

RESEARCH ARTICLE

Nutrient, carbon, and darkening impacts on coastal dissolved phosphorus bioavailability—a mesocosm study

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Abstract

Coastal eutrophication results from increased riverine loads of inorganic nutrients, including phosphorus (P), which may co-occur with increased dissolved organic carbon (DOC) loading. These DOC molecules are often pigmented, causing water darkening, but they also contain dissolved organic P (DOP), which could exacerbate eutrophication. However, it is unclear how the bioavailable DOP (BDOP) pool responds to the individual and interactive effects of increased DOC, higher inorganic nutrient concentrations, and water darkening in coastal ecosystems. To explore these interactions, we conducted bioassays to estimate BDOP in a fully factorial mesocosm experiment manipulating the supply of labile DOC (glucose), inorganic nutrients and pigmented compounds that cause darkening. Whereas the evidence for labile DOC (glucose) effects on BDOP was weak, inorganic nutrient enrichment caused increases in BDOP concentrations in clear-water mesocosms. By contrast, in experimentally darkened waters, the addition of inorganic P did not contribute to BDOP but mainly persisted in its inorganic form. Our results suggest that water management efforts aimed at preventing or reversing coastal darkening could increase the removal of excess inorganic P from the water due to light-enhanced algal uptake. However, the total dissolved bioavailable P pool may not decrease but rather shift from dominance by inorganic to organic forms. Therefore, mitigating both coastal darkening and eutrophication in these ecosystems is essential for reducing total bioavailable P to a level that supports their ecological balance and functionality.

Phosphorus (P) is a critical nutrient in aquatic ecosystems, influencing primary productivity, biogeochemical cycles, and community composition (Elser et al. 2007). It is often the limiting nutrient in freshwater and coastal systems, where its availability controls microbial and phytoplankton growth

(Schindler 1977; Elser et al. 2007; Nausch and Nausch 2011). In natural waters, P exists in various forms, including dissolved inorganic phosphorus (primarily as orthophosphate), particulate phosphorus, and dissolved organic phosphorus (DOP) (Karl and Björkman 2015). While inorganic P is immediately available for uptake by aquatic organisms, DOP has variable bioavailability and often requires enzymatic transformation into assimilable forms, making this an important but less direct source of P for microbial communities (Björkman and Karl 2003).

The concentrations and forms of bioavailable P in coastal ecosystems are driven by a diversity of external and internal sources and mechanisms, which are in turn sensitive to ongoing environmental change. For example, inorganic nutrient loading has decreased in many northern rivers over the last decades (i.e., “oligotrophication”), partly due to eutrophication

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mitigation efforts and reductions in atmospheric deposition (Huser et al. 2018; Mason et al. 2022; Isles et al. 2023). Yet, such changes often co-occur with increases in dissolved organic matter (DOM) runoff (i.e., “browning”), which has contributed to the darkening of coastal waters (Weyhenmeyer et al. 2014; Isles et al. 2021; Frigstad et al. 2023). Co-occurring oligotrophication and browning may not only change the relative pool sizes of inorganic and organic P forms delivered to coastal systems, but also alter the limnological conditions under which plankton communities use these resources. More broadly, coastal aquatic ecosystems, such as along the Baltic Sea, experience significant variability in nutrient dynamics driven by terrestrial inputs and water stratification (Nausch and Nausch 2011; Nausch et al. 2018; Rönspiess et al. 2021), with additional challenges posed by internal P loading from sediments, particularly under anoxic conditions, which can exacerbate eutrophication (Conley et al. 2009). In such ecosystems, organic P compounds, often transported from surrounding catchments, can constitute a major component of the total dissolved phosphorus (TDP) pool (Baldwin 2013; Nausch et al. 2018; Yeh et al. 2020).

The cycling of organic P is tightly linked to microbial and algal processes, influenced by nutrient stoichiometry, light, and carbon availability (McGuirk Flynn 2008; Duhamel et al. 2012). In oligotrophic systems, where inorganic P is scarce, microbial communities often rely on DOP, particularly during periods of high metabolic demand or when allochthonous DOM inputs are elevated (Cotner and Wetzel 1992; Karl and Björkman 2015). In contrast, eutrophic systems, characterized by high concentrations of inorganic P, often see a reduced reliance on DOP (Berthold and Schumann 2020; Duhamel 2024), although stoichiometric shifts may still drive DOP utilization (Stepanuskas et al. 2002; Schindler et al. 2008). Moreover, phytoplankton can also scavenge bioavailable DOP (BDOP), particularly during periods of nutrient limitation, through enzymatic activity that transforms BDOP into inorganic P (Maranger et al. 2018; Feng et al. 2023). These complex interactions underscore the dynamic nature of P cycling (Lebret et al. 2018; Maranger et al. 2018; Feng et al. 2023).

However, past research on the role of DOM in the P cycle is limited and has not disentangled the two main effects of DOM: its pigmentation that causes water darkening and its content of organic carbon that boosts microbial processes. Moreover, interactions between DOM and inorganic nutrient loading on BDOP dynamics have remained unexplored. In this context, our study aims to investigate the dynamics of DOP bioavailability under varying environmental conditions, specifically focusing on light availability, carbon enrichment, and inorganic nutrient enrichment. Our broadest hypothesis is that these factors interact to shape P dynamics and microbial community responses. More specifically, we tested three primary, non-mutually exclusive hypotheses, each of which explores a distinct aspect of P bioavailability in aquatic environments:

1. Light availability plays a central role in regulating BDOP concentrations by modulating autotrophic demand and competition for P.
Prediction: Under dark water conditions, we expect BDOP to accumulate, whereas under light conditions greater autotrophic biomass and demand will deplete this nutrient pool.
2. The availability of organic energy from DOC regulates BDOP by stimulating microbial activity and the breakdown of complex C and P structures, making P more bioavailable.
Prediction: adding labile DOC will lead to increased BDOP.
3. The supply of inorganic nutrients indirectly drives BDOP concentrations by promoting the preferential uptake of inorganic P.
Prediction: adding inorganic nutrients will lead to the accumulation of BDOP.

