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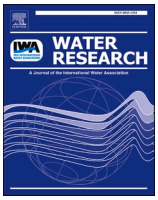

Plastic pollution affects ecosystem processes including community structure and functional traits in large rivers

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ABSTRACT

Plastics in aquatic ecosystems rapidly undergo biofouling, giving rise to a new ecosystem on their surface, the 'plastisphere.' Few studies quantify the impact of plastics and their associated community on ecosystem traits from biodiversity and functional traits to metabolic function. It has been suspected that impacts on ecosystems may depend on its state but comparative studies of ecosystem responses are rare in the published literature. We quantified algal biomass, bacterial and algal biodiversity (16S and 18S rRNA), and metabolic traits of the community growing on the surface of different plastic polymers incubated within rivers of the Lower Mekong Basin. The rivers selected have different ecological characteristics but are similar regarding their high degree of plastic pollution. We examined the effects of plastics colonized with biofilms on ecosystem production, community dark respiration, and the epiplastic community's capability to influence nitrogen, phosphorus, organic carbon, and oxygen in water. Finally, we present conceptual models to guide our understanding of plastic pollution within freshwaters. Our findings showed limited microalgal biomass and bacterial dominance, with potential pathogens present. The location significantly influenced community composition, highlighting the role of environmental conditions in shaping community development. When assessing the effects on ecosystem productivity, our experiments showed that biofouled plastics led to a significant drop in oxygen concentration within river water, leading to hypoxic/anoxic conditions with subsequent profound impacts on system metabolism and the capability of influencing biogeochemical cycles. Scaling our findings revealed that plastic pollution may exert a more substantial and ecosystem-altering impact than initially assumed, particularly in areas with poorly managed plastic waste. These results highlighted that the plastisphere functions as a habitat for biologically active organisms which play a pivotal role in essential ecosystem processes. This warrants dedicated attention and investigation, particularly in sensitive ecosystems like the Mekong River, which supports a rich biodiversity and the livelihoods of 65 million people.

1. Introduction

Several studies have shown the ubiquitous and pervasive presence of plastic pollution in the aquatic environment, and the occurrence and transfer of plastic debris between different pools have led to the recognition of a "plastic cycle", which is akin to global biogeochemical cycles (Rochman and Hoellein, 2020). While research primarily has focused on plastic pollution in marine ecosystems, there is a growing acknowledgment of the impacts of plastic pollution on freshwater ecosystems. The relevance of inland waters as pathways for plastics from land to ocean is widely recognized. Various models have been devised to estimate the fluxes. Mai et al. (2020) reported values ranging from 57,000 to 265,000 metric tons per year across 1518 main rivers. Alternatively, Meijer et al. (2021) estimates suggest that 1656 rivers contribute to 80 % of global

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annual emissions, with figures ranging between 0.8 million and 2.7 million metric tons per year. While the consensus acknowledges the role of lakes and rivers in the transportation of plastics, less attention has been given to their capacity to act as sinks for plastic accumulation (Gallitelli and Scalici, 2022; Hoellein and Rochman, 2021). However, recent findings suggest that freshwater systems can accumulate plastics (micro and macro) at similar or even higher rates than marine systems, underscoring their equal susceptibility to plastic pollution (Blettler et al., 2018; Cera et al., 2023; Nava et al., 2023).

Plastics and microplastics, which can result from the breakdown of larger plastic objects or can be directly produced in micrometer sizes (Hartmann et al., 2019), present a danger to aquatic organisms from the cellular to the population level of organization (Scherer et al., 2018). Currently, our understanding of the biologic impacts caused by these pollutants is relatively limited. The toxic effects of plastics are influenced not only by the type of species affected and the size and shape of the plastic debris, but also by polymer-dependent or by-product toxicities (e.g., phthalate esters, PAEs) (Coffin et al., 2022; Rochman, 2015). The consequences of plastic pollution can be far more extensive than expected and could encompass a variety of functional processes in aquatic ecosystems. For instance, a recent study demonstrated that plastic-derived leachate is chemically distinct and more bioavailable than natural organic matter of lakes, strongly promoting bacterial growth (Sheridan et al., 2022).

Plastic debris provides a substrate for biofilm colonization and thus, habitats for rafting organisms (i.e., epiplastic community) (Wright et al., 2020). Similar to other surfaces introduced into aquatic ecosystems, plastic debris quickly becomes biofouled. This new artificial ecosystem on the surface of plastics, which has been defined as the 'plastisphere', includes microbial communities composed of diverse prokaryotes, microalgae, and fungi, and can also include macroorganisms (Barros and Seena, 2021; Wright et al., 2020; Zettler et al., 2013). The colonized substrates can harbor pathogens and studies showed that plastic debris may serve as carbon sources for organisms that grow on their surfaces or favor organisms with specific metabolic traits. For example, Su et al. (2022) demonstrated that biofilm formation on plastic provides conditions that promote denitrifiers, resulting in higher denitrifying activity and N_2O production compared to the surrounding bulk water. Additionally, the presence of photosynthetic organisms within the plastisphere (Nava and Leoni, 2021) suggests potential effects on primary productivity. However, the significance of these processes in global productivity remains unknown and, presently, it is challenging to extrapolate or scale up this evidence to broader contexts (Jacquin et al., 2019; Troost et al., 2018; Zhao et al., 2021).

While numerous studies have investigated the biodiversity of the plastisphere, few have ventured into understanding the impact of these communities on the functionality and metabolism of aquatic ecosystems (e.g. Chaudhary et al., 2022; Vincent et al., 2022). In this study, we aimed to address this gap by quantifying the broader effects of plastic debris on epiplastic biofilm community development and by assessing the resulting consequences on ecosystem metabolic traits (i.e., net ecosystem production (NEP), gross primary production (GPP), respiration (R), and community dark metabolism (CDR)) from 3 rivers with contrasting trophic state and water clarity (Bassac (BAS); Mekong (MKG); and Tonle Sap (TS)) within one of the most biodiverse and functionally important regions of the world, the Lower Mekong River basin. Notably, the Mekong River is one of the largest contributors to plastic mass load to the ocean (Haberstroh et al., 2021; Van Emmerik et al., 2023). Over a 30-day period, we incubated four different plastic polymers (polyethylene (PE_30d); polypropylene (PP_30d); polystyrene (PS_30d); polyamide (PA_30d)) and collected additional macroplastics of an unknown submergence time (PE_unk), characterizing the algal biomass, bacterial and algal biodiversity, and metabolic traits of the community growing on their surface. The overarching objective is to understand whether plastics with different polymeric compositions can represent a new habitat for rafting microorganisms and to what extent these pollutants can influence microbial and microalgal biodiversity and energy fluxes in aquatic ecosystems. We hypothesize that the enhanced surface area created by plastic pollution promotes the growth of microorganisms, which exhibit autotrophic or heterotrophic traits depending on environmental factors such as clarity and nutrient levels. Consequently, plastic pollution is expected to influence water quality parameters, potentially leading to changes in oxygen levels, and causing variable effects on net ecosystem productivity. In particular, this study aims to quantify: i) whether the type of plastic or the environmental conditions of the site is the dominant driver in determining the amount of photosynthetic biomass within the biofilm, the prokaryotic and eukaryotic diversity, and microorganism specific metabolic traits; ii) the effects of the plastisphere on ecosystem production (respiration, gross, and net), community dark respiration processes, and the capability of the epiplastic community in driving changes in the chemical species of nitrogen, phosphorus, organic carbon and oxygen within water; and iii) the reciprocal relationships among species diversity, biomass development, and primary productivity, in the context of spatial variability.

2. Methods

2.1. Study site

The Mekong River is one of the most biodiverse rivers in the world, harboring a variety of aquatic organisms, including several critically endangered species, and sustaining 65 million people that live in the basin and depend on this biodiversity (Dudgeon et al., 2006; The Mekong River Commission et al., 2019). With a length of 4909 km, the Mekong River is the 12th longest in the world and the 3rd longest in Asia, draining a total area of 795,000 km^2 (Soukhaphon et al., 2021). It originates in China from the high altitude of the Tibetan Plateau and flows south through five countries (Myanmar, Lao PDR, Thailand, Cambodia, and Vietnam). The Lower Mekong Basin, which stretches from Lao PDR to the Vietnam Delta, accounts for 80 % of the overall Mekong drainage system and is distinguished by important tributaries such as the 3S rivers (Sekong, Sesan, and Srepok) and Tonle Sap system. This basin has a pronounced flow variation between the wet (May–October) and dry (November–April) season, resulting in a noticeable flood pulse that serves as the foundation for its exceptional productivity and biodiversity (Piman et al., 2013; Sor et al., 2021).

The research has been developed in the Lower Mekong Basin, specifically at the confluence of the Mekong, Tonle Sap and Bassac rivers adjacent to Phnom Penh (Cambodia), and three sampling sites were selected for macroplastic substrates incubation, one from each river (i.e., Bassac River, BAS; Mekong River, MKG; and Tonle Sap River, TS; Fig. 1). We selected these sites due to their ecological significance for supporting the rich fisheries and biological biodiversity in the region, while also considering water quality conditions and proximity to our laboratory. Long-term data from the three sites were gathered from the Ministry of Water Resources and Meteorology (Cambodia) specifically focusing on electrical conductivity (EC), dissolved oxygen (DO), total suspended solids (TSS), total phosphorus (TP), nitrate (NO_3^-), and ammonium (NH_4^+; Fig. S1). These data reveal considerable variations among the sites, especially during the dry season when our experiments were conducted. Specifically, the mean monthly data indicates lower water clarity in TS, followed by BAS (as evidenced by TSS). MKG exhibited higher EC and DO levels, while nutrient concentrations showed variability among sites.

2.2. Experimental design

Semi-natural field experiments were performed to evaluate the bacterial and algal colonization of plastics with different polymeric compositions and to quantify their influence on productivity. Four distinct pristine macroplastic substrates (polyethylene and polypropylene film, polystyrene foam, and polyamide net) were employed in

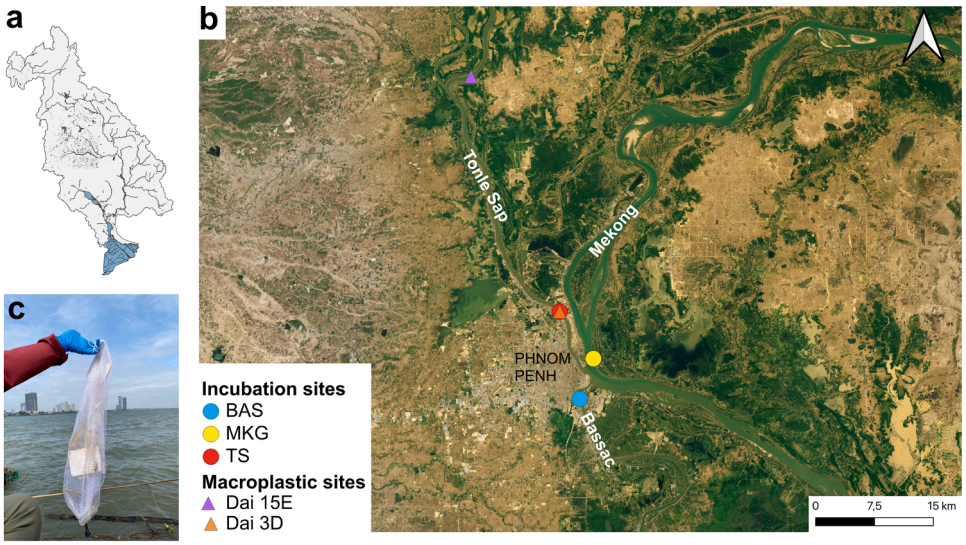

Fig. 1. The map displays: a) the Lower Mekong Basin (LMB); b) the specific locations of the three incubation sites situated at the confluence of Bassac (BAS), Mekong (MKG), and Tonle Sap (TS) River near Phnom Penh, Cambodia and the two Dai Fisheries (Dai 15E, Dai 3D) where macroplastic samples were collected (note: the TS sampling site and Dai 3D coincide); and c) accompanying picture showcasing the incubated nets with the different plastic substrates.

