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# Prokaryotic metabolic response to water-soluble polymers enhances oxygen drawdown in freshwater systems: A polyethylene glycol case study

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#### ABSTRACT

Water-soluble polymers (WSPs), such as polyethylene glycol (PEG), are emerging contaminants with widespread industrial and commercial applications. Despite their extensive use, the environmental fate and impact of WSPs in freshwater ecosystems remain largely unexamined. We hypothesize that PEG exposure will increase microbial oxygen consumption rates, thereby exacerbating deoxygenation. Short-term incubation experiments were conducted on water samples collected from the River Test, Romsey, UK, to assess PEG's effect on microbial: oxygen consumption, leucine uptake and assimilation efficiency, to indicate rates of prokaryotic respiration and production, and eco-physiological status. A range of PEG molecular weights (100-1000 g/mol) and concentrations (50-1600 mg/L) were used. Our results demonstrate that PEG enhances microbial respiration, with an increase in oxygen consumption across all tested molecular weights and concentrations. Even at the lowest concentration (50 mg/L), PEG exposure resulted in a 40-80 % increase in oxygen consumption compared to controls. Leucine assimilation efficiency also increased, suggesting that PEG serves as a bioavailable carbon source, thereby promoting microbial growth and enhancing eco-physiological status. However, the relationship between PEG molecular weight, concentration, and oxygen consumption was non-linear, likely influenced by factors such as aggregation effects, microbial enzymatic selectivity, and threshold-driven metabolic responses. These findings suggest that WSPs may play an underappreciated role in altering oxygen dynamics in freshwater systems, with potential implications for eutrophication, aquatic ecosystem functioning and enhanced carbon dioxide production. Given the increasing global production and environmental presence of WSPs, further research is needed to assess their potential long-term environmental impacts to inform disposal strategies.

#### 1. Introduction

Liquid plastics, also known as synthetic water-soluble polymers (WSPs), have been detected in freshwater ecosystems [1–3]. However, their environmental impacts remain largely unexamined. In contrast, microplastics (MPs; emergent pollutants with similar origins), and their ecological consequences have received extensive research attention and widespread media coverage [2,4,3]. This gap in our understanding is particularly concerning given that freshwater ecosystems constitute some of the most biodiverse regions on Earth [5,6] and serve as essential sources of potable water, food, and recreational opportunities for human populations. Should WSPs prove harmful to freshwater species, their

presence will further compound the existing and mounting pressures imposed on these ecosystems by climate change, as well as urban and agricultural expansion [5,7–9]. As emerging pollutants, WSPs may therefore have far-reaching consequences for both environmental and human health.

The imperative to evaluate the potential environmental risks associated with WSPs is compelled by their likely widespread distribution resulting from their extensive application. WSPs possess unique properties that render them highly effective as adhesives, thickeners, gelling agents, and emulsifiers [10,11]. Consequently, they have been adopted across diverse sectors, including household products [12,13], agriculture, agrochemicals [12,13], and wastewater treatment as flocculants

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[12,14,15]. Their broad utility has driven rapid market growth, with the WSP sector currently valued at approximately \$125.2 billion per year [13,16]. Prominent industrial WSPs include polyacrylamide (PAM), polyacrylic acid (PAA), polyethylene glycol (PEG), polyethyleneimine (PEI), polyethylene oxide (PEO), polypropylene glycol (PPG), polyquaternium (PQ), polyvinyl alcohol (PVOH), and polyvinyl pyrrolidone (PVP) [1,12].

WSPs' disposal is a regulatory grey area, in contrast to the stringent regulations developed to govern MP disposal, such as the REACH framework (Regulation [17] 2006; [1,18,12]). Although WSPs are generally removed via wastewater treatment processes, eventually partitioning into sewage sludge, their fate, transport, and toxicity in the natural environment remain uncertain [16]. Once released, WSPs may undergo various physical and chemical transformations, including flocculation and degradation [19,16,20], and may induce changes in water chemistry, such as alterations in pH, solubility, and charge [21, 22]. In the absence of disposal regulations or removal protocols, the contamination of freshwater, river, lake, and wetland systems by WSPs continues to escalate [23].