Our study investigates both the separate and interactive effects of these three factors on BDOP by conducting a fully factorial mesocosm experiment that manipulates water color, labile carbon inputs, and inorganic nutrient levels. This approach enables us to disentangle the effects of DOM pigmentation and carbon content on BDOP dynamics, while examining their interactions with inorganic nutrients, thus potentially providing novel insights with relevance to the management of coastal ecosystems affected by coastal darkening and eutrophication.

Methods

Site and experimental setup

This study was conducted as part of a larger project examining nutrient dynamics using coastal mesocosms (Garnier et al. 2023). We set up 24 outdoor mesocosms at the Forsmark field station on the Swedish Baltic Sea coast. Each mesocosm consisted of a 400 L flexible, foldable plastic tank (FlexiTank, 1.1 m deep, 0.68 m diameter), containing a 7 cm layer of substrate (3 cm sand + 4 cm natural soft sediment) and filled with 350 L of unfiltered seawater. The resulting salinity was 5.62 PSU (± 0.07 SD across mesocosms). The sediment, collected from nearby coastal areas (60°24'16.8"N, 18°11'2.3"E), was sieved (5 mm mesh) to remove large debris and large organisms, then aerated to ensure oxygenation before being added to the mesocosms. Zooplankton and zoobenthos were introduced to all mesocosms as part of the standardized setup, following methods detailed in Garnier et al. (2023). Prior to the start of our experiment on August 11th, 2020 (Day 0), we randomly added sediments, seawater, zooplankton, and zoobenthos in equal amounts to all mesocosms (from Day -7 to Day -1) to establish initial communities and substrate conditions.

Experimental design

This study shared the same setup and site as Garnier et al. (2023). We used a full-factorial design with two levels of each factor—nutrients, light availability, and carbon addition. This

produced eight treatment combinations, each replicated three times across 24 mesocosms.

Nutrient enrichment in the experiment was designed to mimic eutrophic conditions typical of high-nutrient regions in the Baltic Sea, with initial additions of nitrogen (ammonium nitrate [NH_4NO_3], $60 \mu\text{mol N L}^{-1}$) and phosphorus (potassium dihydrogen phosphate [KH_2PO_4], $3.8 \mu\text{mol P L}^{-1}$), followed by smaller pulses (10% of the initial concentration) every second or third day, always before sampling (see Garnier et al. (2023) for details). Carbon enrichment was applied by adding D-glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) at a concentration of $79.5 \mu\text{mol C L}^{-1}$ (1 mg C L^{-1}), synchronized with each nutrient addition. Cumulative nutrient and carbon additions across the experiment amounted to $715.5 \mu\text{mol C L}^{-1}$ (8.6 mg C L^{-1}), $108 \mu\text{mol N L}^{-1}$ ($1512 \mu\text{g N L}^{-1}$), and $6.84 \mu\text{mol P L}^{-1}$ ($212 \mu\text{g P L}^{-1}$), maintaining a C : N : P ratio of 106 : 16 : 1.

Light availability was manipulated by adding 80 mL (i.e., 0.23 mL L^{-1}) of Sera Blackwater Aquatan (Sera GmbH) water conditioner to each tank. This commercial product, derived from peat extracts, contains humic substances, trace electrolytes, and minor amounts of organic acids (as described in the product's Safety Data Sheet; Sera GmbH, 2023). It has previously been applied in aquarium and experimental contexts to reduce light penetration without introducing major nutrient artifacts (van Dorst et al. 2020; Garnier et al. 2023). At the chosen concentration, the addition resulted in a light attenuation coefficient [$K_d(\text{PAR})$] of approximately 5. In dark treatments, this resulted in only 5% ($\pm 8\%$ SD) of incoming light reaching the bottom compared to 14% ($\pm 6\%$ SD, $K_d \approx 2$) in clear-water controls. To maintain reduced light conditions, a maintenance dose corresponding to 10% of the initial concentration addition was applied at mid-experiment.

To evaluate potential side effects of the conditioner, we measured dissolved organic carbon (DOC), as well as inorganic and organic nutrient concentrations the day after each addition. Nutrient concentrations remained unchanged (ANOVAs for inorganic N and P: $F_{1,13} = 0.030$, $p = 0.866$ and $F_{1,13} = 0.003$, $p = 0.954$, respectively), while DOC concentrations increased modestly in dark treatments ($+2.18 \text{ mg C L}^{-1}$) compared to controls (ambient 4.88 mg C L^{-1} ; ANOVA: $F_{1,13} = 16.949$, $p = 0.001$). Thus, the main effect of the conditioner was reduced light availability, with a minor but measurable increase in DOC. Further details on setup and experimental design are provided in Garnier et al. (2023).

Sampling and filtration

Water samples were collected from each mesocosm on three occasions, at the start (Day 1), middle (Day 10), and end (Day 20) of the experiment. Samples were coarsely filtered using a $70 \mu\text{m}$ mesh sieve. We then transferred water samples from each mesocosm to sterile 1 L polypropylene bottles, which were stored in the dark and kept cool (approximately 4°C) for no more than 24 h before further processing in the laboratory.

Measurements for basic abiotic parameters such as temperature, dissolved oxygen, and conductivity were performed in situ at 40 cm depth using a portable multiparameter probe (AP-2000, AquaRead Ltd.), following the same approach as Garnier et al. (2023). Additionally, the chlorophyll *a* (Chl *a*) concentration was measured as a proxy for phytoplankton biomass. For this, 500 mL of the coarse-filtered water samples were filtered onto glass-fiber filters (Whatman GF/F). The filters were stored at -20°C until analysis, when Chl *a* was extracted in 96% ethanol for 12 h and measured fluorometrically (excitation 433 nm, emission 673 nm) in darkness with a spectrophotometer (LS 30 Perkin Elmer).

