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The interaction between plastics and microalgae affects community assembly and nutrient availability

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The presence of plastics and microplastics in water environments has raised concerns for potential negative impacts. The broader ecological implications for ecosystem functioning are, however, still unknown. The interaction between phytoplankton community and plastics has, for example, been overlooked. Here, we investigated the role of plastic as a substrate for biofilm growth and how this affects the dispersal of terrestrial microalgae, potentially altering the assembly of pelagic communities. When exposing an artificially assembled microalgae community to pristine and biofouled plastic under laboratory-controlled conditions, we found that only biofouled plastic affected the final community structure and the content of available nutrients in water. This is due to the exchanged algal species between the biofilm and the pelagic community. The results from this batchwise pilot scale study indicate that plastic can act as a substrate for benthic and pelagic species, potentially affecting ecosystem functions, which have been overlooked so far.

Plastic pollution is of global environmental concern[1,2]. Aquatic ecosystems are strongly affected by this pollution: up to 20 million metric tons of plastic enter water bodies every year, and these ecosystems accumulate plastic as progressive accumulators or as temporary capacitors[3–5]. In addition, widespread sources and high mobility of plastic through the atmosphere and in the landscape generated contamination with plastic also in pristine and remote aquatic environments, indicating a potential threat at a planetary scale[6–8].

The effects of plastic pollution in water environments are commonly assessed following the traditional ecotoxicology approaches, focusing on the direct effects on water organisms depending on the exposure[9]. However, this approach overlooks several other environmental implications of plastic pollution on microalgal communities. For example, plastics can affect the quality of the habitat through light shading[10,11], leaching toxic chemical additives[12,13] or modulating the release and uptake of macronutrients from sediments[14,15].

Plastics also have distinct chemical composition, size, shape, surface properties[12,16], thermal properties[17], bulk density and mechanical properties[18] compared to other naturally dispersed solids in aquatic environments. These properties of plastic make this substrate likely to host a specific microbiological community: the communities found on environmental plastic are in fact markedly different from other natural substrates (e.g., wood, rocks and cellulose) and the surrounding water[19–21]. While the biodiversity of this community has been investigated in marine and freshwater systems[22,23], the role of plastic-specific biofilm in affecting the exchanges of resources and organisms with the surrounding environment has been widely overlooked[24–26]: only a few studies recently pioneered the role of plastic as a substrate selecting a specific community and the potential implications for ecosystem functioning[21,27].

Given these premises, we are interested in studying the role of plastic as a xenobiotic habitat for naturally occurring microorganisms[28] and its role in potentially transfering these organisms in the pelagic ecosystem. Plastic is in fact commonly dispersed in urban settings and can host specific microbial communities; then, owing to its low density and long-term persistence in the environment[29], it can be transported in freshwater bodies favouring the dispersal of these organisms[22,26]. These aspects entail possible indirect impacts on ecosystem structure and functioning, as organism distribution and resource availability in the aquatic environment may be impacted. We focus here on phytoplankton communities, due to their key role in pelagic ecosystem primary production and in supporting the aquatic food web[11]. After postulating that plastic serves as a substrate for biofilm-forming microalgae[25,30,31], we question whether this plastic-associated biofilm community can exchange nutrients and organisms with the surrounding physical environments and the pelagic community. As some microalgae are capable of expressing both pelagic and biofilm-forming phenotypes[32,33], this exchange can potentially affect nutrient availability and natural microalgae assembly, with possible broader implications for ecosystem structure and functioning[34].

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In this pilot study, we aim to test the following hypotheses: (1) plastic can favour the dispersal of biofilm-forming microalgal species, potentially affecting pelagic community assembly; and (2) biofouled plastic can alter the natural nutrient availability and chemical composition of water.

We addressed these hypotheses through an articulated laboratory experiment: we exposed an artificially assembled freshwater microalgae community composed of five species with distinctive traits prevailing in pelagic communities to pristine (*plastic* treatment, for the sake of syntheses tags of the treatments, will be indicated using italic font) and biofouled (*biofilm* treatment) plastic fragments. In these treatments, we added an environmentally relevant concentration of plastic fragments made of polypropylene (PP), one of the most commonly detected polymers in freshwater bodies[6,35]. Biofouled plastics were obtained by previously exposing pristine plastic fragments to two freshwater benthic, biofilm-forming species distinct from those used in the assembled community (see "Methods" section for more details). Through this approach, we measured whether: (i) the biofouled plastics could favour the dispersal of benthic species in the pelagic realm; (ii) the changes in the pelagic community biodiversity were mediated by the biofilm on plastics or by the plastic itself; (iii) the pelagic community delivered species to the xenobiotic substrate of plastic; (iv) the development of the benthic community on plastic biofilms may affect the quantity of dissolved nutrients.

Controls included: (i) treatments with growth medium and without assembled community or any plastic, to check for the partitioning of nutrients with the glassware (*blank* treatment), (ii) the growth medium with the assembled community to control for the development of the undisturbed microalgae assemblage (*control* treatment, Fig. 1) and (iii) plastic fragments covered with biofilm community in pure growth medium to observe the effect of their detachment without any competition with a pelagic community (*dispersal* treatment).

The obtained results clearly confirm that plastic and microplastic can be xenobiotic substrates for benthic species, leading to competition with the natural pelagic community and posing risks for the depletion of available nutrients. This highlights a key indirect environmental effect, which is evident only with the presence of a biofilm community on plastic, and not related to plastic itself. We also observed that this substrate may favour the formation of biofilms by the algae composing the pelagic community, potentially further altering the assembly process.

Results and discussion

Algal growth and photosynthetic efficiency

The algal growth measured by chlorophyll fluorescence in the batches is shown in Fig. 2. The growth of algae was not significantly different in the *plastic* and *biofilm* treatments in comparison to the *control*. An initial phase of low growth rates (0–5 days) was followed by exponential growth between day 5 and day 10 and a final stable phase with low growth rates between day 10 and day 15. A similar behaviour was observed for the *dispersal* experiment, demonstrating that microalgae in the biofilm consortium could easily detach and develop in the pelagic phase by dispersal following shaking and movements.

