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Effects of increased allochthonous dissolved organic carbon on the growth of planktonic biota in freshwater ecosystems: A meta-analysis

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Abstract

Water browning, induced by allochthonous dissolved organic carbon (DOC) input, has become a widespread phenomenon in boreal lakes over the past decades. Directly quantifying aquatic organisms' responses to increased DOC concentrations is essential for projecting carbon cycle processes in freshwater ecosystems. In this study, we assessed the impacts of DOC addition on the growth of three freshwater planktonic groups: phytoplankton, zooplankton, and bacteria, and explored potential drivers behind variations in effect size. Background DOC concentrations vary between 0.5 and 25 mg L^{-1}, while total phosphorus concentrations span from 0.0003 to 1.55 mg L^{-1}. Based on a meta-analysis of 804 observations from 47 publications, we found that DOC addition had a significant positive effect on bacteria, while it had a small but negative impact on both phytoplankton and zooplankton. In different climate zones, DOC addition often stimulated bacterial growth, but it exerted either positive or negative effects on phytoplankton and zooplankton. Additionally, the effect sizes of both phytoplankton and zooplankton showed a significant negative relationship with the magnitude of DOC enrichment, while bacteria exhibited positive responses. Furthermore, the effect sizes of these three taxa correlated negatively with background total phosphorus concentrations and positively with the DOC : total phosphorus ratio. A significant negative correlation between effect size and experimental duration was observed for bacteria. In summary, this synthesis indicates that excessive DOC loading can inevitably inhibit phytoplankton and zooplankton growth. Future studies should focus on the interactions between DOC addition and global change factors to improve forecasts of carbon-climate feedback in aquatic ecosystems.

Surface water browning accompanied by increasing dissolved organic carbon (DOC) has raised great concern in northern ecosystems over the past decades (Rasmussen et al. 1989; Monteith et al. 2007; Creed et al. 2018; Kritzberg et al. 2020). Enhanced loads of terrestrial (allochthonous) organic matter, driven by climate change, precipitation-driven runoff, land-use change, and reduced sulfate deposition, contribute to the browning of northern lakes (Freeman et al. 2004; Evans et al. 2012; de Wit et al. 2016; Kritzberg 2017). In fact, terrestrial-derived organic matter is considered to constitute the majority of total aquatic organic matter in these regions (Wilkinson et al. 2013; Berggren et al. 2014). Excessive allochthonous DOC inputs can affect the physical and chemical properties of water bodies, such as light and ultraviolet (UV) attenuation, thermal stratification, and the availability of oxygen and nutrients. These changes can, in turn, impact community composition, ecosystem functioning and services. However, current studies about the effects of DOC enrichment on aquatic organisms have primarily been conducted through small-scale surveys (Seekell et al. 2015*a*; Blanchet et al. 2022; Kelly and Jones 2023), leading to contradictory conclusions (Kelly et al. 2014, 2016; Creed et al. 2018; Isles et al. 2021). Therefore, we perform a global meta-analysis to draw more general conclusions about the impacts of DOC addition on aquatic taxa in freshwater ecosystems.

Owing to the dual effects of increased DOC on light and nutrient availability, there is ongoing debate regarding the role of allochthonous DOC inputs as a trophic basis for productivity in freshwater ecosystems. Moderate terrestrial DOC enrichment can supply carbon and nutrient subsidies for aquatic organisms, as sustained photolysis can convert some

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

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dissolved organic matter into dissolved inorganic matter, enhancing the bioavailability of these molecules (Moran and Zepp 1997; Cory et al. 2014). Additionally, terrestrial DOC, due to its chromogenic properties, can decrease UV penetration and thereby protect organisms from harmful UV irradiation (Williamson et al. 2016). Some studies have demonstrated that the addition of DOC enhances the biomass of phytoplankton, zooplankton, and bacterial production (Arvola et al. 1996; Kelly et al. 2016; Fonseca et al. 2022). However, as allochthonous DOC loads increase, the nutrient subsidy associated with the DOC is eventually offset by the increased light attenuation caused by chromogenic organic matter (Seekell et al. 2015*b*). Reduced light availability can decrease the depth of mixed layer, restrict the photic zone, and result in a lower whole-water column temperature as the volume of the cold hypolimnion increases (Read and Rose 2013; Kelly and Jones 2023). Furthermore, lower ambient temperatures can reduce the standing stock biomass of phytoplankton due to the decreased energy demand of individual organisms (Brown et al. 2004; Yvon-Durocher et al. 2015). In addition, reduced light penetration can shift the base of production from autotrophic to heterotrophic dominance, potentially leading to a decline in energy transfer efficiency and a decrease in biomass production of consumers (Creed et al. 2018). Recent findings suggest that the addition of allochthonous DOC can also decrease the productivity of both phytoplankton and zooplankton (Kelly et al. 2014; Deininger et al. 2017), which contrasts with the expectation that allochthonous carbon serves as a resource subsidy. In summary, these conflicting results underscore the urgent need for a comprehensive analysis to determine which factors might regulate the response of organism to DOC inputs.

