

Nutrient metrics to compare algal photosynthetic responses to point and non-point sources of nitrogen pollution

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ABSTRACT

Point- and non-point source nutrients are likely to have different ecological impacts in receiving waters, due to differences in the concentration and proportions of nutrient fractions. However, the direct comparison of their ecological impacts in receiving waters has barely been quantified. We undertook algal bioassays with algal communities from river sites and showed that there was a photosynthetic yield (F_v/F_m) response to nutrient enrichment when river nutrient concentrations were relatively low, but not at higher nutrient concentrations. To combat this variability in the photosynthetic state of algae, we developed a standardized algal bioassay (3-day), using a cultured species of algae which was starved of nitrogen, to compare the photosynthetic response to three nitrogen sources: treated wastewater, aquaculture farm discharges, and soil erosion-derived nutrient sources. This study showed that the nutrient parameter that had the highest correlation with algal photosynthetic response was total dissolved nitrogen (TDN), i.e., the sum of dissolved inorganic and organic nitrogen, rather than dissolved inorganic nitrogen alone. This was true across all three nutrient sources ($R^2 = 0.58\text{--}0.78$). Additionally, the same concentrations of TDN from soil erosion-derived and aquaculture samples resulted in a significantly higher algal photosynthetic response, compared to the treated wastewater. This indicates that TDN from soils and aquaculture farms was significantly more bioavailable to the cultured algae than treated wastewater. When a range of parameters were correlated with algal responses, organic and inorganic nutrients, and organic carbon were the parameters that had the highest explanatory power for soil erosion-derived and aquaculture samples ($R^2 = 0.75\text{--}0.87$). The importance of organic compounds in these equations points to the potential of microbial transformation of organic nutrients into more bioavailable forms during the 3-day bioassay. This highlights the need to understand the relationship between algal and microbial communities in natural systems for nutrient source impact assessment. This study provides an improved understanding and metrics for comparing the algal growth response to different nutrient sources.

Abbreviations and Notations: TOC, Total organic carbon; DOC, Dissolved organic carbon; TN, Total nitrogen; TKN, Total Kjeldahl nitrogen; TP, Total Kjeldahl phosphorus; TDN, Total dissolved nitrogen; TDP, Total dissolved phosphorus; NO₃-N, Nitrate; NO_x-N, Nitrate/nitrite; NH₄-N, Ammonium; DIN, Dissolved inorganic nitrogen (NO_x-N + NH₄-N); DON, Dissolved organic nitrogen; DOP, Dissolved organic phosphorus; PO₄-P, Phosphate; PN, Particulate nitrogen; PP, Particulate phosphorus; Conc., Concentration; Eqn., Equation; Aqua_PF, Aquaculture prawn farms; Aqua_BF, Aquaculture barramundi farms; JM media, Jaworski's media; (F_v/F_m)_{treatments}, Photosynthetic yield of the treatment; (F_v/F_m)_{NO₃-N}, Photosynthetic yield of the NO₃-N addition control.

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1. Introduction

Point source discharge, i.e., discharge from wastewater treatment plants, and non-point source discharge, i.e., catchment (=watershed) runoff from urban area, hillslope/streambank/gully erosion, and agriculture activities, are important sources of nutrients that can cause water quality degradation in both freshwater and marine ecosystems. Impacts may include algal blooms, fish kills, and loss of coral and seagrass beds (Diaz and Rosenberg, 2008; Malone and Newton, 2020).

Compared to non-point sources, point source discharge of nutrients are more likely to be regulated by governments, especially in developed countries. This is because the end-of-pipe discharge is relatively easily measured and differentiated from other sources to waterways, as well as being potentially easier to control (Loague and Corwin, 2005). Non-point source discharge at the catchment scale has typically been estimated from single land use type (Johnes, 1996), but it is difficult to identify the sources or hotspots of nutrient inputs across catchments. Due to these difficulties, non-point sources are typically not regulated, often relying on voluntary methods for control of nutrient discharge into waters. For example, the use of best management practices for agricultural land uses, and catchment restoration activities, such as hillslope and riparian buffer revegetation, and wetland construction (King and Kuch, 2003; Srinivas et al., 2020). Catchments vary in their dominant nutrient sources from either point or non-point discharges (Diaz and Rosenberg, 2008; Lu et al., 2023a; Mesfioui et al., 2012). However, non-point sources of nutrients can often be the dominant sources in catchments, so there are significant gains to be made in aquatic ecosystem health from reductions in these sources. This can provide a range of other environmental co-benefits, including reducing sediment loads, improving carbon sequestration, mitigating flood risk, and increasing terrestrial and aquatic biodiversity (Cole et al., 2020; Mueller et al., 2019).

Catchment restoration to reduce nutrient inputs to waterways is often prohibitively expensive, so it is critical to prioritize management actions to target the dominant sources of nutrients. Efforts have been made to compare nutrient load contributions of point and non-point sources to waterways. However, the link between these nutrient loads and their corresponding aquatic ecosystem impacts, such as algal growth response, has primarily been investigated via modelling approaches (Santhi et al., 2001; Yadav and Pandey, 2017). One limitation of these water quality models for lakes and rivers is that the response of algal growth to nutrients is overly simplistic, typically only including uptake of dissolved inorganic nutrients, such as ammonium, nitrate, and phosphate. However, the ability of smaller molecules of dissolved organic nitrogen (DON), e.g., urea, free amino acids, to support algal growth is well established (Flynn and Butler, 1986). Studies of lake sediment or wastewater-derived DON showed algal responses during 2–5 weeks of incubations (Fan et al., 2018; Liu et al., 2012; Yao et al., 2020). Organic nutrients and carbon sources from soil erosion have also been shown to promote algal photosynthesis using algal bioassays (Franklin et al., 2018; Garzon-Garcia et al., 2018). Therefore, there is scope to use this algal bioassay approach to directly compare the impacts of point and non-point sources of nutrients on algal responses, as an indicator of ecosystem impacts, and identify the nutrient parameters that best describe this response.