Table 1
Summary of the experiments performed with indication of the analyses done, the type and polymeric composition of the plastic substrates used, the method adopted, the site in which the experiment was conducted and the total number of samples analyzed. Each experiment was conducted with separate replicates of the substrates, except for experiment 6, in which nutrients were measured in bottles upon completion of experiments 4 and 5.

Experiment number	Analysis	Plastic polymeric composition	Incubation period before collection (days)	Method of collection	Site	Number of samples
1	Photosynthetic biomass measured as chlorophyll *a*	PE PP PS PA PE	30 Unknown	Scraped with sterile toothbrushes	Mekong (MKG) Bassac (BAS) Tonle Sap (TS)	**17** (4 substrates × 3 sites) + 5 PE_unk
2	Bacterial 16S rRNA Algal 18S rRNA	PE PP PS PA PE	30 Unknown	Scraped with sterile swabs	Mekong (MKG) Bassac (BAS) Tonle Sap (TS)	**17** (4 substrates × 3 sites) + 5 PE_unk
3	High-frequency dissolved oxygen sensors	All plastics together (PE, PP, PS, PA)	30	All plastic substrate together in containers with filtered waters	Mekong (MKG)	**4** ((1 sample (all plastics) + 1 reference) × 2 replicates)
4	Ecosystem production (light/dark)	PE PP PS PA	30	Transfer of each plastic substrate with the same area to individual light/dark bottles (3 days measurements) with filtered water	Mekong (MKG) Bassac (BAS) Tonle Sap (TS)	**45** ((4 substrates + 1 reference) × 3 sites × 3 replicates)
5	Community dark respiration (dark)	PE PP PS PA	30	Transfer of each plastic substrate with the same area to individual dark bottles (3 days measurements) with filtered water	Mekong (MKG) Bassac (BAS) Tonle Sap (TS)	**45** ((4 substrates + 1 reference) × 3 sites × 3 replicates)
6	Nutrient transformation (light/dark)	PE PP PS PA	30	Measurement of nutrient concentration in light/dark bottles after completing experiment 4/5	Mekong (MKG) Bassac (BAS) Tonle Sap (TS)	**90** ((4 substrates + 1 reference) × 3 sites × 3 replicates × 2 treatment (light/dark)
7	Macroplastic counts	/	/		Tonle Sap (TS): Dai Fishery 3D, Dai Fishery 15E	/

the study, subsequently referred to as PE_30d, PP_30d, PS_30d, and PA_30d, respectively. These polymers were selected to reflect the types of plastics commonly encountered in the study sites, each exhibiting varying thickness and rigidity. Each substrate, with an average dimension of 82 ± 14 cm^2, was securely attached to a polyamide net bag with a mesh size of 0.4 cm using staples. No fixed orientation was applied to the substrates. Additionally, a weight was attached at the end of each bag to ensure submergence throughout the experiment. Subsequently, the bags were submerged within each river on piers near the bank, positioned at a depth of 10 cm below the water surface, and left submerged for an incubation period of 30 days (Fig. 1c). Furthermore, two additional macroplastics of an unknown submergence time were collected at each site (except for BAS, where only one macroplastic was taken) for comparison with the incubated plastics. The polymeric composition of these

macroplastics was determined to be PE (Table S1). The spectra displayed signals indicative of consistent weathering; however, it is not possible to accurately determine the duration of their presence in the three rivers (thereafter, 'PE_unk' representing polyethylene of unknown incubation time).

Polymeric characterization of all plastic polymers was conducted through micro-Raman Spectroscopy utilizing a Horiba Jobin Yvon LabRAM HR Evolution spectrometer. Raman spectra were baseline-corrected and processed using the 'Fityk' program and the R package 'RamanMP' (Nava et al., 2021). The final identification of macroplastics was based on individual assessment of each spectrum (Fig. S2). Previous data about plastic pollution in the Mekong River and consideration of the polymers more abundantly found in freshwater ecosystems worldwide informed the selection of which plastic polymers were to be incubated (Haberstroh et al., 2021; Nava et al., 2023).

After the elapsed incubation time, the substrates were collected using stainless steel tweezers and transferred to sterile aluminum trays containing river water from each location. Any loosely attached materials were discarded and only those firmly adhered to the substrates were retained for further analysis. The substrates were then divided into groups for analysis of the biofilm community and ecosystem metabolic traits: chlorophyll *a* measurement, DNA extraction and taxonomic marker gene sequencing, and primary productivity assessment. Table 1 provides a summary of the experiments and detailed information on analyses conducted, types of plastic used, duration of the experiment, sampling sites, and replicate numbers.

2.2.1. Photosynthetic biomass measured as chlorophyll a (experiment 1)

We analyzed chlorophyll *a* on the surface of plastic substrates, as an indicator of autotrophic organism biomass. Biomass was removed from a known surface area using a sterile toothbrush, transferred on ice to the laboratory at Cambodia's Inland Fisheries Institute and frozen until analysis. Pheophytin-corrected chlorophyll *a* was determined via ethanol extraction and quantified using a Turner Designs AquaFlor fluorometer.

2.2.2. DNA extraction, amplification and sequencing of bacterial and algal communities (experiment 2)

The analysis of 16S rRNA was employed to identify and classify bacteria on the surface of plastics, while 18S rRNA was used for the identification and classification of algal eukaryotes. Samples for DNA analysis were collected using a sterile swab and frozen at -20°C before extraction. The FastDNA® Spin for Soil kit (MP Biomedicals, Solon, OH, USA) was utilized to extract DNA, following the instructions provided by the manufacturer. To amplify the V5-V6 hypervariable regions of 16S rRNA, bacterial primers 783F and 1046R were used, with Illumina adapters (6 bp) added at the 5′ end (Huber et al., 2007). The V4 hypervariable region of the 18S rRNA gene was amplified using the eukaryotic primers 528F and 706R (Cheung et al., 2010). For the amplification of the 16S rRNA gene, 2 × 50 μL PCR reactions were prepared for each sample with GoTaq® G2 Green Master Mix (Promega Corporation, Madison, WI, USA) and 1 μM of each primer. The cycling conditions involved an initial denaturation at 94°C for 4 minutes, followed by 27 cycles of 94°C for 50 seconds, 47°C for 30 seconds, and 72°C for 45 seconds. A final extension step was performed at 72°C for 5 minutes. PCR amplification of the 18S rRNA gene was performed as described in Di Mauro et al. (2020). Following the purification of amplicons using the Wizard® SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI), their quantity was determined using the Qubit® fluorometer (Life Technologies, Carlsbad, CA). Subsequently, the regions were subjected to sequencing using the MiSeq Illumina platform (Illumina, Inc., San Diego, CA) employing a 2 × 300 bp paired-end protocol. Operational Taxonomic Units (OTUs) were defined by clustering sequences at 97 % identity for both the 16S and 18S rRNA gene fragments. To ensure data quality, the forward and reverse reads were merged only if they exhibited zero mismatches and were discarded if the maximum number of expected errors was higher than 0.5 per read. Additionally, any suspected chimeras and global singleton sequences (i.e., sequences appearing only once in the entire dataset) were eliminated (Di Mauro et al., 2020; Pittino et al., 2018). Taxonomic classification of the OTU representative sequences was obtained by RDP classifier for 16S rRNA, and by SILVA SSU Ref NR 99 database for 18S rRNA.

2.2.3. Water quality dissolved oxygen dynamics using a high-frequency sensor (experiment 3)

To understand the cumulative effect of plastics on dissolved oxygen, we placed pieces of all the different plastic polymers (i.e., PE_30d, PP_30d, PS_30d, PA_30d) that had been colonized by microorganisms from the Mekong River site into 18L containers with filtered water. These containers were exposed to ambient surface irradiance over a diel cycle for three days. Dissolved oxygen levels (mg L^{-1}) were measured at a high frequency (every 10 minutes) using an optical oxygen probe (PME miniDOT Logger). The experiment had two replicated treatments: 1) container with plastics added as described above and 2) a reference container without added plastics.

2.2.4. Ecosystem production (experiment 4), community dark respiration (experiment 5) and nutrient transformations (experiments 6)

We obtained metabolism data by measuring the oxygen dynamics in light-dark bottle incubations (experiment 4). Plastics were incubated *in situ* for 30 days at each site to capture the maturation phase of the biofilm and allow for comparison with prior studies that utilize this time frame (Cheng et al., 2021; Silva et al., 2023). Following the 30-day incubation, each of the four plastic substrates was placed in both light and dark 250 mL glass BOD bottles. Each bottle condition (plastic type × light-dark) was replicated three times. The bottles were filled with pre-screened water (screen size of 125 μm) from each river location to remove invertebrates and debris. To assess the potential effects of water on metabolic rates, we also included reference samples with only screened water. All bottles were incubated outside the laboratory, exposed to the ambient day and night cycles of surface irradiance. We measured the dissolved oxygen (DO, mg L^{-1}) using an optical dissolved oxygen and temperature probe (ProSolo DIGITAL YSI) along a diel cycle in the morning and evening at dawn and soon after dusk, respectively. From light bottles incubated in light-dark conditions, we calculated daily estimates (mmol O_2 m^{-3} h^{-1}) for respiration (R), net ecosystem production (NEP), and gross primary production (GPP; GPP = NEP + R).

Dark bottles were used to estimate community dark metabolism by measuring oxygen consumption (experiment 5). This experiment simulates reactions occurring well below the influence of the photic zone, such as those at the river bottom. Our visual observations confirm the accumulation of plastics in close proximity to or along the river bottom. It was assumed that the entirety of dissolved oxygen depletion resulted from microbial aerobic respiration, which was the prevalent oxygen-consuming process in our systems, with each unit reduction in O_2 corresponding to an equivalent unit of CO_2 production. The computed CO_2 production during the incubation period was then multiplied by the ratio of the atomic mass of carbon to oxygen (0.375) using the following equation:

$$CDR = \frac{\left([O_2]_i - [O_2]_f\right)}{t} \cdot 0.375$$

where CDR = community dark metabolism, $[O_2]_i$ = initial oxygen, $[O_2]_f$ = final oxygen, and t = time.

Furthermore, we analyzed changes in the chemical species of nitrogen (NH_4^+, NO_3^-), phosphorus (SRP), and organic carbon (DOC) by measuring their concentrations at the beginning and end of the light/dark bottle incubation (experiment 6). Firstly, the samples were filtered through Whatman GF/F glass fiber filters. For DOC analysis, Shimadzu TOC-L was used (Shimadzu Corporation, 2003). Phosphorus and nitrogen species were analyzed colorimetrically following the Standard

Methods for the Examination of Water and Wastewater; SRP was analyzed using the ascorbic acid method (Murphy and Riley, 1962), NH_4^+ using the phenol-hypochlorite method (Solórzano, 1969), and NO_3^- using the hydrazine reduction method (Kamphake et al., 1967). Data of nutrients in dark bottles of TS are missing.