The responses of aquatic organisms to allochthonous DOC addition may be context-dependent for several reasons. First, a previous study showed that DOC concentrations in boreal lakes increase in a nonlinear manner along latitudinal and altitudinal temperature gradients (Weyhenmeyer and Karlsson 2009). Additionally, temperature can modulate the impacts of allochthonous DOC on bacterial biomass and plankton phenology (Nicolle et al. 2012; Lonborg et al. 2019). Considering the climate-driven variations in DOC concentrations and the temperature-dependent nature of biological responses, we hypothesize that planktonic biota display a range of responses to DOC enrichment across different climatic zones, which are characterized by varying annual mean temperatures. Second, high concentrations of DOC tend to have negative effects on the biomass of phytoplankton and zooplankton, in contrast to the medium addition of DOC (Cooke et al. 2015; Bergström and Karlsson 2019). Thus, the responses of organisms to DOC addition may be contingent on the magnitude of DOC enrichment. Third, previous studies have shown that the quantity and quality of food, influenced by the lake's trophic state—characterized by total phosphorus (TP) level—regulate zooplankton biomass (Persson et al. 2007). Specifically, in oligotrophic lakes, it is the quantity of food that constrains zooplankton growth, while in eutrophic lakes, it is the quality. Given the commonality of phosphorus (P) limitation in freshwater ecosystems (Schindler 1977; Elser et al. 2007), we deduce that the ambient water P concentration significantly affects the extent of the response of organisms to the addition of DOC. Additionally, DOC : TP ratio influences the phytoplankton's response to DOC enrichment (Kelly et al. 2018; Bergström and Karlsson 2019; Isles et al. 2021). Whole-lake manipulation experiments have indicated that lakes with lower DOC : TP ratios can promote higher peak biomass of phytoplankton at a given DOC concentration (Bergström and Karlsson 2019). Alternatively, phytoplankton in water with low DOC : TP ratios may have a high probability of responding negatively to the addition of allochthonous DOC due to light limitation. Finally, manipulation experiments involving allochthonous DOC addition typically utilize humic (humic acid, fulvic acid, and humin) and nonhumic (sugars, amino acids, and lipids) substances. Due to their chemical composition, nonhumic substances like sugars are generally assimilated more rapidly by heterotrophic bacteria, resulting in a fast turnover rate and high bioavailability that supports a large proportion of bacterial growth (Kirchman 2003). In contrast, humic substances, which contain a complex array of compounds with varying chemical activities and concentrations, are large-molecule compounds with lower bioavailability (Fan et al. 2018). However, DOC absorption of light causes photolysis and subsequent photochemical processes that can oxidize complex organic molecules into smaller units, enhancing bioavailability (Bertilsson and Tranvik 2000; Tranvik and Bertilsson 2001). Therefore, both the type of DOC additive and the duration of exposure to light are crucial in determining the responses of aquatic organisms.

Here, we presented a global and comprehensive meta-analysis to quantify the responses of organism growth (e.g., biomass and abundance) to allochthonous DOC addition, as achieved through experimental manipulations. We aimed to answer the following questions: (1) What is the direction and magnitude of the responses of aquatic plankton (phytoplankton, zooplankton, and bacteria) to DOC addition on a global scale? (2) Which drivers, such as climatic zone, the magnitude of DOC enrichment, ambient TP concentration and DOC : TP ratios, experimental duration, and DOC composition, influence the response of aquatic taxa to DOC addition?

Materials and methods

Database

Relevant articles related to allochthonous DOC addition from 1993 to 2021 were found using keyword combination searches in Web of Science and China National Knowledge Infrastructure. The keywords were (organic carbon addition or brown*) and experiment* and (phytoplankton or zooplankton or alga* or bacteria*). Data that met the following screening criteria were included in the meta-analysis: (1) the data must originate from experiments conducted within freshwater ecosystems, either laboratory or field experiments, and long-term monitoring data will not be included; (2) each study must provide the mean value of the growth-related variables, the duration of the experiment, and the sample size for the treatment group (with the addition of allochthonous DOC) and the control group (without the addition of allochthonous DOC); (3) at least one response variable that is relevant to growth must be reported for phytoplankton, zooplankton, or bacteria (Supporting Information Table S1). As all the variables pertain to plankton growth, we have included all relevant variables in our analysis. Furthermore, if a specific response variable was measured continuously at multiple time points, we collected all the observation data to analyze the changes in the response throughout the experiment's duration.

Based on these criteria, we screened to obtain 47 published papers with 804 observations on phytoplankton, zooplankton, and bacteria in this study (*see* Supporting Information for detailed references). These study sites were distributed globally, with a primary focus on North America and Europe (Fig. 1). For each experiment, the following information was extracted if provided by the study: experimental location, ecosystem type, method or source of allochthonous DOC addition, presence or absence of fish, presence or absence of grazer, and the concentrations of DOC (mg L^{-1}) and background TP (mg L^{-1}). In the control group, DOC concentrations range from 0.5 to 25 mg L^{-1}, averaging 5.45 mg L^{-1}. TP concentrations range from 0.0003 to 1.55 mg L^{-1}, with an average of 0.19 mg L^{-1}.

The Köppen-Geiger system is a highly suitable means for aggregating complex climate gradients into a simple yet ecologically meaningful classification scheme. It is divided into five main climate classes and 30 subtypes, based on threshold values and the seasonality of monthly air temperature and precipitation. These main classes include tropical, arid, temperate, cold, and polar climates (Beck et al. 2018). Based on the information collected regarding the experimental sites' locations, we classified the corresponding climate zones using the Köppen-Geiger system.

Data were extracted directly from tables and text or indirectly from graphs using WebPlotDigitizer. The mean values, standard deviation and sample size of each response variable for the control and treatment groups were extracted from the publications. If the standard deviation was not reported, we calculated it using the R package metagear (Lajeunesse 2016).