Cell counting and chlorophyll-*a* measurements have historically been used to determine the potential for algal communities to respond to nutrient inputs. However, more recently rapid bioassays have been developed, i.e., 3-day incubations with measurements of algal photosynthetic yield as an indicator of the algal growth response to nutrient inputs (e.g., Saeck et al., 2016; Franklin et al., 2018). Photosynthetic yield is a fluorescence-based measure of photosynthetic efficiency: maximum quantum yield of PSII (Fv/Fm), with a maximum value ranging from 0.40 to 0.72 for healthy cells depending on different algal species (Santabarbara et al., 2019; Tan et al., 2019). The photosynthetic yield has been used as an indicator of nutrient responses in microalgae,

with higher photosynthetic yields pointing to higher growth rates (e.g., Burford et al., 2011; Geider et al., 1993; Lippemeier et al., 2001). One of the challenges with using natural algal communities in these bioassays is that their nutrient status can vary substantially such that there is a lack of consistency in their response to nutrient enrichment (Saeck et al., 2016). Therefore, more standardized methods of using algae species that are in a consistently nutrient stressed state are needed to measure the nutrient source impacts on algal growth responses.

To compare the impacts of point- and non-point sources of nitrogen on ecosystem measure, such as algal growth, the present study examined the effect of three nutrient sources on algal responses using standardized algal bioassays. The three nutrient sources were discharge from wastewater treatment plants and aquaculture farms (discharged via pipes), as well as the simulated catchment runoff from soil erosion. Specifically, this study 1) examined the variation on photosynthetic responses of natural algal communities to nitrogen source addition. This led to 2) investigating the effectiveness of using nitrogen-starved algal cultures to examine the algal response to nutrient source additions. Based on validation the use of this approach, this study 3) compared nutrient characteristics of both point sources (wastewater and aquaculture discharge via pipes) and non-point sources (simulated catchment surface runoff from soil erosion), and linked this with algal photosynthetic responses; and then 4) identified the best explanatory nutrient parameters for algal photosynthetic responses to different point and non-point nutrient sources of nitrogen.

The present study focuses on the algal response to nitrogen enrichment because 1) nitrogen limitation of primary producers is common in both freshwater and marine ecosystems and nitrogen enrichment in marine environments is likely to trigger relatively higher primary production than phosphorus enrichment, based on a global analysis (Elser et al., 2007), 2) nitrogen loads in waterways have been identified as outside the global safe operating space for humanity, and therefore nitrogen has been identified as the most important nutrient affecting the future functioning of ecosystems (Rockström et al., 2009), and 3) it has been established that microalgae may store phosphorus in cells rather than increase their growth rates or photosynthetic yields in response to phosphorus enrichment (Xiao et al., 2023; Xiao et al., 2020), which may become a confounding factor for algal response to nutrient source additions. The present study aims to provide new and useful metrics to compare the ecosystem response (e.g., algal growth responses) to point and non-point sources of nitrogen pollution. This is particularly helpful for catchment managers wishing to determine the links between restoration effects to reduce nutrient loads and water quality benefits for waterways (Abd-Elaty et al., 2022; Badrzadeh et al., 2022).

2. Methods

Within the study, algal bioassays were initially used to determine if natural communities of algae in the study area could be used to determine the effect of nitrogen source addition on photosynthetic yield. Based on these findings, a second stage was conducted by developing a rapid algal bioassay method to examine the effectiveness of using a cultured freshwater algal species (Chlorophyte, *Monoraphidium* spp., isolated from a local waterway). This species was starved of nitrogen only (with phosphorus present in excess), to assess the effect of nitrogen inputs from wastewater, aquaculture, and soil slurries, on photosynthetic yield. Finally, the measured nutrient parameters from the nutrient sources were analyzed to determine which parameters best explained the algal photosynthetic response to point and non-point sources.

2.1. Stage 1: Bioassays testing the effect of nutrient additions on riverine and marine microalgae

This first stage of the study tested the photosynthetic yield response of algae from river and marine sites (collected along a continuum with different background nutrient levels) to the addition of nitrogen

standards at a known concentration. This approach has previously been used to assess the potential for algae to respond to nutrients (e.g., [Burford et al., 2012](#); [Muhid and Burford, 2012](#); [Wang et al., 2014](#)), and quantify the algal response to a range of nitrogen concentrations and sources ([Franklin et al., 2018](#); [Saeck et al., 2016](#)).

Water samples were collected by boat from five sites along a high to low surface nutrient gradient (top 30 cm) of: 1) Brisbane River mouth to Moreton Bay on 21 June 2022; and 2) Logan River to the Broadwater of Moreton Bay on 11 July 2022, in southeast Queensland, Australia ([Fig. 1](#)). Samples were collected at the water surface using acid-washed buckets and stored under cool conditions during transport from the field to the laboratory. Within a few hours, three replicate subsamples were taken from each bulk sample. For the total nutrients and organic carbon, samples were collected into polypropylene containers, whilst for dissolved nutrients and organic carbon, samples were filtered through a 0.45 μm polyethersulfone membrane filter (Sartorius, Germany) into polypropylene containers. All samples were immediately frozen until analyzed within one month.