The changes in water-phase nutrient concentrations and photosynthetic efficiency of the pelagic community were measured during the initial phase (day 5), at the highest growth rate (day 8) and at the end of the experiment (day 15). Community composition was instead assessed on day 8 and day 15. The photosynthetic yield obtained from pulse-amplitude-modulated fluorimeter measurements (details in "Methods" section), showed a similar trend in all the treatments but *dispersal*: no significant differences were observed between the *control*, the *plastic* and the *biofilm* treatment at any of the sampling times. As expected, maximal photosynthetic efficiency was generally reached in the periods of maximum growth rate (at 5 and 8 days), while a decrease in photosynthetic efficiency was observed at day 15 towards the end of the experiment, indicating stress

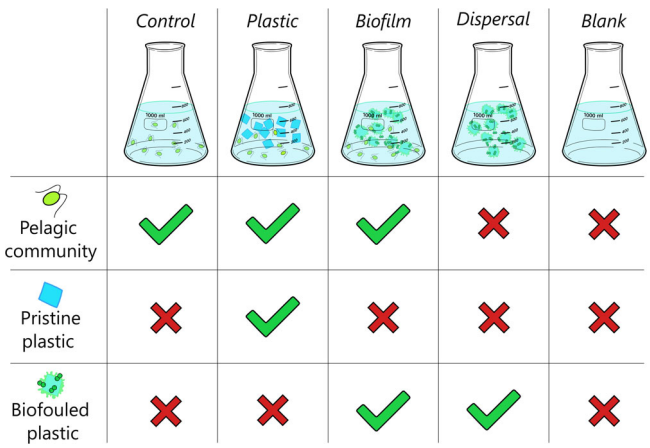

Fig. 1 | Sketch of the experimental design of the study. The presence or absence of the different factors (i.e., a pelagic community, pristine plastic and biofouled plastic) is described for every treatment using a green checkmark (if present) or a red cross (if not present). All treatments were performed in 4 replicates. More details are described in the "Methods" section.

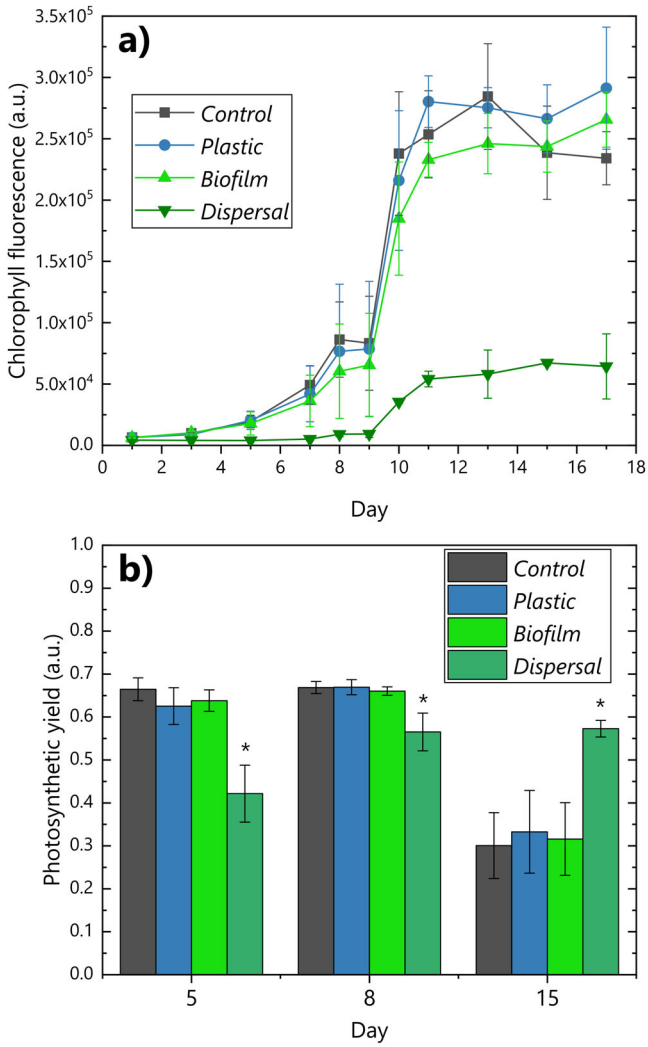

Fig. 2 | Algal growth and photosynthetic yield in the different treatments. **a** Chlorophyll fluorescence values, analysed in water, of all treatments from day 1 to day 15 of the experiment. A further analysis was performed after 17 days to observe if a plateau in growth was reached. **b** Photosynthetic yield of the different treatments at days 5, 8 and 15. Data are expressed as average values ± standard deviation after 4 replicates. Significantly different values from the *control* at the same interval time are shown by an asterisk ($p < 0.05$).

induced by competition for light or suffering of the community likely due to nutrient limitations[36,37]. In contrast, the *dispersal* experiment maintained efficient photosynthesis until the end of the experiment. This was due to the significantly lower pelagic biomass yield (and hence possibly lower stress) achieved by the community germinated from biofouled plastics. Low pelagic biomass may have reduced stress from the competition; it, however, appeared to trade-off with the formation of a biofilm layer at the bottom of all the containers used in the experiments for the *dispersal* (not detectable by the chlorophyll analysis in water from Fig. 2a). Beside detaching in the pelagic phase, in fact, the epibenthic species attached to plastic also formed a visible biofilm community at the bottom of all microcosms in the *dispersal* and *biofilm* treatments (i.e., all treatments with biofouled plastics).

