hitachi TM3000 TableTop Scanning Electron Microscope to observe changes to surface structure throughout the course of the study (Neu et al., 2018). Images were taken at the following magnifications: x100, x200, x300, x500 and x1000. SEM micrographs were generated for each polymer type from the photic and aphotic zone for every sampling occasion.

2.6. Statistical analysis

2.6.1. PPCs

Statistical tests were performed to assess the significance of differences between PPCs on samples in the photic and aphotic zones of Knypersley reservoir, and between each polymer in both of these zones throughout the six-week study. Anderson-Darling Normality Tests confirmed that data was non-normally distributed (p=<0.05), necessitating the use of non-parametric statistical tests. Differences in photic and aphotic pigment concentrations were compared using a Mann-Whitney U test, and differences between polymers in each zone were determined using a Kruskal-Wallace test. Probability percentages (p) of <0.05 were considered and this analysis was completed using Minitab 19.

Using the programme Canoco version 4.5 (Ter Braak and Smilauer, 2002), the pigment data was log transformed (x +1) and Detrended Correspondence Analysis (DCA) revealed a short axis DCA axis 1 gradient length (<1) suggesting that and Principal Components Analysis (PCA) would be appropriate to summarize the pigment composition on the different plastic samples over the six-week study period (Lepš and Smilauer, 2003). Using the software programme R (Version 4.04) and the VEGAN package (version 2.5–7) an analysis of similarity (ANOSIM, Bray-Curtis distance measure, 999 permutations) was used to explore the statistical differences between the pigment assemblages and the plastic types in the photic and aphotic zone and also over time. ANOSIM analysis creates the R statistic representing the strength of the factors on the samples. An R value close to 1 indicates high separation between factor levels, a value of 0 represents the null hypothesis indicating no difference. For this analysis, probability percentages (p) of <0.05 were considered.

2.6.2. Diatom assemblages

Diatom assemblages were converted to relative abundances and the similarities between the diatom assemblages on the polymer types, over time and in the aphotic and photic zone were explored using Detrended Correspondence Analysis (DCA) using the programme Canoco version 4.5 (Ter Braak and Smilauer, 2002). Using the software programme R (Version 4.04) and the VEGAN package (version 2.5–7), an ANOSIM (Bray-Curtis distance measure, 999 permutations) was run to investigate the statistical differences between the diatom assemblages on the polymer types, between the aphotic and the photic zone and also over time. ANOSIM analysis creates the R statistic representing the strength of the factors on the samples.

