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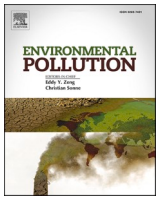

Temporal succession outweighs substrate characteristics in shaping riverine plastisphere biofilm on textiles

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ABSTRACT

Biofilms developing on plastic surfaces (the plastisphere) are increasingly recognized for their ecological significance, yet the drivers of community biomass and biodiversity remain poorly understood. Moreover, plastisphere research has focused on a narrow range of polymers, leaving widely distributed substrates such as textiles understudied. Here, we assessed the relative importance of temporal succession and substrate properties (polymeric composition and color) in shaping microalgal and bacterial community composition and photosynthetic abundances, and evaluated whether substrate-specific selection results in long-term community divergence or is restricted to early colonization. We conducted a mesocosm experiment simulating a lotic system to examine biofilm development on polyester textiles and non-synthetic analogue (cotton), each in two colors (black, white). Prokaryotic and microalgal eukaryotic community composition (16S, 18S rRNA), together with picophotosynthetic abundances assessed by flow cytometry, were monitored over five time points spanning 7 to 35 days. Microbial diversity, community composition, and inferred prokaryotic functions did not differ significantly among polymer types or colors, indicating that substrate characteristics played a limited role in overall community assembly and the predominance of opportunistic colonization, despite the presence of indicator species. In contrast, microalgal abundances differed among substrates, with higher abundances observed on cotton, suggesting that substrate properties may still influence specific aspects of biofilm development. Temporal succession emerged as the primary driver of community change, with significant effects observed for prokaryotic communities even over short timescales. These findings emphasize that temporal dynamics must be explicitly considered in plastisphere studies, as differences in colonization time may confound interpretations of substrate effects.

1. Introduction

Aquatic biofilms are complex communities characterized by a pronounced three-dimensional structure that adheres to solid surfaces through an extracellular matrix (Wijewardene et al., 2022). They encompass both autotrophic (e.g. diatoms, green algae, cyanobacteria) and heterotrophic components (e.g. bacteria, protozoa, fungi), which are functionally interconnected (Kouzuma and Watanabe, 2015; Stevenson et al., 1996). These structures play a vital role in aquatic environments as a key mode of microbial life and as fundamental modulators of local chemical conditions, including nutrient availability and redox dynamics (Gubelit and Grossart, 2020). In addition to biofilms that develop on naturally occurring substrates (e.g., sediments, stones, plants, and floating wood), increasing attention is being directed toward

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a novel form of biofilm that grows on "artificial" substrates, known as the plastisphere, which forms on the surfaces of various plastic polymers (Nava et al., 2024a; Su et al., 2024; Zettler et al., 2013). The widespread presence of plastics in aquatic ecosystems is now well-recognized, with certain areas being heavily impacted (Eriksen et al., 2014; Meijer et al., 2021; Nava et al., 2023). Therefore, there is a high availability of plastic surfaces for diverse organisms to colonize, creating new spatial niches that have the potential to alter ecological dynamics. For instance, a recent study revealed that the plastisphere communities on biofouled plastics caused a significant reduction in oxygen levels in river water, leading to hypoxic or anoxic conditions, with impacts on the ecosystem metabolism (Nava et al., 2024b). A deeper understanding of the communities that form on plastic substrates, along with the processes driving their development, is essential to assess the potential ecological impacts.

The development and succession of microorganisms in the plastisphere community are influenced by a complex interplay of stochastic and deterministic processes (Sun et al., 2021; Zhang et al., 2024b). Environmental and niche-determining factors play a crucial role in shaping the plastisphere community (Nguyen et al., 2022; Wright et al., 2021). However, determining the relative contribution of the different processes to biomass and biodiversity development remains challenging. Evidence regarding community similarity across different plastic polymers is mixed, with variations often linked to the specific type of polymer (Silva et al., 2023; Zhang et al., 2024a), as well as intrinsic properties like hydrophobicity, texture, and the presence of additives (Dey et al., 2022; Ogonowski et al., 2018; Pinto et al., 2019; Ward et al., 2022a). However, most plastisphere research has focused on a narrow range of polymers (e.g., polyethylene, polypropylene) with similar properties, while other common plastics, such as fabrics, have been largely neglected (Silva et al., 2023). Various factors may influence colonization dynamics, but their individual contributions are often difficult to disentangle. For example, studies conducted in lakes suggest that plastic color can affect the community structure and functional diversity of the plastisphere. Differences in algal growth and diversity observed on plastics of different colors have been attributed to visual resemblance to algae that may alter zooplankton grazing behavior, and the presence of additives that can reduce algal biomass (Martí et al., 2020; Wen et al., 2020).

While there is broad consensus that communities developing on plastics differ significantly from those in the planktonic realm (Di Pippo et al., 2022; Dussud et al., 2018a; Oberbeckmann et al., 2016), less is known about how these communities compare to those on other non-synthetic substrates in aquatic systems. This gap of knowledge leaves open the question of whether these differences are specifically related to the synthetic nature of plastics or simply reflect opportunistic colonization of any available substrate (Oberbeckmann et al., 2018; Wright et al., 2021).

To address these questions, we conducted a mesocosm experiment in a simulated lotic system to study the temporal development of the plastisphere on a less-studied substrate, polyester textile, in two colors, and compared it with biofilms developing on a non-synthetic, cellulosic analogue (cotton textile). Our goal was to identify the primary factors shaping microalgal and bacterial community composition (including temporal succession, substrate properties like color and polymeric composition, and their interactions) and to investigate how the photosynthetic community abundance responds to these drivers, providing insights into potential effects on ecosystem functioning. We formulated two alternative hypotheses: (1) substrate properties have little influence on the long term community composition, with differences only occurring at early successional stages when microorganisms first colonize the substrate; or (2) substrate-specific selection drives community divergence over time, resulting in more pronounced differences at later successional stages, whereas early-stage communities show limited differences due to some stochasticity during colonization.

2. Materials and methods

2.1. Study site and experimental setup

The experiment was conducted at the Lunz Mesocosm Infrastructure (LMI, Benthic Flumes, WasserCluster Lunz - WCL) located in Lunz am See, Austria (47° 15′ N, 15° 04′ E; Fig. 1). This infrastructure comprises six streamside channels, each measuring 40 m in length and 0.4 m in width, continuously supplied with stream water from Oberer Seebach. Oberer Seebach drains a pristine calcareous 20 km^2 large catchment with elevations ranging from 600 m to 1878 m above sea with oligotrophic conditions.

From mid-June to mid-July 2021, sets of fabrics, each with a surface area of 18.5 × 4.3 cm, were positioned along two flumes and served as substrates for the growth of the textile-associated biofilm. These fabrics were made of either cellulosic (100 % cotton) or synthetic (100 % polyester) material and were colored either black or white. The setup thus represented a 2-level 2-factor factorial design. Before deployment, each fabric and color combination was thoroughly hand-washed in separate 5 L buckets of tap water with 10 mL of liquid detergent (Splendid Vollwaschmittel, liquid) and allowed to soak overnight for approximately 16 h. Following washing, fabrics were rinsed under microbiologically pure water for 10 min using gloves to avoid contamination. The experiment included three treatment replicates spaced 1 m apart (Fig. 1) and five time points (t_1 = 7 days after fabric deployment, t_2 = 14 days, t_3 = 21 days, t_4 = 28 days, t_5 = 35 days). At each time point, fabrics were removed and collected from downstream to upstream (to minimize background disturbance) and split into aliquots for subsequent analyses. Water flow in the two flumes was adjusted to an approximate discharge of ~0.7 L s^{-1}. Channel slope was set to 0.0025 m m^{-1}, which corresponds to a typical slow-flowing lowland river. The side walls and bottom of the channel were covered with a black ethylene propylene diene monomer rubber (EPDM) lining. Prior to the experiment, the EPDM lining was cleaned by mechanical scrubbing using floor scrubbers while the flumes were operated at high flow with running stream water, allowing detached biofilm and particles to be flushed from the system. No chemical cleaning agents were applied. Hence, only a minimal biofilm was present on the surfaces of the flumes when the fabrics were immersed. The first 2-3 m of all channels were used as a sedimentation chamber. The water from this chamber was passed through a medium-sized sieve (mesh size = 1.2 mm) and a flow straightener consisting of 2 × 1 cm and 40 cm long straight pipes across the entire width and height of the flume to ensure stable flow conditions. Water temperature and light intensity were recorded every 15 min using a HOBO Data Logger (mean ± standard deviation, SD temperature: 11.5 ± 1.7 °C; mean ± SD illuminance: 9999 ± 21,243 lux). At each time point, we measured concentrations of dissolved organic carbon (DOC), nitrogen in nitrate (NO_3–N), ammonium (NH_4–N), and nitrite (NO_2–N) and soluble reactive phosphorus (SRP) at the inlet and outlet of the two flumes in which the fabrics were submerged. During the entire experimental period, water chemistry remained relatively stable. DOC concentrations ranged 0.8-2.0 (mean ± SD = 1.2 ± 0.4) mg L^{-1}, and NO_3–N concentrations ranged 0.65-0.96 (mean ± SD = 0.80 ± 0.11) mg L^{-1}. Concentrations of NH_4–N, NO_2–N, and SRP were consistently low, remaining below 3.3 μg L^{-1}, 2.1 μg L^{-1}, and 2.9 μg L^{-1}, respectively, and were below the analytical detection limits on many sampling days (see Table S1 in Supplementary Information for details).