Community composition

The initial cell density of the inoculum before the start of the experiment (day 0) was in the range of 1–10 cells ml^{-1} for all pelagic species (Supplementary Fig. 1 and Supplementary Table 1): such a low initial biomass allowed to simulate, at best, the spontaneous assembly of the pelagic communities reducing potential bias due to the inoculum. The pelagic assemblages were similar in the *control* and the *pristine* treatments, with the community dominated by *Chlamydomonas noctigama* (representing 85% of all cells after 15 days) and *Raphidocelis subcapitata* (with about 14% of the cells in water after 15 days), while *Spirogyra* sp. and *Planktothrix prolifica* presented less than 10 cells ml^{-1} after 15 days. After day 8, *Synura* sp. was completely outcompeted in all treatments and replicates (Supplementary Fig. 1). No significant decrease in the algal biomass or significant difference in community assemblage in the *plastic* treatment was observed (Fig. 3a, b), indicating that in the conditions of the experiment, non-biofouled plastic (mm-size) did not significantly affect the natural community assembly. In other words, algal growth was not directly affected by the physical disturbance of plastic or by the potential releases of toxic substances by this material. The similar assembly of the community observed between the *plastic* and *control* treatment, furthermore, shows that our approach led to the assembly of similar communities in case of limited disturbances or competitions.

In the *biofilm* and *dispersal* treatments, instead, the presence of diffused *Klebsormidium flaccidum* and *Aphanocapsa musciola* individuals in the pelagic form is evident after 8 days and more marked after 15 days of experiment with generally similar cell density values (Fig. 3c, d). The development of this community, however, showed a generally higher variance in comparison with the pelagic community, as its assembly relies on the random process of cells detaching from the plastic substrate.

This developed community and the community present in the biofilms covering the plastic fragments likely competed for resources with the pelagic community of the *control* treatment. Significantly lower abundance of *C. noctigama, P. prolifica* and *Spirogyra* sp. was in fact observed in the *biofilm* treatments compared to the *control* (Fig. 3a, b). This was evident starting at day 8 of the experiment and became more marked at day 15. These results support the hypothesis that plastic can be a novel xenobiotic substrate promoting the dispersal of organisms from biofilm, influencing the assembly of the pelagic community. This highlights the need to comprehend the relevance of the indirect effects of plastic pollution potentially altering ecosystem functioning.

Nutrient and minor element concentration

The principal component analysis plot for the concentration of all nutrients and trace elements analysed is shown in Fig. 4, and the average values of all measured chemical variables are listed in Supplementary Table 2. In the principal component plot, all the *blank* samples are closely clustered (regardless of the sampling time), indicating a minor effect of the containers in the depletion of nutrients. An overall decrease in the concentration of dissolved elements was expectedly observed in all the other treatments, as

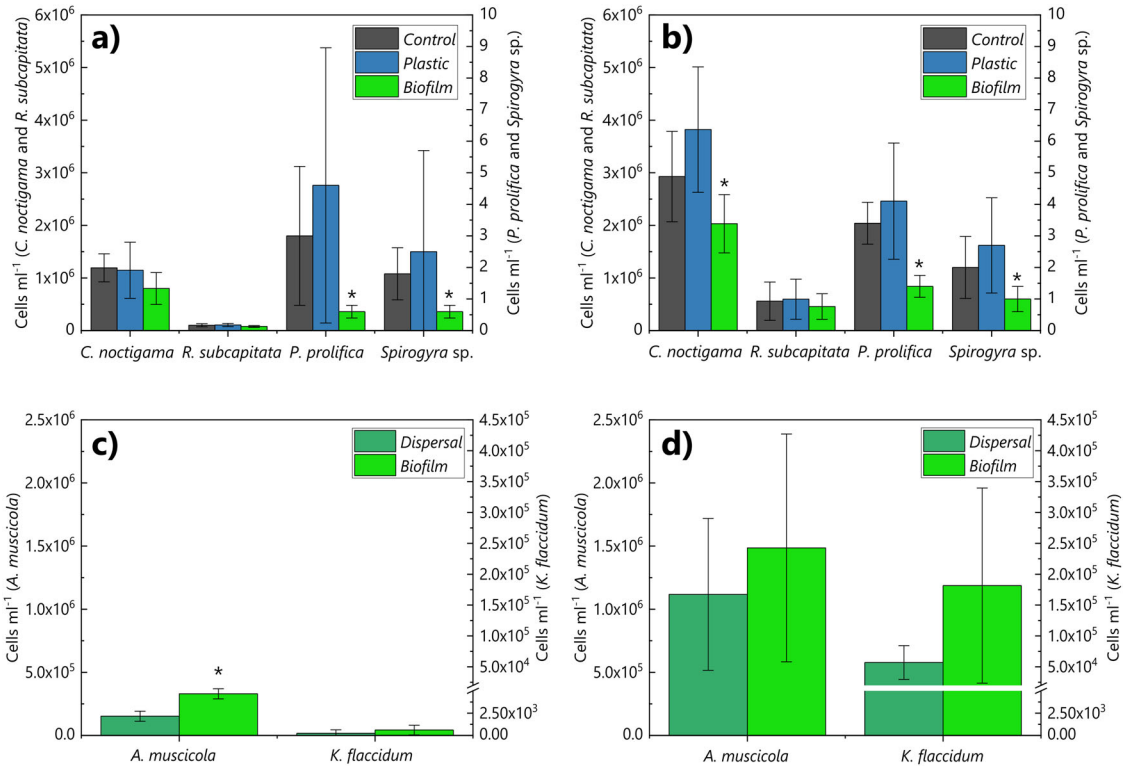

Fig. 3 | Algal community composition in the different treatments. Cell density of the pelagic community in the *control*, *plastic* and *biofilm* treatments after 8 days (**c**) and 15 days (**d**) of the experiment (in cells ml^{-1}). Values significantly different from the *control* ($p < 0.05$) are highlighted by an asterisk. **c**, **d** show the algal cell density developed in the water of the biofilm community in the *biofilm* and *dispersal* treatments after 8 days (**a**) and 15 days (**b**) of the experiment. Data are shown as average ± standard deviation among the 4 replicates. Values significantly different from the *dispersal* ($p < 0.05$) are highlighted by an asterisk.