Studies indicate that WSPs are biologically active and could, therefore, adversely affect aquatic organisms [16]. For example, the PAM degradation product acrylamide has been linked to carcinogenic and neurotoxic effects [24–27], while PEG and PEO have been associated with allergic reactions [25]. Acute toxicity tests reveal that high concentrations of PAM-based flocculants can cause short-term physical effects in juvenile rainbow trout (*Oncorhynchus mykiss*), and histopathological studies have shown that exposure to these polymers induces structural alterations in fish gills, potentially compromising respiratory function [28].

At the community level, enhanced flocculation may alter nutritional fate, morphological parameters, and light intensity [29-31]. Additionally, evidence suggests that PAM may also interfere with phosphorus removal during wastewater treatment, thereby affecting nutrient dynamics and contributing to eutrophication [30,20]. Although WSPs are not typically considered primary drivers of eutrophication, which is largely driven by nitrogen and phosphorus enrichment, they could indirectly exacerbate this harmful phenomenon by serving as substrates for microbial growth. Increased biological oxygen demand can lead to deoxygenation, with severe ecological consequences such as fish kills and loss of biodiversity [32,33]. Furthermore, increased respiration rates enhance the generation of the greenhouse gas carbon dioxide. Consequently, the presence of WSPs in freshwater systems may contribute to eutrophic conditions by influencing both oxygen dynamics and nutrient cycling and exacerbate climate change through greenhouse gas generation.

We hypothesize that PEG serves as a substrate for freshwater microbial metabolism, thereby enhancing oxygen consumption rates. To investigate this, we examined the metabolic response of river microbial communities to enrichment with PEG, a WSP that we have detected in 7 UK rivers with a range of 100-1000 Da molecular weight (MW) and 2.1-33.5 µg/L concentration [34]. To maximize the likelihood of detecting measurable metabolic responses, we applied PEG at concentrations of 50-1600 mg/L. Although these concentrations exceed those observed in river water, they are environmentally plausible in the vicinity of WSP point sources such as wastewater effluents or industrial discharges and thus represent upper-bound or worst-case exposure scenarios. Microbial communities were targeted since they drive key biogeochemical processes such as nutrient cycling and organic matter decomposition and are highly sensitive to changes in water chemistry. Shifts in microbial metabolism were determined by oxygen consumption rates, radio-tracer bioassays to measure leucine uptake and respiration [35] and changes in cell abundance. Leucine uptake provides estimates of prokaryotic production rates, and the ratio of leucine used for anabolism versus catabolism (the leucine assimilation efficiency; LAE) indicates changes to eco-physiological status [36,37] and thereby, indicates levels of microbial stress. By studying river microbial

communities' responses to PEG, we aim to provide insights into whether WSP pollutants induce ecosystem stress and, thereby, inform effective risk assessments, the requirement for WSP disposal legislation and remediation strategies.

#### 2. Materials and methods

Freshwater and its associated microbiome were collected from the River Test in Romsey, UK, during May-September 2024. Surface river water was sampled in an 8 L acid-cleaned carboy and immediately transported back to the laboratory in a sealed cool box. (Full workflow of materials and methods summarised in Fig. 1.)

#### 2.1. Incubation experiments

The whole water collected was gently gravity-filtered through a 20 µm mesh to remove larger organisms before experimentation. The filtered water was then diluted 1:3 with artificial freshwater medium (ISO medium: 25 mL of each stock solution; calcium chloride dihydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O, 11.76 g), magnesium sulphate heptahydrate (MgSO<sub>4</sub>·7H<sub>2</sub>O, 4.93 g), sodium bicarbonate (NaHCO<sub>3</sub>, 2.59 g), and potassium chloride (KCl, 0.23 g) added to 1 L of Milli-Q water). This dilution step was used to lower background concentrations of dissolved organic matter and thereby limit competing heterotrophic respiration, increasing the likelihood of detecting PEG-specific metabolism. The diluted samples were then incubated at the in-situ river temperature (6 °C) in the dark for 24 h to allow the microbial community to acclimate to the experimental conditions and to prevent autotrophic production of new organic material during the incubation period. For initial proof-of concept total oxygen consumption study, incubation experiment was set up by placing 25 mL of pre-prepared river water in acid-cleaned borosilicate glass bottles before spiking with PEG of allocated molecular weights of 100, 200, 400 and 1000 Da with concentrations of 50, 400 and 1600 mg/L (Sigma Aldrich, UK) with 25 mL of artificial water

Further investigation of total oxygen consumption, incubation experiment was set up by placing 25 mL of pre-prepared river water in acid-cleaned borosilicate glass bottles before spiking with PEG of allocated molecular weights of 100, 200, 400 and 1000 Da with concentrations of 50 mg/L with 25 mL of artificial water media with each treatment having three replicates. Samples were incubated at 6  $^{\circ}\text{C}$  in the dark 24 h before analysis.