2.2. Fabric sampling

The sample holder was carefully removed from the respective flume, allowed to drain for 5–10 s, and then placed in a designated box. Each fabric was handled using two tweezers and transferred to a clean, pre-labeled aluminum tray. Subsequently, 20 mL of DNA-free water was added to the aluminum tray using a pipette, and the tray was covered and placed in a cooler. On the same day of collection, each fabric was

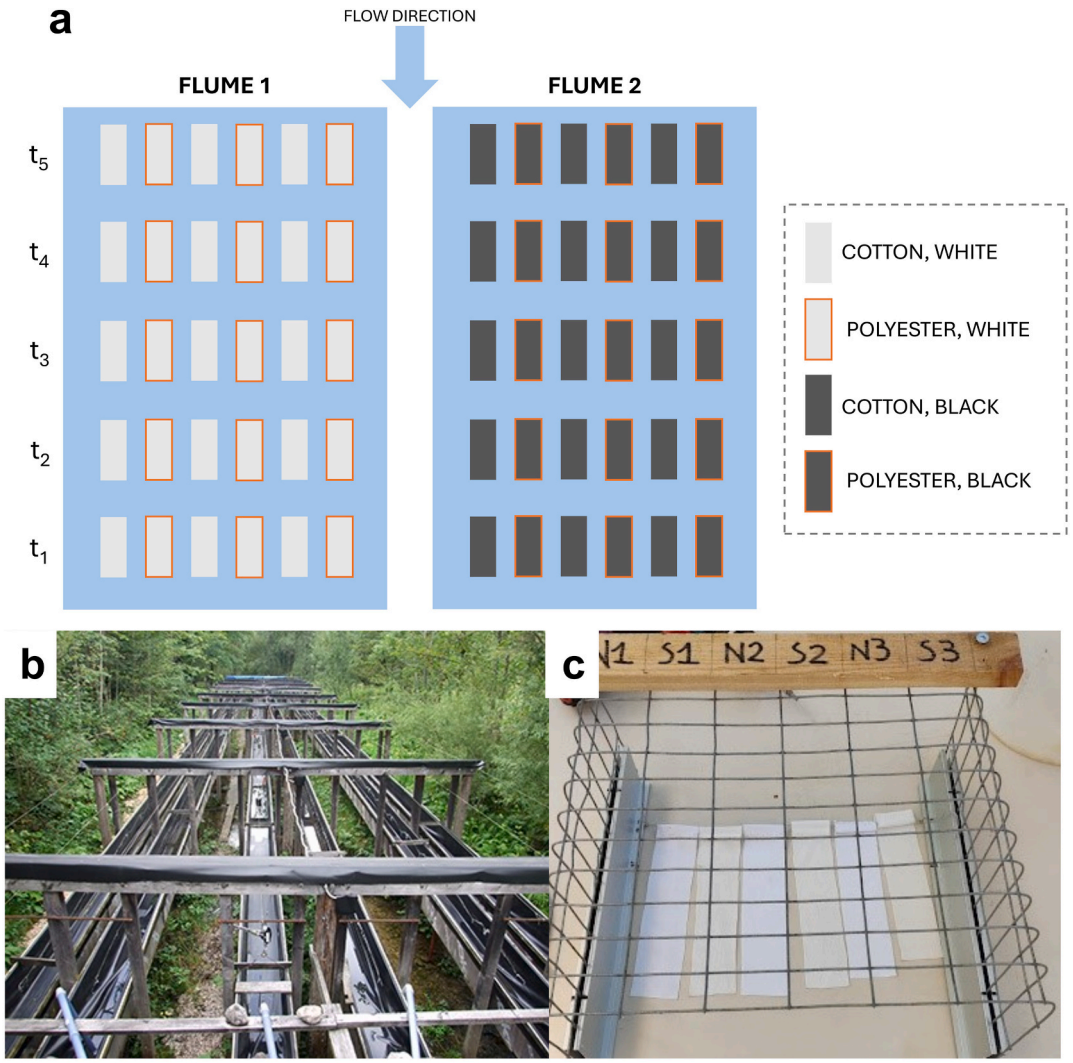


Fig. 1. (a) Schematic representation of the experimental design, showing the substrates used and their arrangement within the system; (b) Photograph of the Lunz Mesocosm Infrastructure (LMI, Benthic Flumes, WasserCluster Lunz - WCL), where the experiment was conducted; (c) Example photograph of the substrates used during incubation.

brushed and cleaned in its tray using a new brush. The resulting water/biofilm mixture was poured into a 50 mL plastic tube. The aluminum tray was rinsed with approximately 20 mL of DNA-free water from a squeeze bottle, and the rinse water was also transferred to the tube. The volume was then adjusted to 50 mL. The sample was sonicated, and 2 mL were transferred to an Eppendorf tube for flow cytometry analysis (Section 2.3.2). Until then, the samples were stored at 4 °C. The remaining 33 mL from each of the three replicate samples were pooled (total volume 99 mL) to obtain sufficient biomass for DNA extraction and subsequently filtered through sterilized Whatman GF/F glass microfiber filters with 0.45 μm pore size. Filtration was continued until the filters became clogged, and the filtered volume was recorded. The filters were then folded in half (inward), wrapped in aluminum foil, and stored at −20 °C until further processing for sequencing (Section 2.3.1).

2.3. Laboratory analyses

2.3.1. DNA extraction, amplification and sequencing of bacterial and algal communities

We identified and classified bacteria and algae on fabric surfaces using 16S rRNA and 18S rRNA analysis, respectively. DNA extraction was performed from half filter per sample using the FastDNA® Spin for Soil kit (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The V5-V6 hypervariable regions of the 16S rRNA gene were amplified using bacterial primers 783F and 1046R, with Illumina adapters added at the 5′ end (Huber et al., 2007). Eukaryotic primers 528F and 706R were used to amplify the V4 hypervariable region of the 18S rRNA gene (Cheung et al., 2010). PCR amplification and Illumina sequencing were performed as previously described (Nava et al., 2024b). Amplicon Sequence Variants (ASVs) were inferred using the DADA2 pipeline (Callahan et al., 2016). Forward and reverse reads were trimmed after 180 and 150 bases, respectively, for bacteria, and after 200 bases for algae. A quality filter with a maximum number of expected errors equal to 0.5 per read was applied. The two reads were then merged and taxonomically assigned with SILVA v132 databases. For bacteria, all non-bacterial ASVs were discarded, as described in Gandolfi et al. (2024) and the remaining ASVs were classified again with the latest version of the RDP classifier (ver. 2.14, August 2023). For algae, all non-algal ASVs were manually discarded. The FAPROTAX database was used to annotate the metabolic functions (Louca et al., 2016). We tested for homogeneity of multivariate dispersion among groups using the betadisper procedure, followed by post-hoc Tukey's HSD comparisons. Mean pairwise Euclidean distances calculated on Hellinger-transformed ASV abundances were used to quantify between-group dissimilarity for polymer type (cotton vs. polyester) and color (white vs. black) across sampling times. To evaluate patterns in

bacterial community composition, we conducted a redundancy analysis (RDA) on Hellinger-transformed ASV data. ASVs present in only a single sample were removed because they can inflate the variance explained by multivariate tests (Borcard et al., 2018). The RDA model included polymer, time, and color as explanatory variables, and its overall significance was evaluated using permutation tests with 999 iterations. Marginal effects of each variable were tested to assess their individual contributions while accounting for the others. The significance of individual RDA axes was also assessed by sequential permutation tests. To identify species associated with specific treatments (substrate, sampling time, and color), the Dufrêne–Legendre Indicator Species Analysis was performed, calculating indicator values based on both species fidelity and relative abundance using 99,999 permutations.