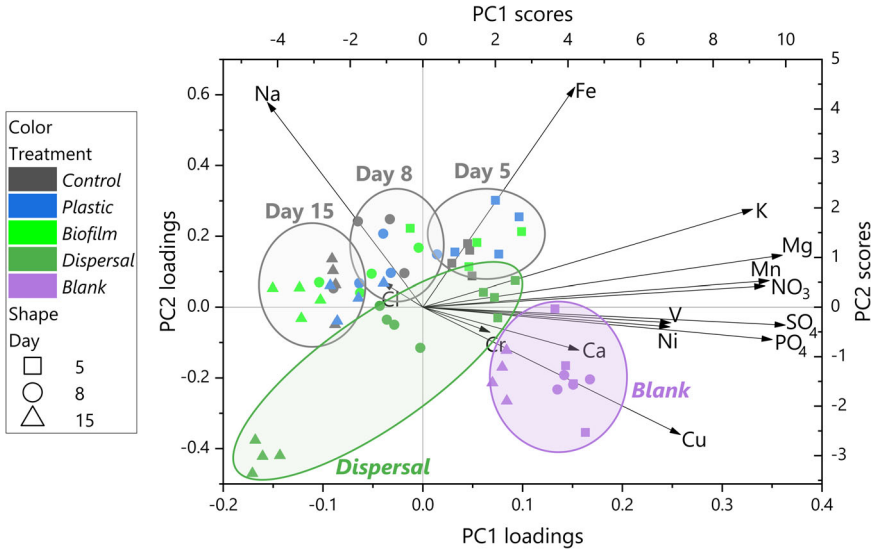

Fig. 4 | Principal component analysis biplot of components 1 and 2 for all analysed major nutrients and minor elements. The first 2 principal components (PC) explained 46% and 12% of the total variance, respectively. The three grey circles highlight the *control*, *plastic* and *biofilm* treatments at 5 days, 8 days and 15 days of the experiment respectively; the green circle highlights the *dispersal* treatments and the violet circle the *blank* at all sampling intervals.

visible by the trend of component 1 loadings and scores. Some major nutrients, such as PO_4 and NO_3, became completely depleted in all the treatments after 15 days of the experiment (Fig. 5a and Supplementary Table 2), demonstrating nutrient limitation at the end of the experiment. Time of the sampling is also a strong determinant of variability in water chemistry for the *control*, *plastic* and *biofilm* treatments: most of the samples are in fact clustered by the sampling day. However, on day 15, a shift of the *biofilm* treatment away from the *control* and *plastic* cluster is observable, highlighting a change in the chemical composition of water at the end of the experiment. *Dispersal* treatments, instead, show a drift on the first two components in all the sampling periods, highlighting a more marked difference in the chemical composition likely due to the markedly different community and a different amount of biomass compared to the other treatments.

At day 15, *biofilm* treatments displayed an interesting and distinct behaviour from the *control* (Fig. 4 and Supplementary Table 2): while NO_3 is depleted in all experiments at this time point (Fig. 5a), such a depletion was faster in the *biofilm* and *dispersal* treatments compared to the *control* for other elements such as Mg, K and Mn (Fig. 5b–d). The *plastic* treatment, instead, did not show any difference from the *control*. These effects are likely caused by the different algal assemblages developed in the pelagic community as a result of dispersal by organisms on the biofouled plastics in the *biofilm*. The *dispersal* treatment, which expresses a pelagic community with completely distinct species from that of the *control*, had a quicker depletion of several cations while presenting a slower depletion of other nutrients in comparison to the *control*: for example, at day 8 the NO_3 concentration was significantly higher from the *control*. The decrease in cations may be due to the algae maintaining their activity in the biofilm phase, both on the plastic fragments and at the bottom of the containers used in the experiments. Research has shown that biofilms tend to be more efficient in adsorbing or sequestering essential and non-essential metals compared to pelagic microorganisms, as a storage or detoxification mechanism[38–40].

These results support the second hypothesis of our study, as only biofouled plastic shows to potentially alter the concentration of elements in water. It is acknowledged that this effect is only visible in the communities treated with biofouled plastics at the stationary phase under conditions of major nutrient limitation. This suggests that biofilm-covered plastics have the potential to affect ecosystem processes by acting on the balance of competitive interactions in the pelagic phase, rather than as a direct chemical interaction between plastics and the water phase[26,27] (as plastic alone did not affect community composition and nutrient concentration). This reinforces the view that environmental plastics can affect ecosystems mainly indirectly, by altering natural habitats and key ecological processes[11].

Colonisation of plastic: a substrate exchanging species

As a secondary objective of this pilot study, we sought to explore the effectiveness of plastic to serve as a substrate for biofilm consortia created or participated also by the microalgae originally present in the pelagic community. Plastic fragments were collected from all treatments in which they were present (i.e., *plastic*, *biofilm* and *dispersal*), and their surface was analysed for the presence and composition of the biofilm. Measurement endpoints included morphology, coverage level and chlorophyll fluorescence (see details in the "Methods" section).