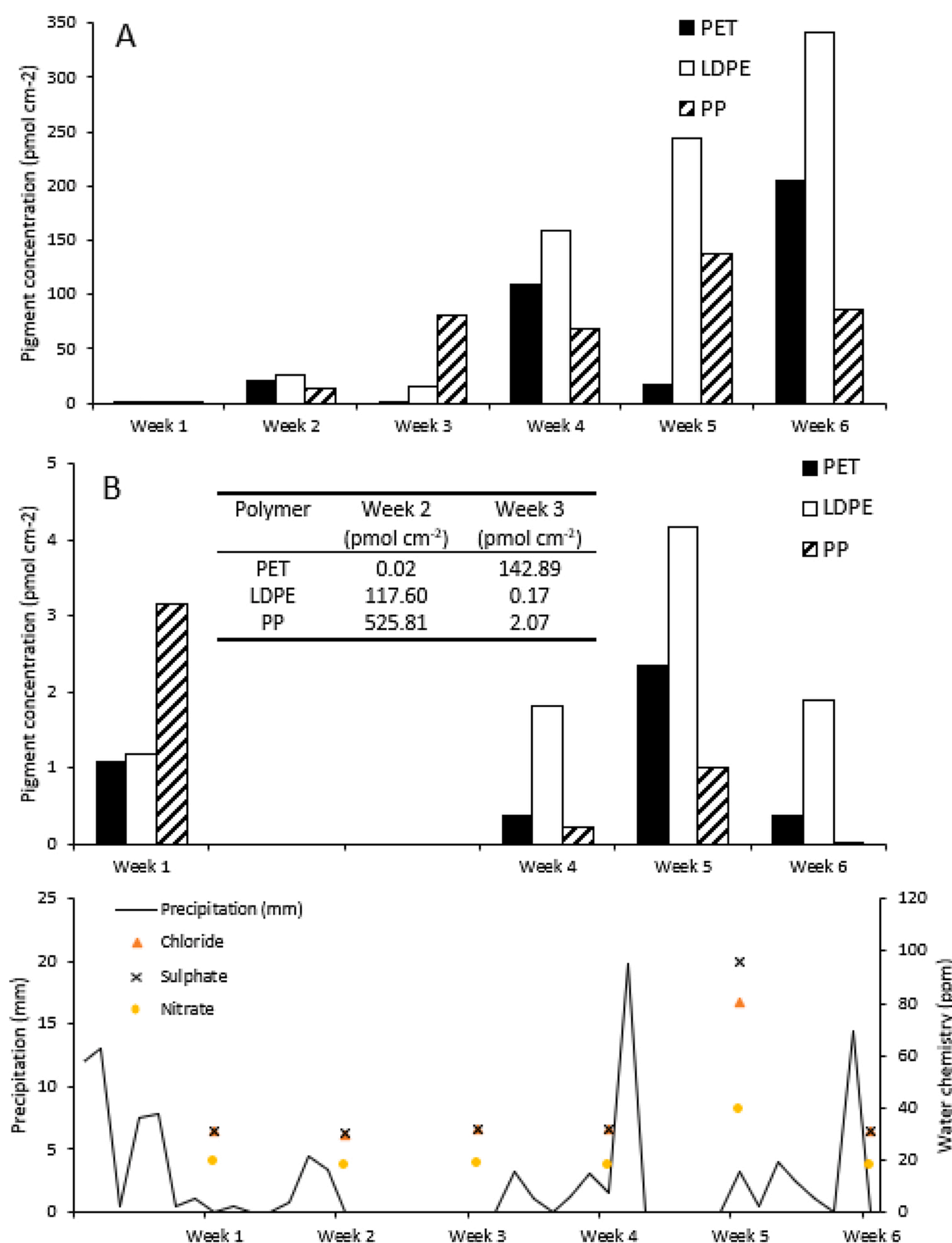
3. Results

3.1. Photic and aphotic photosynthetic pigment concentrations

Generally, PPCs showed a progressive increase from week 1 to week 6 in the photic zone. In the aphotic zone this not observed, with concentrations peaking in week 2 (Fig. 2). A large rainfall event is recorded prior to the collection of the week 4 samples, followed by an increase in chloride, sulphate and nitrate in week 5, which does not influence PPCs in either zone (Fig. 2).

Overall, total PPCs were higher in the photic zone, at 1.530 nmol cm^{-2} compared to 0.806 nmol cm^{-2} in the aphotic zone, however, this difference was not significant (Mann-Whitney U $p = 0.298$). Kruskal Wallace tests comparing PPC concentrations between polymers in each depth zone identified no significant difference between PPCs on polymers in the photic zone ($p = 0.567$) or aphotic zone ($p = 0.751$).

Fig. 3 shows the Principle Components Analysis of the pigment compositions throughout this study. Plastic samples located close to each other in the PCA diagram reflect similar pigment assemblages. Pigments are displayed as blue arrows. The longer arrows that are closer to the 1st and 2nd axis represent the most important pigments in the aphotic and benthic zone. Arrows close to each other demonstrate similar patterns and trends over time and on the plastic samples. The pigment assemblages demonstrate some significant differences between the aphotic and photic zones (ANOSIM R value of 0.267 at the 0.05 level) and also developed into significantly different assemblages over the six week study period in the photic zone (ANOSIM R value = 0.4535; $p = 0.005$) but not the aphotic zone. However, there were no significant differences in the assemblages across polymers. A low ANOSIM R value of -0.026, suggests that plastic type did not result in different assemblages. There is also evidence for the presence of Nitrogen fixing cyanobacteria in the biofilms in both the aphotic and photic zone, indicated