Data statistical analysis

The natural log-transformed response ratio (LRR) was used to measure the effect size as follows:

$$\mathrm{LRR} = \ln\left(\frac{X_\mathrm{t}}{X_\mathrm{c}}\right)$$

where X_t is the mean value in the treatment and X_c is the mean control value (Hedges et al. 1999). The LRR was used due to its robustness to small sample sizes and its ability to detect true effects (Lajeunesse and Forbes 2003). The variance (υ) of each individual effect size was computed as follows:

$$\upsilon = \frac{S_\mathrm{t}^2}{n_\mathrm{t}\overline{X}_\mathrm{t}^2} + \frac{S_\mathrm{c}^2}{n_\mathrm{c}\overline{X}_\mathrm{c}^2}$$

where n_t and n_c are the sample sizes of the treatment and control groups, respectively, and S_t and S_c are the standard deviations of the treatment and control groups, respectively. For each observation, we used the escalc function in R package metafor to calculate the effect size (Viechtbauer 2010). We estimated the mean effect size (LRR_{++}) with 95% confidence

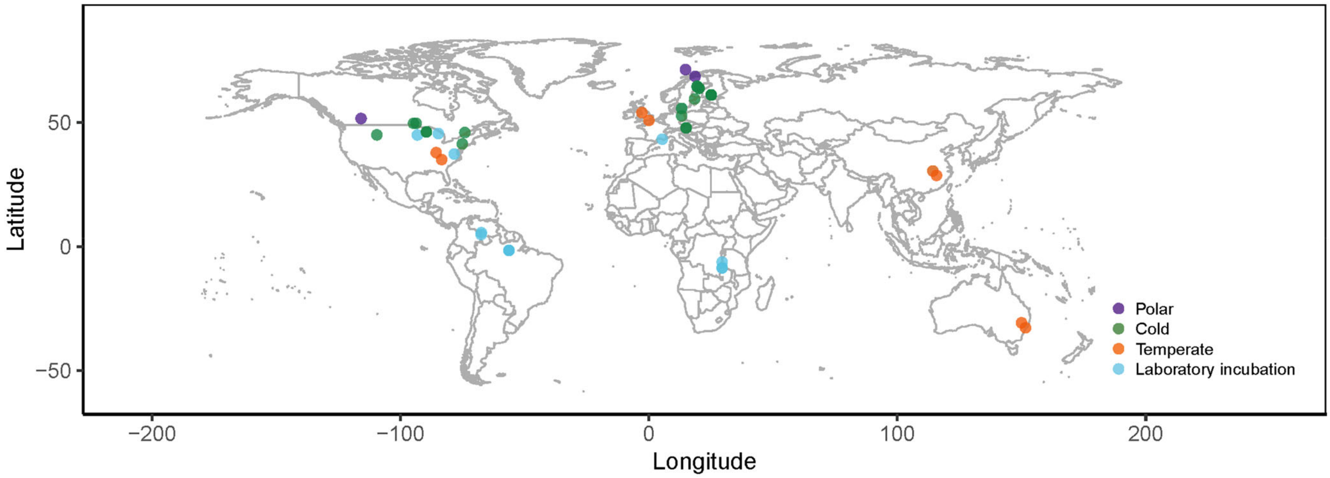

Fig. 1. Global distribution of allochthonous DOC addition experiments used in this analysis. A total of 47 sites across the world were used in this study. We grouped these sites, with the exception of those involving laboratory incubations, into different climate zones according to the Köppen-Geiger classification, as indicated by various colors. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

intervals of treatments using a variance-weighted mixed-model. An LRR < 0 for a certain response variable indicates negative effects on organisms, whereas an LRR > 0 suggests the opposite trend. The weighted average effect sizes are considered statistically significant if the 95% confidence intervals do not include zero. Additionally, we conducted multiple comparisons using Tukey's honest significance test (function glht in package multcomp) to evaluate the differences in effect sizes across various climate zones and among different DOC additives.

As data used for meta-analysis typically exhibit a multi-level structure that induces nonindependence among samples, we utilized multilevel meta-analysis models. In this study, taking into account the structure of our dataset, we constructed a four-level random effects model and calculated the overall effect of DOC addition (Feng et al. 2022). Level-1 heterogeneity is caused by sampling errors; level-2 heterogeneity is between-study heterogeneity; level-3 heterogeneity is the variation in each observation; level-4 heterogeneity arises from the different designs of experiment (i.e., different combinations of fish [presence or absence], grazer [presence or absence], and DOC additive [humic or sugar]). Therefore, we included these three factors as a random factor in a nested structure, to account for interstudy differences and thereby mitigate the potential dependency within our data (Nakagawa and Santos 2012; Cauvy-Fraunié and Dangles 2019). First, random-effects models were used to assess the overall response of each taxon following allochthonous DOC addition, with results weighted by inverse variance weights. Then, we performed mixed-effects meta-regressions, incorporating ΔDOC (the extent of DOC enrichment), TP concentrations in the control, background DOC : TP ratios, and experimental duration as fixed factors. We set the level of significance at 0.1 for statistical analysis (Wasserstein et al. 2019). We assessed the heterogeneity of effect sizes using the Q-statistic to determine if the models accounted for a significant proportion of the variation. Total heterogeneity can be categorized into two types of tests: the residual heterogeneity test (Qe) and the moderator test (Qm). In addition, a significant Qm statistic signifies the extent to which moderators contribute to effect size heterogeneity. To assess the robustness of our study, we conducted a test for publication bias. This test involves regressing the effect size on the standard error, which is weighted by its inverse variance, using Egger's regression (Egger et al. 1997) (Supporting Information Fig. S1). In the absence of publication bias, the weighted regression slope is expected to approximate zero. Trim and fill analysis was also employed to adjust for any asymmetric funnel plots that may be due to publication bias (Duval and Tweedie 2000) (Supporting Information Fig. S2). We compared the results obtained from multilevel random effects, simple random effects, Egger's regression, and trim and fill method (Supporting Information Table S2). Statistical analyses were conducted using the R package metafor (Viechtbauer 2010).