2.3.2. Flow cytometry

To assess microalgal abundances weekly over the sampling time, algal cell numbers (pico-cyanobacteria, PCY; and pico-phytobenthos, PEUK) were counted with a flow cytometer (CytoFLEX, Beckman Coulter Life Sciences, Indianapolis, IN, USA) using their autofluorescence. For each sampling event, a fresh subsample of 2 mL of brushed biofilm was collected following the same procedure for all samples and analyzed within 24 to 72 h. Manual gating was used to distinguish populations of phytobenthic and cyanobacterial cells (detection: 690 ± 25 nm versus 660 ± 10 nm). Each sample was measured in triplicate after additional vortexing between each replicate. Samples from the initial time point (t_1) were excluded from analysis due to their low biomasses and issues during the sample processing that affected their quality and prevented accurate quantification.

2.4. Statistical analyses

16S and 18S rRNA data were analyzed using the package 'phyloseq' (McMurdie and Holmes, 2013). Diversity indices (Shannon and Inverse Simpson) were calculated on ASVs following rarefaction, using the 'vegan' package (Oksanen et al., 2025). Rarefaction curves were generated to assess sequencing depth. To account for unequal sequencing depth, 16S rRNA samples were rarefied to 10,000 reads. Four samples with fewer than 10,000 reads were removed prior to diversity analyses (Fig. S1a). 18S rRNA samples were rarefied to 1200 sequences per sample, yielding 21,600 sequences (Fig. S1b). To assess how diversity indices responded to experimental factors, linear models were fitted with the diversity indices as response variables, using polymer type and substrate color as categorical predictors and time as a continuous predictor.

To assess the effects of polymer type, color, and time on biofilm cell density of PEUK and PCY, we fitted generalized linear mixed models using the 'glmmTMB' package (Brooks et al., 2017). Cell density was modeled as a Gamma-distributed response with a log link to account for its strictly positive, continuous nature. Time was modeled using a natural cubic spline with 3 degrees of freedom to capture potential nonlinear temporal dynamics. Polymer and color were included as fixed effects, and replicate was included as a random intercept to account for repeated measurements within replicates. For each group, five candidate models were considered. These included a main-effects model with additive contributions of time, polymer, and color, as well as two models incorporating interactions: time × polymer + color, and time + polymer × color. The fourth model included time and polymer without color, and the fifth model included time only. Model selection was performed using the Akaike Information Criterion corrected for small sample sizes (AICc) via the model.sel() function from the 'MuMIn' package. Models with ΔAICc <2 were considered competitive. Residual diagnostics were assessed using the 'DHARMa' package (Hartig, 2022), examining uniformity, dispersion, and potential deviations from model assumptions. Significance of fixed effects and interactions was evaluated using Type II Wald χ^2 tests using the 'car' package (Fox and Weisberg, 2019).

3. Results

3.1. Taxonomic composition and alpha diversity of the textile-associated biofilm

Between 4149 and 104,579 sequences of the V5-V6 hypervariable regions of the 16S rRNA were identified across our samples. Overall, after removing singletons (defined here as ASVs occurring only once across the entire dataset), we detected 6999 unique ASVs. The three dominant phyla across all samples were Pseudomonadota, Actinomycetota, and Bacteroidota, with average relative abundances (± SD) of 58.3 ± 8.3 %, 20.3 ± 8. 5 %, and 7.0 ± 2.5 %, respectively (Fig. 2a). Among the most abundant classified families, we identified within the phylum Pseudomonadota: Paracoccaceae (formerly Rhodobacteraceae, 10.8 ± 4.1 %), mainly comprising the genus *Tabrizicola* and other unclassified members; Comamonadaceae (9.7 ± 5.9 %), among which the most abundant genus was *Variovorax*; Sphingomonadaceae (3.0 ± 2.3 %), mainly represented by the genus *Sphingorhabdus*; and Hyphomicrobiaceae (2.8 ± 1.1 %). In the phylum Bacteroidota, Chitinophagaceae represented 3.7 ± 0.8 %, and in the phylum Verrucomicrobiota, Verrucomicrobiaceae accounted for 2.6 ± 1.2 %, especially belonging to genus *Luteolibacter*. Lastly, in the phylum Actinomycetota, the family Ilumatobacteraceae represented 3.7 ± 1.1 %, mainly with the genus *Ilumatobacter*. On the contrary, the phylum Cyanobacteria was represented by low abundances (0.9 ± 0.4 %).

From the 18S rRNA analysis, 788 unique ASVs related to the microalgal community were identified, based on a total of 591,636 sequence reads. Due to low-read abundance, two samples (replicate of black polyester, t_3; replicate of black cotton, t_5) were excluded from further analyses. Charophyta was the most abundant phylum, though with considerable variability among samples (49.1 ± 28.0 %), followed by Chlorophyta (35.0 ± 24.1 %). Ochrophyta had an average occurrence of 15.3 ± 11.2 %, while all other phyla exhibited mean occurrences below 0.5 % (Fig. 2b). In total, 30 distinct genera were taxonomically classified. The genus *Spirogyra* (family Spirogyraceae, phylum Charophyta) was the most dominant, comprising up to 87.3 % of the microalgal community in certain samples. This genus was particularly prevalent during the early colonization stages (t_1 to t_3), with an average relative abundance of 61.6 ± 29.4 %, which declined to 26.3 ± 12.7 % by t_4 and t_5. Within Chlorophyta, the genus *Chlamydomonas* was the most frequently detected, alongside recurring representatives of the order Sphaeropleales, such as *Mychonastes* and *Desmodesmus*. Among Ochrophyta, diatoms (class Bacillariophyceae) were represented by several genera, including *Amphora*, *Cymbella*, *Fragilaria*, *Gomphonema*, *Navicula*, *Nitzschia*, *Pinnularia*, *Planothidium*, and *Staurosira*. Additionally, members of the class Chrysophyceae, such as *Mallomonas* and *Ochromonas*, were also identified.

Richness and diversity indices, including Shannon and Inverse Simpson, generally increased over time within the bacterial community. Early colonization stages were characterized by lower evenness and fewer observed ASVs, both of which slightly increased as colonization progressed (mean ± SD Shannon index at $t_1 = 6.03 \pm 0.11$ and $t_5 = 6.41 \pm 0.15$; Inverse Simpson at $t_1 = 191 \pm 35.8$ and $t_5 = 244 \pm 33.9$). Linear models indicated that neither polymer type (Polyester; $\beta = -0.045 \pm 0.12$ SE, $t(12) = -0.37$, $p = 0.71$) nor color (White; $\beta = -0.025 \pm 0.12$, $t(12) = -0.22$ $p = 0.83$) had a significant effect on Shannon diversity, which showed a small, non-significant increase over time ($\beta = 0.011 \pm 0.006$ SE, $t(12) = 1.64$, $p = 0.13$). A similar pattern was observed for log-transformed Inverse Simpson diversity: polymer type ($\beta = -0.046 \pm 0.12$ SE, $t(12) = -0.37$, $p = 0.72$), color ($\beta = -0.102 \pm 0.12$ SE, $t(12) = -0.84$, $p = 0.42$), and time ($\beta = 0.006 \pm 0.007$ SE, $t(12) = 0.91$, $p = 0.38$) all had non-significant effects. Both models explained little variation in diversity and were not statistically significant.

Similarly, 18S ASV richness indices generally showed a slight increase over time, with the Shannon index rising from 2.8 ± 0.8 at t_1 to 3.8 ± 0.5 at t_5, and the Inverse Simpson index from 7.5 ± 7.6 at t_1 to