2.3. Macroplastic counting (experiment 7)

High concentrations of plastic pollution have been documented in the geographical area of this study (Haberstroh et al., 2021). To gauge the surface area provided by the plastic for the plastisphere community and assess the subsequent potential magnitude of changes to metabolic and water quality traits from plastics, we collected and counted macroplastics from the Tonle Sap River. To conduct the macroplastic sampling, we leveraged the stationary trawl fishing platforms (locally referred to as 'Dai' fishery) occurring along the Tonle Sap River. We sampled from Dai 3D located within the city of Phnom Penh and Dai 15E located approximately 35 km to the north in Kandal Province, a more rural area (Fig. 1b). Dai nets were deployed for one hour, after which the litter was separated from the fishing catch, sorted by litter type, and reported as item hour^{-1}. Three replicates were collected for each site (Fig. S3).

The litter was initially sorted into plastic and non-plastic materials, and subsequently categorized into litter subtypes based on item type (e.g., plastic bags, bottles, straws), similar to other litter sorting approaches (Cheshire et al., 2009; Davidson et al., 2023; Hapich et al., 2022). For example, many of the item types were similar to the UNEP/IOC marine litter scheme (Cheshire et al., 2009), however, we added plastic bags (small), plastic bags (medium), and plastic bags (large) as separate categories (Table S1) to more accurately determine surface area. Following the sorting process, we photographed each category and estimated the surface area. Table S1 presents a comprehensive list of all 18 plastic type categories considered.

2.4. Statistical analyses

All statistical analyses were completed in R (version 4.2.2), using the packages 'ggplot2' (Wickham, 2009), 'vegan' (Oksanen et al., 2015), 'phyloseq' (McMurdie and Holmes, 2013), 'indicspecies' (Cáceres and Legendre, 2009), 'forecast' (Hyndman and Khandakar, 2008), 'tidyr' (Wickham et al., 2023) and 'zoo' (Zeileis and Grothendieck, 2005). To assess the statistically significant differences among the various sites and substrates for chlorophyll *a*, diversity indices, and metabolic traits of the bacterial community, the Kruskall-Wallis test was utilized (Kruskal and Wallis, 1952). For the high-frequency data of dissolved oxygen obtained from the optical oxygen probe, a screening process was applied to remove potential outliers. This screening was achieved using the running lines smoother method proposed by Friedman in 1984, employing the 'supsmu' function from the 'forecast' package. We performed an analysis of covariance (ANCOVA) to compare Dissolved Oxygen (DO) rate among replicated treatments (with plastic addition and without plastic addition). Time was included as a covariate in the model, with the interaction term also incorporated. To assess the productivity data (R, NEP, GPP), we employed a Dunnett's test (Dunnett, 1955). This test was utilized to compare each of the response variables from the various plastic incubation treatments with the reference (water). For the assessment of bacterial and algal alpha diversity in the Operational Taxonomic Units (OTUs) resulting from DNA analyses, we calculated diversity indices using the 'phyloseq' package. These diversity indices include Chao1, an abundance-based estimator of species richness that considers rare species; Shannon, an estimator that evaluates both species richness and evenness, with greater weight on species richness; Simpson, measuring the probability that two randomly selected individuals from a sample belong to the same species (Kim et al., 2017). Before conducting these calculations, data were rarefied using the 'vegan' package. To explore the beta diversity of OTUs for both 16S and 18S rRNA, nonmetric multidimensional scaling (NMDS) analysis based on Bray-Curtis dissimilarities calculated on normalized data was employed. ANalysis Of SIMilarity (ANOSIM) was applied to determine whether significant differences in the OTU community composition were present among the sites and the different substrates.

3. Results

3.1. Photosynthetic biomass

Photosynthetic biomass, represented as total chlorophyll *a* concentration, found on the surface of the different plastic polymers was generally low, with average values of 0.13 ± 0.18 μg cm^{-2} (median 0.07 μg cm^{-2}). The greatest concentration was observed on PS_30d in TS. Although not statistically significantly different, we observed comparatively higher values of photosynthetic biomass on average on PS_30d, and lower on PE_unk (Fig. 2a).

Considering the different sites, the highest mean value was identified in MKG, while the lowest in BAS, excluding the outlier value observed on PS_30d in TS. However, the observed differences are not statistically different (Fig. 2b).

3.2. Community composition and biodiversity

3.2.1. Alpha diversity and taxonomic community structure

Using Illumina sequencing, we obtained 8652-116,006 sequences of the V5–V6 hypervariable regions of the 16S rRNA gene per sample. Overall, these sequences clustered in 3220 Operational Taxonomic Units (OTUs). The two most abundant phyla were Proteobacteria and Actinobacteria. On average, Proteobacteria accounted for 55.8 ± 11.1 % of the bacterial communities across all samples, while Actinobacteria constituted approximately 20.0 ± 6.7 % (Fig. 3a). Cyanobacteria represented only 3.3 ± 3.8 % of the total community. Examining the relative abundances at the family level, the top three families in each sample represented a substantial proportion of their total microbial community, ranging from 21.6 % (on PP_30d in BAS) to 66.0 % (on PE_unk in TS). The families which showed broader distribution and relevance throughout the dataset were Nocardiaceae, which accounted for an average relative abundance of 7.9 % ± 5.4 %, and Comamonadaceae and Rhodobacteraceae, which exhibited relative abundances of 6.2 % ± 5.1 % and 6.0 % ± 4.2 %, respectively (Fig. 3b).

When focusing on the sites, MKG exhibited the highest number of unique bacterial OTUs within the epiplastic community (440). Regarding the different plastic substrates, the biofilm on plastics with an

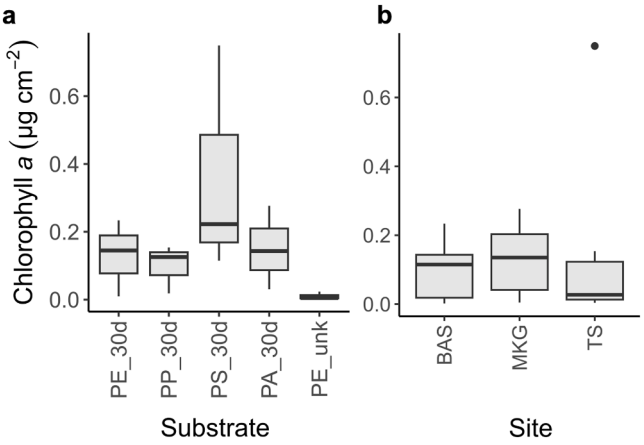

Fig. 2. Chlorophyll *a* concentration on surface area observed among (a) plastic polymers (PE_30d, polyethylene; PP_30d, polypropylene; PS_30d, polystyrene; PA_30d, polyamide; PE_unk, polyethylene collected from the sites); (b) sites of incubation BAS (Bassac River); MKG (Mekong River); TS (Tonle Sap River).

unknown submergence time (PE_unk) displayed the highest count of unique OTUs (284). The greatest number of shared OTUs was observed in the biofilm across the sites between MKG and BAS (681), and for the substrates between PE_30d and PE_unk (71; Fig. 3c,d).

When examining the different plastic substrates, the Shannon, Simpson and Chao1 indices displayed slightly higher values for PA_30d, indicating a diverse and evenly distributed community. In contrast, lower values were observed for PS_30d; however, these differences are not remarkable, and no significant differences were highlighted by Kruskal–Wallis test among substrates. When comparing the different sites, higher values were found in BAS for Shannon and Chao1 and in MKG for Simpson and lower in TS; nevertheless, no significant differences were observed in this case as well (Fig. S4).

In the analysis of the 18S rRNA gene, the number of sequences specific to microalgal organisms was substantially lower when compared to the bacterial 16S rRNA gene, ranging from 1172 to 20,015 sequences per sample. These sequences were grouped into 206 OTUs. Among these, the two most dominant phyla were Ochrophyta and Chlorophyta, constituting approximately 63.6 % ± 19.8 % and 30.4 % ± 19.3 % of the microalgal communities across all samples, respectively (Fig. 4a). Classification at the order level was only possible in a limited number of cases, resulting in an average relative abundance of unclassified sequences of 96.9 ± 2.1 %. The highest number of unique OTUs was found in TS (16), considering the site, and in PA_30d and PE_unk, with only 2 unique OTUs, considering the different incubated substrates (Fig. 4b,c).

The observed number of OTUs was higher in PE_unk, with average values of 85.2 ± 5.8. Slightly higher values of the Shannon and Simpson indices were observed for PS_30d. When considering different sites, higher values were observed for all metrics in BAS, except for Chao1, which reached its highest value in MKG (Fig. S5). Similar to the results reported for the bacterial community (16S rRNA), there were no