Results

Overall effects of allochthonous DOC addition on the growth of freshwater biota

Data were extracted from 47 published papers, totaling 804 observations. These included 304 observations on phytoplankton, 331 on zooplankton, and 169 on bacteria.

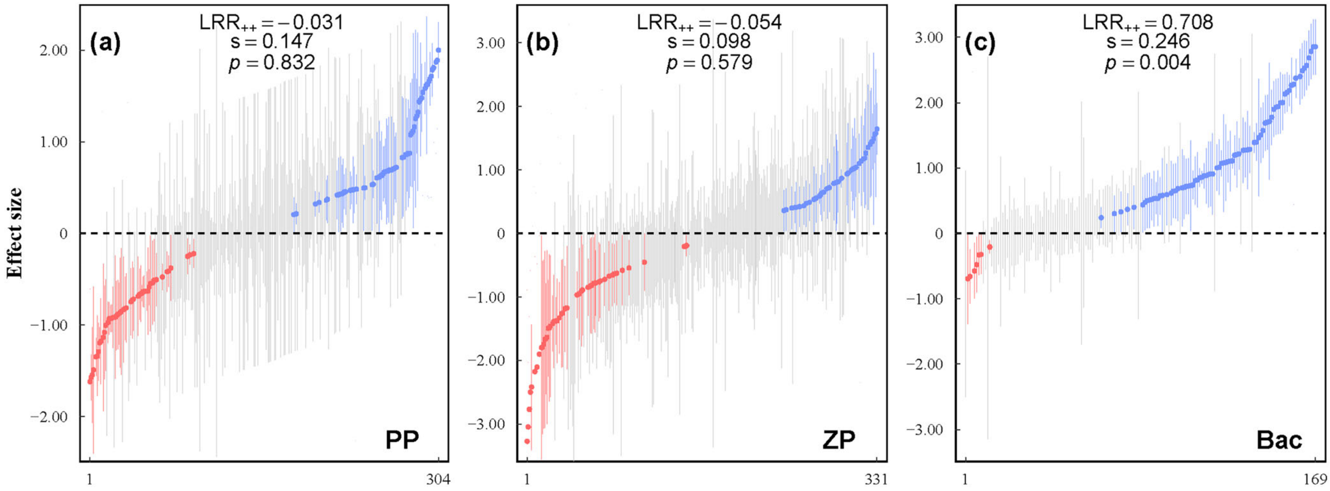

Fig. 2. Responses of (**a**) phytoplankton (PP); (**b**) zooplankton (ZP); (**c**) bacteria (Bac) to the addition of allochthonous dissolved organic carbon. Effect sizes and their corresponding 95% confidence intervals are presented in ascending order for each sample. Colors are used to denote the significance and direction of the effect sizes: red for significant negative, blue for significant positive, and gray for insignificant. LRR_{++} represents the mean effect size (weighted response ratio), and "s" denotes the standard error. The "*p*" value indicates the significance of the overall effect size being different from zero ($p < 0.1$).

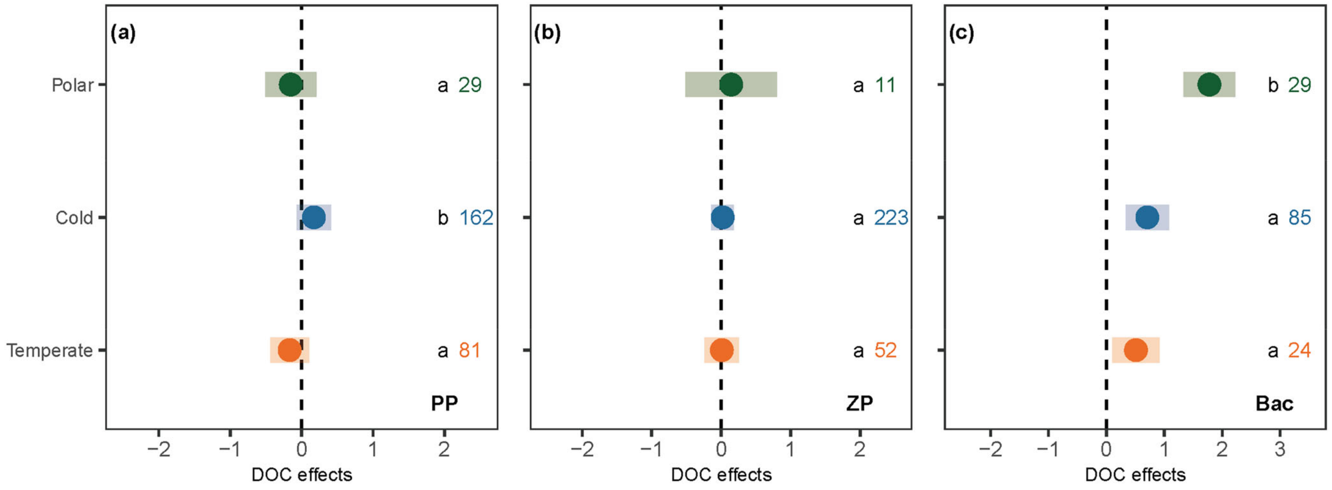

Fig. 3. Weighted response ratio for allochthonous dissolved organic carbon (DOC) addition across different climate zones. The mean effect sizes along with their respective ± 95% confidence intervals for (**a**) phytoplankton (PP); (**b**) zooplankton (ZP); and (**c**) bacteria (Bac) are depicted. The letters a and b denote significant differences between groups across various climate zones.