The parameters measured were total and dissolved organic carbon (TOC and DOC), total Kjeldahl nitrogen (TKN), total dissolved N (TDN),

nitrate/nitrite (NO_x-N), ammonium (NH₄-N), total Kjeldahl phosphorus (TP), total dissolved P (TDP), and phosphate (PO₄-P). The dissolved organic N and P (DON and DOP) were calculated by subtracting dissolved inorganic N (DIN = NH₄-N + NO_x-N) and PO₄-P from the TDN and TDP concentrations, respectively. Total nitrogen (TN) was calculated from the sum of TKN and NO_x-N concentrations. Samples were analyzed using colorimetric and American Public Health Association standard methods ([Greenberg et al., 1992](#)).

The following treatments were used for the algal bioassay:

- Control – no nutrients added (four replicates from each site)
- Treatment – 0.13 mg L⁻¹ as added nitrogen after dilution to the ambient water, resulting in a 30–500 % DIN increase to control sites with no nitrogen added (four replicates from each site). The added nitrate (NO₃-N) concentration was determined based on a previous study in Moreton Bay that showed how much nitrogen addition was needed to stimulate a response in bioassays ([Saeck et al., 2016](#)).

All bioassay containers were incubated for three days in a controlled temperature room (28°C) on a 12:12 light:dark cycle and with a light

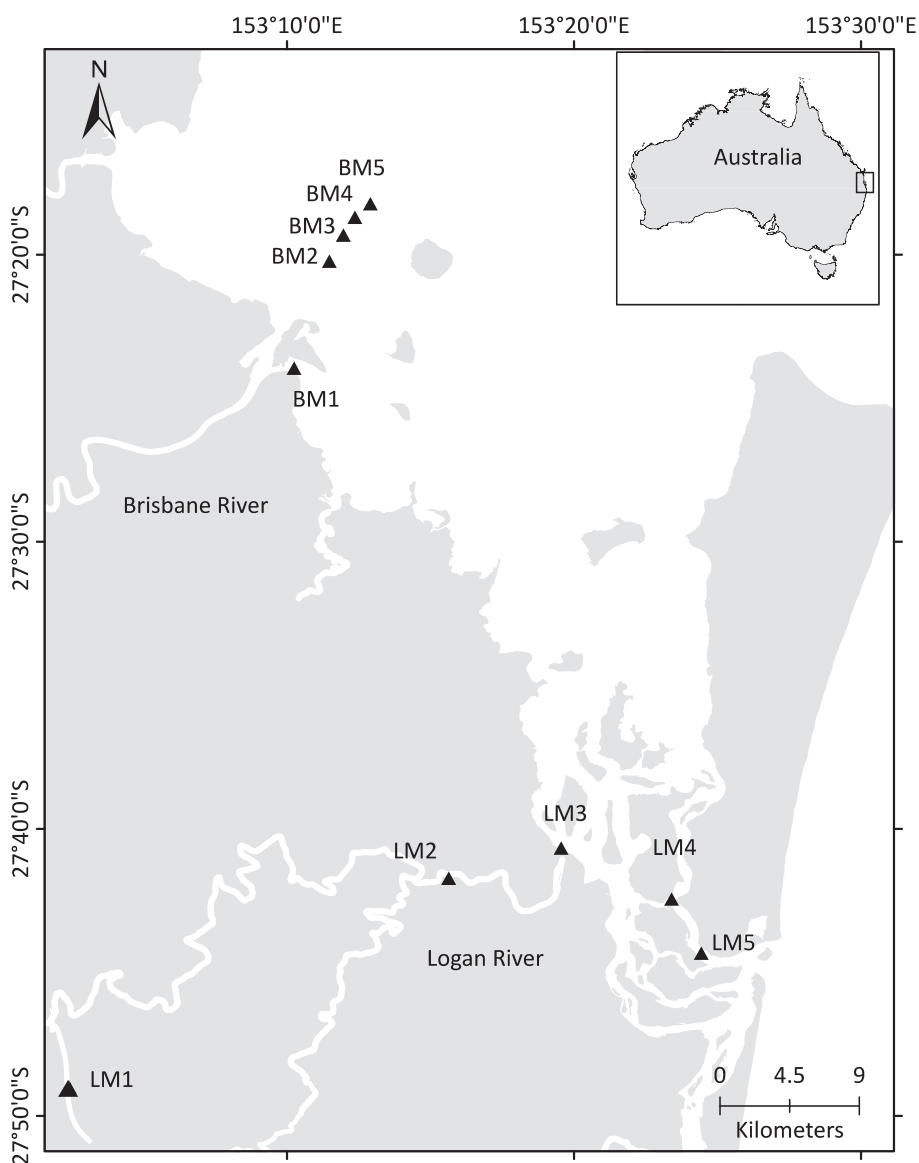


Fig. 1. Sampling sites in transects from 1) the Brisbane River mouth to Moreton Bay (BM1 to BM5) and 2) the Logan River to the Broadwater of Moreton Bay (LM1-LM5) (five sites from each transect).

intensity of $60 \mu\text{mol s}^{-1} \text{m}^{-2}$ (Maltsev et al., 2021). After three days, the photosynthetic yield, i.e., maximum quantum yield of PSII (F_v/F_m), was determined for each sample using a PHYTOPAM-PAM II Phytoplankton and Photosynthesis Analyzer (Walz, Germany). Samples were kept in the dark for 20 min prior to readings.