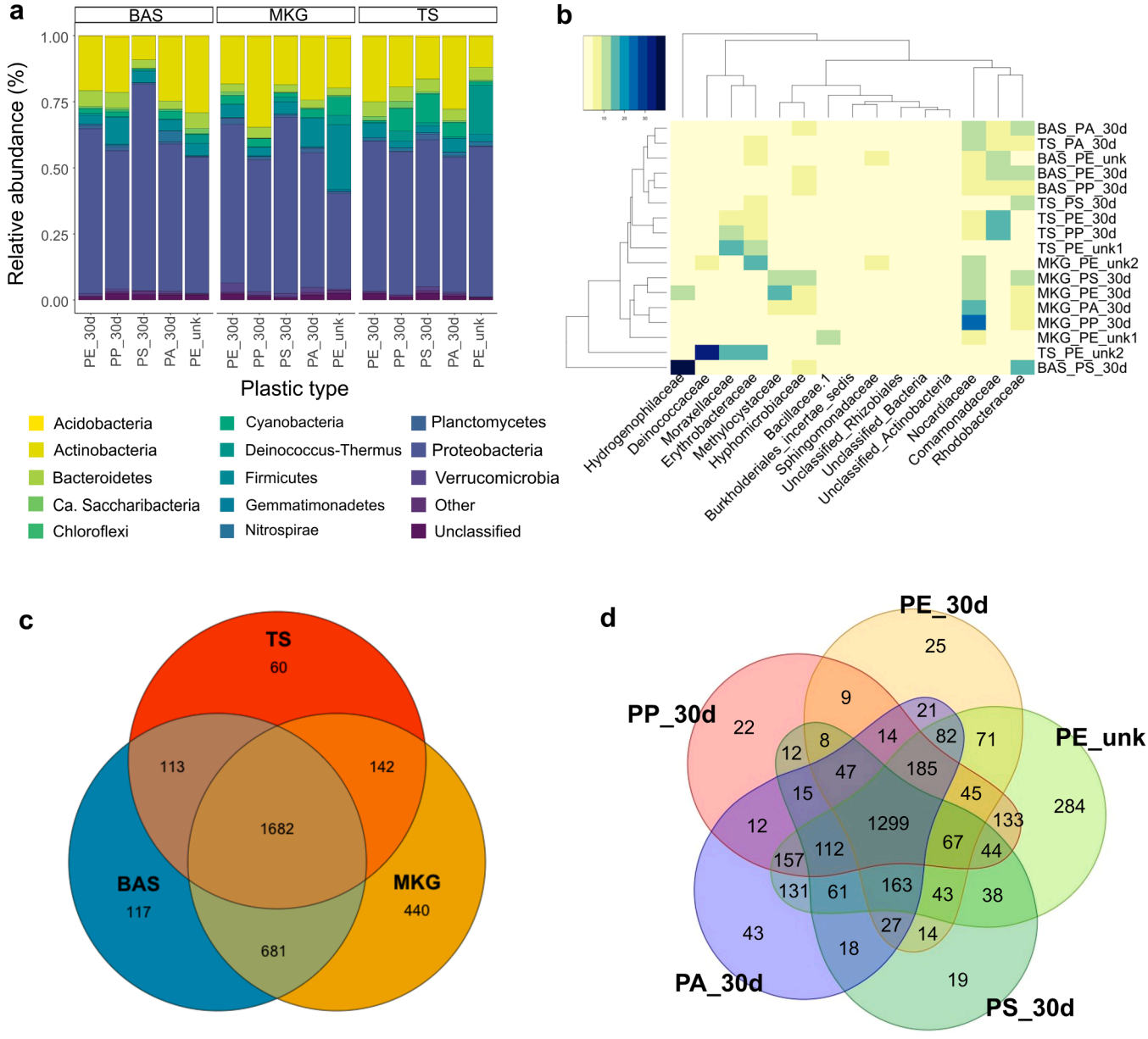

Fig. 3. The taxonomic composition obtained from the 16S rRNA analysis: a) The relative abundance of phyla categorized based on the different substrates (PE_30d, polyethylene; PP_30d, polypropylene; PS_30d, polystyrene; PA_30d, polyamide; PE_unk, polyethylene collected from the sites) and sites (BAS, Bassac; MKG, Mekong; TS, Tonle Sap). b) A heatmap displaying most abundant families and the clustering among them and among the samples. c) Shared OTUs based on the sites; d) Shared OTUs based on the different substrates.

significant differences in terms of richness and evenness within the microalgal community observed across the three river sites or among the various plastic substrates.

3.2.2. Beta diversity

The ordination of samples through NMDS analysis, based on the composition of the bacterial (three-dimensional solution, STR = 0.09) and algal community (three-dimensional solution, STR = 0.05), indicated different beta diversity depending on the site (Fig. 5). The ANOSIM permutation test confirmed the significant differences in the microbial and microalgal communities among the three river sites for both 16S and 18S rRNA OTUs ($p < .001$). In contrast, no significant difference in composition of the community was observed when considering the colonized polymer. However, some samples exhibit similarities. For instance, the PA_30d samples for 16S rRNA appear closer in proximity. Others are more divergent (e.g., PE_unk for 16S rRNA, PS_30d for 18S rRNA), highlighting additional complexities in the distribution of communities across different polymers.

3.2.3. Metabolic traits of bacterial community

Functional Annotation of Prokaryotic Taxa (FAPROTAX) analysis was utilized to explore the variations in microbial groups with specific functional traits across the analyzed sites and the different substrates (Fig. 6). The results suggested the prevalence of chemoheterotrophic organisms, with a notable abundance of aerobic taxa. In relation to the carbon cycle, organisms capable of methylotrophy, particularly

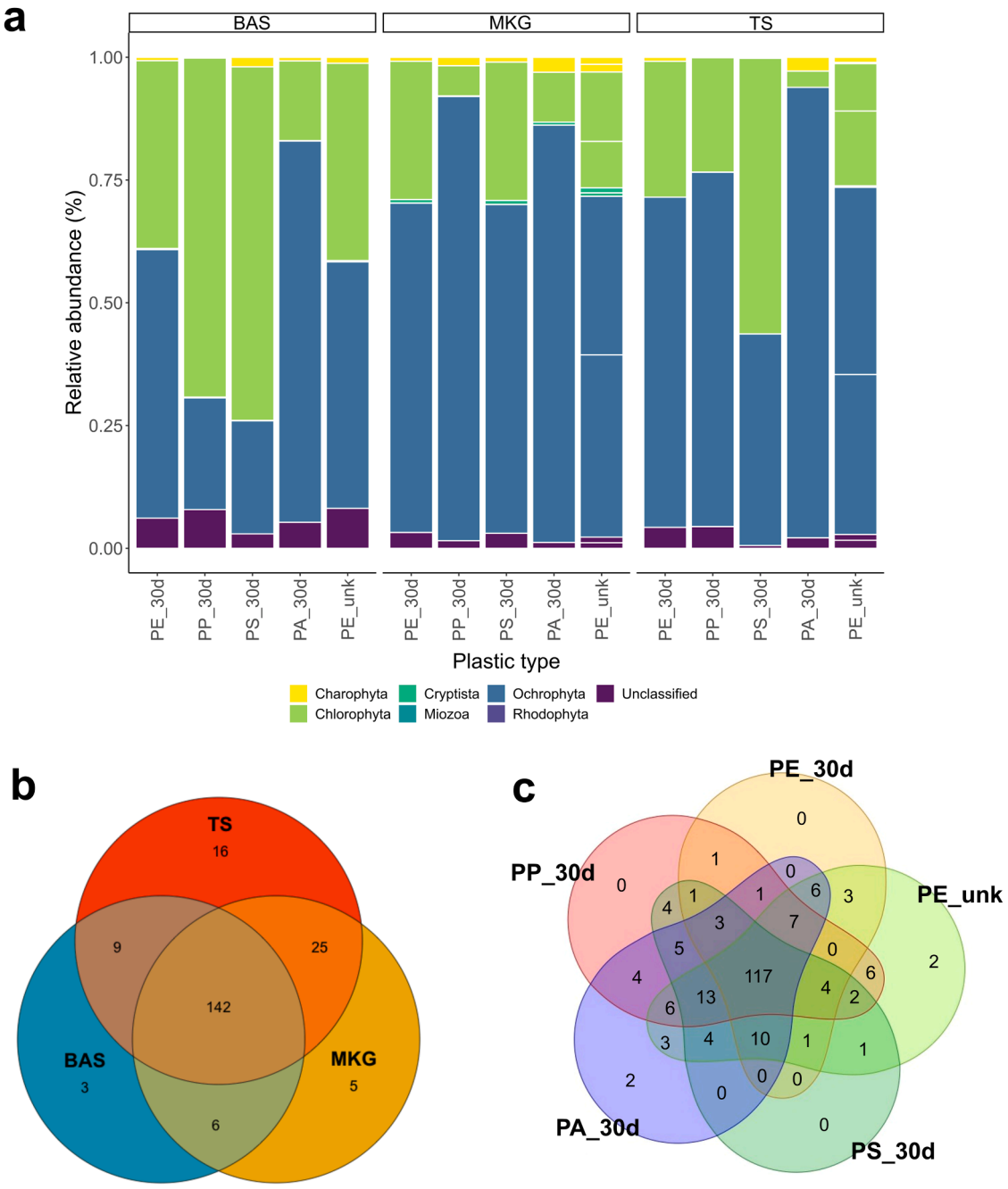

Fig. 4. The taxonomic composition obtained from the 18S rRNA analysis: a) Barplot of relative abundance of algal phyla categorized based on different substrates (PE_30d, polyethylene; PP_30d, polypropylene; PS_30d, polystyrene; PA_30d, polyamide; PE_unk, polyethylene collected from the sites) and sites (BAS, Bassac; MKG, Mekong; TS, Tonle Sap); b) Shared OTUs based on the sites; c) Shared OTUs based on the different substrates.

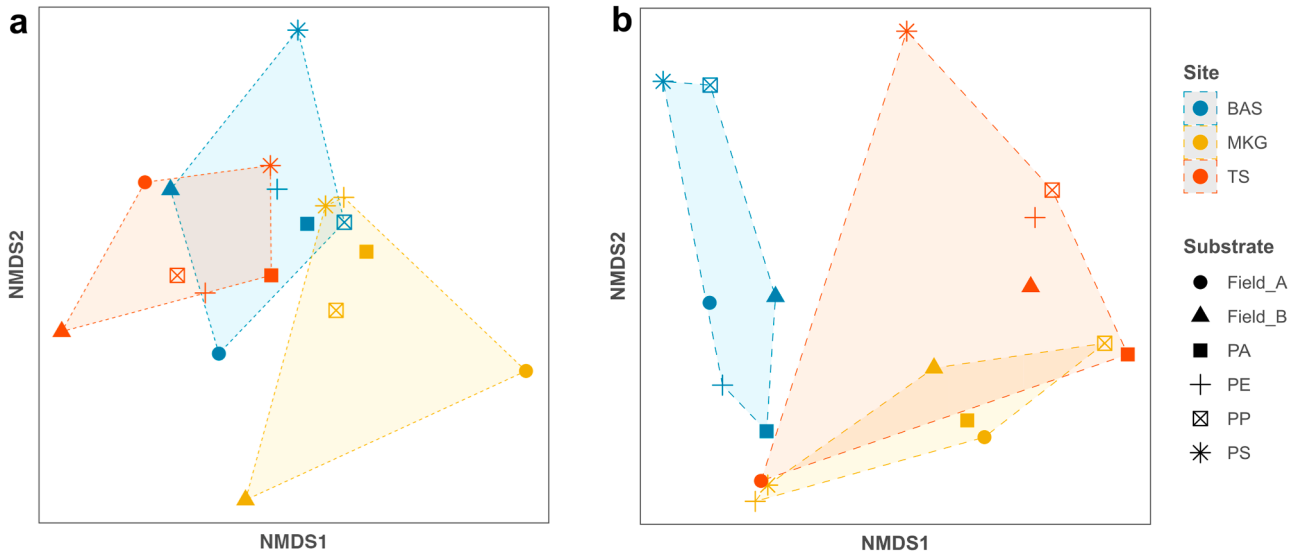

Fig. 5. Plot of non-metric multidimensional scaling (NMDS) run on: a) bacterial OTUs (three-dimensional solution, STR = 0.09); b) algal OTUs (three-dimensional solution, STR = 0.05). Different shapes represent the different substrates (PE_30d, polyethylene; PP_30d, polypropylene; PS_30d, polystyrene; PA_30d, polyamide; PE_unk, polyethylene collected from the sites); while the color corresponds to the different sites (BAS, Bassac; MKG, Mekong; TS, Tonle Sap). Convex hulls are drawn for the site variable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

associated with methanotrophy, were found in abundance, especially within the family Methylocystaceae. Overall, 48 OTUs exhibited this function, with the highest relative abundance detected in MKG on the PP substrate. Furthermore, a considerable relative abundance of organisms capable of hydrocarbon degradation was observed, with 49 OTUs associated with this function present in all the samples analyzed.