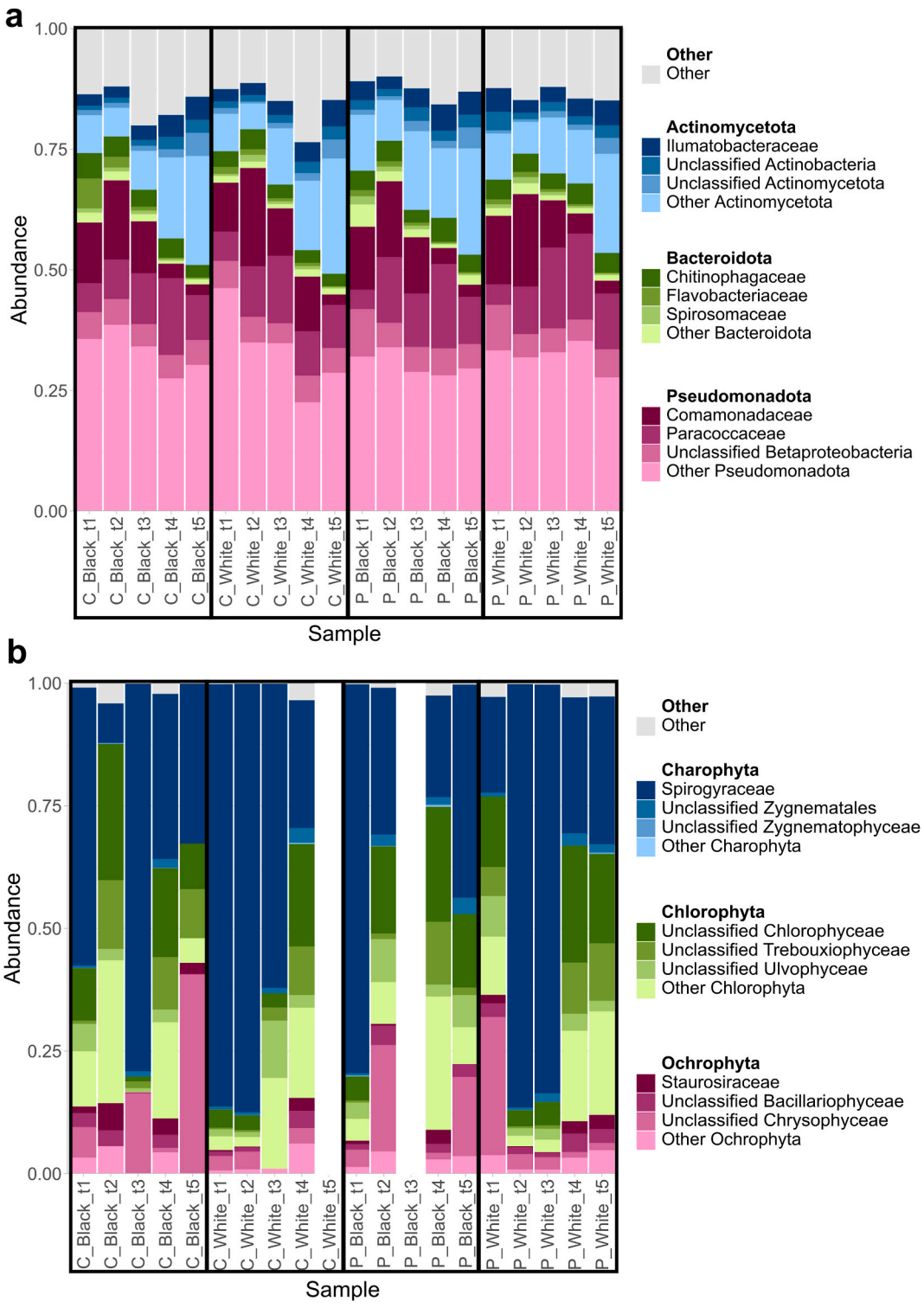


Fig. 2. Percent stacked bar chart showing the relative abundances of a) bacterial and b) microalgal ASVs across different samples: black and white cotton (C), and black and white polyester (P). Colors represent the top taxonomic rank (Phylum), while gradients of shades indicate the nested taxonomic levels (Family). The white bars in the 18S ASV plot correspond to samples with a limited number of sequences that were excluded from the analyses. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

20.0 ± 9.2 at t_5. In contrast, species richness was comparable across substrates and colors: black and white samples had Shannon indices of 3.0 ± 1.0 and 2.9 ± 1.0, and Inverse Simpson indices of 12.5 ± 8.2 and 12.4 ± 11.0, respectively; cotton and polyester samples showed average Shannon indices of 2.8 ± 1.1 and 3.1 ± 0.6, and Inverse Simpson indices of 12.9 ± 11.8 and 12.0 ± 7.6, respectively. The linear model for Shannon diversity showed a slight, non-significant increase over time ($\beta = 0.040 \pm 0.02$, $t(14) = 1.69$, $p = 0.11$), with no detectable effects of polymer type or color ($\beta = 0.014 \pm 0.46$, $t(14) = 0.31$, $p = 0.76$; $\beta = -0.020 \pm 0.47$, $t(14) = -0.42$, $p = 0.68$, respectively). In contrast, Inverse Simpson diversity increased significantly over time ($\beta = 0.052 \pm 0.21$, $t(14) = 2.45$, $p = 0.028$), while polymer type and color remained non-

significant ($\beta = -1.780 \pm 4.18$, $t(14) = -0.42$, $p = 0.677$; $\beta = -1.04 \pm 4.21$, $t(14) = -0.25$, $p = 0.809$, respectively). The Shannon model explained very little variation in diversity (adjusted $R^2 = 0.008$), whereas the Inverse Simpson model accounted for a moderate fraction of variation (adjusted $R^2 = 0.15$). In both cases, residuals were approximately normally distributed, and multicollinearity was negligible (VIF < 1.05).

3.2. Beta diversity of textile-associated biofilm

Beta-dispersion analysis on 16S rRNA community data (betadisper) showed significant differences in multivariate dispersion among the different sampling times (ANOVA on distances to group centroids: $F_{4,15} = 7.55$, $p = 0.0015$), indicating unequal within-group variability in community composition across sampling times. In contrast, dispersion did not differ significantly among color ($F_{1,18} = 0.0001$, $p = 0.99$) or

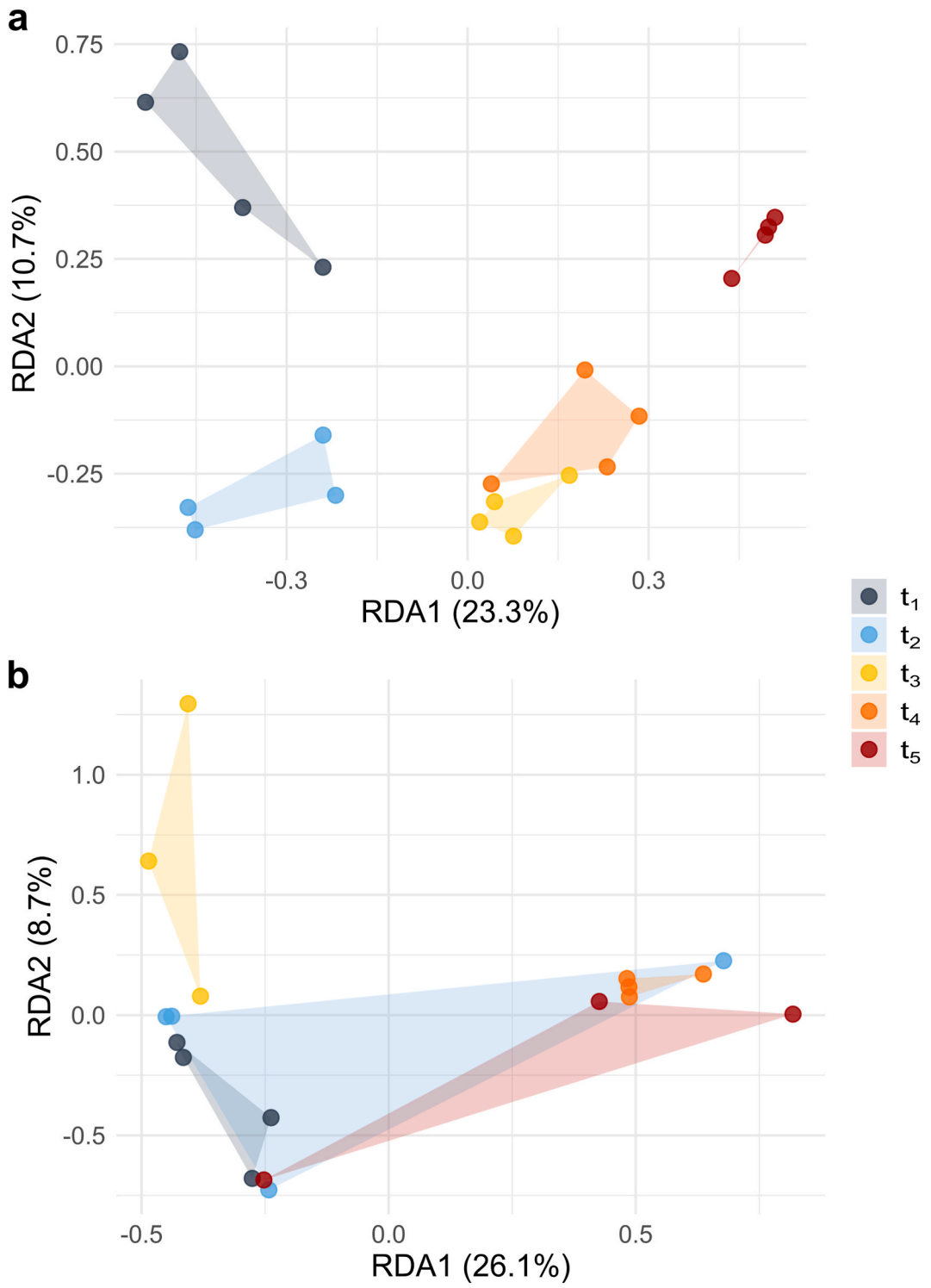


Fig. 3. Redundancy analysis (RDA) of microbial communities based on (a) 16S and (b) 18S rRNA gene data. Sample colors indicate incubation time points: t_1 (7 days), t_2 (14 days), t_3 (21 days), t_4 (28 days), and t_5 (35 days). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