2.2. Stage 2: Bioassays for testing the effect of various nutrient sources using cultured algae

Stage 2 used a strain of a cultured freshwater green alga, i.e., *Monoraphidium* spp., to test the photosynthetic yield response to three nutrient sources and added nutrient standards. *Monoraphidium* spp. was previously isolated from the Wivenhoe reservoir in Queensland, Australia. *Monoraphidium* was subcultured in Jaworski's media (JM media) (Jaworski et al., 1981) diluted to 1:10 (with the exception of phosphorus, which was diluted 1:30; referred to as JM(1/3P)/10 media thereafter) for three generations. The diluted JM(1/3P)/10 media was used to balance the N:P ratio to the Redfield ratio (Redfield, 1958) for *Monoraphidium* and provide nutrients in the range of concentrations that can be found in polluted rivers (e.g., 1.4 mg L^{-1} as TN).

Prior to conducting experiments, *Monoraphidium* algal cells were pre-starved of nitrogen by filtering onto a cellulose acetate filter (pore size $0.45 \mu\text{m}$; Sartorius, Germany) under a low vacuum ($<5 \text{ kPa}$) under sterile conditions. Then sterile MilliQ water was used to wash the algal cells on the filter to remove residual nutrients. Each filter was then transferred into a sterile falcon tube with nitrogen-free JM(1/3P)/10 media. The media was shaken by hand to remove the algal cells from the filter, then the media was transferred to sterile Schott bottle. This harvesting process was repeated with multiple filters until the cell density was sufficiently high, i.e., $\text{OD}_{750} = 0.6$, estimated by absorbance at a wavelength of 750 nm (OD_{750}) with a spectrophotometer. The harvested *Monoraphidium* was then diluted using the nitrogen-free JM(1/3P)/10 media to set up the bioassays ($\text{OD}_{750} = 0.02$) and apportioned into bioassay containers with 100 mL culture in each (this vessel size was tested to ensure there were no artifacts compared with larger vessels). Cultures were then incubated at the same growth conditions as previously described. For the treatment with no nitrogen starvation (positive control), the same harvesting protocol was used except that full growth media was added to the Schott bottle.

In order to determine the photosynthetic yield response under different nutrient concentrations and forms, nutrient standards of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{NO}_3\text{-N} + \text{PO}_4\text{-P}$ at concentrations ranging from 0.1 mg L^{-1} to 3 mg L^{-1} were added to the N-starved *Monoraphidium* (Table A.1). There were also treatments with three types of nutrient sources, i.e., nine samples from wastewater treatment plants with tertiary treatment and chlorination, eight samples from aquaculture farms, and ten samples of simulated catchment runoff (soil slurries from different soils) (Table A.2). The samples from the nine wastewater treatment plants were collected at the point of discharge into the Brisbane River and its tributaries. The aquaculture farm samples were collected from four shrimp farms (brackish water) and four barramundi farms (brackish and freshwater farms) throughout Queensland. Samples were collected from ponds with mature stock or from discharge water coming from the ponds. Both wastewater and aquaculture samples were transported under cool conditions to the laboratory before the bioassay set-up (within 24 h).

Soils were collected from three different catchments of eastern Australia, i.e., Brisbane River catchment, Bowen River catchment in a dry tropics Great Barrier Reef catchment, and Hawkesbury-Nepean River catchment (Table A.2). Samples were collected as surface (top 10 cm) and subsurface soils (lower than 10 cm) using soil corers and stored in cool conditions during transportation. Simulated catchment runoff samples were prepared as soil slurries ($<63 \mu\text{m}$ soil particles) made with soil and deionized water (ten soils in total of surface and subsurface soil from five locations; Table A.2) following the methods of Franklin et al. (2018) and Garzon-Garcia et al. (2018). These were

prepared on the day of setting up the bioassay. In summary, soil samples were dried, sonicated and mixed with water to stimulate catchment runoff (slurries) from soil erosion. Slurries were then settled for a pre-determined time for harvesting $<63 \mu\text{m}$ (dominant sediment sizes found in Australian rivers) soil particles and a range of total suspended solids concentrations that can be found in catchment runoff during storm events. The soil slurries were diluted using DI water, i.e., 10 %, 25 %, 50 %, and 100 % of the original soil slurries. Subsamples were analyzed for the same nutrient and organic carbon parameters as outlined in Section 2.1 for river/marine water samples.

The experiments were done over five months, but each experiment consistently had the following three treatments: negative control: nitrogen starvation; positive control: nitrogen starved culture with $\text{NO}_3\text{-N}$ addition (1 mg L^{-1}); as well as the nutrient source treatments (Table A.2).

Incubations commenced when the photosynthetic yield of *Monoraphidium* culture was measured to be 0.35 or less, indicative of stressed cells. A total of 15 mL of each nutrient source was added to a pre-treated clean dialysis tube (molecular weight cut-off 14000 , width: 15 mm ; Sigma-Aldrich), using standard protocols for dialysis tubing and methods of Franklin et al. (2018). Tubes were then sealed and added to the algal bioassay containers containing the pre-nitrogen starved *Monoraphidium* culture. The rationale for the use of dialysis tubing is that soil particles and organic matter are kept separate from algal cells to prevent flocculation of cells and to prevent physical disruption of cells. The dialysis membrane still allows exchange of dissolved nutrients. The treatments were incubated in the conditions outlined in Section 2.1 on custom-designed rocking tables, rocking $12 \text{ times min}^{-1}$ to ensure diffusion of nutrients across the dialysis tubes to the *Monoraphidium* culture. After three days of incubation, the photosynthetic yield of *Monoraphidium* was determined as outlined in Section 2.1.