The increase of biofilm is observable in both *plastic* and (more markedly) in the *biofilm* and *dispersal* treatments (as measured by chlorophyll fluorescence and optical microscopy, Supplementary Fig. 2). This trend is confirmed observing the scanning electron microscopy micrographs (Fig. 6): the fragments added in the *plastic* treatment, which is free from biological film at the beginning of experiment (Fig. 6a), showed sparse extracellular matrix material, and the presence of individual cells of *C. noctigama* anchored to the plastic surface (Fig. 6b, c). This species is known to potentially form biofilms: it can form aggregates, lose their flagella and increase the production of extracellular polymeric substances when under stress conditions[32]. Filaments of *K. flaccidum* are instead evident in biofouled plastics from both the *biofilm* treatment and the *dispersal* experiment, as well as smaller agglomerates of *A. muscicola* (Fig. 6d). These observations have multiple implications: (i) species present in the control system may also attach to the plastic surface; (ii) several species from the pelagic community may colonize this new habitat and favour their survival in stressful conditions, as well as obtain protection from grazing, favouring in turn their dispersal[41]; (iii) at the analysed experimental conditions, beside colonizing the pelagic environment, species composing the biofilm further colonize the plastic surface. These observations further corroborate the hypothesis that plastic is a novel substrate that can favour the dispersal of benthic species in the water column, and possibly acting as a novel habitat to be colonized by algae composing the pelagic community owing to its surface properties and its likely residence time in the water column[21,30].

Ecological implications and future research steps

The results described here show the potential of plastics for influencing fundamental ecological processes underpinning the development and characteristics of phytoplankton. They, in particular, highlight the emerging role of plastic as a donor and recipient of organisms in the biofilms that, by exchanging with the surrounding environment, can contribute to the dynamics of the microalgae pelagic consortium and increase the competitive

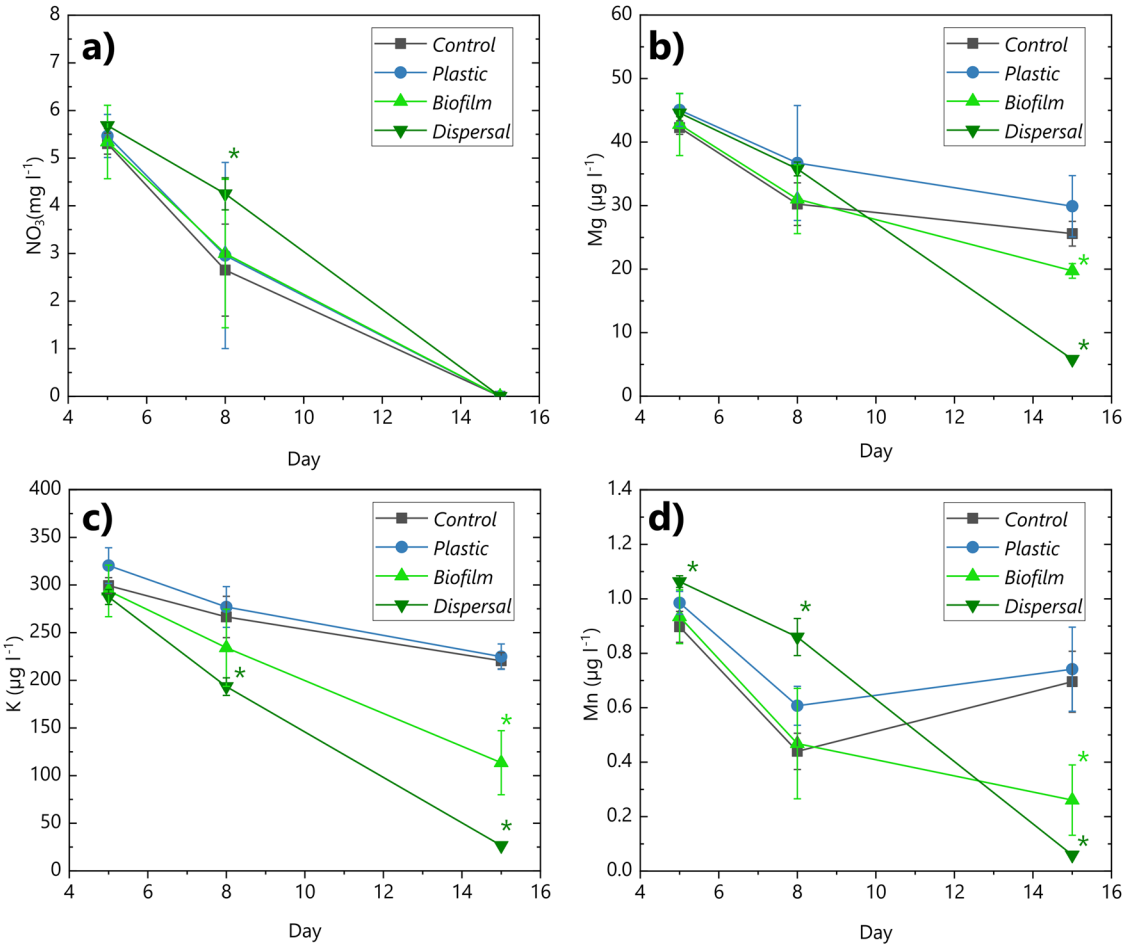

Fig. 5 | Time trends of nutrient and minor element concentrations after 5, 8 and 15 days of the experiment. Namely, NO_3 is depicted in (**a**), Mg in (**b**), K in (**c**) and Mn in (**d**). Data are shown as average ± standard deviation among the 4 replicates. Values for every sampling period showing statistically significant ($p < 0.05$) difference from the *control* are highlighted by an asterisk.

pressure on the pelagic community by favouring the presence of benthic species in the pelagic realm[22,42]. We demonstrated here that plastic can serve as a substrate, favouring the dispersal of microalgae, affecting community dynamics and nutrient availability. This process has been previously hypothesized following the analysis of environmental epibenthic communities found in plastic samples[11,22,26], but has never been empirically demonstrated before in laboratory conditions.

The results from our experimental setup showed that plastic, owing to its abundance and density, may favour the dispersal of very prolific (often prone to produce blooms) benthic algae from terrestrial sources in contact with the pelagic community while growing on plastics. Here, they can compete for resources, thus reducing their productivity while being less suitable for grazing (due to being attached to plastic). All these processes will potentially have repercussions on the food web, and the energy flows to higher trophic levels.