polymer ($F_{1,18} = 0.3689$, $p = 0.55$). Post-hoc Tukey comparisons indicated significantly greater dispersion at t_1 relative to t_3 and t_5, with a marginally non-significant difference between t_1 and t_4 (see Supplementary Table 2 for full pairwise comparisons). These results indicate a progressive reduction in community heterogeneity over time, consistent with increased compositional homogeneity at later sampling points. Patterns of mean between-group dissimilarity were consistent with this temporal trend. Mean Hellinger distances between polymer types were highest at the initial time point (t_1, 0.891 ± 0.026) and decreased at later times, reaching lower values at t_3 (0.547 ± 0.027) before slightly increasing at t_4 (0.670 ± 0.109), indicating increased similarity between microbial communities colonizing cotton and polyester. A similar pattern was observed for color, with mean distances declining from t_1 (0.844 ± 0.099) to t_3 (0.555 ± 0.024) and modestly increasing at t_4 (0.690 ± 0.100), suggesting that communities on white and black substrates became more compositionally similar as colonization progressed. RDA of Hellinger-transformed 16S rRNA community data showed that the constrained model including polymer type, sampling time, and color explained a significant proportion of community variation (permutation test: $F_{6,13} = 2.24$, $p = 0.001$), with an adjusted R^2 of 0.28. Marginal permutation tests indicated that sampling time was the only variable that significantly explained community composition after accounting for polymer type and color ($F_{4,13} = 2.79$, $p = 0.001$), whereas neither polymer ($F_{1,13} = 1.26$, $p = 0.16$) nor color ($F_{1,13} = 1.0$, $p = 0.37$) had significant independent effects. Axis-wise permutation tests showed that the first two constrained axes were significant (RDA1: $F_{1,13} = 6.15$, $p = 0.001$; RDA2: $F_{1,13} = 2.84$, $p = 0.002$), together explaining ~34 % of the constrained variation. Consistent with beta-dispersion results, early samples were more widely distributed in ordination space, while later time points showed tighter clustering. Because multivariate dispersion differed among sampling times, the RDA was interpreted primarily as illustrating directional changes in community composition over time, rather than as evidence for distinct compositional groupings among time points. The indicator species (IndVal) analysis identified 39 ASVs typical of cotton and 21 of polyester (Fig. S2). For substrate color, 4 ASVs were typical of white and 17 of black (Fig. S3). Over time, the number of indicator ASVs increased from 50 at t_1 to 325 at t_5 (Fig. S4).

Beta-dispersion analysis on 18S rRNA data showed no significant differences in multivariate dispersion among the different treatments (time: $F_{4,15} = 7.55$, $p = 0.0015$; color: $F_{1,16} = 0.038$, $p = 0.847$; polymer: $F_{1,16} = 0.495$, $p = 0.4918$). The RDA model for the microalgal community (Fig. 3b) explained 18.9 % of the total variance (adjusted $R^2 = 0.165$) but was marginally non-significant ($F_{6,11} = 1.56$, $p = 0.068$). Indicator species analysis identified one significant ASV each associated with polyester and white substrates (classified as *Spirogyra*), and three with black substrates ($p < 0.05$). Across sampling times, indicator ASVs were detected at all time points except t_3, with counts of 12 at t_1, 6 at t_2, 10 at t_4, and 58 at t_5 (Fig. S6).

3.3. Metabolic traits of bacterial community

Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used to explore variations in microbial functional potential across the samples. Fig. 4a presents functions with relative abundances exceeding 0.01 %, while Fig. 4b shows the same data after excluding the two most dominant functions, chemoheterotrophy and, in particular, aerobic chemoheterotrophy, to highlight less abundant processes. The microbial communities exhibited a strong and conserved potential for anaerobic or facultative anaerobic nitrogen metabolism, indicating that nitrogen turnover is a central ecological process in this system and forming a tightly clustered functional group. The community also displayed substantial metabolic potential for the degradation of complex organic compounds, especially aromatic, consistent with adaptation to an organic matter–rich environment. Host-associated functional categories were consistently detected at low abundance, suggesting they play a minor ecological role in this environmental context. Interestingly, a clustering of nitrogen fixation and methylotrophy functions was observed, highlighting the presence of a range of diverse potential carbon and nutrient acquisition strategies. Overall, sample clustering indicated broadly similar functional potentials across treatments and time points, with the only exception of samples collected at the end of the experiment, consistently with the observed taxonomic shifts over time. Generally, differences were largely driven by relative abundances rather than the presence or absence of specific functions.

3.4. Abundance of the photosynthetic components

The assessment of photosynthetic picobenthos (PCY and PEUK) through flow cytometry revealed a logistic trend in community development, despite the lack of data for time point t_1 (Fig. 5). We observed a steeper increase in abundances between t_2 and t_3, followed by a more steady-state condition. PCY dominated the photosynthetic community, showing greater abundance compared to the other PEUK present. Initially, this difference was less pronounced, but it became more evident over time, with variations observed among the different categorical variables considered.

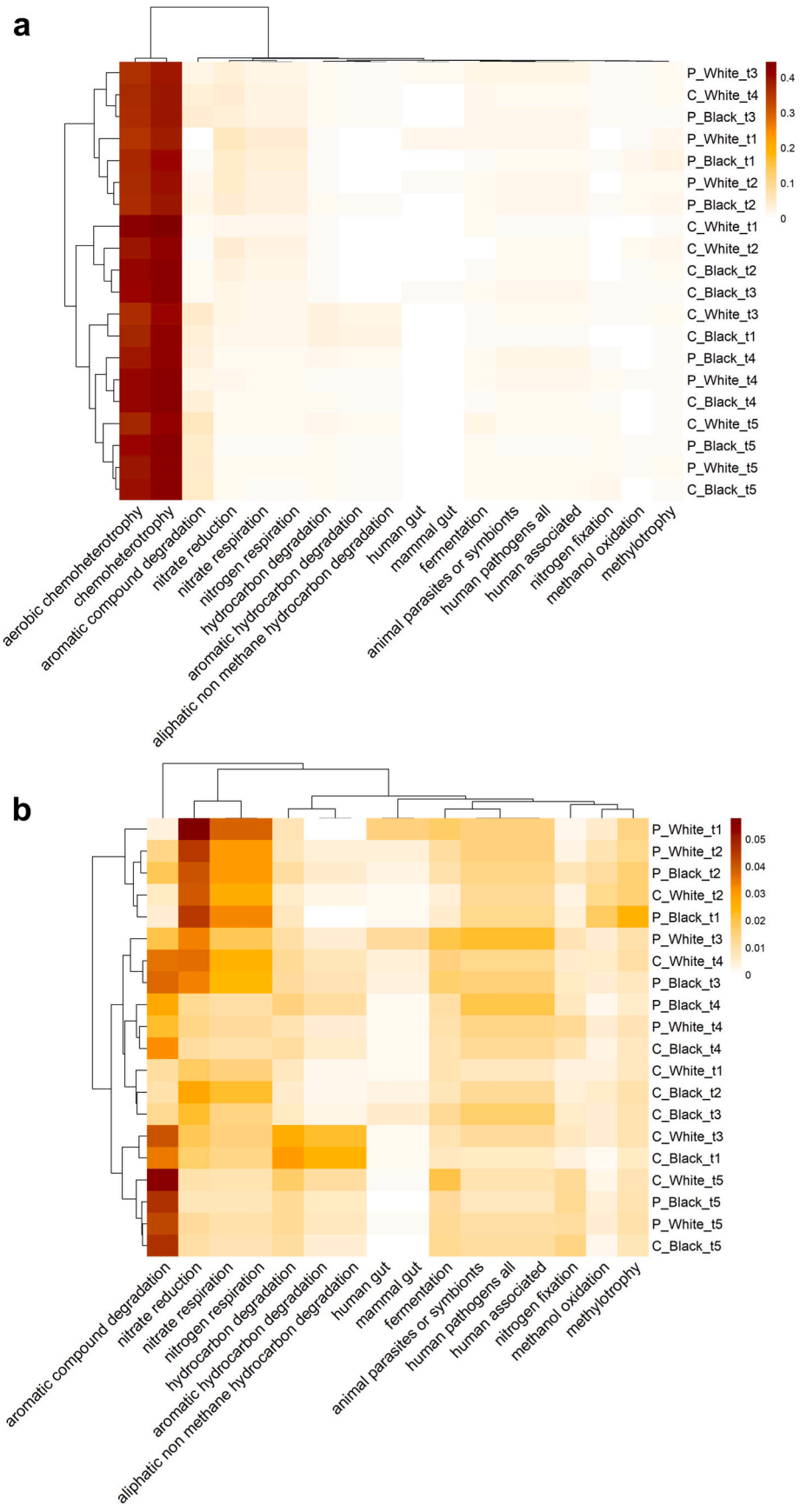
Model selection for PEUK identified the model including time and the interaction between polymer type and color as best supported (time + polymer × color; AICc weight = 0.40). Residual diagnostics confirmed an adequate fit. In this model, PEUK colonization varied significantly over time ($\chi^2(3) = 352.2$, $p < 0.001$). Polymer type also had a significant effect of smaller magnitude ($\chi^2(1) = 6.55$, $p = 0.011$), with higher cell densities on cotton compared to polyester. Although the main effect of color was not significant ($\chi^2(1) = 3.09$, $p = 0.079$), the significant polymer × color interaction ($\chi^2(1) = 4.13$, $p = 0.042$) indicates that the effect of color depended on polymer type. Specifically, cell densities differed between black and white substrates for cotton, but not for polyester. The next two models in the ranking (i.e., the main-effects model: time + polymer + color; and model without color: time + polymer) were also strongly supported (ΔAICc <1). Their slightly lower fit compared to the interaction model suggests that polymer type was a consistently important predictor, while the contribution of color was weaker but still relevant. Models including only time or the time × polymer interaction were considerably less supported (ΔAICc >3).