Leucine incorporation incubation experiments were set up by placing 250 mL of pre-prepared river water in acid-cleaned borosilicate glass bottles, before spiking with PEG of allocated molecular weights of 100, 200, 400 and 1000 Da with concentrations of 50, 400 and 1600 mg/L (Sigma Aldrich, UK) together with 750 mL of artificial water media, and incubating at 6  $^{\circ}{\rm C}$  in the dark for 24 h. After this period, all treatments were assayed for prokaryotic leucine assimilation and respiration according to the following protocols.

#### 2.2. Total oxygen consumption

River water samples were poured at a 45° angle into 22 mL Supelco® gas-tight glass vials and gently filled until a reverse meniscus formed to limit any formations of excess air. Each vial was fitted with a SP-PSt3 oxygen planar sensor spot (PreSens, Germany), allowing an oxygen determination limit of 15  $\mu g/L$  and a reported accuracy of >0.5 %. Vials were incubated at in-situ temperature conditions (6 °C) using a waterbath. Dissolved oxygen concentration (DO) and temperature was measured using a OXY10 Oxygen optode (Presens, Germany), which is regularly calibrated (0 and 100 % saturation). Initial measurements were taken immediately at the start of incubation and subsequently every 45 min for 6 h and then the following next day at 22, 23 h, with the final measurement at 24 h. Mass balance calculation of carbon recovery for PEG mineralized was evaluated using stoichiometric composition of

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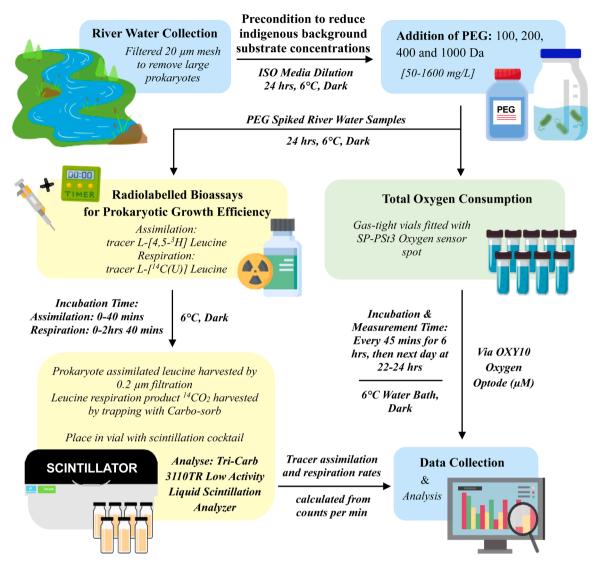


Fig. 1. Schematic workflow of experimental design of total oxygen consumption (Section 2.2), prokaryotic leucine assimilation (Section 2.3), and prokaryotic leucine respiration (Section 2.4).

one repeating unit of PEG ( $C_2H_4O$ ) that requires 1.25 mol of oxygen per 1 mol of carbon (See more in Supplementary Information, eq. 1a-g).

#### 2.3. Prokaryotic leucine assimilation

Prokaryotic leucine assimilation was measured via an adapted time series bioassay method [35], using L-[4,5-3H] leucine (specific activity 81.5 Ci/mmol, Hartmann Analytic, Germany). Three replicates of 2 mL microcentrifuge tubes (Starlab, Milton Keynes) were preloaded with <sup>3</sup>H-Leucine, and when filled with 1.6 mL of sample, achieved a final concentration of 0.6 nM <sup>3</sup>H-leucine. Incubations were terminated after 0, 10, 20, 30, and 40 min by the addition of glutaraldehyde (1 % final concentration, 16 µL). After 1 hour of fixation, samples were filtered via 0.2 µm pore-size polycarbonate filter and washed twice with 3 mL of Milli-Q. Filters were then placed into scintillation vials with 5 mL of scintillation cocktail (Merdian, Gold Star) and radioactivity was measured as counts per minute (CPM) through liquid scintillation counting (Tri-Carb 3110TR Low Activity Liquid Scintillation Analyzer, PerkinElmer, Beaconsfield, UK). Relative leucine assimilation rates were determined from the linear regression of CPM against incubation time. Errors were calculated by measuring the standard deviation between rates derived at each time point.