For analysis of total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrate (NO_3), ammonium (NH_4), and phosphate (PO_4 , represented by soluble reactive P [SRP]), water samples were filtered into 50 mL Falcon tubes using prerinsed $0.2 \mu\text{m}$ -pore syringe filters and stored at -20°C until analysis. The TDN and TDP concentrations were analyzed by measuring color on a photometer segmented flow analyzer (QuAatro 39, Seal Analytical) after an in-line digestion step with alkaline acidic persulfate at 110°C and 0.9 MPa (method no: Q-115-10 Rev. 4). Analysis of NO_2 and NO_3 was conducted using a cadmium reduction coil to form an azo dye (method: MT3B Q-126-12 Rev 1), NH_4 used the salicylate method (method: Q-033-04 Rev. 8) and PO_4 the molybdenum blue method (method: MT3A Q-125-12 Rev 1). Dissolved inorganic nitrogen (DIN) concentration estimates were calculated as the sum of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations. Moreover, we calculated DON and DOP by subtracting the DIN and phosphate from TDN and TDP, respectively. In some cases, DON slightly exceeds TN and DOP exceeds TP. These discrepancies result from independent measurements of total and dissolved fractions and are within the combined analytical uncertainties of the methods for N. They do not affect the interpretation of treatment differences.

In the laboratory, we filtered approximately half of the 1 L water samples collected from the mesocosms through a membrane nylon filter ($0.2 \mu\text{m}$ pore size, Whatman) to eliminate the highest number of cells possible. We then collected subsamples for DOC analysis in pre-acid-washed 40 mL amber borosilicate glass vials filled to the brim and immediately fixed with $20 \mu\text{L}$ of 10% HCl to reach a pH of 2 and closed with silicone septum caps. Samples were kept cool at 4°C until analysis at the Jan Veizer Stable Isotope Laboratory, University of Ottawa. The remaining filtered samples were immediately used for bioassay preparations.

Bioavailability bioassays and bioavailable dissolved organic phosphorus estimations

The bioavailability assays were conducted using a modified version of the bacterial regrowth bioassay described by Rulli et al. (2022), adapted from the "spike-response" methods by Soares et al. (2017) and Stepanauskas et al. (2002), to estimate DOP bioavailability. This method captures immediately

bioavailable nutrients through microbial growth responses under nutrient-limited conditions. Adaptations were made to accommodate a high-throughput setup using sterile 96-well deep-well plates as an efficient way to analyze multiple samples while maintaining alignment with the principles of the original bioassay protocol.

Following Rulli et al. (2022), bioassays were conducted by adding 0.2- μm filtered mesocosm water and a microbial inoculum (2% v/v) from the corresponding mesocosm to each well to preserve site-specific microbial communities. To ensure strong phosphorus-limited conditions, we added a mixture of micronutrient growth medium (5% v/v), together with C (as $\text{C}_6\text{H}_{12}\text{O}_6$, 20 mg C L^{-1}), and inorganic N (as NH_4NO_3 , 2000 $\mu\text{g N L}^{-1}$) solutions. Four replicates were prepared for each sample, with half of the wells receiving a P spike (as Na_2HPO_4 , 10 $\mu\text{g P L}^{-1}$) to verify limitation and calculate BDOP concentrations. Non-spiked and spiked samples were prepared in distinct plates to avoid contamination. Control samples (blanks) were included in each plate and prepared similarly but with Milli-Q water instead of mesocosm water. This approach assumes that the cellular P quota is the same in spiked and non-spiked samples, that is, that the added phosphate spike does not affect the P content per cell. This assumption is based on and supported by the findings of Soares et al. (2017), who applied similar methodology but added a series of five different spike concentrations to determine if the growth response per unit added P was dependent on the spike itself. Thus, Soares et al. (2017) found that the growth response was proportional to the spike concentration, representing a linear relationship that crossed the origin.

Samples were incubated in a dark constant chamber at 20°C for 3 days. Subsamples were taken on Days 1, 2, and 3, fixed with 3% glutaraldehyde and stained with SYTO 13 nucleic acid stain for cell counts analysis using a BD Accuri C6[®] flow cytometer following the protocol outlined in Rulli et al. (2022). The cell numbers peaked 48 h after the start of the incubation, that is, $t = 48$. Therefore, bacterial cell counts, the bacterial growth yields, at Day 2 were used to estimate BDOP concentrations. To calculate the amount of macronutrient assimilated per cell, we divide the concentration of P-spike added by the number of bacterial cells produced from the spike addition (spiked minus non-spiked cells), assuming that the entire spike was assimilated (Stepanuskas et al. 2002). Bioavailable DOP concentrations were then calculated as the blank-corrected (non-spiked) cell counts multiplied by the known macronutrient assimilation per cell. As in previous applications of spike-response assays (e.g., Stepanuskas et al. (2002) and Soares et al. (2017)), BDOP values should be interpreted as standardized, relative measures of DOP bioavailability rather than precise fractions of chemically defined DOP.

Statistical analysis

All data processing and analyses were done with the statistical software R 4.2.0 (R Core Team 2024).

For the bioavailable phosphorus (BDOP), dissolved organic phosphorus (DOP), and phosphate (PO_4) variables, we used linear mixed-effects models to evaluate the fixed effects of darkening, nutrient enrichment, and carbon addition over the experiment's duration, using data from Days 10 and 20, with the sampling date treated as a random effect. The models were fitted using the *lmerTest* R package (Kuznetsova et al. 2017). Response variables were tested for normality assumptions, using QQ-plots and Shapiro–Wilk's tests, as well as heteroscedasticity and collinearity. Where normality assumptions were not met, natural logarithmic transformations were applied as indicated in the results tables. Model refinement was guided by the removal of non-significant three-way interaction terms ($p > 0.05$) when appropriate. When models were refined, we assessed the models' fit using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). For both BDOP and DOP, the simplified or refined models showed improved fit compared to the initial models based on lower AIC and BIC values.

Marginal means for post hoc comparisons of BDOP, DOP or PO_4 concentrations across different treatment combinations were estimated using the *emmeans* R package (Lenth 2024) based on model fits including both Day 10 and Day 20 data and plotted to summarize the effects of the main treatments and their interactions. These estimated marginal means were derived from the fitted linear mixed-effects model and adjusted for the presence of covariates in the model using the Tukey method.

Day 1 is a starting point and thus we assume that bacterial abundance would be similar in all mesocosms. Therefore, we estimated BDOP concentrations only for control mesocosms on Day 1, and for all mesocosms on Days 10 and 20, which formed the basis for the mixed-model analyses. Moreover, one sample (all three replicate mesocosms for Dark only treatment) on Day 10 was excluded from data analysis due to slightly negative values recorded, which indicates that BDOP was below detection level as discussed by Rulli et al. (2022).