Some pelagic microalgae can develop sessile forms and take part in biofilm consortia. This has been described as an adaptation for facing competition for resources or escaping grazing[41]. Our results indicate that floating plastic in the environment can also favour these strategies, holding the potential to act as a refuge while dispersing the organisms in the biofilm. Because of the ubiquitous occurrence of plastic debris in fresh and marine waters[6,8], the marked physical and chemical properties of this substrate and its persistence in water bodies[29,43], the potential implications of this phenomenon are worth scientific consideration.

The one described here is a highly reductionist pilot study, however, the focus on dispersal and competition processes and the systematic positive assessment of the hypotheses set here can open a new perspective on the environmental relevance of plastic as a carrier of biofilms and their broader environmental and ecological implications. Future research should focus on expanding knowledge in this area.

There are, in fact, several variables which will need investigation to improve the comprehension of the environmental relevance of this process. For example, while there is increasing knowledge on the selection of a specific microbial community of plastic in comparison to natural substrate[21,23], future studies should evaluate if plastic properties also alter the attachment and detachment of organisms in the biofilms in comparison to other substrates. It is also not known how the plastic type and surface properties (e.g., hydrophobicity) can influence biofilm attachment, development and growth[19,25,44]. As a further complication, environmental plastic undergoes ageing processes which alter the initial chemical properties[13,45], potentially affecting the adhesion dynamics of cells composing the biofilms. A more complex simulation of the natural biofilm community on plastic and of the planktonic community will enforce the environmental relevance of this process[20,46,47] as also other features besides plastic substrate (e.g., nutrient concentrations, temperature and initial microbial community) impact the final formation of biofilms on plastic and other substrates[48,49]. Future investigations assessing a similar hypothesis with a more complex community will help in rating the environmental relevance of this process and the further ecosystem implications.

Methods

Algal strains, reagents and solutions

Five microalgal strains from the Norwegian Culture Collection of Algae (NORCCA) were selected as common representatives of freshwater

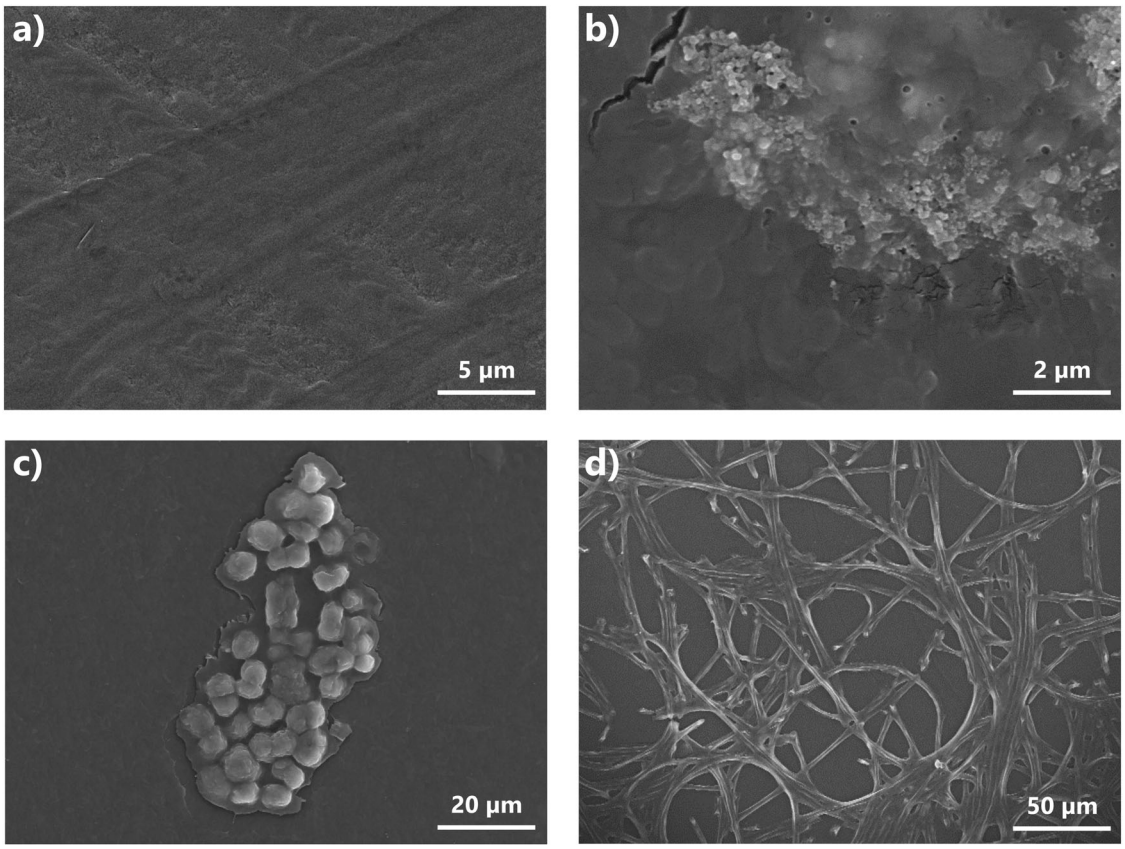

Fig. 6 | Scanning electron microscopy micrographs of the PP fragments. A pristine fragment before the incubation in the *plastic* treatment is shown in **a**, while **b** and **c** show details of biofilm formation after the 15 days of the experiment. **d** shows an example of a PP fragment in the *biofilm* treatment.

phytoplankton communities, covering major lineages and functional groups (Supplementary Fig. 3). All the strains were non-axenic, containing an accompanying uncharacterized microbiome. The selected algae are (codification in brackets shows the name in which these strains are deposited at the NORCCA collection at the Norwegian Institute for Water research):