Overall, bacteria exhibited a significant positive response ($LRR_{++} = 0.708$, $p < 0.1$; Fig. 2c) to the addition of allochthonous DOC. In contrast, allochthonous DOC addition did not significantly affect phytoplankton ($LRR_{++} = -0.031$, $p > 0.1$; Fig. 2a) or zooplankton ($LRR_{++} = -0.054$, $p > 0.1$; Fig. 2b; Supporting Information Fig. S3, Table S3). Taking into account the impact of fish and larger grazer on effect size, we have conducted relevant analyses. Our findings indicated that the presence or absence of fish or grazer does not influence the effect size of phytoplankton, zooplankton, and bacteria (Supporting Information Fig. S4).

Effects of climate zone on effect size

In cold zones, phytoplankton exhibited a positive response to the addition of allochthonous DOC. Conversely, in the temperate zone, phytoplankton displayed a negative response to allochthonous DOC addition (Fig. 3a). Bacteria responded positively to the addition of DOC in temperature, cold, and polar zones, with significant responses observed (Fig. 3c). The responses of zooplankton to allochthonous DOC input were nonsignificant across climate zones (Fig. 3b). The detailed background information for each climate zone is presented in Supporting Information Table S4.

Effects of DOC enrichment levels, background TP concentrations, and DOC : TP ratios on effect size

Given that the Akaike information criterion values are similar for both quadratic and linear regressions, we opt to use linear regression to illustrate the impact of environmental variables on the effect size (Supporting Information Table S5). The effect sizes of phytoplankton and zooplankton exhibited a significantly negative correlation with the extent of DOC addition, whereas the effect size of bacteria demonstrated a significantly positive correlation ($p < 0.1$; Fig. 4a–c). Additionally, a significant negative relationship between the control TP concentrations and effect sizes was identified for phytoplankton, zooplankton, and bacteria ($p < 0.1$; Fig. 4d–f). In contrast, a significant positive relationship between background DOC : TP ratios and effect sizes was observed for phytoplankton and zooplankton ($p < 0.1$; Fig. 4g,h). LRR-bacteria exhibited a nonsignificant relationship with the background DOC : TP ratios ($p > 0.1$; Fig. 4i). Similar results were observed in both the humic substance and sugar addition experiments (Supporting Information Figs. S5, S6). In addition, we divided the data into two subgroups: low and high DOC : TP ratios. We found that significantly lower effect sizes were observed in the low DOC : TP subgroup for both phytoplankton and zooplankton. Furthermore, in this subgroup, there was a notably steep negative correlation between effect sizes and the magnitude of DOC addition (ΔDOC) for both taxa (Supporting Information Fig. S7).

Effects of experimental duration and DOC composition on effect size

A nonsignificant correlation between effect size and experimental duration was observed for both phytoplankton and zooplankton ($p > 0.1$; Fig. 5a,b). In contrast, effect size for bacteria exhibited a significant negative correlation with the duration of experiment ($p < 0.1$; Fig. 5c). Similar results were observed in the humic substance addition experiments, but this pattern was not evident in the sugar addition experiments (Supporting Information Figs. S8, S9).