To assess initial environmental conditions across the mesocosms, we performed two-way and three-way ANOVAs on key variables. For DOC, DON, and DOP concentrations measured on Day 1, we employed a three-way ANOVA to test the effects of Dark, Carbon, and Nutrient, and their interactions. For other variables measured on day -1 , we conducted two-way ANOVAs with Dark and Carbon as the primary factors. The significance threshold was set at $p < 0.05$, and data were log-transformed when necessary to meet assumptions of normality and homoscedasticity, verified by Shapiro–Wilk and Levene's tests, respectively.

Results

Initial conditions and general observations

The initial environmental conditions across all mesocosms are summarized in Table 1. Baseline values did not

Table 1. Initial conditions prior to treatment manipulations (Day -1 or 1 [before additions]) of all mesocosm ($n = 24$). Concentrations mean, standard deviation (SD), and standard error (SE) measurements of chlorophyll *a* (Chl *a*), dissolved oxygen (DO), nitrate (NO₃), ammonium (NH₄), dissolved inorganic nitrogen (DIN), phosphate (PO₄), total nitrogen (TN), total phosphorus (TP), dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), and dissolved organic carbon (DOC). Numbers marked in bold indicate treatment means that were significantly different from other treatment means ($p < 0.05$) on the same sampling day (two- or three-way ANOVA).

Variable	Mean	SD	SE	Day
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	3.16	0.36	0.07	-1
DO (mg L^{-1})	9.24	0.19	0.03	-1
NO ₃ ($\mu\text{g L}^{-1}$)	2.39	1.50	0.31	-1
NH ₄ ($\mu\text{g L}^{-1}$)	21.63	16.38	3.34	-1
DIN ($\mu\text{g L}^{-1}$)	23.91	16.90	3.45	-1
PO ₄ ($\mu\text{g L}^{-1}$)	1.64	1.11	0.23	-1
TN ($\mu\text{g L}^{-1}$)	255.67	88.32	18.03	-1
TP ($\mu\text{g L}^{-1}$)	23.38	8.05	1.64	-1
DON ($\mu\text{g L}^{-1}$)	269.34	83.11	16.96	1
DOP ($\mu\text{g L}^{-1}$)	30.63	11.99	2.45	1
DOC (mg L^{-1})	9.30	4.08	0.83	1

significantly differ between treatments ($p > 0.05$), except for DIN and DON on Day 1 (see Supporting Information Fig. S2). Yet, these concentration differences were low and negligible when comparing with the overall concentration variability during the course of the experiments (see Supporting Information Table S2 for complete raw data). Moreover, during the experiment, we observed variation in several parameters in response to the treatment conditions (Supporting Information Figs. S1, S2). Notably, Chl *a* concentrations, initially similar between dark and clear mesocosms, were 1.4 times higher in clear waters on Day 10 and approximately twice as high in nutrient-amended mesocosms by Day 20 compared to those without nutrient additions. The Chl *a* concentration values ranged widely, from $1.47 \mu\text{g L}^{-1}$ (± 0.14 SE) to $41.79 \mu\text{g L}^{-1}$ (± 1.49 SE), with the lowest and highest values recorded in carbon-only and nutrient-plus-carbon treatments on Day 10 (Supporting Information Fig. S1a).

Response of the P pools to the main effects of the treatments

Unless otherwise noted, results reported below are based on model estimates averaged across Days 10 and 20, with time-specific patterns shown in Fig. 1. The main effect of the Dark treatment (averaged data) was significant for BDOP and PO₄ (Table 2), suggesting an overall positive influence on their concentrations. However, model-based contrasts (pairwise comparisons) revealed that this effect was not statistically significant for BDOP ($t = -2.618$, $p > 0.05$; Supporting Information Table S1)

or in the absence of nutrient and carbon enrichments for PO₄ ($t = -1.818$, $p > 0.05$; Supporting Information Table S1). This implies that the effect of Dark is context-dependent and influenced by interactions with other factors, particularly nutrient availability for BDOP, as further supported by the significant Dark : Nutrient interactions (see Response of the P pools to the interaction effects of the treatments section). The lack of a significant effect on DOP implies that the Dark treatment specifically influenced the bioavailable P pools rather than total organic P.

Nutrient enrichment significantly affected all phosphorus pools—BDOP, PO₄, and DOP—with particularly strong main effects on PO₄ and DOP (Table 2), implying that the addition of nutrients substantially influenced DOP and PO₄ concentrations. When averaged across Days 10 and 20, DOP concentrations were twofold higher under Nutrient treatment compared to control (Fig. 1b), while PO₄ concentrations were approximately 27-fold higher in nutrient-enriched treatments (Fig. 1c). For BDOP, however, the pairwise comparisons revealed that averaged concentrations in nutrient-enriched mesocosms were not significantly higher than those of control mesocosms ($t = -2.618$, $p > 0.05$; Supporting Information Table S1). This implies that while nutrients enhanced P bioavailability overall, their effect on BDOP was context dependent.

In contrast, the carbon enrichment did not significantly affect the BDOP, DOP, or PO₄ concentrations across time (Table 2). Thus, carbon additions alone had minimal direct influence on P dynamics.

Response of the P pools to the interaction effects of the treatments

The Dark : Nutrient interaction had a significantly negative effect on BDOP concentrations, indicating that the effect of nutrient enrichment was dependent on light availability (Table 2). Specifically, BDOP concentrations averaged across time for Dark : Nutrient treated mesocosms were approximately threefold higher than in the controls (Fig. 1a). Moreover, BDOP concentrations in nutrient enriched waters were 30% higher in clear compared to dark water conditions (Fig. 2c). Yet, adjusted post hoc contrasts revealed no statistically significant pairwise differences between these treatments across the dataset (Supporting Information Table S1). Interestingly, in Carbon-enriched mesocosms under clear water conditions, a marginally significant difference was observed between treatments with and without Nutrient addition ($t = -2.912$, $p = 0.047$; Supporting Information Table S1; Fig. 2a), with higher values when nutrients are added. This result implies that Carbon enrichment may modulate the interplay between light availability and nutrient effects on P dynamics. Because the marginal means in Fig. 2 are averaged across both Day 10 and Day 20, some treatment differences may appear less pronounced than in the endpoint data alone (Fig. 1). We chose this approach to capture longer-term trends across the incubation and reduce the influence of variability