Polymer	Week 2 (pmol cm^{-2})	Week 3 (pmol cm^{-2})
PET	0.02	142.89
LDPE	117.60	0.17
PP	525.81	2.07

Fig. 2. Average total photosynthetic pigment concentration (pmol cm^{-2}) per week on the PP, LPDE, and PET samples over the 6 six-week sampling campaign for the photic (A) and the aphotic (B) zone. PPCs for aphotic weeks 2 and 3 are presented to 2 d.p. in pmol cm^{-2} in the table inset. Precipitation (mm) and chloride, sulphate, and nitrate concentrations (ppm) are also shown throughout the six-week sampling campaign (see Table S1 for values).

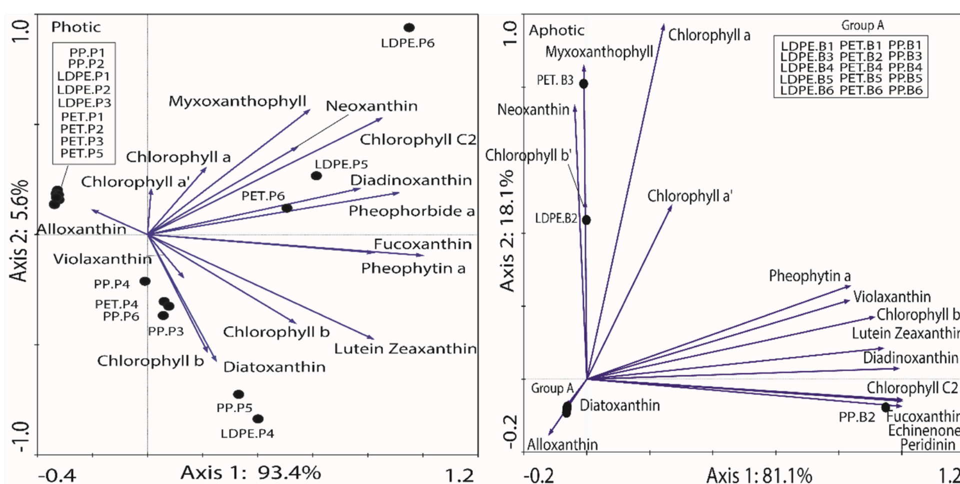


Fig. 3. Principal Components Analysis (PCA) of the total concentration of pigments identified on all three plastic types in the photic and aphotic zone and showing the pigments most strongly associated with the first and second axis throughout the six-week period. The pigment assemblage identified on each plastic sample, each week, are summarized by the black circles (labelled by polymer type and week).

by the presence of the pigment Myxoxanthrophyll.

3.2. Diatom communities

There was no significant difference in the diatom assemblages that developed on the different plastic types over the course of the study (ANOSIM R value = -0.144; p = 0.9838). However, diatom assemblages demonstrated statistically significant differences between the aphotic and the photic zones (ANOSIM R value = 0.4697; p = 0.0015), and also throughout the six weeks of the study in both the photic (ANOSIM R value = 0.7366; p = 0.0066) and the aphotic zone (ANOSIM R value = 0.9506; p = 0.0034) (Fig. 4). For example, by week 2, all plastic types in the aphotic zone were characterised by an assemblage dominated by *Discostella*, *Navicula*, *Amphora* and *Fragilaria* species, by week 4 the assemblage was predominantly *Aulacoseia* and *Cyclostephanos* species and week 6 was characterised by *Achnanthes* and *Gomphonema* species. In the photic zone, the transitions in assemblages over time were very similar with the exception of the centric species *Cyclostephanos*, *Discostella* and *Aulacoseira*, which are present in much lower abundances relative to the aphotic zone. These centric species comprise less than 10 % of overall diatom abundance in the photic zone samples but up to 50 % in the aphotic zone samples.

3.3. SEM imaging

Changes to surface structure were apparent across all polymer types in both the photic and aphotic samples by the end of the six-week study (Fig. 5). This change was more extensive in samples in the photic environment than the aphotic, which was expected due to the exposure of these samples to UVR. Supplementary Figs. 3 and 4 show the development of surface changes for each polymer as the six weeks of this study progressed.