- *Raphidocelis subcapitata* (NIVA-CHL 1), a cosmopolitan pelagic alga belonging to green algae (Chlorophyceae), with croissant-like cell shape (generally of 5–10 µm in size)[50,51] widely used in ecotoxicological studies[30];
- *Chlamydomonas noctigama* (NIVA-CHL 25), representing a cosmopolitan species of flagellated green algae (Chlorophyceae), present in freshwaters with a preference for pelagic habitat[32,52];
- *Synura* sp. (NIVA-5/09), a colonial planktonic algal species with drop-shaped cells belonging to Ochrophyta division, with dimensions of ca. 20–30 µm[53];
- *Spirogyra* sp. (NIVA-CHL 189), a common and widely distributed benthic freshwater filamentous algae of Charophyta with a cell size of about 100–120 µm, widely used for wastewater treatments[54];
- *Planktothrix prolifica* (NIVA-CYA 129), a pelagic filamentous cyanobacteria species presenting filaments of about 10 µm in diameter and with a variable length up to about 1000 µm, likely to form blooms in freshwater bodies[55].

While there is no claim that this type of community perfectly represents a natural freshwater system, this kind of approach is commonly used to provide sufficient information for testing community-level responses to environmental stressors in laboratory settings[56–58].

Two other algal species were selected to create a simulated biofilm community on plastic fragments: the strains of *Klebsormidium flaccidum* (NIVA-CHL 80, a filamentous Charophyta with size up to several mm in length) and *Aphanocapsa muscicola* (NIVA- CYA 474, a coccoid Cyanobacterium with round cells of size <1 µm usually forming colonies) were selected as an example of biofilm-forming algae common in benthic freshwater communities and terrestrial environments that are capable to accumulate on plastic from terrestrial source or sedimented plastic in aquatic environments[26,59,60].

All the algal species were kept in single-use plastic flasks containing Z8 growth media[61] at 15 °C in the algal collection for several generations. Cell density in the inocula was controlled to maintain continuously a log phase of single species prior to their inoculation in the microcosms.

All solutions were prepared using ultrapure water (18.2 MΩ × cm resistivity) obtained from a Sartorius (Germany) Arium™ pro-VF. If not differently specified, labware and plastic samples were rinsed with ultrapure water. Algal growth medium was prepared following the Z8 formulation[61], with the addition of the vitamins thiamine (at a final concentration of 0.1 mg l^{-1}), biotin (1 µg l^{-1}) and cobalamin (1 µg l^{-1}). Nitric acid for labware washing and sample acidifications was obtained by dilution of HNO_3 Suprapur (Sigma–Aldrich, United States of America).

Standards for (trace) elements and anions analysis were obtained by the dilution of Merck (United States of America) standards. All solid reagents were of reagent-grade level.

Plastic samples

Plastic samples were obtained from commercial objects in PP by cutting "confetti-like" fragments from single-use transparent food containers (Pro-Pac, Germany) in squares of about 5 × 5 × 0.3 mm. A thorough characterization of this material can be found elsewhere[25].

The biofouled fragments were obtained by adding the pristine PP fragments in a co-culture of *Klebsormidium flaccidum* and *Aphanocapsa muscicola* species. Briefly, 100 plastic fragments were added to 100 ml Erlenmeyer flasks filled with 80 ml of Z8 growth media and algae cells were

Table 1 | Description of the setup for the different treatments, including notes and the rationale for the specific treatment

Treatment	Description	Notes and rationale
Control	A simulated freshwater algal community of 5 species.	Assessing the community assembly in a simplified freshwater ecosystem without any disturbance.
Plastic	*Control* community with PP fragments.	Assessing the phytoplankton community response to plastic disturbance.
Biofilm	*Control* community with the addition of previously biofouled PP plastic.	Assessing the effect of biofilm species dispersal after the interaction with the natural algae community.
Dispersal	Biofouled plastic in the growth media without the *control* community.	Assessing the dispersal potential of species from the biofilm without competition with a pelagic community and the use of nutrients by the community developed from biofilms.
Blank	Diluted growth medium without plastic and inoculum.	Assess the depletion of nutrients induced by the adsorption or precipitation processes of the container, acting as a simplification of the effect of a natural substrate (e.g., sediments).

inoculated, reaching an initial cell density of about 1000 cells ml^{-1}. Then, the flasks were put in an incubator with continuous light (100 µmol m^{-2} s^{-1} of intensity) at 15 °C under horizontal shaking and the incubation lasted 10 days (see Binda et al. [25] for more details). To obtain similar conditions of plastic fragments and rule out potential issues due to the leaching of plastic additives on the growth of the algal communities in the *plastic*, *biofilm* and *dispersal* treatments, also the pristine plastic fragments were soaked in the same solution without the inoculation of algae species.

Experimental setup and incubation of plastic samples

The experimental design included a randomized block design of 4 replicates for the *control* and the two treatments (including pristine and biofouled plastic). In addition, 4 replicates of the further experiments (i.e., the *dispersal* experiment and the *blank*) were randomly performed. The details and rationale of the different treatments and experiments are summarised in Table 1.

All the treatments were performed in 100 ml Erlenmeyer flasks, autoclaved and rinsed with ultrapure water prior to the experiment. Then, 100 ml of growth media (prepared by diluting Z8 growth media with vitamins at a 1:50 ratio) were added to the flasks. Four aliquots of this solution were analysed for their chemical composition. After that, 50 µl of every species inoculum were added to the experimental batches (of control, pristine and biofouled plastic), and 6 aliquots of the inoculated media were randomly collected to assess the cell abundance of algae at the beginning of the experiment. The inoculated media was left overnight in the dark in an incubator, and then 15 pristine (or biofouled) plastic fragments were added to the treatments including the addition of plastic (i.e., *plastic*, *biofilm* and *dispersal* experiments). The number of plastic was selected to represent 840 mm^2 of plastic surface as a substratum in the batches, an environmentally relevant scenario in polluted freshwater bodies considering the number of environmental plastic reported[62,63]. All plastic added to the treatments was rinsed three times with ultrapure water to remove potential residues of adsorbed nutrients on its surface and loosely bound organisms not well attached to the biofilm matrix.