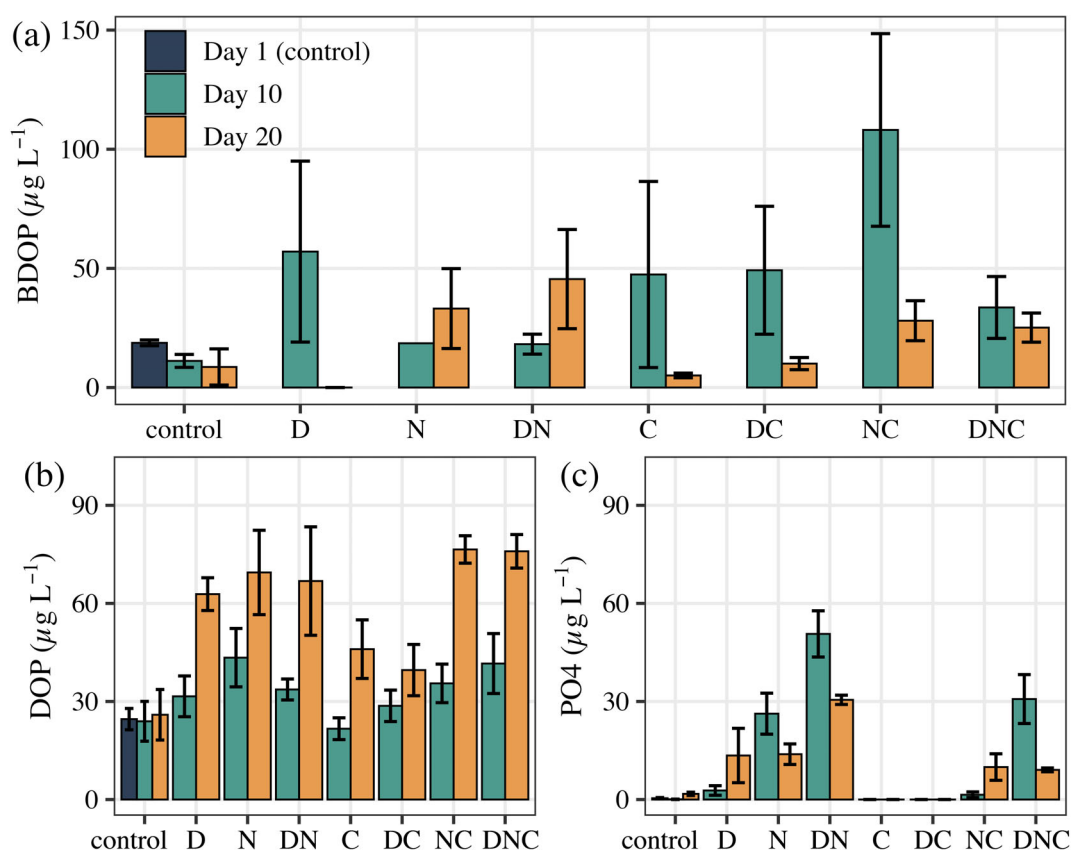


Fig. 1. Concentrations of (a) bioavailable dissolved organic phosphorus (BDOP), (b) DOP and (c) PO_4 shown over time for each mesocosm treatment: control, D (dark water), N (nutrient enrichment), C (carbon enrichment), and their combinations. Day 1 is shown only for the control, as representative of the general starting conditions in all mesocosms. Error bars denote \pm SE (std. error).

at a single time point. The overall conclusions regarding treatment effects are consistent between the raw data and the model-based means.

While PO_4 did not show statistically significant light-nutrient interactions ($p > 0.05$, Table 2), adjusted post hoc comparisons revealed strong significant pairwise differences

between Nutrient-enriched under Dark treatment and control mesocosms ($t = -11.228$, $p < 0.001$; Fig. 2f; Supporting Information Table S1), with higher concentrations when nutrients are added in the dark. Moreover, the pairwise comparisons also showed significant differences between the combined Nutrient- and Carbon-enriched and control mesocosms

Table 2. Results of mixed-effects models on the effects of water color (dark), inorganic nutrient enrichment (nutrient) and organic carbon enrichment (carbon) on BDOP, DOP, and PO_4 concentrations. Numbers marked in bold are significant ($p < 0.05$). BDOP, DOP, and PO_4 concentrations were transformed (natural logarithm) prior to analysis to fit a normal distribution.

Variable	BDOP (ln)			DOP (ln)			PO_4 (ln)		
	$F_{df=1}$	p		$F_{df=1}$	p		$F_{df=1}$	p	
Main effect									
Dark	8.269	0.004	+	0.006	0.941	-	4.542	0.033	+
Nutrient	4.192	0.041	+	14.636	<0.001	+	117.358	<0.001	+
Carbon	0.730	0.393	+	0.000	0.993	-	1.308	0.253	-
Interaction effect									
Dark : Nutrient	4.905	0.027	-	0.758	0.384	-	0.572	0.449	-
Dark : Carbon	1.767	0.184	-	1.254	0.263	+	1.430	0.232	-
Nutrient : Carbon	0.409	0.523	+	0.326	0.568	-	3.464	0.063	-
Dark : Nutrient : Carbon							4.955	0.026	+