SEM images of each polymer prior to deployment in Knypersley Reservoir show the initial surfaces of PET and PP to be smoother than that of LDPE (Fig. 5). Fig. 5 also shows the clear presence of fissures in the polymer surface of PET and PP samples. The extent of changes to the surface structure on the aphotic samples was less apparent that on the photic samples.

4. Discussion

4.1. Biofilm development on plastic polymers

Much of the existing literature shows photic biofilms to be more productive, thicker and contain a higher total algal biomass compared to aphotic biofilms (Rao et al., 1997; Bengtsson et al., 2018; Pinto et al.,

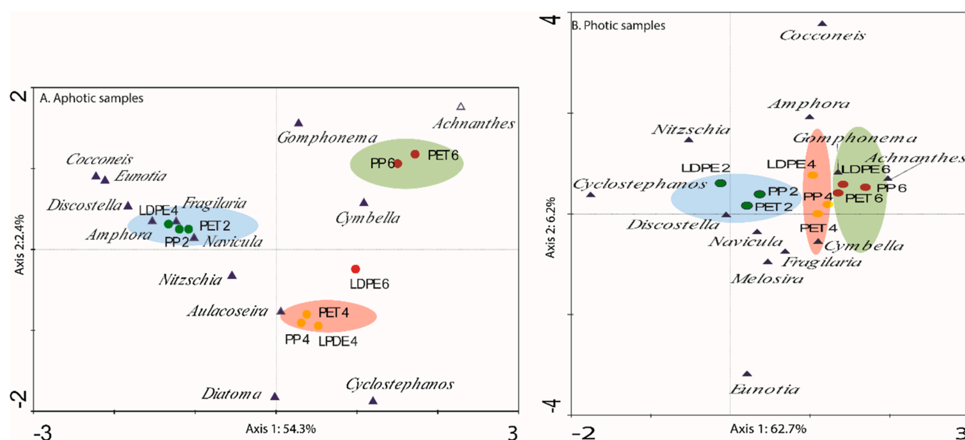


Fig. 4. DCAs of the diatom assemblages present on the plastic types over the six-week study period. Diatom species are marked as a blue triangle and labelled in italics. Plastic types are labelled and marked by green circles (week 2), yellow circles (week 4) and red circles (week 6). Circles in the DCA located close to each other have similar diatom assemblages.