Organisms with the ability to fix nitrogen were abundant, and OTUs with this function (genera *Bradyrhizobium, Magnetospirillum, Methylocystis, Methanospirillum,* and *Azospirillum*) were detected across all samples, ranging from a relative abundance of 20 to 705. The OTU with nitrogen fixation function with the highest relative abundance was observed in MKG, particularly on the PS substrate. On the other hand, three OTUs associated with nitrification (family Nitrososphaeraceae) were found, and they were present in 7 out of the 18 analyzed samples. Significant differences in relative abundance were identified across sites for methylotrophy ($p < .05$), nitrate reduction ($p < .05$), nitrate

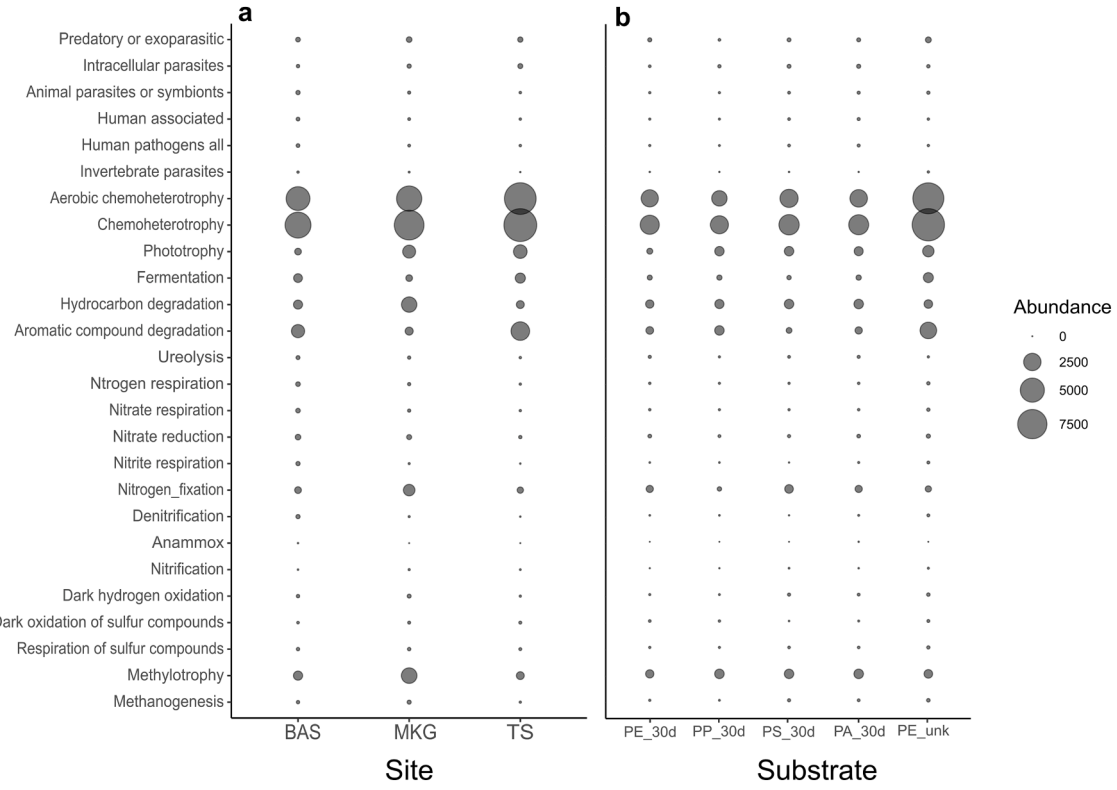

Fig. 6. Relative abundance of OTUs linked to the predicted functions using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database, categorized by a) site and b) substrate.

respiration ($p < .05$), nitrogen respiration ($p < .05$), ureolysis ($p < .05$), aromatic compound degradation ($p < .05$), and hydrocarbon degradation ($p < .05$). However, no significant differences were observed based on substrate type.

The analysis also revealed the presence of potential pathogens, with 5 OTUs linked to human pathogens (e.g. genera *Roseomonas, Stenotrophomonas*) and 11 OTUs associated with animal pathogens or symbionts (e.g. genera *Rhodococcus, Roseomonas, Stenotrophomonas*).

3.3. Water quality dissolved oxygen dynamics

Using high-frequency measurement sensors, we observed a significant decrease in dissolved oxygen levels over time in treatments involving biofouled plastics (Fig. 7). In contrast, the reference treatment with river water exhibited a comparatively slower rate of decrease in dissolved oxygen concentration. ANCOVA analysis revealed highly significant effects of treatment (with plastics and without; F=1256.9, $p < .001$) on dissolved oxygen concentration. Furthermore, the interaction between treatment and time was also significant (F=229.8, $p < .001$), indicating a statistically different regression slope for treatments with and without plastic addition.

3.4. Ecosystem production

3.4.1. Light-dark bottle incubation and changes in nutrient content

Dissolved oxygen concentration measured in the light-dark bottles (Fig. S6) generally decreased over time. Throughout the experiment, dissolved oxygen concentrations exhibited a gradual decline in all experimental sites containing incubated plastics, displaying a more pronounced reduction compared to reference bottles containing only river water.

Reference bottles displayed an average hourly oxygen consumption rate (R) of -1.1 ± 0.6 mmol O_2 m^{-3} h^{-1}, ranging from -1.8 to -0.2 mmol O_2 m^{-3} h^{-1} (Fig. 8). In contrast, bottles with plastics incubated for 30

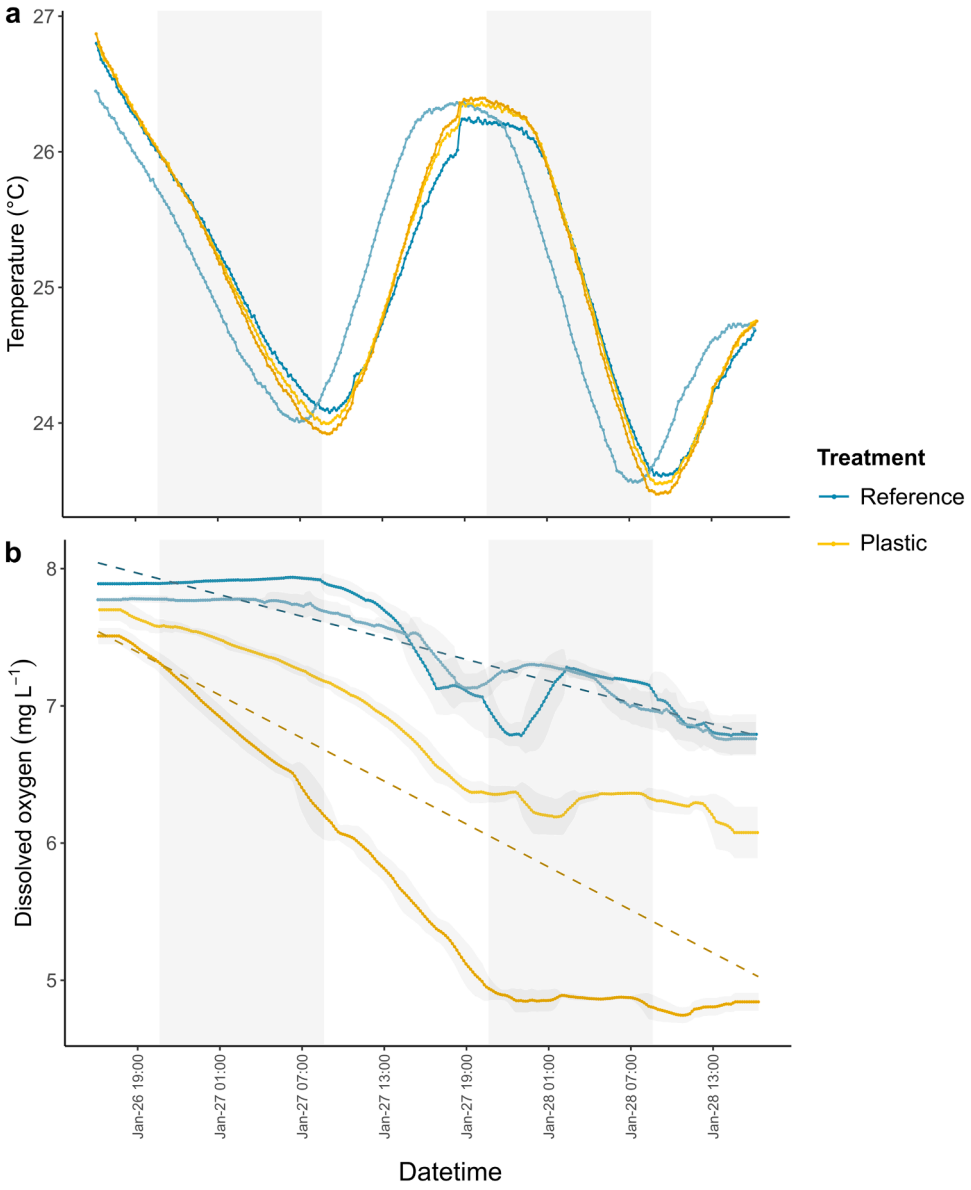

Fig. 7. High-frequency measurement of: a) temperature (°C); b) Dissolved oxygen (mg L^{-1}) recorded using an optical oxygen probe (PME miniDOT logger), over a 48-hour period; dashed lines indicate the regression lines for each treatment. The reference condition (blue lines) represents 18L containers with filtered Mekong River water and no plastics, while the treatment condition (yellow lines) consists of containers with an equitable amount of biofouled macroplastics. Two replicates for each treatment were performed. Gray bands indicate nighttime, while white bands represent daytime. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

days exhibited a mean R value of -2.9 ± 1.5 mmol O_2 m^{-3} h^{-1}, reaching a maximum consumption of -7.5 mmol O_2 m^{-3} h^{-1}. The difference in R rates between bottles with plastics and those without was found to be significant when tested through the t-test ($t(32)=5.47$, $p < .001$). When considering the different plastic substrates, R rates were highest in PA_30d and PE_unk and lowest in PE_30d. The highest oxygen consumption was observed in TS, and the lowest was in MKG (Fig. 8). Statistically significant differences were evident in R when comparing sites, whereas, across substrates, no notable distinctions were observed. Compared to the reference bottle with water, we observed statically significant differences for PP_30d in BAS ($p < .05$), and for all the plastics in MKG, while no statistically significant differences were observed in TS, where the variability among replicates was generally higher (Fig. 8).