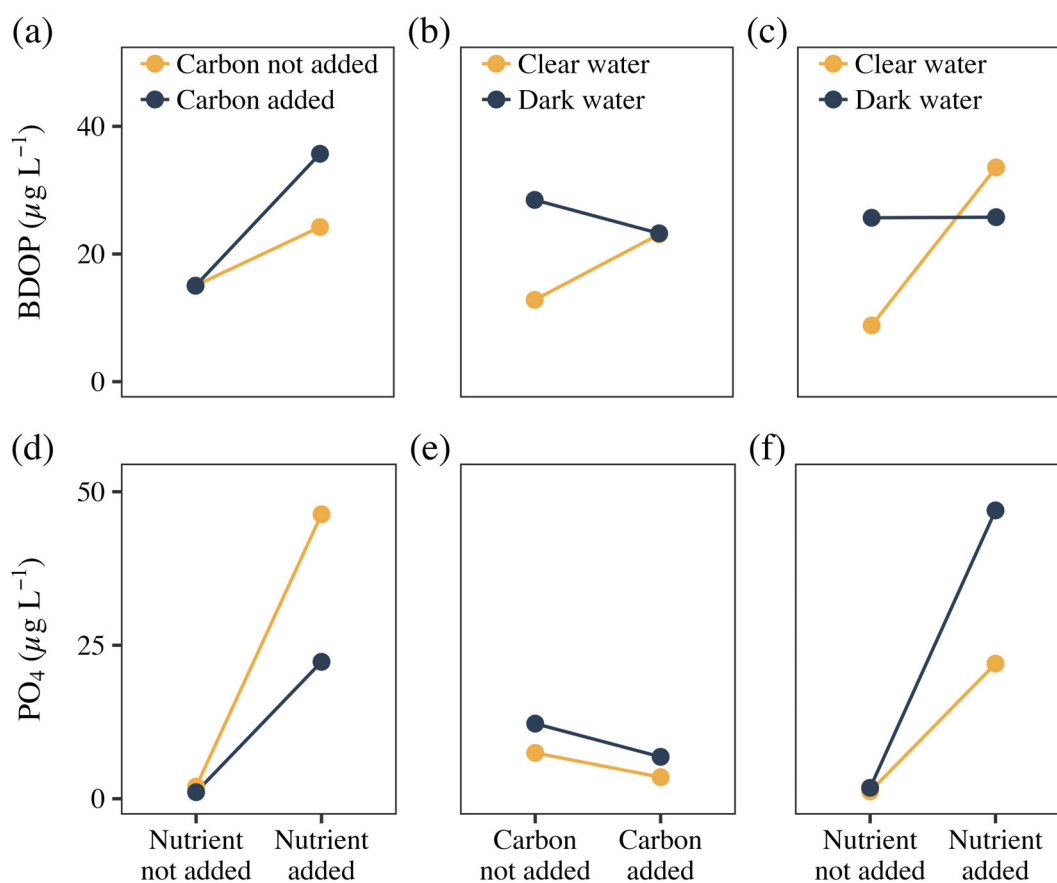


Fig. 2. Estimated marginal means for (a–c) BDOP and (d–f) PO_4 concentrations from the linear model of the average measurements between Day 10 and Day 20. The marginal means for each of the three treatments (carbon, nutrient, and darkening) encompass the measurements of all the tanks that received the said treatment (in isolation or in combination). For example, in panel d, the marginal mean of “nutrient added and carbon added” is the average PO_4 concentrations of the treatments NC and DNC, whereas the marginal mean of “nutrient added and carbon not added” is the average PO_4 concentrations of the treatments N and DN. Profile plots are shown for (a, d) nutrient and carbon enrichment interactions, (b, e) carbon enrichment and water color interactions, and (c, f) nutrient enrichment and water color interactions.

($t = -6.532$, $p < 0.001$) as well as other treatments (Fig. 2d,e; Supporting Information Table S1).

A significant three-way interaction between Dark treatment, Nutrient addition, and Carbon enrichment was observed for PO_4 concentrations (Table 2), implying complex interactions among light conditions, nutrient availability, and carbon enrichment in shaping PO_4 dynamics.

Discussion

Recent environmental changes in northern latitudes, including decreased inorganic nutrient loading and increased dissolved organic matter (DOM) runoff, have reshaped phosphorus cycling in coastal systems. Rising DOM concentrations are often attributed to recovery from historical acid deposition, increases in terrestrial runoff, land-use change, and enhanced plant growth and/or microbial activity in soils (Kritzberg et al. 2020). This change may alter microbial community structures and shift the balance between organic and inorganic nutrient supply. Despite these changes, the separate

contributions of DOM pigmentation and organic carbon content to bioavailable DOP (BDOP) dynamics remain unclear. Our study provides new insights into how these factors, along with inorganic nutrient loading, interact to influence P availability, both as DOP and inorganic P, in coastal ecosystems. Our results show for the first time how water darkening, carbon supply and eutrophication interact to shape the pools of bioavailable P, both in the form of DOP and of phosphate. We discuss here that light availability, influenced by water darkening, likely modulates the effectiveness of eutrophication mitigation measures. Actions targeting water darkening can modify the extent to which such measures reduce bioavailable P levels in eutrophic coastal waters.

Phosphorus dynamics under dark vs. clear water conditions

We hypothesized that BDOP concentrations would be higher under dark water conditions due to reduced competition from autotrophs (Berggren et al. 2010b; Solomon

et al. 2015). In line with this reasoning, we expected a decline in BDOP in clear water conditions, where higher light availability fosters autotrophic BDOP use, either through direct assimilation (Isles et al. 2021) or through phosphatase-facilitated transformation of BDOP to orthophosphate and subsequent uptake (Duhamel et al. 2012; Feng et al. 2018), leading to greater competition with heterotrophic microorganisms for BDOP (Elser et al. 1990; Cotner and Wetzel 1992). The positive main effect of the Dark treatment (Table 2) aligns with our hypothesis that darker waters promote higher concentrations of BDOP. Beyond the effects on competition for P, shifts in microbial community composition in low-light environments may have further influenced the effects of the Dark treatment on BDOP concentrations (Suominen et al. 2022). For example, in darker waters, microbial communities often exhibit increased heterotrophic activity, as reduced light limits autotrophic competition and potentially selects for microbes that are adept at utilizing organic P sources (Jones 1992; Berggren et al. 2010a). This, and other shifts in microbial functional composition, could enhance the cycling and retention of BDOP, thereby increasing concentrations in dark conditions.

The rise in BDOP concentrations in low-light conditions (Table 2) could also be due to reduced photodegradation of organic P compounds in darker waters (Liu et al. 2017). Studies have shown that reduced UV exposure, for example, in dark conditions, could result in higher BDOP accumulation in aquatic systems by reducing photo-mineralization to PO_4 Liu et al. (2017). However, as discussed by Younes et al. (under revision), it is also possible that photoreactions may be facilitated by inputs of pigmented compounds, such as the darkening conditioner used in this study. Speculatively, the increased presence of pigment in the water (Dark treatment) may trigger the production of free radicals (Alleson et al. 2016) that in turn degrade bioavailable DOP. Following this reasoning, BDOP concentrations would decrease instead of increase in dark water conditions. Therefore, it is difficult to conclude whether or not photo-mineralization affected the results in this study.