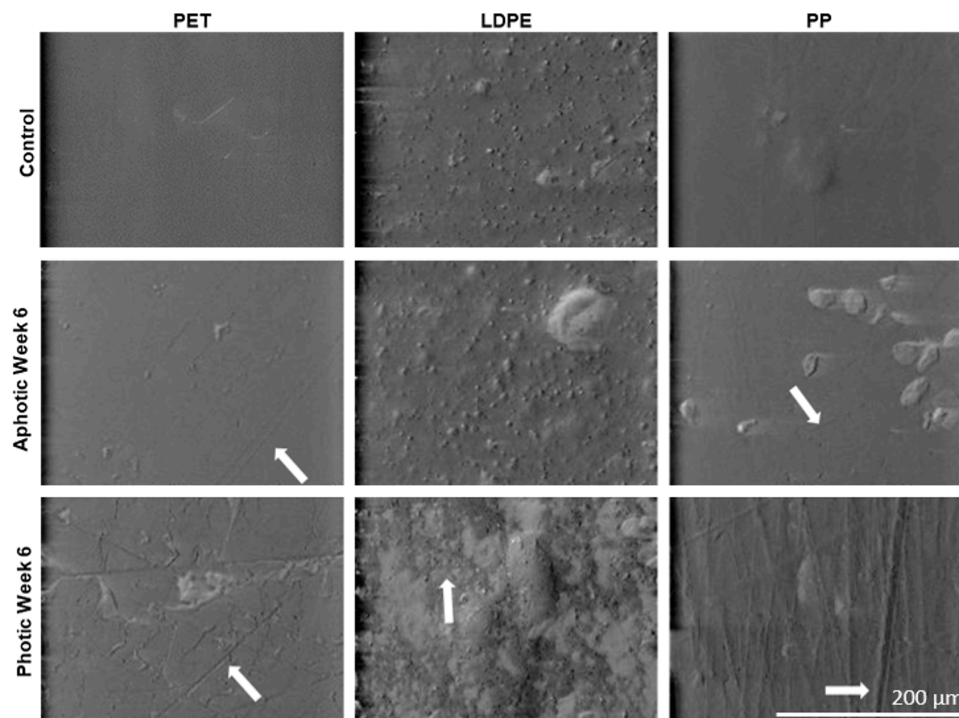


Fig. 5. SEM images at 500X magnification showing the surfaces of each polymer prior to deployment in Knypersley reservoir and after six weeks of exposure in the aphotic and photic environment. Arrows direct the reader to fissures in the polymer surface. Scale bar represents 200 µm.

2019). Although this study did not estimate biomass or thickness of the algal biofilms, we demonstrate higher total PPCs on photic samples compared to aphotic samples. Light is the most important variable determining algal biomass, photosynthesis, community structure and production (Hill et al., 1995). The higher proportion of light available in the photic zone, relative to the aphotic zone where light is attenuated with depth, increases photosynthesis and production leading to higher algal biomass and thicker biofilms.

Fucoxanthin and chlorophyl C2, pigments produced by diatoms, dominated aphotic and photic PPC samples, and Fucoxanthin is also closely related to Axis 1 (Fig. 3), suggesting that diatoms comprise a large proportion of the algae in the biofilms on the polymer samples. These findings in the freshwater environment parallel those of Nenadovic et al. (2015), who studied the diatom diversity on a variety of artificial substrates in a aphotic marine environment for 30 days and found all artificial substrates developed biofilms consisting predominately of diatoms. It also parallels other research showing that biofilms under dim-light (Barranguet et al., 2004) and lower temperatures of between 7−11 °C (Villanueva et al., 2011) were predominately characterised by diatoms. There is no evidence that the biofilm diatom assemblages or pigment concentrations are impacted by the recorded changes in water chemistry.

Unlike Eich et al. (2015), who found that diatom assemblages in the biofilms of PE and biodegradable plastic in the marine pelagic and aphotic zone were significantly different after 33 days, polymer type had no impact on diatom assemblage development in this study. However, statistically different assemblages did develop on all polymers between the photic and the aphotic zones, and diatom assemblages developed into statistically different assemblages throughout the six weeks of the study in the photic zone and the aphotic zone.

These findings highlight that higher abundances of anthropogenic litter in lake ecosystems have the potential to increase algal biomass which could have implications for nutrients cycling and primary production in freshwater systems. Furthermore, beyond aquatic pollution, plastic materials have extensive industrial applications in photic aquatic environments, including navigational buoys and boat fenders. Though individually these plastic items represent a small surface area, cumulatively they do not, and are frequently found in concentrated hotspots in near-shore environments.

Surface texture is known to be important for the structure of biofilm communities (Patil and Anil., 2005). The results from this study suggest that the different surface textures present on the polymers did not affect the biofilm communities that initially colonised each polymer type. Furthermore, similar transitions in the diatom assemblages over time suggest that the diatoms present on the polymer biofilms were determined by other factors such as the ability of species to colonise and compete, changes in the presence of nutrients within the biofilm and the lake, and also the presence of other microorganisms in the biofilm including bacteria and other types of algae.

The findings presented here, alongside those of similar work in the marine environment, highlight that the colonisation of plastic debris by algal biofilms is similar to that of natural substrates. As the abundance of plastic in aquatic environments increases, so too will the surface area for biofilm communities to colonise, with potential implications for ecosystem functions that warrant further investigation.