Model selection for PCY identified the main-effects model including additive contributions of time, polymer, and color as best supported (AICc weight = 0.53). Residual diagnostics indicated an adequate fit. In this model, cyanobacterial colonization varied significantly over time ($\chi^2(3) = 392.8$, $p < 0.001$). Both polymer type ($\chi^2(1) = 7.51$, $p = 0.006$) and color ($\chi^2(1) = 4.47$, $p = 0.034$) had significant effects, with higher cell densities observed on cotton compared to polyester and on white compared to black substrates. The next most-supported models (i.e., time + polymer without color and time + polymer × color) received strong support (ΔAICc <1.5). Models including only time or the time × polymer interaction were considerably less supported (ΔAICc >4.7).

4. Discussion

4.1. Temporal dynamics outweigh substrate-specific effects in shaping biofilm communities

Our results showed no statistically significant differences in microbial diversity or overall community composition, both for prokaryotes and photosynthetic eukaryotes, between polyester and cotton substrates, nor between colors. This suggests that the broader community-level differences observed in our experiment are likely driven by opportunistic colonization, with the particular characteristics of the tested substrates playing a limited role, although other substrate types could potentially lead to different outcomes (Caillon et al., 2021). However, while community composition was largely similar across substrates, we did detect differences in the abundances of microalgae (based on flow cytometry analyses), indicating that substrate properties



(caption on next page)

Fig. 4. Heatmap of the microbial functional potential across the different samples (rows), as predicted by FAPROTAX (*Functional Annotation of Prokaryotic Taxa*). Colors indicate the relative abundance of each function. Panel (a) shows all functions with a relative abundance greater than 0.01 %, while panel (b) shows the same data after excluding the two most frequent functions ('chemoheterotrophy' and 'aerobic chemoheterotrophy'). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

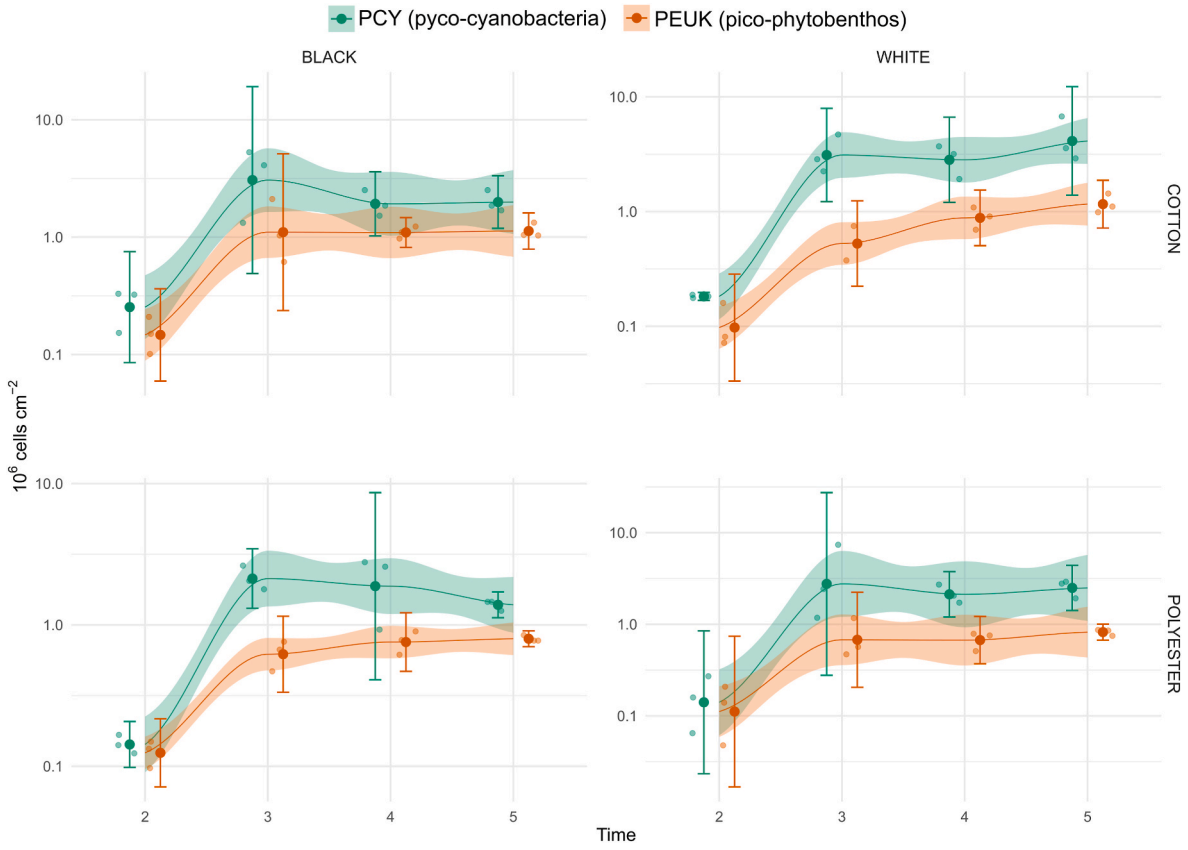


Fig. 5. Abundances of pico-phytobenthos, PEUK (orange) and pico-cyanobacteria, PCY (green) analyzed through flow cytometry over time ($t_2 = 14$ days, $t_3 = 21$ days, $t_4 = 28$ days, $t_5 = 35$ days) categorized by color (black, white) and polymer type (cotton, polyester). Note that data for t_1 is not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

may still influence aspects of biofilm development. Moreover, the presence of significant indicator species in the bacterial community indicates that substrate-specific microhabitat conditions selectively favored certain taxa, without causing a major shift in community structure as a whole. The extent to which substrate type drives differential biofilm colonization has long been debated, and previous research has highlighted substratum heterogeneity as a major source of variability in periphyton production (Besemer, 2015; Vadeboncoeur and Lodge, 2000). Much of this variability has been linked to the capacity of certain substrates (such as macrophytes or sediment surfaces) to release nutrients that fuel periphyton and microbial growth, unlike inert materials such as rocks (Vadeboncoeur and Lodge, 2000). However, additional variables may also have contributed to the observed patterns, as several factors are known to influence biofilm development and to interact with one another as well as with environmental conditions, thereby adding layers of complexity. For instance, surface roughness promotes bacterial adhesion and biofilm accumulation because irregular polymeric surfaces offer increased surface area and protective micro-depressions that facilitate colonization (Katsikogianni and Missirlis, 2004). Another relevant factor is hydrophobicity, which has been reported to exert contrasting effects: it may promote eco-corona formation and the attachment of pioneer microbes, while in other cases bacteria preferentially colonize more hydrophilic surfaces due to higher wettability and surface energy (Kingsbury et al., 2026). In addition, polymer additives can influence substrate chemistry and, consequently, microbial colonization processes (Wiesinger et al., 2021). Textile dyes and associated residues are increasingly recognized as emerging environmental concerns, with several studies demonstrating their potential toxicity (Dave and Aspegren, 2010; Lofrano et al., 2016). In our experimental setup, we hypothesize that such differences were of limited relevance, consistent with the lack of observable differences among treatments. However, these factors were not explicitly evaluated in this study and therefore warrant further investigation. Furthermore, although the experimental design included replication, the downstream 16S and 18S rRNA diversity analyses were conducted on pooled samples of three replicates (see Section 2.2). As a consequence, biological variability among replicates could not be quantified, and the statistical power to detect effects of polymer type and color was reduced. However, pooling multiple samples increased the likelihood of capturing the overall microbial diversity within each treatment.