The Dark treatment also exhibited a positive main effect on PO_4 (Table 2), indicating increased concentrations under low-light conditions. This pattern may also arise from reduced photoautotrophic activity, as algae require light to assimilate inorganic P for growth, leading to decreased PO_4 uptake in darker environments (Marzetz et al. 2020). Additionally, the observed increase in PO_4 could be influenced by enhanced heterotrophic microbial activity, which often dominates under low-light conditions and can facilitate the mineralization of organic P into its inorganic form (Cotner and Wetzel 1992). Together, these dynamics underscore the complementary roles of light-driven autotrophic uptake and heterotrophic microbial processing in regulating PO_4 concentrations. Understanding these interactions provides critical insight into how shifts in light availability and nutrient inputs reshape P cycling in aquatic ecosystems.

Nutrient enrichment effects on the P pools

Anthropogenic nutrient enrichment, particularly inorganic solutes, plays a key role in shaping P availability in aquatic ecosystems, with effects that may vary based on environmental factors. We hypothesized that inorganic nutrient additions would increase BDOP concentrations, as the plankton community would preferentially use PO_4 (Elser et al. 1990; Cotner and Wetzel 1992), allowing BDOP to accumulate. Indeed, nutrient enrichment significantly increased BDOP, PO_4 and DOP concentrations (Table 2), highlighting the strong influence of nutrient inputs on P cycling (Schindler et al. 2008). Specifically, the increase in DOP supports findings that nutrient addition can enhance organic P availability by stimulating shifts toward more accessible P sources, aligning with patterns observed in boreal lakes (Vähätalo and Wetzel 2004). However, the effects of nutrient enrichment alone on BDOP concentrations appear context dependent, with significant increases only observed when combined with carbon additions (Fig. 2). While the overall impact of nutrients was significant, the lack of clear differences in pairwise comparisons (Supporting Information Table S1) suggests that environmental factors such as light availability or water color modulate microbial P processing. This observation is consistent with observations from other aquatic systems, where microbial community responses to nutrient additions varied under different light and organic matter conditions (Vähätalo et al. 2003; Guenet et al. 2010). Overall, our findings suggest that while the addition of nutrients stimulates the overall P cycle, its influence on bioavailable P pools like BDOP may be nuanced, reflecting a balance between nutrient supply and other ecological factors such as microbial demand and light availability. Additionally, the temporal dynamics of BDOP responses may contribute to the observed variability in treatment effects. Given that BDOP represents a rapidly cycling pool that can be quickly consumed by microorganisms (Cotner and Wetzel 1992), the lack of significant nutrient-only effects in pairwise comparisons may reflect rapid microbial turnover that obscures treatment differences at our sampling timepoints. Alternatively, our 20-day experimental duration may have been insufficient for detectable BDOP accumulation under certain treatment conditions, particularly where complex biogeochemical interactions require longer time scales to manifest. The substrate-dependent responses observed in Fig. 2, where nutrient effects on BDOP were only significant when combined with carbon additions, further suggest that the timing and magnitude of BDOP responses depend on the interactive effects of multiple environmental factors rather than single drivers alone.

Carbon enrichment effects on bioavailable dissolved organic phosphorus

Despite high DOC concentrations in terrestrial drainage water, rates of microbial metabolism in boreal freshwaters and coastal systems often remain C-limited due to the low

bioavailability of terrestrially derived DOC (Tranvik 1988; Stepanauskas et al. 2002; Berggren and del Giorgio 2015; Rulli et al. 2022). Therefore, sources of bioavailable C can strongly affect microbial activities, including P uptake (Jansson et al. 2006) and transformation (Karl 2014). We expected that labile C enrichment would positively affect BDOP concentrations, especially in dark waters, based on the assumption that increased C availability could stimulate microbial activity, promoting the breakdown of complex C and P structures and thus making P more bioavailable (Cotner and Biddanda 2002; Berggren et al. 2010c). However, we did not see a statistically significant effect of C enrichment on BDOP, suggesting that the anticipated influence of C enrichment on BDOP was not prominent under our experimental conditions.

Although not statistically significant, there was a tendency for BDOP to increase with C amendments in clear water conditions (Fig. 2b). Interestingly, this increase coincided with higher Chl *a* concentrations (Supporting Information Fig. S1a), suggesting a positive response in phytoplankton biomass to added C. Positive DOC effects on phytoplankton are debated, but recent evidence supports CO₂ fertilization of primary production due to bacterial mineralization of bioavailable organic C (Jansson et al. 2012; Zagarese et al. 2021). Thus, if phytoplankton indeed promoted BDOP availability, contrary to our expectation, this mechanism may explain why BDOP and Chl *a* both increased in light conditions following C additions. Generally, phytoplankton communities are known for efficient assimilation of BDOP (Elser et al. 1990; Cotner and Wetzel 1992), not excretion, although specific taxa might excrete substantial amounts of DOP (Karlsson et al. 2002; Feng et al. 2018). Nonetheless, death and lysis of phytoplankton cells could release significant amounts of BDOP from DNA, phospholipids, and other P-containing organic molecules (Cotner and Wetzel 1992). Moreover, increased phytoplankton biomass might lead to increased zooplankton grazing (Levine et al. 1999), and thus increased excretion and release of BDOP from zooplankton as such and from sloppy feeding. However, this dynamic might depend on bottom-up controls, as increased grazing by zooplankton is contingent on sufficient nutrient availability supporting phytoplankton productivity (Garnier et al. 2023). While we did not include fish in the mesocosms analyzed here, which can further regulate top-down controls by preying on zooplankton, this setup allowed us to isolate bottom-up processes influencing BDOP dynamics. These processes together suggest potential feedback where carbon availability indirectly sustains BDOP in the water column under light conditions.

Inorganic nutrient enrichment effect on P dynamics in dark and clear water conditions

The significant interaction between nutrient enrichment and light conditions highlights the complexity of BDOP dynamics in aquatic ecosystems (Table 2). While nutrient enrichment was expected to increase BDOP concentrations

more in dark than clear conditions—because reduced light availability limits phytoplankton competition for nutrients (Jansson et al. 2012; Feng et al. 2018)—our findings showed the opposite pattern (Fig. 2c). Bioavailable dissolved organic phosphorus accumulated more in clear water, suggesting that phytoplankton activity under light conditions may positively contribute to BDOP production (Li et al. 2014; Akbari et al. 2024). This emphasizes the role of light-mediated microbial and phytoplankton interactions in regulating bioavailable P pools.