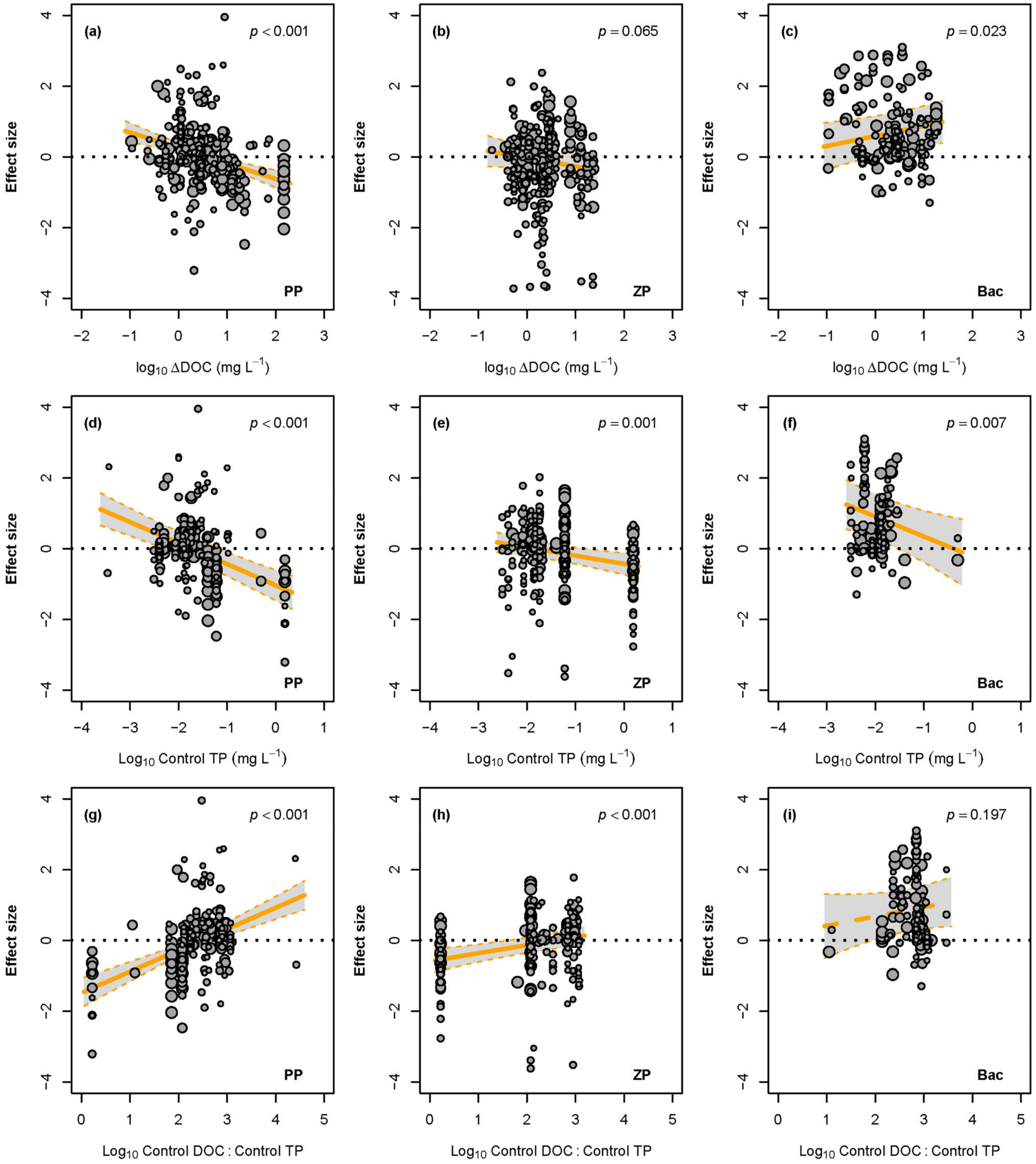

Fig. 4. Effects of dissolved organic carbon (DOC) enrichment magnitude, background total phosphorus (TP) concentration, and background DOC : TP ratios on the growth of freshwater biota using a mixed-effect meta-regression. Size of data points is proportional to the inverse sampling variances. Solid regression lines with 95% confidence intervals indicate significant trends ($p < 0.1$), whereas dashed lines denote nonsignificant trends. ΔDOC (mg L^{-1}) = treatment DOC − control DOC. Bac, bacteria; PP, phytoplankton; ZP, zooplankton.

Phytoplankton exhibited significant differences in response to different DOC composition, while zooplankton and bacteria showed nonsignificant response (Fig. 6). The addition of humic substances resulted in a greater effect size for phytoplankton than sugars. The detailed background information for each DOC additive is presented in

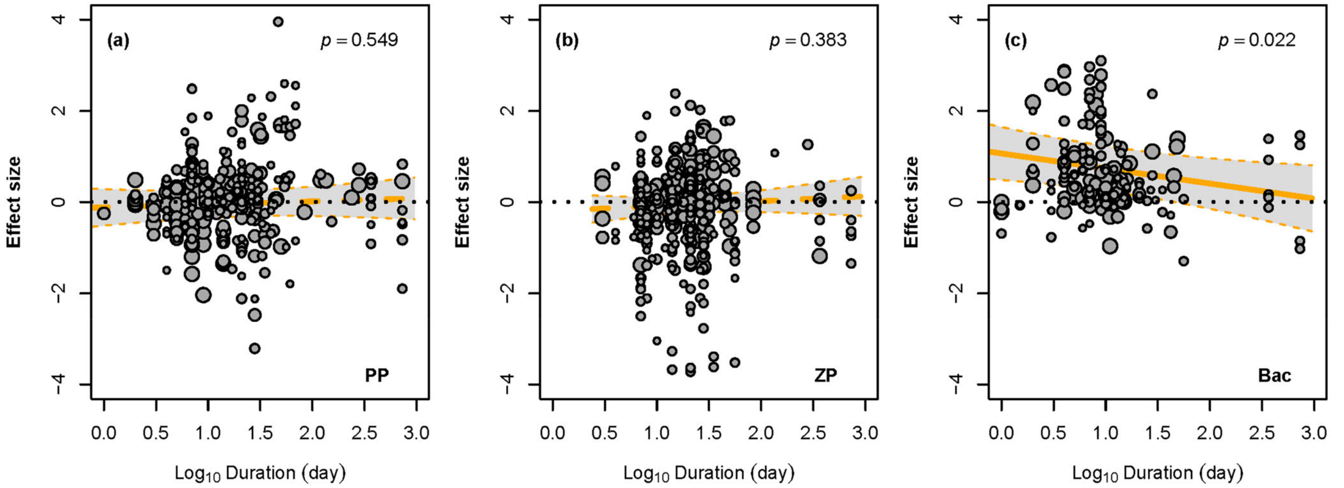

Fig. 5. Effects of experimental duration on the growth of freshwater biota. Size of data points is proportional to the inverse sampling variances. Solid regression lines with 95% confidence intervals indicate significant trends ($p < 0.1$), whereas dashed lines denote nonsignificant trends. Bac, bacteria; PP, phytoplankton; ZP, zooplankton.