Net Ecosystem Production (NEP) calculations produced negative values across the experimental bottles, indicating a net heterotrophic

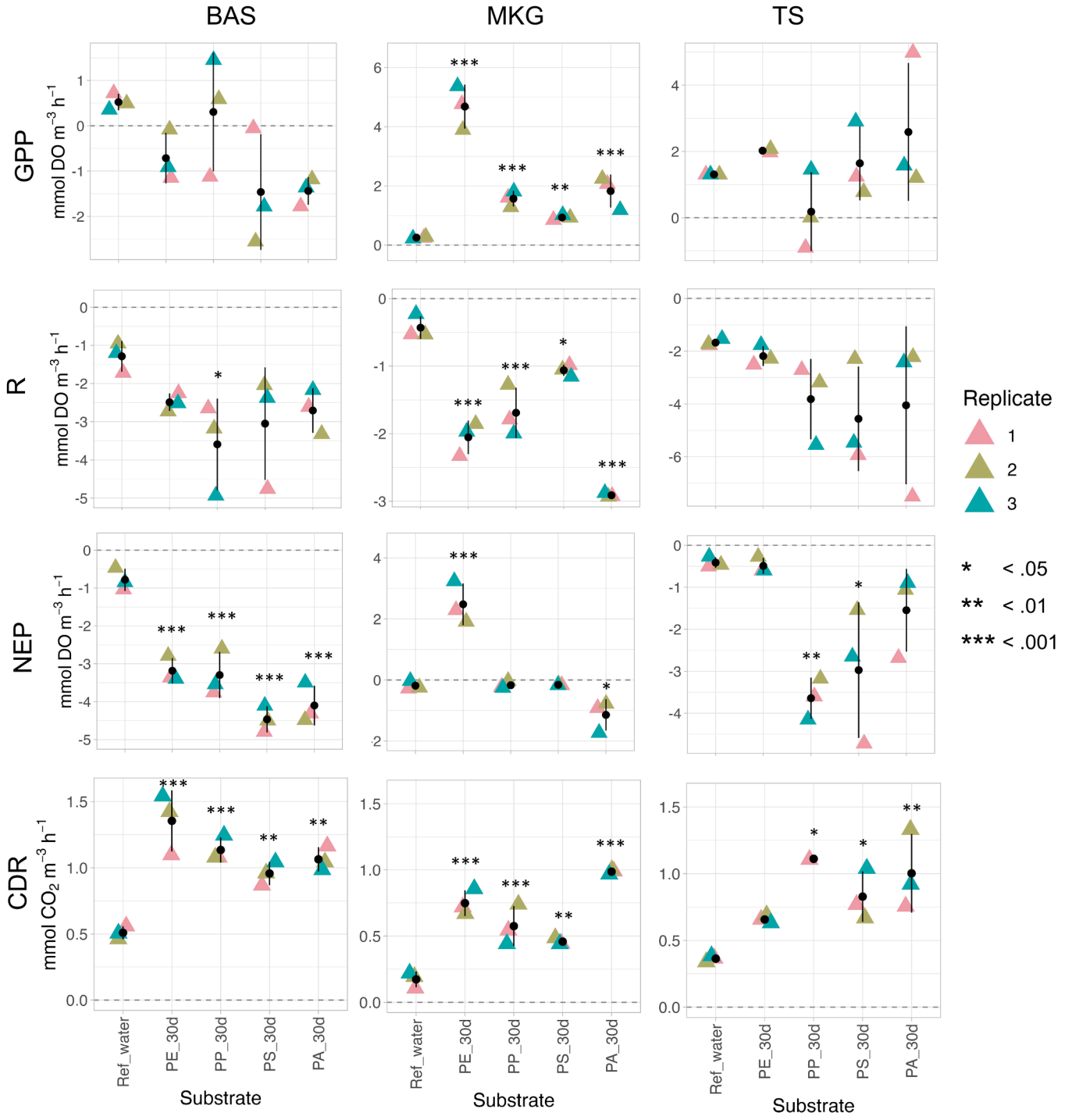

Fig. 8. Hourly rate of dissolved oxygen (mmol DO m^{-3} h^{-1}) and carbon dioxide (mmol CO_2 m^{-3} h^{-1}) change in relation to Gross Primary Production (GPP), Community Respiration (R), Net Ecosystem Production (NEP) and Community Dark Respiration (CDR) for Bassac (BAS), Mekong (MKG), and Tonle Sap (TS). Colored dots represent the measurements with each bottle (replicates) and black dots and lines represent mean and standard deviation. Asterisks indicate statistically significant differences between each substrate and the reference bottle with water (Ref_Water) through Dunnett's test: * = $p < .05$, ** = $p < .01$; *** = $p < .001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

condition, except for PE_30d in MKG where the values were positive (Fig. 8). Mean NEP rates in reference bottles across sites were -0.5 ± 0.3 mmol O_2 m^{-3} h^{-1} (ranging from -1.0 to -0.03 mmol O_2 m^{-3} h^{-1}), while in bottles with plastic samples, the mean NEP was -2.0 ± 2.1 mmol O_2 m^{-3} h^{-1} (ranging from -6.7 to 3.2 mmol O_2 m^{-3} h^{-1}). The two mean values were found to be statistically significantly different (t(40)=3.9, $p < .001$). The highest NEP values were observed in PE_30d, while the lowest values were recorded in PS_30d. When considering different sites, MKG exhibited the highest NEP whereas TS showed the lowest. Similarly to R rate, no statistically significant differences were found in NEP across substrates on average, but variations were observed when considering the different sites. Compared to the reference bottles, we observed statically significant differences for all the substrates in BAS ($p < .001$), for PE_30d and PA_30d in MKG ($p < .05$) and for PP_30d in TS ($p < .01$; Fig. 8).

The community dark metabolism exhibited, on average, the highest value in BAS (1.0 ± 0.4 mmol CO_2 m^{-3} h^{-1}) and the lowest in MKG (0.6 ± 0.3 mmol CO_2 m^{-3} h^{-1}). Across different substrates, similar average values were observed. Statistically significant differences were highlighted when comparing results to the reference condition for all the substrates in all sites, except for PE_30d in TS (Fig. 8).

3.4.2. Nutrient transformation

We quantified the influence of biofouled plastics on nutrients in light-dark bottles following the completion of the incubation. Fig. 9 shows the variation in concentration compared to the reference bottles with water. Although no clear patterns emerged across the different sites, certain trends were observable. Ammonia concentrations were consistently lower than the reference in TS and BAS, irrespective of light or dark conditions and for all substrates, except for PE_unk in BAS and PE_30d in TS. In contrast, MKG exhibited more variable ammonia concentrations, with positive mean values observed in light and dark bottles

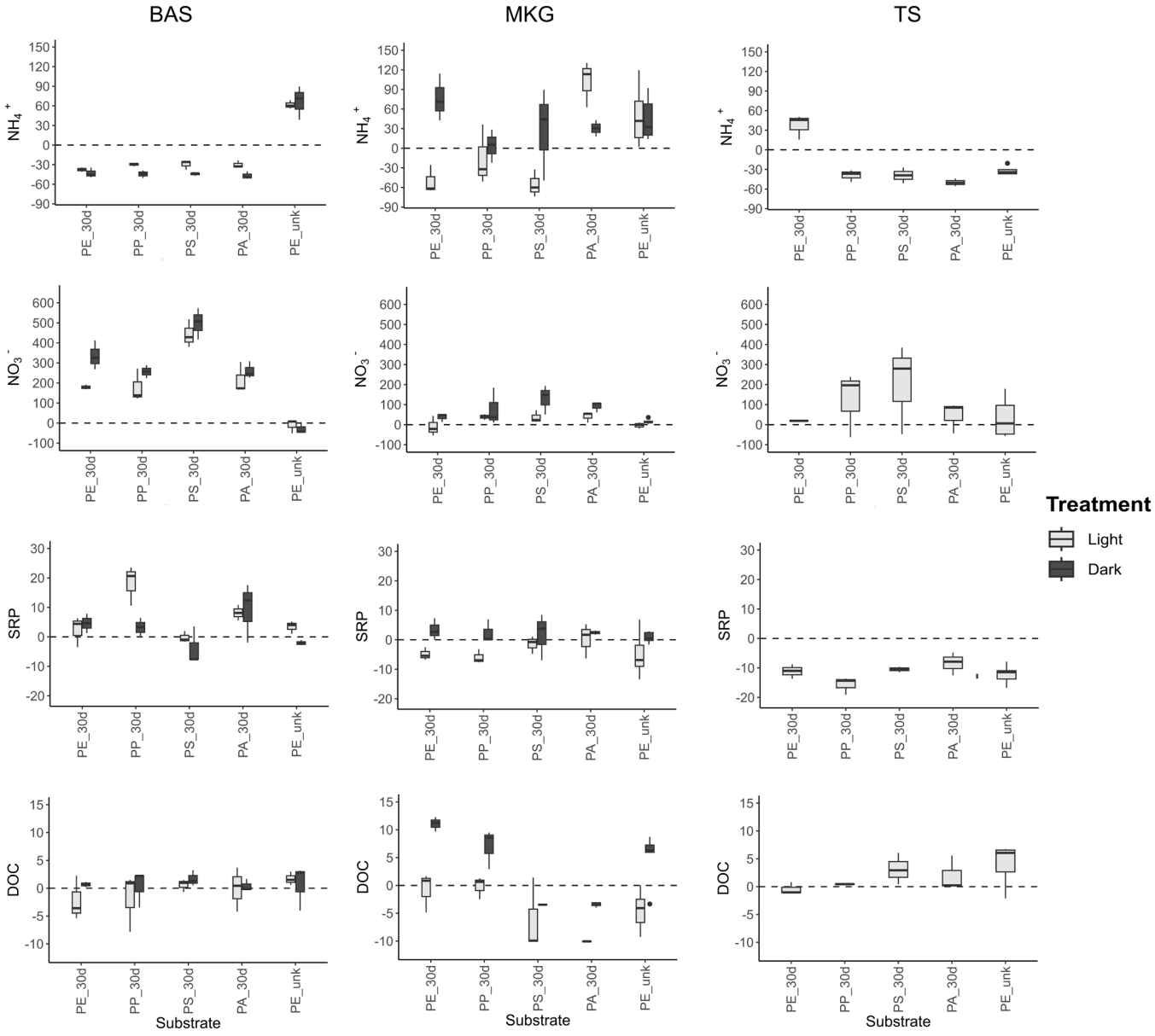

Fig. 9. Absolute difference in main nutrient concentrations (NH_4^+, ammonium; NO_3^-, nitrate; SRP, Soluble Reactive Phosphorus; DOC, Dissolved Organic Carbon) compared to the reference conditions (water samples) at three distinct sites: Bassac (BAS), Mekong (MKG), and Tonle Sap (TS). Box plot statistics: The lower and upper hinges correspond to the first and third quartiles. The upper (lower) whisker extends from the hinge to the largest (smallest) value, no further than 1.5 times the *InterQuartile Range* from the hinge. The dashed line represents 0, indicating no changes observed in comparison to the reference samples with water. Dark bottles of TS are not available.

containing PA_30d, PE_unk, and dark bottles of PE_30d and PS_30d. Nitrate concentrations were consistently higher than the reference, except for PE_unk in BAS, suggesting an enrichment of nitrate. Ammonia and nitrate concentrations in BAS generally showed opposing trends, suggesting the potential occurrence of nitrification in this site, which can lead to an increase in nitrate and a decrease in ammonia (see below). However, these processes can only be hypothesized based on the variance of compound concentrations. Soluble Reactive Phosphorus (SRP) exhibited variations across different substrates and sites. In BAS, concentrations were generally higher than the reference, with the exception of PS_30d and PE_unk. In MKG, where photosynthetic organisms were more abundant (as indicated by the average chlorophyll concentration in Section 3.1), SRP was more actively consumed in light bottles, resulting in lower concentrations compared to dark bottles. In TS, concentrations consistently remained lower than the reference. Dissolved Organic Carbon (DOC) concentrations did not exhibit considerable variation compared to the reference in BAS and TS. However, MKG samples in light bottles displayed negative values compared to the reference, suggesting DOC consumption, while dark bottles showed positive values, indicating DOC production. This observation hints at the presence of a possible labile DOC form in this site.

3.5. Macroplastic abundance and upscaling analysis

The hourly total count of macroplastics in the two Dai fisheries located at one of our experimental sites on the Tonle Sap River (TS) was approximately 206 ± 175 at Dai 3D and 88 ± 4 at Dai 15E. On average, the highest abundance was observed in the 'other plastic smaller than hand' category, with a mean value of 58 ± 52 items per hour across all sites and replicates, followed by 'food wrappers' at 17 ± 14 items per hour. Our conservative estimation of the available surface area suggests that there were roughly 14,271 cm^2 of surface area of plastics observed within one hour. When we scaled the NEP data derived from our experiments in TS (-6.90 × 10^{-4} mmol O_2 h^{-1}) to the estimated surface area, we obtained a value of -0.14 mmol O_2 h^{-1}, equivalent to a consumption of 107.5 mg O_2 d^{-1}. We currently lack data regarding the persistence of these polymers in the system and their actual colonization; however, given the extensive plastic pollution in the Lower Mekong River Basin (Haberstroh et al., 2021; Van Emmerik et al., 2023), this level of oxygen consumption could be ecologically relevant (Fig. S2).