Incubation time, on the other hand, emerged as a highly significant factor shaping the biofilm community. Flow-cytometric measurements confirmed a temporal effect on the pico-photosynthetic fraction, and sequencing data revealed clear compositional shifts over time, although these were statistically significant only for the prokaryotic community. Each weekly sampling point displayed distinct prokaryotic assemblages, indicating rapid temporal succession even within short timeframes (7-35 days). Such rapid community turnover may mask more subtle substrate-specific signals, particularly in dynamic riverine systems where hydrological variability and nutrient flux can accelerate microbial assembly

processes (Cruaud et al., 2020). The microalgal community showed less pronounced temporal differentiation, with some overlap across time points (especially at week 2). These findings are consistent with previous observations in plastisphere research, where substrate type is often found to have limited influence, while temporal dynamics are frequently reported as key drivers (Oberbeckmann et al., 2018; Silva et al., 2023; Wright et al., 2021; Zhang et al., 2024a). For instance, Wallbank et al. (2022) monitored biofilms developing on low-density polyethylene, nylon-6, and glass over 2, 6, and 12 weeks in a marine system, and they observed significant shifts in microbial composition over time across all substrates, without evidence of substrate-specific microbial communities. Similarly, Wright et al. (2021), in a meta-analysis of 35 plastisphere studies, found no consistent effects of substrate type, while some temporal variation was evident. In contrast, Ward et al. (2022b) detected both temporal and substrate-specific differences: sampling time strongly influenced community composition, with clusters forming from day 8 to day 70, and microbial communities on PVC differed from those on other plastics, likely due to the high content of plasticizers in this polymer. These findings indicate that, while general trends of temporal succession dominate across many plastics, certain substrates (especially chemically distinct ones like PVC) can exert specific effects on microbial communities, reflecting the chemical and structural diversity within this broad class of plastic materials. One reason for the generally limited influence of substrate type may lie in the physical structure of biofilms. Since only the basal layers are in direct contact with the substrate, much of the microbial community may be unaffected by its physicochemical properties. Kirstein et al. (2019) examined the tightly attached microbial fraction of the plastisphere (i.e., those communities in direct contact with the plastic surface) and proposed the existence of plastic-specific microorganisms or assemblages. These taxa, often belonging to the rare biosphere, may exploit the physicochemical properties of the plastic, suggesting a potential niche for specialized organisms. Biofilms are well known for their pronounced spatial heterogeneity: bacteria inhabiting different vertical layers experience distinct concentrations of nutrients, oxygen, metabolic byproducts, and extracellular signaling molecules (Sauer et al., 2022). This creates microenvironments that may promote niche differentiation and functional specialization within the three-dimensional biofilm matrix, a level of complexity that likely masks more subtle substrate effects when examining whole-biofilm communities.

Species turnover during biofilm development is driven by a combination of biotic and abiotic factors, with changes in community composition occurring over a broad range of temporal scales. While some systems exhibit successional shifts over years or even decades, others (particularly those subject to frequent disturbances) can undergo marked changes within just a few days (Datta et al., 2016; Fierer et al., 2010; Lyautey et al., 2005; Pollet et al., 2018). In our study, time emerged as the primary driver of microbial community composition, particularly for the prokaryotic community, among the factors considered, in the absence of variation in flow, which is a well-known determinant of biofilm dynamics (Besemer et al., 2009). This underscores the importance of considering temporal dynamics in plastisphere research, as differences in community composition may stem from distinct colonization stages rather than true substrate-specific effects, potentially resulting in misleading interpretations if time is not explicitly accounted for. It is important to note, however, that 16S/18S rRNA amplicon sequencing and flow-cytometric counts were not performed on water samples from the mesocosms during the five sampling events. As a result, it is not possible to fully assess whether the observed temporal shifts in the plastisphere reflect biofilm succession or merely mirror changes in the surrounding water community. Nevertheless, our system appeared largely stable over time with respect to the measured physical and chemical parameters (see Section 2.1 and Supplementary Materials), suggesting that no major environmental disturbances occurred. At the same time, several studies have shown that plastisphere communities are often distinct from the surrounding water microbiome (e.g., Basili et al., 2020; Dussud et al., 2018b; Li et al., 2021). For example, Yang et al. (2020) analyzed microbial communities on plastics and in water over time and found that, although both communities varied temporally, the composition on plastics consistently differed from that in the water. This indicates that plastics provide a novel niche that selectively harbors only a subset of microbial populations from the surrounding environment, with temporal dynamics that are distinct from the source water community.

When bacterial community similarity was assessed at each time point, communities on cotton and polyester were initially more distinct, showing higher average dissimilarity at the start of the experiment. Over time, this dissimilarity decreased, indicating convergence in community composition between the two substrates; a similar pattern was observed for substrate color. This pattern suggests that microbial communities tend to stabilize and converge over time, regardless of substrate features. Such temporal convergence aligns with previous observations that most surfaces in natural environments undergo predictable stages of microbial succession, often leading to similar community profiles across different substrates at later stages (Wright et al., 2021).

4.2. *Taxonomic composition is independent of substrate but with recurrent plastisphere species*

We did not find a clearly distinctive community between the “cellulosic” and plastic substrates, although some indicator species were identified and may warrant more targeted investigation. As discussed above, the differences in substrate characteristics might not be strong enough to drive completely distinct communities, though they may explain smaller-scale variations. Although numerous studies have examined microbial diversity on plastics, consistently identifying recurrent taxa remains challenging due to multiple confounding factors. Moreover, most research has focused on marine systems, leaving the identity of common and unique taxa in freshwater environments, especially lotic systems, largely unresolved. Nonetheless, some patterns and recurrent taxa emerge, pointing to either broadly adaptable generalist species or subtle substrate specificity species which are however difficult to identify without a precise taxonomic classification.

In our samples, the most abundant bacterial phyla were consistent with previous reports (Wright et al., 2021). The dominant phylum was Pseudomonadota, primarily represented by the family Paracoccaceae (formerly Rhodobacteraceae), which has been consistently reported as a core member of the plastisphere in both marine (Liu et al., 2025; Roager and Sonnenschein, 2019; Zettler et al., 2013) and freshwater ecosystems (Silva et al., 2023; Zhang et al., 2023). Members of Paracoccaceae are well-known for their role in biofilm formation, particularly in marine environments, where they often act as primary colonizers of both abiotic and biotic surfaces. Their production of extracellular polymeric substances facilitates the attachment of other microorganisms, thereby promoting biofilm maturation (Elifantz et al., 2013; Jacquin et al., 2024; Kviatkovski and Minz, 2015). Although typically associated with early colonization, they have also been observed at later stages of biofilm development (Bos et al., 2023; Elifantz et al., 2013). In our study, this family was present at all colonization times, with notably higher average abundances after 21 and 28 days. The most abundant genus in this family was *Tabrizicola*, ranging 1.0-5.9 % in the samples. Members of this genus are currently described as aerobic anoxygenic phototrophs (Tarhriz et al., 2019), able to increase their bacterial growth efficiency through light-derived energy, while growing as heterotrophs. This feature can confer an advantage over other heterotrophs, especially under carbon-limited conditions (Koblížek, 2015). The second dominant family within Pseudomonadota was Comamonadaceae, which is likewise frequently reported among the most abundant taxa in plastisphere communities. In freshwater systems, this family has been proposed as a potential marker of riverine origin (Donnarumma et al., 2024; McCormick et al., 2016), reflecting its common association with particle-attached lifestyles and its metabolic versatility in utilizing

diverse organic substrates. Notably, certain members of Comamonadaceae, such as *Ideonella sakaiensis*, are known PET degraders (Yoshida et al., 2016). In our dataset, two ASVs were assigned to the genus *Ideonella*, though additional ASVs from this genus may be present but remain unclassified. Finally, the third most abundant family of phylum Pseudomonadota was Sphingomonadaceae, mainly represented by the genus *Sphingorhabdus*, which accounted for 0.7-8.8 % per sample. Members of this genus are known biofilm-formers and were previously described as main members of the microplastic-associated plastisphere in lentic systems (Di Pippo et al., 2020).