#### 2.4. Prokaryotic leucine respiration

Prokaryotic leucine respiration was measured via an adapted time series bioassay method [35], using L-[14C(U)] Leucine (specific activity 338 Ci/mmol, Hartmann Analytic, Germany). 70 mL of sample was pipetted into 160 mL glass crimp neck serum bottles that were acid cleaned and rinsed three times with sample. Immediately after being spiked with <sup>14</sup>C-Leucine, achieving a final concentration of 0.6 nM, serum bottles were crimp sealed using Teflon coated silicone septa with crimp caps (Fisher Scientific, UK). Samples were incubated in the dark with in-situ temperature conditions (6 °C) over varied time points (0, 40, 80, 120, 180 min). Incubation was terminated using 1 mL of 10 % HCl through each septum via hypodermic needle and syringe. Respiration glass bottles were then bubbled with CO<sub>2</sub>-free air (using Drechsel bottle of 1 M NaOH) and evolved <sup>14</sup>CO<sub>2</sub> was collected over 2-hour period with 4 mL Carbo-sorb filled scintillation vials via a Cole-Parmer Masterflex L/S Economy Drive respiration pump. 16 mL of scintillation cocktail was then added to the vials and radioactivity was measured as counts per minute (CPM) through liquid scintillation counting. <sup>14</sup>C-leucine respiration was then calculated through [35]'s method usage of slope of linear regression line over the incubation time [35]. LeuCF<sub>emp</sub> of 1.15 kg C [mol Leu]<sup>-1</sup> was used as freshwater leucine-to-carbon conversion factor [36]. Errors were calculated by measuring the standard deviation

between rates derived at each time point.

#### 3. Results

#### 3.1. Oxygen consumption following PEG addition

Across all molecular weights (MW = 100–1000 Da) and concentrations (50–1600 mg/L), PEG exposure increased microbial oxygen consumption relative to controls (Fig. 2a). Even at the lowest concentration (50 mg/L), consumption rose by approximately +45–82 % compared with unamended treatments (MW:  $p=7.57\times10^{-3}$ ; concentration: p=0.418;  $R^2\leq0.13$ ).

When the experiment was repeated using 50 mg/L of PEG with triplicate incubations (Fig. 2b), the same general increase was observed, though absolute oxygen consumption was an order of magnitude lower than in the initial experiment. The 400 Da PEG showed the highest mean consumption (2.61  $\mu$ M; approximately 3x control;  $p=4.26\times10^{-18}$ ;  $R^2=0.17$ ), while the 100 Da and 1000 Da PEG treatments were lower (1.54  $\mu$ M and 0.85  $\mu$ M, respectively). The rank order therefore differed between experiments, indicating some temporal or community variation.

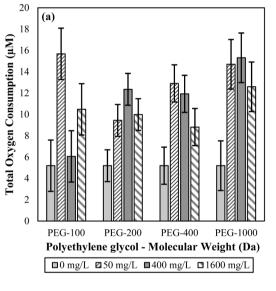
Hourly oxygen consumption rates (Fig. 2c) increased by +40-64 %

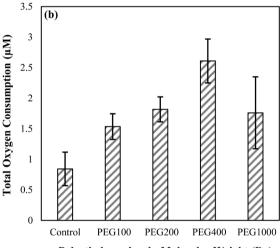
across PEG treatments compared with the control (control:  $p=2.45\times 10^{-4}$ ,  $R^2=0.87$ ; PEG 100–1000 Da:  $p=1.34\times 10^{-6}$ ,  $R^2\geq 0.90$ ). No clear linear relationship was observed between PEG molecular weight and oxygen consumption rate.

A carbon mass balance estimated that 0.0244–0.0622 % of the added PEG (100–1000 Da) was mineralized to  $CO_2$  during incubation (Supplementary Information 12b).