4.2. Biofilms and plastic fate

Changes to surface morphology of plastic materials have the potential to weaken polymer structure, increasing their susceptibility to fragmentation in the environment (Cooper and Corcoran, 2010). The presence of UV light is known to accelerate this (Webb et al., 2013), and the more extensive changes in surface structure observed in the photic samples imaged in this study are therefore not surprising. However, the presence of biofilms on plastic material in aquatic environments is known to decrease their buoyancy (Chen et al., 2019), removing it from the photic zone in which it is likely to break down at its fastest rate. Research documenting the degradation of plastic debris in aphotic environments is lacking (Corcoran, 2015). Though the changes to surface morphology of plastics observed in the aphotic zone were not as stark as those in the photic zone, this work indicates that, in the absence of UV light, surface degradation of aphotic plastic debris is initiated within six

weeks of submersion. Moreover, residence times of neutrally buoyant plastics have been found to exceed that of plastics in surface waters (Cable et al., 2017). It was not possible in this study to determine the influence of biofilms on surface degradation.

It was beyond the scope of this investigation to quantify the extent of changes to surface structure or to determine whether the morphological changes observed contributed to microplastic generation, or changes to polymer integrity, tensile strength or buoyancy. However, though qualitative our observations are of note, building on those reported by (Eich et al., 2015) and raising questions regarding the role of biofilms in plastic degradation that should guide future work.

The surface changes reported here have the potential to cleave microplastic particles from the polymer surface, and any surface degradation of plastics may accelerate the leaching of chemicals associated with plastic production (Rochman et al., 2015). The biofilms that form on plastic debris may therefore act as a concentrated temporary sink for any microplastic particles or chemical agents that are released from the plastic they colonise, contaminating the food source of grazing communities at the bottom of aquatic food webs.

4.3. Recommendations for future work

The findings presented in this manuscript describe one of the most temporally comprehensive studies of freshwater biofilm development on anthropogenic materials, identifying multiple knowledge gaps to guide future research into the relationships between plastic pollution and the biofilm communities that colonise it. Building on the findings presented here, future work should:

1) Quantify the degradation of plastic pollution throughout freshwater environments at high temporal resolutions (section 3.4).
2) Incorporate multiple polymers into experimental design (section 3.1).
3) Consider the evolution and influence of non-algal components of the biofilm (e.g. bacterial and fungal) on plastic pollution in freshwater environments (section 4.2).
4) Quantify the relative role of biofilms in biogeochemical cycles on natural and anthropogenic substrates in freshwater systems.

5. Conclusion

Plastic waste is a diverse and pervasive anthropogenic pollutant in aquatic environments that provides a surface for the development of algal biofilms. Here we present some of the first work to systematically characterise the plastic-biofilm relationship in freshwater environments. We find that plastic polymer does not have a significant influence on biofilm development, but that the nature of diatom and pigment assemblages on plastic debris in photic and aphotic environments is significantly different. The presence of algae on plastic pollution in lakes could have implications for biogeochemical cycles and particularly carbon cycling in lakes as an additional substrate for algal colonisation. As a group of polymers that can have positive, negative and neutral buoyancies, we recommend further work to quantify the role of plastic debris in biogeochemical cycling throughout the water column. Beyond biofilms, we find that the surface degradation of plastic debris occurs early following submersion, even in the absence of UV radiation, with implications for plastic litter breakdown and the generation of microplastic particles that may concentrate in the biofilm. We conclude that plastic represents a novel habitat for biofilm communities, that is currently understudied in freshwater plastic pollution discourses. Research building on these findings should consider the entire biofilm community (bacterial, algal and fungal) and expand the geographical reach of this pilot work. Plastic pollution represents an abundant and diverse substrate in aquatic environments that will be colonised by biofilms. Understanding the plastic-biofilm relationship across the breadth of polymers and lentic and lotic freshwater environments is necessary to determine the impact of plastic pollution biofilms at the global scale.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the University of Nottingham HPLC lab, and in particular Teresa Needham and Suzanne McGowan, for their assistance in pigment analysis. We would like to thank the Canal and Rivers Trust for granting access to Knypersley Reservoir, and to Keele University's Luke Hobson and Jobe Elwell for their assistance in the fieldwork conducted for this work. We also thank Keele University's Adam Jefferies for his support in the laboratory. The precipitation data presented in Fig. 2 is UK public sector information licensed under the Open Government Licence v3.0.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.hazl.2021.100038.

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