2.3. Algal bioassay results standardization

The experiments had to be done over five months, so in order to standardize the algal photosynthetic yield response between each experiment (Eqn.(1)) photosynthetic yield values for each treatment, $(F_v/F_m)_{\text{treatment}}$, were divided by the values for the $\text{NO}_3\text{-N}$ addition control, $(F_v/F_m)_{\text{NO}_3\text{-N}}$, from each experiment (the value of $(F_v/F_m)_{\text{NO}_3\text{-N}}$ were consistently 0.4 ± 0.02 across experiments):

$$\text{Standardized photosynthetic yield response} = (F_v/F_m)_{\text{treatment}} / (F_v/F_m)_{\text{NO}_3\text{-N}} \quad (1)$$

3. Data analyses

Data were analyzed using R software (R Development Core Team, 2010; Vienna, Austria, <https://www.r-project.org/>). All data were tested for normality and natural log-transformed as required. Nutrient concentrations were compared between the five sites out from the Brisbane River and Logan River transects using one-way ANOVA. The photosynthetic yield response of algae from the transect sites, with and without $\text{NO}_3\text{-N}$ addition, was analyzed using two-way ANOVA. Additionally, the photosynthetic yield responses of N-starved *Monoraphidium* to nutrient source additions were compared using one-way ANOVA. When a treatment effect ($p < 0.05$) was detected, mean photosynthetic yield values were compared using Tukey's post-hoc tests.

To compare the dissimilarity of the three nutrient sources, i.e., aquaculture, wastewater, soil slurry, non-metric multidimensional scaling (NMDS) analysis was applied using all the measured nutrient and organic carbon parameters (TOC, DOC, TN, TDN, $\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, PN, TP, TDP, $\text{PO}_4\text{-P}$, and PP). The Vegan package in R was used (Oksanen et al., 2022). To determine the optimal explanatory variables for the photosynthetic yield response of *Monoraphidium* to different nutrient sources, multivariate analysis was applied using the Leaps package in R (Lumley, 2020). The explanatory parameters used were organic carbon and nutrient parameters (TOC, DOC, TN, $\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, DON, TDN, PP,

TP, TDP, PO4-P, DOP and PP) and ratios of TOC:TN, POC:PN, and DOC:TDN. The performance of different models was also compared using anova(model#1, model#2) in R (Lumley, 2020) to determine the model with the highest R^2 and lowest number of parameters.

4. Results

4.1. Stage 1: Response of riverine and marine algae to nutrient addition

Overall, for transects out from the Brisbane River and Logan Rivers, the sites further out in the Bay had lower ambient nutrient concentrations, lower turbidity, and higher salinity than the inshore and riverine sites (Fig. 2). For the Brisbane River, DIN and PO4-P concentrations decreased significantly in the transect into the bay ($p < 0.05$; Site BM5 < BM4 <= BM3 < BM2 = BM1; Fig. 2). Concentrations of TOC, DOC, TN, and TP were significantly higher in Sites BM1 and BM2, compared to the other three sites further to the bay ($p < 0.05$; Fig. 2).

The algal photosynthetic yield response in control samples decreased out into the Bay, whilst nutrient addition resulted in higher photosynthetic yield at sites further out in the Bay compared with the inshore and riverine sites (Fig. 3). Specifically, for the Brisbane River transect, the photosynthetic yield did not increase significantly with NO3-N addition compared with the control at the river sites BM1, BM2, and BM3, compared to their corresponding control with no nitrogen added ($p > 0.05$; Fig. 3). In contrast, Sites 4 and 5 (BM4 and BM5) had significantly higher photosynthetic yields with NO3-N addition (220–420 % DIN increase to the control with no nitrogen added), compared to their corresponding control ($p < 0.05$).

For the Logan River to Moreton Bay transect, the LM1 site (freshwater site, upstream of large point sources) without nutrient addition had the lowest photosynthetic yield amongst the five Logan River sites (Fig. 3). The photosynthetic yield did not significantly increase with NO3-N addition (Site LM1 had a 30 % DIN increase to the control with no nitrogen added) for this site ($p > 0.05$). Concentrations of TOC, DOC, TP, and PO4-P in Site LM1 were significantly lower than that in Sites LM2, LM3, and LM4, while TN and DIN concentrations at LM1 were only significantly lower than that in Site LM2 ($p < 0.05$; Fig. 2). From Site LM2 to Site LM5, there was a decreasing trend for both nutrient and organic carbon concentrations, and photosynthetic yield values of the control river/marine water samples (Figs. 2 & 3). There was a significant increase in the photosynthetic yield after NO3-N addition at Sites LM3, LM4, and LM5 ($p < 0.05$).

4.2. Differences in nutrient characteristics between three nutrient sources

The NMDS analysis showed that overall, there were distinct differences in the nutrient characteristics and concentrations of individual parameters of the point and non-point nutrient sources, i.e., the wastewater, aquaculture, and soil slurry water (Figs. 4 & A.1). Both wastewater and aquaculture samples had a significantly higher proportion of dissolved inorganic nutrients, i.e., higher DIN/TN and PO4-P/TP, compared to the soil slurry samples ($p < 0.05$). In contrast, soil slurry samples had a significantly higher proportion of organic carbon and particulate forms of nutrients, compared to both wastewater and aquaculture samples ($p < 0.05$).