4. Discussion

In this study, we showed that plastic debris with different polymeric composition represents a viable substrate for supporting and sustaining a variety of microorganisms and in particular a diversified bacterial community. This epiplastic community, in turn, alters water quality conditions and metabolic traits of ecosystem production within freshwaters. Indeed, plastics represent available surfaces in aquatic ecosystems which can create new potential niches and contribute to biogeochemical dynamics in aquatic ecosystems (Jacquin et al., 2019; Wright et al., 2020). The community growing on these substrates can influence metabolic traits, but these processes remain poorly investigated and many aspects still need to be disentangled (Chaudhary et al., 2022). The complexity stems from the interplay of a multitude of variables, including diversity of environmental conditions, as well as the characteristics of the substrates themselves (such as polymeric composition and surface roughness) (Barros and Seena, 2021; Vincent et al., 2022). Consequently, it is fundamental to increase our understanding of ecological interactions with plastic pollution across various environmental conditions to identify overarching patterns and unique attributes.

4.1. Phototrophic algal and cyanobacterial biomass

In this research, photosynthetic biomass colonizing different plastic types placed within rivers with a range of water quality conditions was relatively and consistently low. This aligns with findings from previous studies on plastisphere communities in lotic systems (Chaudhary et al., 2022; Vincent et al., 2022) and the values are indicative of oligotrophic-mesotrophic conditions observed on natural substrates (Porter et al., 2008). Particularly, Cyanobacteria were observed at low abundances in all samples. This agrees with several previous studies on bacterial assemblages retrieved on plastics in lotic systems, in which Cyanobacteria were not listed among the main taxa dominating the communities (Chaudhary et al., 2022; Li et al., 2021; Shuai Wang et al., 2020; Wu et al., 2020). Only occasionally Cyanobacteria were reported as abundant members of plastisphere communities in freshwater ecosystems (Miao et al., 2023; Xu et al., 2023).

While not statistically significant, relatively higher values of chlorophyll *a* were observed in MKG, which experienced lower turbidity during this time of year (Fig. S1). Turbidity in the rivers limits the solar radiation and thus the development of the photosynthetic biomass; generally, TS and BAS exhibit higher turbidity, resulting in lower light penetration into the waters, whereas MKG has lower turbidity, allowing for increased light penetration and potential photosynthetic activity (Fig. S1) (Irvine et al., 2011). Compared to the other polymers, photosynthetic biomass was more variable on PS. Previous research suggests that microbial communities on PS substrates differ from those on other polymers, such as PE and PP (Frère et al., 2018; Parrish and Fahrenfeld, 2019). These distinctions may also be associated with variations in the surface characteristics of PS, especially its roughness (Turner, 2020), which alters the effective surface area of a substratum and therefore plays a crucial role in influencing biofilm colonization (Ammar et al., 2015; Vadeboncoeur et al., 2006).

4.2. Heterotrophic bacterial community

The development of a heterotrophic bacterial community on plastic substrates was relatively consistent, despite variations in environmental conditions across the rivers. There was persistent evidence of the presence of bacteria from the Nocardiaceae family, particularly the genus *Rhodococcus*. This genus prevalence within the plastisphere has been documented in previous investigations (Di Pippo et al., 2020; Martínez-Campos et al., 2022). The widespread occurrence of this genus on the surface of plastics is particularly interesting, since culturing efforts have shown that these bacteria can use plastics as a sole carbon source. In particular, (Orr) et al. (2004) isolated a highly hydrophobic strain of *Rhodococcus ruber* (designated C208) capable of utilizing polyethylene as its sole carbon and energy source. Remarkably, this strain effectively degraded the polymer to 8 % of the original dry weight in only 4 weeks. While other genera, some of whose species have been identified as having plastic hydrolyzing capabilities, were also present in the samples (e.g., *Acinetobacter, Clostridium, Pseudomonas*; cfr. Jacquin et al., 2019), it is important to note that the mere presence of these bacteria does not necessarily indicate plastic usage as a carbon source and subsequent polymer degradation and, therefore, the question of whether microorganisms can truly break down plastic debris remains uncertain (Wright et al., 2020).

Additional recurring families present in our samples, such as Comamonadaceae, Erythrobacteraceae and Rhodobacteraceae, are frequently found inhabitants of the plastisphere (Amaral-Zettler et al., 2020; Kim et al., 2017; Pollet et al., 2018). Many Erythrobacteraceae and Rhodobacteraceae members are typically photoheterotrophs, utilizing light as energy source to generate ATP and DOC as carbon source. Moreover, some Comamonadaceae members, such as *Limnohabitans*, have been previously indicated as Aerobic Anoxygenic Phototrophs (AAPs), exhibiting photoheterotrophic growth during daylight hours and being particularly widespread in freshwater systems (Koblížek, 2015). A potential activity from these bacterial families may be hypothesized on the basis of FAPROTAX results, indicating both heterotrophy, mainly but not exclusively aerobic, and phototrophy among

dominant metabolisms. Among the heterotrophic bacterial populations, we observed a notable abundance of Methylocystaceae, aerobic methanotrophs, which potentially suggests the presence of active methanogens. Although archaea generally constitute only a minor component of prokaryotic communities in lotic systems (Battin et al., 2016), these methanogens may hypothetically inhabit the inner anoxic regions of the biofilms (Nadell et al., 2016), although we have not conducted analyses to confirm the presence of this anoxic layer. In turn, the possible presence of methanogens should be supported by the co-occurrence of hydrogen-producing fermenters. Indeed, the high abundance of Hydrogenophilaceae, although in a few samples only, implies the availability of reduced inorganic electron donors, mainly hydrogen. Alternatively, methanotrophs could feed on methane produced in the bottom sediments and diffuse towards the atmosphere. Indeed, river ecosystems have already been described as globally relevant to methane emissions into the atmosphere (Rocher-Ros et al., 2023).

We observed pathogenic bacteria across all locations, a finding consistent with previous research that has highlighted pools of pathogenic microorganisms on plastic surfaces in both marine and freshwater environments, as documented by Jacquin et al. (2019), Junaid et al. (2022), and related studies. While we did not explore this aspect in our study, it is reported in the literature that plastics can act as hotspots for horizontal gene transfer (HGT) involving antibiotic resistance genes (ARGs), antibiotic-resistant bacteria (ARBs), and mobile genetic elements (MGEs). This highlights potential broader-scale implications and risks associated with plastic pollution (Shanshan Wang et al., 2020; Yang et al., 2019). However, evidence supporting the role of the plastisphere in pathogen transmission is scarce, and the association between microplastics and infectious disease dynamics has received limited investigation (Loiseau and Sorci, 2022). Particularly, there is a lack of information to determine whether plastics have a greater influence on the survival and spread of human pathogens in the environment compared to other substrates (Metcalf et al., 2022). In some instances, studies have suggested a lower risk of pathogenic bacteria dissemination associated with the plastisphere compared to the planktonic community, as observed, for instance, in wastewater treatment plants (Galafassi et al., 2021). Consequently, accurately defining the actual risk remains challenging.

4.3. Community assemblages across different substrates and sites

Diversity indices did not reveal significant differences across sites or substrates for either the bacterial or algal communities. Overall, they indicated a notably diverse community of heterotrophic bacteria, which appeared more diversified than the algal community (Fig. S4, S5). Only a small number of OTUs were identified as indicator species shared among different substrates, as determined by the IndVal index (maximum of 6 out of 3220 bacterial OTUs and 2 out of 206 algal OTUs, as shown in Table S2). Based on the results of NMDS analysis, the overall community structure of biofilms was not influenced by plastic types. Instead, communities exhibited clustering patterns based on the rivers, each characterized by contrasting water quality. This underscores the substantial influence of environmental conditions as the primary driver of community composition. This matter has sparked extensive debate, and the consensus among the majority of studies is that there appears to be no discernible substrate preference for different plastic polymers in the core community, particularly in more mature biofilms (Oberbeckmann and Labrenz, 2020; Wallbank et al., 2022). This is likely due to the fact that only the initial biofilm layers come into direct contact with the substrate, and it seems that the observed differences are primarily associated with the rare biosphere within the biofilm (Kirstein et al., 2019; Rummel et al., 2021). Interestingly, the plastics submerged for an unknown period in each site (i.e., PE_unk) appear to diverge in their bacterial communities when compared with the other plastic substrates that were incubated at the sites, as also highlighted by the highest number of unique bacterial OTUs observed (Fig. 3d). This observation may suggest that as biofilms mature, they tend to deviate from the community structure we observed after a 30-day incubation. This emphasizes the necessity of investigating longer-term processes in plastisphere research (Kirstein et al., 2019) perhaps under varied light and nutrient conditions that are representative of the environments where plastics may accumulate in freshwater.

4.4. Impacts on water quality: effects on nutrients and dissolved oxygen

Biofilm-dwelling microorganisms (autotrophic and heterotrophic) engage in close interactions and mutually affect each other's success (Nadell et al., 2016). The aggregation of these organisms within biofilms offers various advantages, notably the efficient capture of nutrients. These nutrients can be acquired from multiple sources, including the surrounding water column, the substrate to which the biofilm is attached, or through internal cycling within the biofilm (Vadeboncoeur and Steinman, 2002). As a result, in contrast to planktonic organisms that inhabit extremely diluted environments, the accumulation and circulation of nutrients among phototrophic and heterotrophic microorganisms within biofilms prove to be significantly more efficient (Wright et al., 2020). Thus, the community that is able to develop on the surface of plastics may exhibit distinct metabolic traits, potentially influencing nutrient cycling and creating 'closed loops' of nutrient cycling (Mincer et al., 2016). Although we did not explicitly investigate the metabolic traits expressed, we examined variations in nutrient concentrations and the metabolic trait associated with the OTUs identified to hypothesize potential processes that may have taken place. An interesting pattern in nitrogen concentrations emerged with an increase in nitrate and a coupled decrease of ammonia across BAS and TS samples. This pattern suggests the presence of the nitrification process, supported by the presence of nitrifying bacteria like the genus *Nitrososphaera*. Although these bacteria were detected with limited relative abundance, the role of the plastisphere in the nitrogen cycle has already been documented in the literature. For instance, Chen et al. (2020) observed that microplastic biofilms can enhance ammonia and nitrite oxidation, thereby increasing the overall nitrification capacity within the system. Similarly, Huang et al. (2022) demonstrated a reduction in ammonia concentrations and an increase in nitrification rates in microcosms with pelagic and benthic plastisphere.

The impact of plastics on water quality extends beyond nutrient cycling, leading to profound effects. Our experiments unveiled a significant decrease in oxygen concentrations associated with the presence of biofouled plastics, overall influencing NEP. The interaction between community composition, the abundance of the photoautotrophic community, and environmental conditions dictates the extent of NEP variation, often showing significant differences (24 out of 36 samples) and more negative values (33 out of 36 samples) compared to reference samples, leading to net heterotrophic conditions. Variations observed across various sites and incubated substrates emphasize the complexity of the process and the variability associated with the interplay of factors shaping the colonization and composition of the epiplastic community. The observed decline in oxygen concentrations can be attributed to the overall limited growth of the autotrophic microalgal community, the well-established heterotrophic bacterial community on plastic surfaces, and to a lesser extent, to the hypothesized nitrification processes which are oxygen-consuming as well.