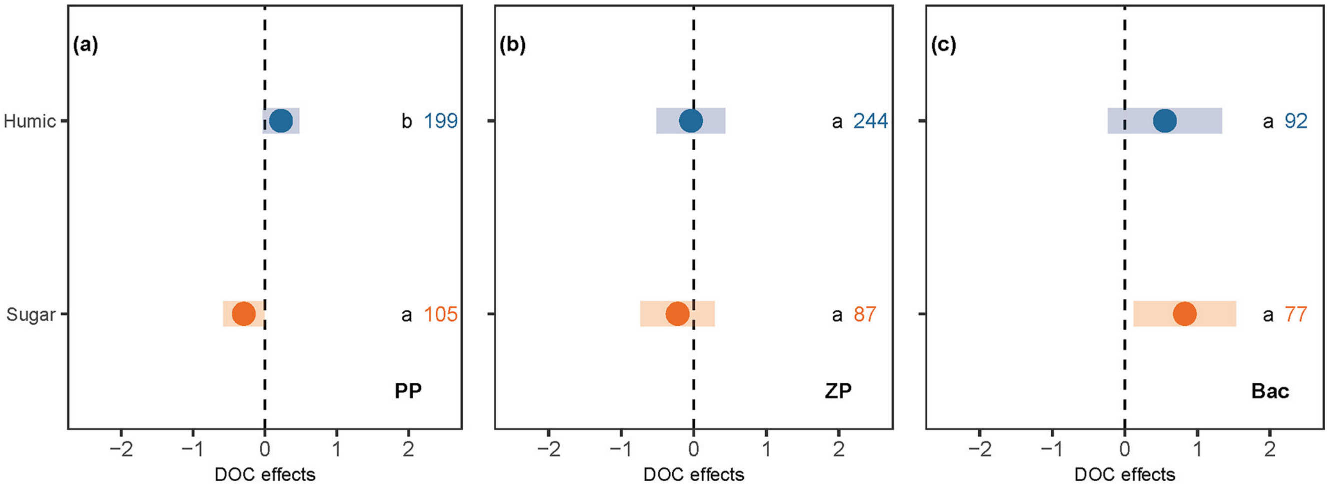

Fig. 6. Weighted response ratio for different dissolved organic carbon (DOC) additives. The errors represent 95% confidence intervals of the weighted response ratio. The letters a and b indicate significant differences between groups with different DOC additives. Bac, bacteria; PP, phytoplankton; ZP, zooplankton.

Supporting Information Table S6. In addition, the statistical results of all meta-analyses are shown in Supporting Information Table S7.

Discussion

Our meta-analysis integrated 47 studies on allochthonous DOC addition worldwide, encompassing a wide geographic range and a variety of response variables. This synthesis provides robust evidence that the addition of DOC can have varying impacts on phytoplankton, zooplankton, and bacteria. Our study constitutes the most comprehensive assessment to date on the effects of allochthonous DOC addition on freshwater biota. However, the analysis presented here is not an exhaustive evaluation of the diverse taxonomic groups within phytoplankton, zooplankton, and bacteria.

Specifically, bacteria were found to be the most sensitive group to the input of allochthonous DOC, while phytoplankton and zooplankton did not exhibit significant overall responses. This variability may be attributed to climatic zones, where phytoplankton respond differently to DOC additions, with positive or negative reactions in different zones. It has been suggested that DOC facilitates primary production in polar zones but has a negative impact in cold zones (Seekell et al. 2015*a*). This discrepancy may be due to the high concentrations of DOC found in boreal lakes compared to the low concentrations in Arctic lakes.

In our study, the relatively high background DOC concentrations and the DOC enrichment levels in temperate zones may lead to negative responses to DOC addition in phytoplankton (Supporting Information Table S4). Excessive addition of sugar may inhibit phytoplankton growth by promoting bacterial proliferation, thereby intensifying the competition for inorganic nutrients between bacteria and phytoplankton (Blomqvist et al. 2001; Hitchcock et al. 2010; Westhorpe et al. 2010). Consequently, in such scenarios, bacteria often outperform phytoplankton in this competitive environment (Currie and Kalff 1984; Bergström et al. 2003). Similarly, when allochthonous humic DOC enrichment reduces light penetration in the water column, it can negatively affect phytoplankton due to light limitation. Additionally, our results demonstrated a significant positive response for bacteria in polar regions compared to warmer ones. On the one hand, the relatively low nutrient concentration in polar regions makes them sensitive to the addition of DOC (Stets and Cotner 2008). On the other hand, the warmer surface layer water can stimulate bacterial growth and metabolism, leading to a trade-off between bacterial proliferation and consumption. Thus, DOC enrichment may elicit a weaker positive response for bacteria in warmer regions.

The input of allochthonous DOC plays a significant role for phytoplankton, as primary production is closely linked to light limitation and nutrient availability (Jones 1992; Williamson et al. 1999). Previous studies have shown that water browning can either enhance (Kissman et al. 2013) or reduce (Godwin et al. 2014; Thrane et al. 2014) phytoplankton primary production. A unimodal peak pattern in primary productivity with increasing DOC concentrations (Seekell et al. 2015*a*) indicates that food web organisms initially benefit from increased energy subsidies, nutrient fluxes, or reduced UV radiation, but are subsequently limited by light availability beyond a certain threshold. In this study, we confirmed that phytoplankton initially benefited from moderate levels of DOC enrichment but was inhibited by excessive levels of DOC input. For zooplankton, the moderate addition of humic substances and sugar is beneficial to their growth. Because humic substances can decrease UV penetration and thereby protect organisms from harmful UV. Additionally, previous studies have shown that the zooplankton community composition shifts from the dominance of calanoid copepods to that of cladocerans as DOC concentrations increase (Cooke et al. 2006; Bergström et al. 2021; Bergström et al. 2022). The proliferation of bacteria due to the addition of sugar or humic substances can provide a supplemental food source for cladocerans that can directly consume bacteria (Karlsson et al. 2004). However, massive DOC loading may negatively affect zooplankton through several pathways. First, high levels of sugar or humic substances can lead to an increase in organic matter in the water. This increase may deplete oxygen levels by enhancing microbial respiration, potentially creating anoxic conditions that are detrimental to zooplankton (Kankaala et al. 2010; Brothers et al. 2014). Second, excessive DOC addition may enhance the input of heavy metals, such as mercury and copper, which can bind with DOC substrates. These heavy metals can be harmful to zooplankton and other aquatic organisms (Bravo et al. 2017; Creed et al. 2018). In our observations, bacteria exhibited increasingly positive responses with the increasing magnitude of DOC enrichment. Due to the lack of constraints from light or oxygen under high DOC conditions, the growth of bacteria may be enhanced as the availability of carbon sources increases.