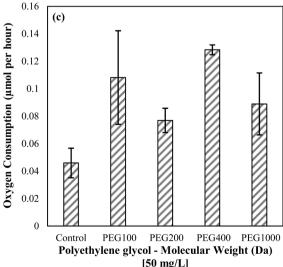
#### 3.2. Prokaryotic leucine uptake and respiration

 $^3$ H-leucine assimilation rates generally increased in the presence of PEG for MW  $\geq 200$  Da at all tested concentrations (Fig. 3a). At 50 mg/L, assimilation reached  $\geq 1.46 \times 10^{-9}$  kg C [mol Leu] $^{-1}$  min $^{-1}$ , significantly higher than the control ( $p=9.87 \times 10^{-5}$ ;  $R^2=0.48$ ). PEG 100 Da produced slightly lower assimilation than the control at 50 mg/L but increased with concentration. Across the full range of molecular weights, mean assimilation for PEG  $\geq 200$  Da reached  $3.61 \times 10^{-9}$  kg C [mol Leu] $^{-1}$  min $^{-1}$  ( $p \geq 0.01$ ;  $R^2 \geq 0.10$ ). Increasing PEG concentration from 50 to 1600 mg/L was associated with declining assimilation rates (p=0.0289;  $R^2 \leq 0.86$ ), showing rate decreases of  $2.17 \times 10^{-10}$  (200 Da),  $2.49 \times 10^{-9}$  (400 Da), and  $1.61 \times 10^{-9}$  kg C [mol Leu] $^{-1}$  min $^{-1}$  (1000



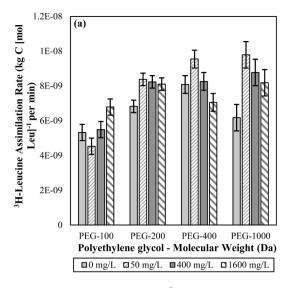


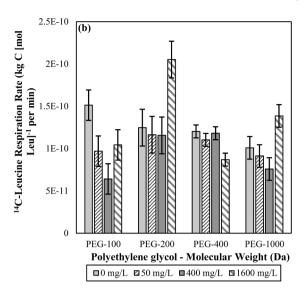
Polyethylene glycol - Moleuclar Weight (Da) [50 mg/L]



**Fig. 2.** Prokaryotic total oxygen consumption with PEG; (a) Initial proof of concept with PEG at varied MW (100, 200, 400, 1000 Da) and concentration (0, 50, 400, 1600 mg/L) (b) Comparison of consumption with PEG at varied MW (100, 200, 400, 1000 Da) with concentration of 50 mg/L (c) Comparison of rate of consumption per hour for PEG at varied MW (100, 200, 400, 1000 Da) with concentration of 50 mg/L (Error bars derived from standard deviation).

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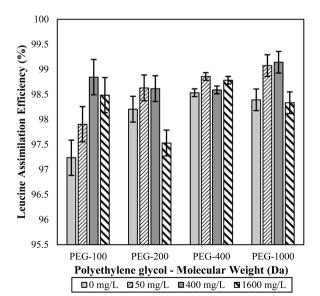
**Fig. 3.** Prokaryotic leucine incorporation rates (a) <sup>3</sup>H-Leuicne assimilation rate with PEG at varied MW (100, 200, 400, 1000 Da) and concentration (0, 50, 400, 1600 mg/L) (b) <sup>14</sup>C-Leucine respiration rate with PEG at varied MW (100, 200, 400, 1000 Da) and concentration (0, 50, 400, 1600 mg/L) (Error bars derived from standard deviation).

Da).

 $^{14}\text{C-leucine}$  respiration (Fig. 3b) showed no consistent linear trend with PEG molecular weight or concentration. Overall respiration was slightly lower than the control (MW:  $p \geq 4.46 \times 10^{-13}$ ; concentration:  $p = 5.05 \times 10^{-6}$ ;  $R^2 \leq 0.91$ ), though several treatments overlapped within the range of replicate variability.

#### 3.3. Leucine assimilation efficiency

Leucine assimilation efficiency (LAE; Fig. 4) was generally but not uniformly elevated in PEG treatments. Across all PEGs and concentrations, LAE increased by +0.29 to 1.91% compared with controls (MW:  $p \geq 2.53\times 10^{-9}$ ; concentration:  $p \geq 2.25\times 10^{-6}$ ;  $R^2 \leq 0.71$ ). However, at higher concentrations (1600 mg/L), the trend was inconsistent: PEG 100 Da and 1000 Da were slightly below the control, whereas PEG 200 Da and 400 Da remained elevated. These deviations indicate that LAE responses were concentration dependent and not uniformly enhanced



**Fig. 4.** Leucine assimilation efficiency percentage of prokaryotes with PEG at varied MW (100, 200, 400, 1000 Da) and concentration (0, 50, 400, 1600 mg/L) (Error bars derived from standard deviation).

across all treatments.