The TOC:TN and DOC:TDN ratios in the wastewater and aquaculture samples were significantly lower than that of the soil slurry samples ($p < 0.05$; Fig. 4). The soil slurry C:N ratios were similar or higher than the Redfield C:N ratio (6.6:1 M ratio) (Redfield, 1958). In contrast, the C:N ratios for the other two nutrient sources (wastewater and aquaculture samples) were significantly lower than the Redfield C:N ratio. The TN:TP ratios in the aquaculture samples were similar to the Redfield N:P ratio (16:1 M ratio), but significantly higher than soil slurry samples with a mean of 8:1 ($p < 0.05$).

4.3. Stage 2: Response of N-starved *Monoraphidium* to increasing concentrations of nutrient standards

The response of N-starved *Monoraphidium* to different concentrations and forms of nutrient standards (NO3-N, NH4-N, or NO3-N + PO4-P) after 3 days varied significantly ($p < 0.05$; Fig. 5). Specifically, the photosynthetic yield for *Monoraphidium* increased significantly with the increasing concentrations of nitrogen standards up to 2 mg L⁻¹ (0.26 mg L⁻¹ available to *Monoraphidium* after the dilution from the dialysis tube to the bioassay containers), compared with the treatment with no nitrogen addition ($p < 0.05$). When NO3-N concentrations were higher than 2 mg L⁻¹, there no significant differences in photosynthetic yield ($p > 0.05$; Fig. 5). There was also no significant photosynthetic yield difference between NO3-N and NO3-N + PO4-P treatments at the same concentration of added nitrogen ($p > 0.05$). The photosynthetic yield of 1 mg L⁻¹ of NH4-N treatment was higher compared to the same concentration of NO3-N ($p < 0.05$). However, there was no significant difference between NH4-N and NO3-N addition treatments at concentrations of 0.5 and 3 mg L⁻¹.

4.4. Standardized algal photosynthetic responses to three nutrient sources

The addition of the nutrient sources significantly increased the standardized photosynthetic yield for *Monoraphidium*, compared to the N-starved negative control for many of the treatments, standardized to 1 mg L⁻¹ NO3-N treatment ($p < 0.05$; Fig. 6). The nutrient concentrations of sources affected the scale of photosynthetic yield response of *Monoraphidium*, i.e., more concentrated soil slurries increased the photosynthetic yield for the same soil type. Additionally, different soil samples affected the scale of response.

4.5. Optimal nutrient characteristics to explain algal photosynthetic yield responses

Each nutrient source was analyzed individually to determine which nutrient and organic carbon parameters best correlated with the algal photosynthetic response (Table 1). The single parameter that best explained the algal photosynthetic response to each nutrient source was TDN concentration, with an $R^2 = 0.56, 0.78, \text{ and } 0.78$, for soil slurries, wastewater, and aquaculture samples, respectively (Table 1). However, for the soil slurries, log-transformed TDN concentrations significantly increased the adjusted R^2 from 0.56 to 0.72. DIN and DON were also significantly correlated with algal responses, but they showed a significantly lower R^2 compared to TDN (DIN $R^2 = 0.42, 0.75, \text{ and } 0.74$, for soil slurries, wastewater, and aquaculture samples, respectively, whereas DON $R^2 = 0.55, -0.03, \text{ and } 0.04$, respectively).

The logarithmic fit to the data showing standardised algal photosynthetic response to increasing TDN was similar for the soil slurry and aquaculture treatments ($p < 0.05$; Fig. 7), so these datasets were combined. The combined dataset of soil slurry and aquaculture samples had significantly higher algal photosynthetic response than the treated wastewater treatment for the same TDN concentrations (Fig. 7).

Multivariate analysis was undertaken to determine the best combination of nutrient parameters to explain the algal photosynthetic response for each nutrient source. Adding additional measured nutrient parameters to TDN did not significantly increase the adjusted and predictive R^2 for wastewater nutrient sources (Table 1). For aquaculture samples, the optimal combination of nutrient parameters to explain the algal photosynthetic response variation were concentrations of TDN, POC, PP, and TOC:TN ratio (Eqn.(2); adjusted $R^2 = 0.87$, predictive $R^2 = 0.80$; $p < 0.05$).

$$y(\text{aquaculture}) = 0.60 + 0.08 \times \text{Conc.}(TDN) - 0.008 \times \text{Conc.}(POC) + 0.51 \times \text{Conc.}(PP) + 0.02 \times (TOC : TN) \quad (2)$$