In addition to Pseudomonadota, the phylum Actinomycetota (Actinobacteria) was also well represented, particularly by members of the family Ilumatobacteraceae, and especially by genus *Ilumatobacter*. While Actinomycetota are frequently reported in plastisphere studies (Lin et al., 2025; Y. Liu et al., 2025; Wright et al., 2021), Ilumatobacteraceae are only occasionally mentioned (e.g. Wang et al., 2024). However, this family is frequently associated with freshwater ecosystems (Mauro et al., 2025), yet most known freshwater Actinobacteria have been described as free-living bacterioplankton rather than particle-associated (Bashenkhaeva et al., 2020).

The third most abundant phylum was Bacteroidota, particularly members of the class Flavobacteriia, which were also well represented in our dataset. Flavobacteriia are frequently reported in plastisphere studies (Donnarumma et al., 2024; Kirstein et al., 2019; Wright et al., 2021) and are known for their close associations with photoautotrophic organisms and their ability to degrade complex organic matter, which may facilitate their persistence on plastic surfaces (Buchan et al., 2014). For instance, Pollet et al. (2018) found that Flavobacteriia, particularly the family Flavobacteriaceae, may play a key role in the functioning of marine biofilms on PVC, reporting relative abundances between 14.3 % and 31.7 % over 75 days of incubation and suggesting that this group could be a keystone component in the formation and functioning of these ecosystems.

Although globally less abundant, the phylum Verrucomicrobiota was particularly represented by the genus *Luteolibacter*, which ranged 1.1-4.7 % of relative abundance in the samples. Despite not being described as a common member of plastisphere communities, this genus was retrieved in oligotrophic lakes and has been suggested as a potential bacterial indicator of good-quality water (Guo et al., 2020; Veach et al., 2016).

Moreover, several functions of the prokaryotic community were inferred that are consistent with findings from previous studies on plastisphere communities, even though their relative abundances were generally low. Among the most frequently predicted functions, in addition to (aerobic) chemoheterotrophy, was the potential capacity of plastisphere-associated microbes to degrade complex organic compounds, especially aromatic, but also C1 compounds even though at a lesser extent, a feature also reported in previous studies (e.g., Deng et al., 2023, 2022). In addition, the role of the plastisphere in nutrient cycling is becoming increasingly recognized (Binda et al., 2025; Nava et al., 2024a). In particular, epiplastic communities have been suggested to influence nitrogen biogeochemistry, with several studies reporting their involvement in nitrification-related processes (Cornejo-D'Ottone et al., 2020; Su et al., 2024). Although we detected the presence of genus *Nitrospira* (on average relative abundance of 0.31 ± 0.18 %), a nitrifying bacterium (Daims et al., 2015), our analyses did not directly indicate nitrification potential. This may be related to the limitations of functional inference based on FAPROTAX assignments and to the inherent constraints of amplicon-based approaches. Consequently, more targeted analyses, such as metabolomic or process-based measurements, would be required to confirm prokaryotic active functions. Nevertheless, the identification of a cluster of taxa associated with nitrogen respiration and nitrogen fixation suggests that plastisphere biofilms may act as active nitrogen recyclers, potentially creating localized nutrient-rich microenvironments that support biofilm-associated organisms (Barros and Seena, 2021; Bryant et al., 2016; Wright et al., 2020).

When examining the microalgal eukaryotic community within the textile-associated biofilm, we notably detected a substantial presence of Charophyta, particularly the genus *Spirogyra*. This taxon appeared dominant in many samples. This filamentous alga is known for its high nutrient requirements and may have thrived under the slow flow velocities observed in our system, as its autogenic needs often restrict its development to stable flow periods (Asaeda and Hong Son, 2001; Stevenson et al., 1996). The temporal pattern of *Spirogyra* colonization was irregular with slightly higher abundances during the early colonization stage. Furthermore, no significant differences in colonization were observed between substrates, possibly because *Spirogyra* typically does not attach firmly to surfaces. Instead, it tends to form loose aggregations that associate with substrates primarily in sheltered areas protected from currents or waves (Stevenson et al., 1996). Although this genus is infrequently reported in plastisphere studies, Binda et al. (2024) conducted an experiment exposing an artificially assembled microalgal community, including *Spirogyra* sp., to pristine plastic under controlled laboratory conditions, demonstrating an increased abundance of *Spirogyra* on plastics compared to controls. This suggests that plastic surfaces can serve as viable substrates for *Spirogyra*, a genus often considered a nuisance in aquatic ecosystems (Liang et al., 2016). From a functional perspective, filamentous species play a crucial role in phytobenthic communities. Growth form is often associated with a species' capacity to dominate benthic assemblages. In particular, the stature or height within the biofilm matrix enhances an individual's ability to access light and nutrients from above (Stevenson et al., 1996). Consistent with this, our samples contained several filamentous taxa. Beyond *Spirogyra*, we identified numerous filamentous cyanobacteria, including *Planktothrix* (typically known for free-floating solitary trichomes, though benthic strains have been documented (Pancrace et al., 2017), as well as *Pseudoanabaena*, *Apatinema*, *Laspinema*, and *Plectolyngbya*. Importantly, some of these genera include potentially toxic species (Catherine et al., 2013), highlighting not only ecological and health risks associated with plastisphere communities but also possible competitive interactions within the biofilm (Allen et al., 2016). Taxa belonging to the phylum Chlorophyta were also highly abundant, although most could not be assigned to lower taxonomic levels, making it difficult to determine their precise identity, ecological role, or comparability with previous studies. Diatoms constituted another major group, dominated by pennate taxa such as *Amphora*, *Navicula*, and *Nitzschia*, species frequently reported on both plastics (Nava and Leoni, 2021) and natural substrates (Stevenson et al., 1996). Many of the most recurrent diatoms belong to the 'motile' guild, representing an alternative colonization strategy to filamentous or stalked forms. Motility allows taxa to reposition within favorable microenvironments, for example through positive phototaxis (Serôdio, 2021), and is consistent with recent findings on functional traits of diatoms colonizing plastics (Taurozzi et al., 2023). In our dataset, motility appears to confer a competitive advantage in biofilm communities, as this trait occurs across distinct phylogenetic groups. Among the Chlorophyta identified, *Chlamydomonas* stands out as a motile genus whose flagella not only facilitate surface contact and adhesion but also enable active movement (Kreis et al., 2018).

5. Conclusions

Our study provides insights into the prokaryotic and eukaryotic communities of textile-associated biofilms in a slow-flowing lotic system, highlighting potential ecological implications for riverine environments where plastics can accumulate. We observed a pronounced temporal increase in microbial abundances, which tended toward a steady state rather than declining, indicating that plastic textiles, similarly to their cellulose counterparts, can sustain biofilm growth over time. This persistence may have consequences for aquatic ecosystem functioning, including productivity and metabolism, as witnessed by the potential dominance of functions such as organic compound degradation, mainly towards aromatic and C1 molecules, and nitrogen cycling. The presence of nuisance and potentially toxic species underscores

additional ecological and health concerns. No significant differences emerged among synthetic and 'natural' substrates, suggesting that colonization is largely opportunistic. Although taxa frequently reported in plastisphere research were present, strong substrate specificity was not detected; any differences, if they exist, may occur at finer taxonomic or functional levels beyond the resolution of current methodologies, warranting further investigation. In contrast, temporal succession played central role in shaping community composition. Community development was rapid, emphasizing the importance of accounting for colonization time when comparing studies or interpreting patterns, as it can represent the primary driver of variation.

CRediT authorship contribution statement

Veronica Nava: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Katrin Attermeyer:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Jakob Schelker:** Writing – review & editing, Methodology, Investigation. **Jovan Kalem:** Writing – review & editing, Investigation. **Flavia Dory:** Writing – review & editing, Software. **Isabella Gandolfi:** Writing – review & editing, Software, Investigation. **Roberto Ambrosini:** Writing – review & editing, Software, Formal analysis. **Afsané Kruszelnicki:** Writing – review & editing. **Claire Gwinnett:** Writing – review & editing. **Barbara Leoni:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used ChatGPT in order to improve the writing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.127881.

Data availability

All data and analysis scripts used for the statistical analyses are available in a Zenodo repository (https://doi.org/10.5281/zenodo.18340890). Raw DNA sequences of 16S/18S rRNA were submitted to Sequence Read Archive (SRA-NCBI), with accession numbers SAMN54195237-SAMN54195276, under BioProject PRJNA1390670.

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