#### 4. Discussion

Our findings demonstrate that PEG, a representative WSP, enhances microbial oxygen consumption in river water across all tested molecular weights and concentrations confirming our hypothesis. This suggests that WSPs are bioavailable, serve as carbon sources, and stimulate microbial respiration, potentially contributing to oxygen depletion and carbon dioxide generation in freshwater systems. Given the rising prevalence of WSPs release into aquatic environments [12,38], their influence on microbial metabolism could exacerbate deoxygenation, particularly in eutrophication-prone systems and enhance production of the greenhouse carbon dioxide.

The implications extend beyond PEG, as many WSPs with varying MWs and chemical compositions may behave similarly. In real-world scenarios, water bodies receiving high inputs of WSPs such as landfill leachate, industrial discharge, and wastewater effluent/discharge could experience enhanced microbial oxygen consumption, exacerbating hypoxia risk. Given that most studies on plastic pollution focus on solid MPs [39], our findings highlight the need to consider the understudied contribution of WSPs to aquatic oxygen depletion and greenhouse gas emissions.

The increased leucine uptake rates observed after 24 h of PEG exposure confirm that PEG can serve as a bioavailable substrate, stimulating microbial growth. As an essential amino acid, leucine is a valuable prokaryotic substrate, and its uptake rates are widely used to estimate prokaryotic production [36,40]. Given its fundamental role in protein synthesis, prokaryotic cells preferentially allocate leucine for anabolic processes rather than catabolic energy generation. Accordingly, the decrease in leucine respiration rates following PEG exposure and the resulting increase in leucine assimilation efficiency suggest that PEG fulfilled the cells' energy demands, allowing more leucine to be directed toward biomass synthesis. Microbial growth efficiency serves as a key indicator of a community's eco-physiological status, for which leucine assimilation efficiency serves as a proxy [37]. The observed increase in leucine assimilation efficiency in the presence of PEG suggests that PEG did not impose physiological stress on the microbial community but instead functioned as a readily bioavailable carbon source, enhancing bacterial carbon use efficiency [41].

Interestingly, our results revealed distinct relationships between PEG concentration and oxygen consumption depending on molecular weight,

suggesting that microbial responses to PEG exposure are complex and influenced by additional factors. One possible explanation for this linearity is the formation of colloidal structures by higher-molecular-weight PEG molecules, which may alter microbial accessibility and reduce degradation efficiency [42]. Another contributing factor could be microbial enzymatic selectivity, where different taxa preferentially metabolize specific polymer sizes, leading to variation in degradation rates [43]. Additionally, threshold-driven responses may play a role, as similar non-linear patterns have been observed in toxicological and nutrient-addition studies, where microbial activity is influenced by concentration thresholds or nutrient availability [44].

This study was designed as a short-term proof of concept to capture immediate microbial responses. The PEG concentrations used (50–1600 mg/L) were intentionally higher than environmental levels in UK rivers (2.1–33.5  $\mu$ g/L; [34]) to elicit measurable metabolic effects within 24 h. Such concentrations are plausible near WSP point sources (e.g., wastewater outfalls, industrial discharges), where dilution is limited. Consequently, our results represent upper-bound responses relevant to impacted reaches.

Rates of oxygen consumption in the presence of PEG (0.05–0.08 mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>) were low in absolute terms but significantly exceeded the control (0.03 mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>). Control rates were an order of magnitude below typical headwater streams (0.3–1.2 mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>; [45]) and two orders below nutrient rich lowland rivers (5-10 mg O<sub>2</sub> L<sup>-1</sup> day<sup>-1</sup>; [46]), reflecting the influence of experimental design rather than intrinsic microbial inactivity. The dilution of natural river water (1 part in 4) and dark incubation, used to reduce background DOC and competing substrates, likely lowered microbial abundance and altered community structure in addition to typical bottle effects [47]. These constraints limit total metabolic capacity and thus absolute O2 demand. Hence, while our mass balance calculation indicated that 0.0244-0.0622 % of the added PEG (100-1000 Da) was mineralised to CO2 during 24 h, in-situ microbial communities would be expected to exhibit proportionally higher metabolic potential. (It should be noted that our mass balance assumes complete PEG degradation, whereas partial persistence of oligomers may have occurred [48] and warrants further investigation.) It is therefore reasonable to infer that PEG could further stimulate oxygen consumption in undiluted river water, potentially exacerbating local oxygen depletion and contributing to eutrophication-like conditions, particularly in warm or nutrient-rich reaches.