In addition to the effects of DOC enrichment levels on effect sizes, we discovered that background TP concentrations are negatively correlated with effect sizes, while the DOC : TP ratios exhibit a positive correlation. In oligotrophic environments with low TP concentrations, DOC addition can stimulate plankton growth both directly and indirectly by providing additional nutrients, energy, or food subsidy. As the trophic state increases, a turbid water state characterized by the dominance of phytoplankton can occur (Scheffer et al. 2001). This phenomenon is corroborated by our results, which demonstrate a positive correlation between chlorophyll *a* and TP concentrations (Supporting Information Fig. S10). Therefore, the addition of DOC in eutrophic conditions may have a negative impact on phytoplankton, particularly with respect to existing light limitation. Furthermore, cyanobacterial blooms are known to flourish with eutrophication (Huisman et al. 2018). This proliferation may suppress zooplankton growth due to the cyanobacteria being less nutritious or inedible for zooplankton. In environments with high TP, bacteria may be restricted by nitrogen due to nutrient imbalances, as has been reported in humic lakes of northern Sweden (Jansson et al. 2001). Additionally, DOC : TP ratio is another important factor in controlling plankton responses to the addition of DOC. Low DOC : TP ratios are typically associated with turbid water, which has high chlorophyll *a* concentrations and low light availability (Supporting Information Fig. S10). Previous studies have indicated that lower DOC : TP ratios can lead to higher peaks in phytoplankton biomass at a given concentration of DOC (Kelly et al. 2018; Bergström and Karlsson 2019). Therefore, phytoplankton in water with low DOC : TP ratios might respond negatively to DOC addition due to severe light limitation. For zooplankton, the low food quality in water with low DOC : TP ratios and high chlorophyll *a* concentrations can inhibit their growth. This inhibition is due to poor water clarity, algal blooms, and high DOC concentrations, which aligns with findings from previous studies (Persson et al. 2007). Finally, the effect sizes of bacteria may show a negative response to DOC enrichment in water with low DOC : TP ratios. This is because bacteria, relieved of their dependence on autochthonous carbon, may become limited by inorganic nitrogen (Hessen et al. 1994; Jansson et al. 2001). These findings further corroborate the relationships between the effect sizes of plankton and TP concentrations, as previously discussed.

In our study, the effect sizes of bacteria showed a negative relationship with experimental duration specifically in the experiments where humic substances were added, which can be attributed to several factors. First, the majority of humic substances in our study originate from natural sources, including humic water and the leachates from organic matter. The photoproducts of humic substances increase with the extension of experimental time, which may not be beneficial for heterotrophic bacteria (Häder et al. 2015; Creed et al. 2018). Second, as the experimental period extends, bacteria continuously mineralize allochthonous DOC, potentially reducing its availability. This could diminish the positive impact of DOC addition and even lead to negative effects on bacteria when DOC becomes scarce. Third, most experiments in our study include large potential grazers (Supporting Information Fig. S4). Furthermore, DOC enrichment can lead to shifts in the zooplankton community composition, transitioning from a predominance of calanoid copepods to cladocerans (Bergström et al. 2022). Cladocerans can feed on bacteria, thereby potentially minimizing the growth of bacterial populations with the increase of experimental duration (Jansson et al. 2000). Additionally, we found that phytoplankton responded negatively to the addition of sugars, but positively to the addition of humic substances. The relatively larger increase in the magnitude of DOC enrichment in the sugar addition experiments, compared to the humic substance addition experiments, may contribute to this result (Supporting Information Table S6). Massive sugar loading can restrict the growth of phytoplankton by intensifying competition for inorganic nutrients, placing phytoplankton at a competitive disadvantage relative to bacteria.

Previous meta-analyses have lacked a comprehensive understanding of the effects of DOC addition on freshwater ecosystems at a global scale. Our study represents the first quantitative analysis of the impact of allochthonous DOC enrichment on various aquatic biota. Our meta-analysis demonstrates that DOC enrichment significantly impacts aquatic organisms, with the direction and magnitude of the response being regulated by climatic zones, DOC enrichment levels, background trophic state, DOC : TP ratios, and experimental duration. Furthermore, manipulation experiments with DOC addition from various climate regions may differentially affect plankton, depending not only on the DOC concentrations themselves, but also on the quality of the DOC and the stoichiometry of DOC to nutrients. This could enable managers to more accurately predict the impacts of DOC enrichment on freshwater ecosystems under future climate change scenarios. Additionally, we identify several issues that require further research. First, many manipulation experiments add large amounts of DOC, exceeding environmental levels, in a short period to elicit a more pronounced response. In lakes, the changes induced by DOC enrichment may occur slowly, potentially taking years to manifest as increased water color and other alterations. Such differences may complicate the extrapolation of simulation experiment results. In addition, the space-for-time substitution method commonly used in field investigations may not resolve how plankton respond to increases in DOC, as changes in DOC and nutrients may be spatiotemporally decoupled (Stetler et al. 2021; Isles et al. 2023). Therefore, we advocate for the use of more near-natural incubation protocols and long-term simulation experiments in future studies to yield more comprehensive and robust findings. Second, given the varied responses of aquatic plankton to DOC enrichment, further research should include multiple trophic levels within the same study to capture the full ecosystem response. Lastly, as multiple global change factors may occur concurrently, a comprehensive understanding of the combined effects of DOC addition with other global change factors, such as warming and eutrophication, is urgently needed to develop multidimensional response surfaces for aquatic ecosystem community structure and function in the context of future climate change.

Data availability statement

All data from the manuscript will be uploaded as soon as this manuscript is accepted.

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Conflict of Interest

None declared.

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