This phenomenon holds substantial implications, particularly due to the marked decrease in oxygen levels, resulting in nearly hypoxic conditions. Prior studies have provided evidence of the capacity of plastic surfaces to harbor a thriving and dynamic biofilm community (Chaudhary et al., 2022; Vincent et al., 2022). Our findings further underscore the significance of this phenomenon, indicating that this process can give rise to net-heterotrophic hotspots with a profound impact on system metabolism, creating a novel and distinctive niche for heterotrophic activity (Arias-Andres et al., 2019). The observed oxygen consumption change could occur with other naturally occurring

substrates, such as stones, wood, or macrophytes. The overall negative or positive NEP value is tied to the type of community that develops on the substrate surfaces and environmental parameters (see Section 5 and Fig. 10). Previous research has indeed documented variable effects on NEP, spanning from negative to neutral or positive values (Chaudhary et al., 2022). The discussion should not only focus on contrasting the effects of different substrates (natural versus plastics) but also on quantitatively evaluating the significance of processes linked to plastic pollution compared to natural ones. This entails assessing the abundance of plastic surface area and measuring its associated impact on water quality. When we extrapolated the oxygen consumption to encompass the extent of macroplastic contamination in regions like the Mekong River Basin, we obtained a value of 107.5 mg O_2 d^{-1}. Placing this value in the context of previously estimated plastic fluxes in the region (approximately 10^6 items $days^{-1}$ during the wet season and 10^4 items $days^{-1}$ during the dry season; Van Emmerik et al., 2023), it becomes evident that plastic pollution could alter carbon and nutrient fluxes in aquatic ecosystems. There are significant challenges related to sustaining fish production and biodiversity in the Lower Mekong River basin. In recent years, numerous press articles have documented fish kills along the river, attributing these incidents to warming waters and nutrient pollution. Biofouled plastic litter and the associated alterations of carbon and nutrient fluxes may play a more dominant role in ecosystem health than previously thought.

Microbial colonization and metabolic activity on plastics may be an underexplored aspect in scientific research. Nevertheless, the transformation of carbon and alterations to gas fluxes could emerge as crucial research areas in the near future, as current efforts to reduce plastic pollution seem to yield limited impact (Borrelle et al., 2020). In light of our results, it is clear that the plastisphere community functions as a habitat for biologically active organisms that play a pivotal role in essential ecosystem processes, thus warranting dedicated attention and investigation.

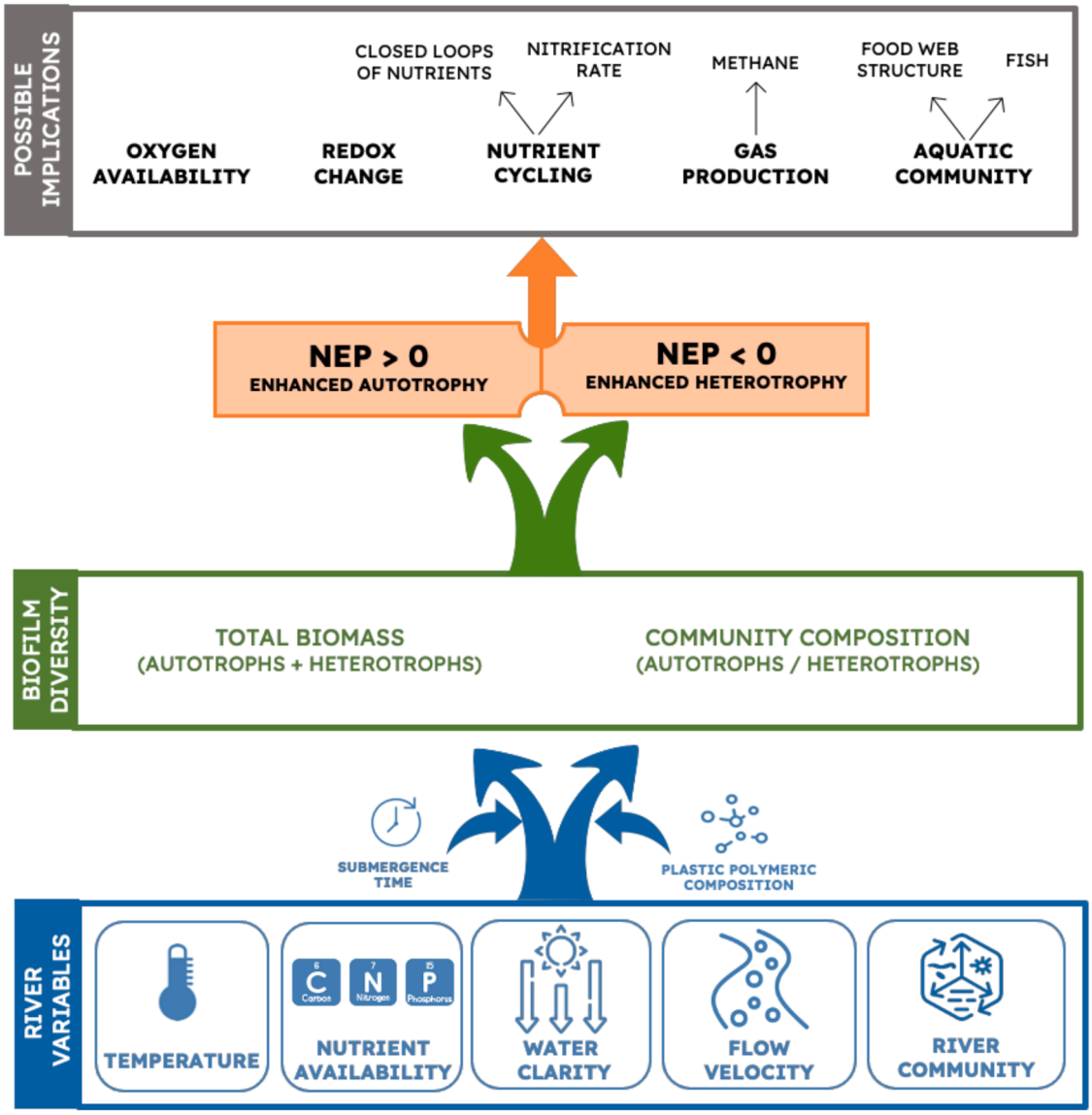

Fig. 10. Conceptual model depicting the influence of river variables (in blue) on the composition (ratio of autotrophs/heterotrophs) and total biomass (autotrophs + heterotrophs) of the epiplastic community (in green). These factors subsequently impact Net Ecosystem Productivity (NEP, in orange), resulting in either positive or negative values based on the community composition. The cascading effects of this process on aquatic ecosystem processes are illustrated in gray. Variables that we tested in the present study are indicated with an asterisk (*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5. Limitations and future perspectives

While plastic pollution is recognized as a widespread and growing problem in freshwater ecosystems on a global scale, it is often viewed primarily as a mere contributor to marine plastic pollution, with issues related to plastic in riverine environments being overlooked. Our research unveils that plastic pollution may exert a more significant and ecosystem-altering impact than initially anticipated, especially in regions with mismanaged plastic litter. This active community and the resultant shift in water quality can carry substantial implications at the ecosystem level, which we anticipate and hypothesize to include the following:

a) The observed decline in oxygen levels and especially heightened heterotrophic activity may lead to hypoxic or anoxic conditions, impacting various aquatic ecosystem processes, thereby altering overall water quality and energy fluxes. This can significantly affect the biological community, especially the fish community, which plays a crucial role in the Lower Mekong Basin and beyond, with subsequent implications for nutrition and livelihoods;
b) Plastic pollution can alter nutrient cycling by retaining nutrients on the plastics, creating 'closed loops' of nutrients;
c) Colonization of plastics by microbial communities can promote the establishment of other organisms, such as macroinvertebrates (Taurozzi et al., 2023), or increase ingestion by organisms attracted to 'flavored' plastics with possible implications for the food web;
d) Plastic materials, coated with a microbial layer, have the potential to be transported over long distances, potentially altering microbial biogeography. Additionally, they may be ingested by various organisms, potentially altering the microbiomes of these species;
e) Plastics, known to emit greenhouse gasses like methane and ethylene (Royer et al., 2018), may undergo enhanced emission processes due to the presence of active methanogens, although a potential for methane removal by methanotrophs before emission to the atmosphere could still exist;
f) Our observations include the presence of potentially pathogenic organisms, which could have implications for human health by introducing or increasing the numbers of pathogenic microbes. In this light, pathogen spreading could also be altered or enhanced.

While our results provide first evidence concerning the broader ecological impacts of plastics and address a significant knowledge gap in the plastisphere field, there are limitations to consider when interpreting our findings. Although we attempted to mimic real-world conditions (e.g., by using diverse polymers commonly found in freshwater ecosystems, particularly the Mekong River), our results stem from a semi-natural incubation and are subject to experimental limitations. This included incubation within mesh bags, restricting the plastics' ability to float freely and potentially affecting the substrates inside (e.g., through shading or flavoring effects), as well as potentially influencing the grazing effects of larger organisms. Additionally, the incubation period was limited, and we lack information on longer-term processes, especially across different seasons. Furthermore, our understanding of the metabolic pathways involved is speculative, and more comprehensive analyses are necessary. However, the results reported here provide a foundational proof-of-concept, opening avenues for further exploration and guiding future research endeavors. Future studies should therefore expand into understanding the impacts to metabolic processes within the plastisphere, utilizing metabolomic techniques to better grasp the actual processes at play. Additionally, research should encompass the study of other components of the epiplastic community, such as fungi, protists and viruses, to gain a more comprehensive understanding of the community developing on plastic surfaces and its influence on ecosystem metabolism. Different spatial and temporal scales should be considered to capture processes at a broader scale in various habitats and conditions. This approach is crucial for determining whether there is a consistent push toward heterotrophy in biofouled plastics or if different community compositions, especially in the ratio of autotrophs to heterotrophs, linked to varying environmental conditions, may result in increased oxygen levels and, consequently, different outcomes than those observed in this study. To synthesize existing knowledge, identify gaps, and guide future research in the field, we have formulated a conceptual framework. This framework aims to explore opportunities for further investigation, emphasizing critical aspects of variability that can offer valuable insights for future research (Fig. 10).

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CRediT authorship contribution statement

Veronica Nava: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Barbara Leoni:** Writing – review & editing, Funding acquisition, Conceptualization, Supervision, Project administration. **Monica M. Arienzo:** Writing – review & editing. **Zeb S. Hogan:** Writing – review & editing, Funding acquisition. **Isabella Gandolfi:** Writing – review & editing, Methodology, Formal analysis. **Valeria Tatangelo:** Writing – review & editing, Investigation. **Emily Carlson:** Writing – review & editing, Methodology, Investigation. **Seila Chea:** Writing – review & editing, Investigation. **Savoeurn Soum:** Writing – review & editing, Investigation. **Rachel Kozloski:** Writing – review & editing. **Sudeep Chandra:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization, Project administration.

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.

Data availability

The datasets generated and analyzed during this study are available at this link: 10.5281/zenodo.11349364

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Supplementary materials

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