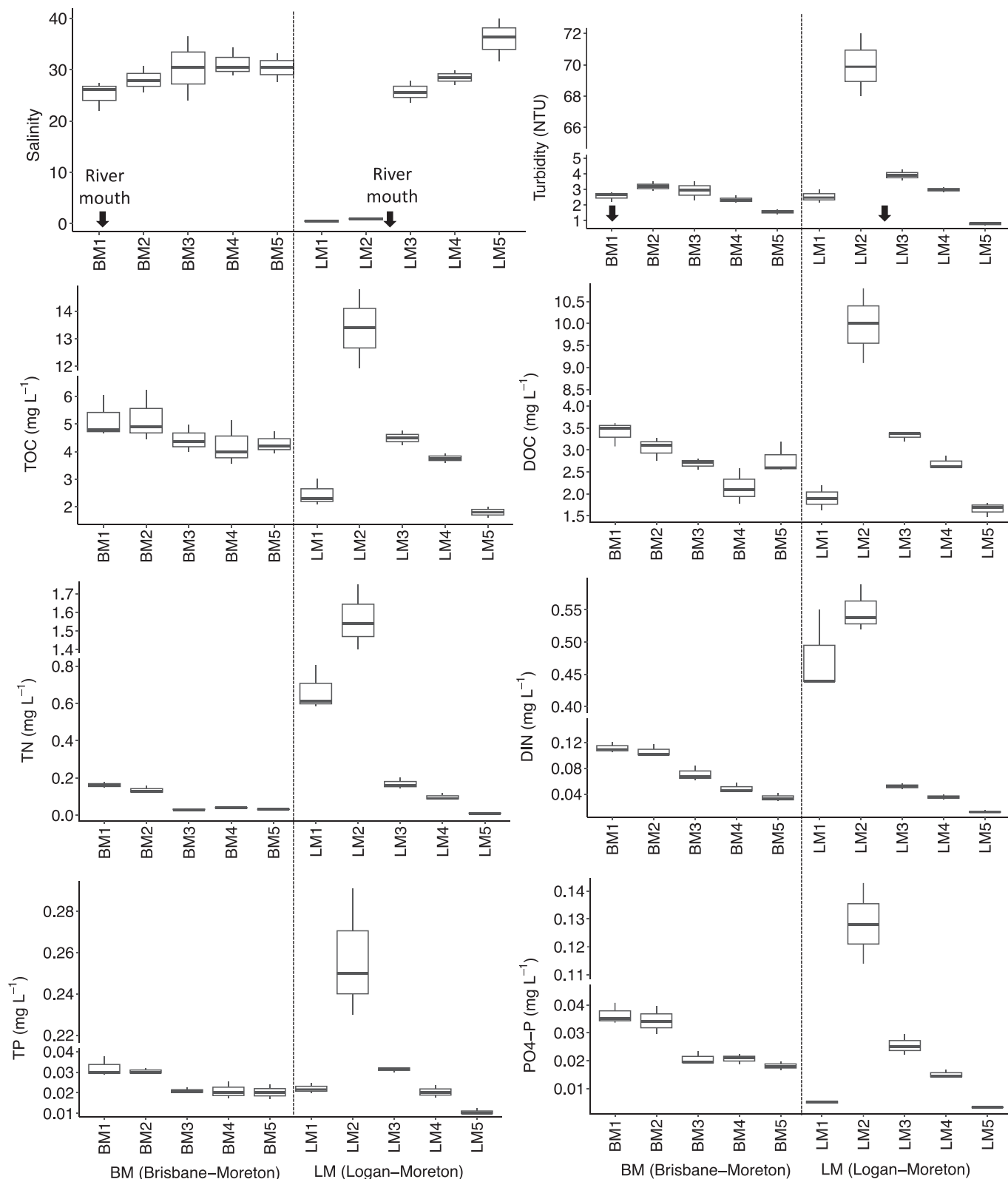


Fig. 2. Box and whisker graphs of water salinity, turbidity (NTU), and concentrations of different forms of nitrogen, phosphorus, and organic carbon (mg L^{-1}) across surface waters of five sampling sites from the Brisbane River to Moreton Bay transect (BM1 to BM5), and Logan River to Moreton Bay transect (LM1 to LM5), respectively. Locations of the river mouth from each transect were indicated by black arrows (at Site BM1 and between Sites LM2 and LM3) on the first two figures.

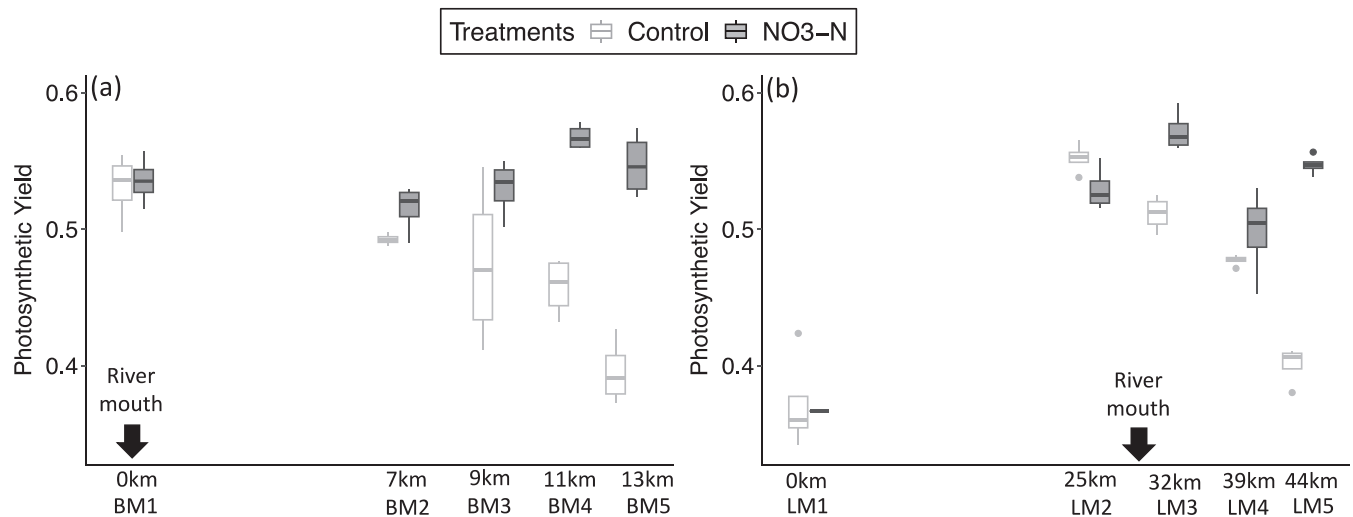


Fig. 3. Box and Whisker graphs of the photosynthetic yield of the river and marine samples after adding $\text{NO}_3\text{-N}$ standard (1 mg L^{-1}) from five sampling sites of the a) Brisbane River to Moreton Bay transect (BM1 to BM5) and b) Logan River to the Moreton Bay transect (LM1 to LM5). Locations of the river mouth from each transect were indicated by black arrows (at Site BM1 and between Sites LM2 and LM3). Distance from the first site for each sampling site of each transect were indicated in the figure.