While our experiments demonstrate that PEG is bioavailable to river microbes, further work is required to quantify realistic in-situ degradation rates. Seasonal variability in temperature, nutrient availability, and community composition (e.g., May-September sampling) likely contributed to differences between experiments, as seen in control rates (Figs. 2a-b). Long-term studies across environmental gradients and at environmentally relevant ( $\mu$ g/L) concentrations are needed to determine chronic impacts, community shifts, and potential toxicity thresholds. Sustained WSP exposure could alter nutrient cycling or promote oxygen depleting microbial pathways characteristic of eutrophic systems [49].

Oxygen depletion in aquatic ecosystems is a growing concern, particularly in the context of climate change. Warmer temperatures accelerate microbial respiration, while altered precipitation patterns such as increased drought frequency and intense rainfall can influence nutrient and pollutant loading [50–52]. In light of our findings, we speculate that pulses of WSP inputs, particularly in areas with high plastic contamination, could further exacerbate oxygen deficits, compounding the effects of climate change-induced eutrophication [53].

Although this study focused on a freshwater system, it could be suggested that similar processes may be occurring in marine environments, particularly in plastic accumulation zones such as the Great Pacific Garbage Patch [54]. WSPs discharged through wastewater treatment plants (WWTPs) may eventually reach coastal and oceanic waters, where their contribution to hypoxia remains largely unquantified [55]. Given that PEG concentrations as low as 50 mg/L influenced

oxygen consumption in this study, areas with higher WSP loads, such as industrial outfalls and plastic pollution hotspots may experience more severe oxygen depletion.

Currently regulatory frameworks for plastic pollution primarily focus on visible macro-plastics and particulate MPs, overlooking the potential impacts of dissolved polymers such as PEG and other WSPs. The European Chemicals Agency (ECHA) defines microplastics as "synthetic water-insoluble polymers of 5 mm or less" but does not include soluble plastics in its regulatory framework [18]. Similarly, the UK's Water Framework Directive (WFD) monitors chemical pollutants but does not explicitly address WSPs [56]. Our findings suggest that WSPs certainly warrant further investigation in the form of long-term, low WSP concentration studies to further evidence causality and going forward, may warrant explicit regulatory consideration due to their demonstrated capacity to fuel microbial oxygen consumption.

#### 5. Conclusion

We hypothesized that WSPs, represented here by PEG, serve as substrates for freshwater microbial metabolism and thereby enhance oxygen consumption. Bioassay experiments using riverine microbial communities supported this hypothesis: PEG increased prokaryotic growth efficiency, stimulated oxygen drawdown, and demonstrated that PEG is a readily bioavailable substrate. These findings reveal an underrecognized mechanism by which dissolved plastics, when present at high concentrations, may contribute to oxygen depletion and elevate eutrophication risk. The implications extend beyond PEG, as many WSPs are likely degraded by microbes in similar ways, particularly in polluted or low-flow water bodies. Identifying WSPs as potential drivers of oxygen loss is therefore essential for advancing scientific understanding and informing environmental policies aimed at protecting water quality and aquatic ecosystem health.

#### Author statement

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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#### Data availability

Data will be made available on request.

# CRediT authorship contribution statement

Drishna Sainju: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Sarah Bercovici: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Robert Lucas: Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. Adam Le Gresley: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. Alice A. Horton: Supervision, Project administration, Methodology, Conceptualization. Claire Evans: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

## Declaration of competing interest

The authors declare the following financial interests/personal

relationships which may be considered as potential competing interests: Drishna Sainju reports financial support was provided by Haleon plc. Drishna Sainju reports financial support was provided by Biotechnology and Biological Sciences Research Council. Drishna Sainju reports a relationship with Haleon plc that includes: funding grants. Drishna Sainju reports a relationship with Biotechnology and Biological Sciences Research Council that includes: funding grants. If there are other authors, they 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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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nexres.2025.101149.

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