Thus, one possible explanation for the interaction we observed is the enhancement of autotrophic activity under light conditions with nutrient enrichment, leading to rapid PO₄ assimilation by phytoplankton. This uptake primarily reduces inorganic P, while any released organic compounds likely originate from phytoplankton metabolic byproducts (Isles et al. 2021). Some studies suggest that microbial processing of such metabolic byproducts enhances their bioavailability and thus contributes to the BDOP generation (Karlsson et al. 2002; Karl 2014). The observed tendency for BDOP to increase under light, nutrient-enriched conditions may be partly influenced by microbial priming, where labile DOC from phytoplankton exudates stimulates microbial activity and the breakdown of complex organic P molecules (Guenet et al. 2010; Weigelhofer et al. 2020). This suggests that phytoplankton-derived DOC may play a more significant role in enhancing BDOP availability compared to external carbon additions, reflecting complex interactions between microbial and autotrophic processes (Karlsson et al. 2002). These dynamics merit further investigation in the context of aquatic nutrient cycling.

Furthermore, there is potential for inorganic P to be converted into BDOP through microbial processes under both light and dark conditions. In light conditions, bacteria can utilize PO₄ to synthesize organic P compounds which are later released into the water, for example, through cell lysis, thereby enriching the BDOP pool through pathways that rely on available inorganic nutrients (Karl 2014; Feng et al. 2018). This transformation is facilitated when microbial communities assimilate inorganic P for growth, subsequently producing organic matter that includes DOP and other metabolic byproducts, which contribute to BDOP dynamics (Karl 2014). These processes may enhance the organic P pool, potentially supporting heterotrophic activity, especially under dark conditions, where phytoplankton competition is reduced (Akbari et al. 2024). In contrast, under light conditions, the presence of both microbial and phytoplankton activity might intensify this process, further boosting BDOP availability (Karl 2014; Feng et al. 2018). This dual pathway suggests that while inorganic P additions could reduce competition for DOP, they may also directly enrich the organic P pool, particularly under conditions where light promotes microbial production of BDOP.

In contrast, under dark conditions, nutrient addition did not lead to the same increase in BDOP (Fig. 2c). Instead, BDOP

concentrations were stable in the presence of added nutrients, suggesting that heterotrophic microbes under low-light conditions may preferentially utilize PO_4 when available (Li et al. 2014), thereby reducing their dependence on BDOP. This observation aligns with previous findings showing that heterotrophic bacteria can shift their nutrient use based on availability, often favoring inorganic phosphorus when it is abundant, particularly in light-limited environments (Berggren et al. 2010c; Akbari et al. 2024). Such a shift in microbial resource use could explain the observed suppression of BDOP in dark, nutrient-rich conditions.

Interaction between Dark and Nutrient treatments differed depending on the P form and highlighted complex interactions between the light environment and nutrient cycling of organic and inorganic P. For example, the negative interaction between Dark and Nutrient treatments suggests that darker waters combined with nutrient enrichment reduce BDOP concentrations compared to their individual effects. This may reflect a positive interaction between light and nutrients, as both are essential for phytoplankton-driven BDOP generation. While the Dark-Nutrient interaction was itself not significant in pairwise comparisons with control (Supporting Information Table S1), the overall trend underscores the importance of light and nutrient availability working together to enhance BDOP dynamics. By comparison, PO_4 dynamics in response to nutrient enrichment were similar across both light and dark conditions, indicating that inorganic P availability increased consistently regardless of light. This may reflect reduced competition for P between phytoplankton and heterotrophic microbes when PO_4 is abundant, as nutrient enrichment can temporarily alleviate P limitation and competition (Cotner and Wetzel 1992; Karl 2014). These dynamics point to the potential for nutrient inputs to modify P cycling across different light conditions, though the pathways for BDOP and PO_4 transformations appear to diverge under clear vs. dark environments (Feng et al. 2018).

Mitigation efforts

Management efforts focused solely on reducing nutrient runoff to surface water are often insufficient for mitigating eutrophication symptoms and restoring the full health of aquatic ecosystems, especially if increased runoff of organic matter and consequent darkening of coastal waters contribute to the degradation of these habitats (Frigstad et al. 2023). Our findings indicate that while nutrient enrichment can initially promote BDOP concentrations in light conditions, water darkening can complicate and modify nutrient dynamics by altering microbial interactions and limiting autotrophic competition. Indeed, the interactions between dissolved organic matter (DOM) inputs and nutrient enrichment seemingly create complex microbial responses, reshaping phosphorus cycling and ecosystem functioning. Regardless, the increase in BDOP under dark water conditions underscores the influence of organic matter runoff on heterotrophic

bacterial productivity (Jansson et al. 2000). Effective management must therefore address both nutrient inputs and organic matter dynamics to reduce eutrophication and protect aquatic ecosystems. Moreover, the pivotal role of phytoplankton in regenerating bioavailable P highlights the importance of controlling nutrient loads in well-lit waters. Strategies should account for potential feedback loops where nutrient and light conditions amplify BDOP production, exacerbating eutrophication. Future research should focus on long-term trends in light–nutrient interactions and their implications for P cycling, guiding comprehensive management approaches that improve ecosystem resilience to environmental changes.

Author Contributions

Study conception: Aurélie Garnier, Magnus Huss, and Martin Berggren. Data acquisition: Mayra P. D. Rulli, Aurélie Garnier, Hani Younes, and Olivia Bell. Developing methods: Mayra P. D. Rulli and Martin Berggren. Data analysis: Mayra P. D. Rulli. Preparation of figures and tables: Mayra P. D. Rulli. Conducting the research, data interpretation, and writing: Mayra P. D. Rulli, Martin Berggren, Aurélie Garnier, Magnus Huss, Olivia Bell, and Hani Younes. All authors contributed to the revisions.

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Conflicts of Interest

None declared.

Data Availability Statement

The original data are found in Supporting Information Table S2. Additional unprocessed raw data are available from the corresponding author upon request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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