All the flasks were inserted in an incubator with 60 µmol m^2 s^{-1} light radiation (measured with a Skye SpectroSense2+ system, United Kingdom) with a 16:8 h of light:dark cycle and a constant 18 °C temperature. The experiment lasted 15 days in total. Flasks were shaken and changed in position every 24 h to ensure a random variability of light irradiation and temperature conditions.

Assessment of algal growth and community composition in water

The algal densities in suspension were measured every 2 days of the experiment to assess biomass growth in all treatments and replicate batches. Chlorophyll fluorescence measurements were used for this scope by analysing 0.5 ml culture aliquots in a 24-well plate using a Spectramax iD3 (Molecular Devices, United States of America) plate reader: fluorescence was measured at 685 nm using the exciting wavelength at 485 nm[25,64].

The photosynthetic yield was assessed by measuring the quantum yield with a pulse-amplitude-modulated fluorimeter (Heinz Walz GmbH, Effeltrich, Germany). Aliquots of 3 ml were collected after 5, 8 and 15 days of the experiment and added to the fluorimeter cuvette. Samples were then acclimated for 4 min in dark conditions, and fluorescence was measured subsequently[65].

Algal community counting was performed after 8 and 15 days of the experiment. 5 ml of unfiltered samples were collected and stabilized using Lugol's iodine fixation. Samples were then left to sediment in counting chambers[66] overnight and counting was performed using an inverted microscope. The cell counting was favoured by the marked algae morphological differences (see Supplementary Fig. 3).

Analysis of nutrients in water

Two aliquots of freshwater media of 5 ml each were collected for chemical analysis at 5, 8 and 15 days of the experiment from all experimental batches to analyse major anions (i.e., Cl, NO_3, SO_4, PO_4), major and minor/trace metals (i.e., Na, Mg, K, Ca, V, Cr, Mn, Fe, Ni, Cu). Water was directly filtered on cellulose filters (0.45 µm pore diameter) and added in 15 ml PP vials (previously rinsed with ultrapure water for anions, while rinsed sequentially with ultrapure water and 2% v/v HNO_3 for metals)[67]. Samples for metal analysis were also acidified with Suprapur HNO_3 to reach 2% in volume. All samples were then refrigerated and kept in the dark until further analysis. Anions were then measured via ion chromatography (IC, EcoIC Metrohm, Switzerland), while metals were analysed via inductively coupled plasma-mass spectrometry (ICP-MS, Icap Q, Thermo Scientific, United States of America). Specific analytical details and quality assurance-quality control protocols can be found elsewhere[68].

Analysis of biofilms on plastic

Plastic samples in the experiments were collected to analyse the growth of biofilm and the morphology of the plastic surfaces. Three plastic fragments for every experiment were collected at 5, 8 and 15 days. In addition, 3 random fragments were analysed before the addition in the experiments (of pristine plastic for the *plastic* treatment, and with a priorly developed biofilm for the *biofilm* treatment).

Collected fragments were rinsed twice in deionized water to remove non-attached biological material and left for 24 h in the air to dry. After drying, the plastic fragments were placed in a 24-well plate (1 fragment per well, using 4 replicates for each incubation batch). Then, two distinct methods were used to determine biofilm growth: (i) fluorescence analysis of chlorophyll and (ii) optical microscopy method to measure coverage rates of plastic fragments. We analysed chlorophyll fluorescence directly on collected plastic fragments using a plate reader (as for water samples). We applied a well scan mode with 32 measures equally distributed on the well surface and we then calculated an average value of fluorescence for every fragment. We then analysed the same plastic samples through optical microscopy to measure the percentage of total plastic surface covered by biofilm. For this, stereomicroscope images of plastic specimens were captured using a Nikon SMZ 745 T stereomicroscope with Infinity 1–3 C camera and Infinity Analyse software. Images were processed using ImageJ software plugged into the colseg color segmentation plugin. This enabled a direct measure of the covered area. Plastic fragments without biofilm were used as sample blanks.

The micromorphology of plastic fragments and biofilm surface features was investigated through a Philips (Netherlands) field emission gun scanning electron microscope, with a 25 keV beam under high vacuum conditions. Samples for electron microscopy were air dried and covered with a ~5 nm thick and uniform gold layer using a Cressington (United Kingdom) 108 auto vacuum sputter coater to improve image quality.

Data treatment

All datasets were evaluated for normality using a Shapiro–Wilk test prior to further analysis. Since data followed normal distributions among replicates, *t*-tests were performed to assess differences between the control and the treatments. The threshold for statistical difference was established as $p < 0.05$. The relationships among the nutrient and other element concentration values in the different experiments have also been analysed by principal component analysis. All statistical tests and analyses were computed using Origin 2018 software (OriginLab Corporation, United States of America).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Experimental data of the measurements presented in this paper are available at the following link: https://zenodo.org/records/10907892[69].

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Author contributions

Gilberto Binda – Study design, formal analysis and preparation of the original draft; Stefano Carnati – Formal chemical analysis, reviewing and editing of the draft; Margarida Costa – Conceptualization, reviewing and editing of the draft; Vladyslava Hostyeva – Formal analysis of algae, data curation; Eva Leu – Conceptualization, formal analysis, reviewing and editing of the draft; Birger Skjelbred – Formal analysis of algae, data curation; Davide Spanu – Formal chemical analysis, data curation; Luka Šupraha – Conceptualization, reviewing and editing of the draft; Sara Trotta – Formal chemical analysis; Christian Vogelsang – Reviewing and editing of the draft, data curation; Luca Nizzetto – Funding acquisition, conceptualization, supervision, reviewing and editing of the draft.

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