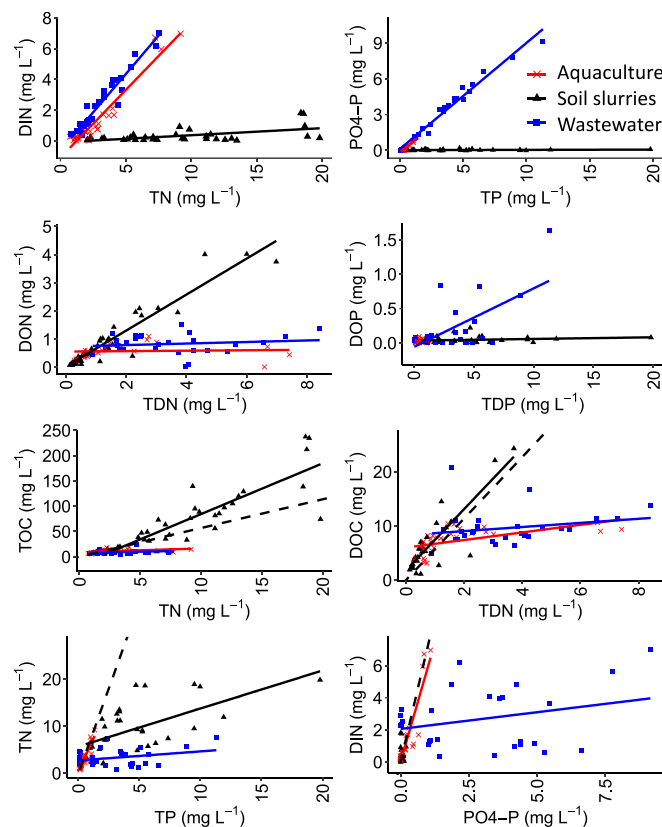


Fig. 4. Nutrient concentrations and correlations for measured parameters from three nutrient sources, aquaculture, wastewater, and soil slurry. Dashed black lines indicate the Redfield (1958) C:N or N:P mass ratios (C:N = 5.68, N:P = 7.23).

where $y(\text{aquaculture})$ is the standardized photosynthetic yield of *Monoraphidium* to the aquaculture sources.

The nutrient parameters that optimally explained the algal photosynthetic response to soil slurries were TDN, DIN, DOC, $\text{PO}_4\text{-P}$, TP, DOP, and ratios of POC:PN and DOC:TDN (Eqn.(3); adjusted $R^2 = 0.75$,

predictive $R^2 = 0.66$; $p < 0.05$).

$$y(\text{soil slurry}) = 1.02 - 0.11 \times \text{Conc.}(\text{TDN}) + 0.24 \times \text{Conc.}(\text{DIN}) + 0.03 \times \text{Conc.}(\text{DOC}) + 3.99 \times \text{Conc.}(\text{PO}_4 - \text{P}) + 5.39 \times \text{Conc.}(\text{TP}) + 6.46 \times \text{Conc.}(\text{DOP}) - 0.03 \times (\text{POC} : \text{PN}) - 0.02 \times (\text{DOC} : \text{TDN}) \quad (3)$$

where $y(\text{soil slurry})$ is the standardized photosynthetic yield of *Monoraphidium* to the soil slurry sources.

5. Discussion

This study showed the value of using a nitrogen-starved algal species to directly compare the relative photosynthetic yield effects of three different nutrient sources, which were standardized to concentrations. It also provides a means of identifying which nutrient parameters are most useful in determining algal responses to these sources. This approach has the potential to be useful for comparison of a wide range of nutrient sources on algal photosynthesis.

The use of algal bioassays with natural water samples to identify nutrient limitation for microalgae has been shown to be useful in previous studies (e.g., Franklin et al., 2018; Garzon-Garcia et al., 2018; Muhid and Burford, 2012). However, in the present study, the riverine and more inshore sites were found to be nitrogen-saturated at the time of experiments, i.e., additional input of nitrogen did not increase algal photosynthetic yield. Therefore, in order to make direct comparison of the potential of algal responses to different nitrogen sources of point and non-point sources, we used a cultured algal species starved of nitrogen. This ensures we had standardization in algal responses to nitrogen additions. These point and non-point source (i.e., soil slurry, aquaculture discharge, and tertiary-treated wastewater) have not previously been compared in terms of a potential ecosystem impact, such as algal photosynthetic response (Lu et al., 2023a). There are a number of benefits of the bioassay approach used in the present study: firstly, short-term (3 day) incubations minimize artificial nutrient limitation for the algal bioassays. Additionally, incubations with dialysis tubes allow the continuous movement of nutrients across the membrane from the source to the algae, again reducing the risk of artificial nutrient limitation (Posselt et al., 2009). The potential for artificial nutrient limitation in longer-term bioassays has previously been demonstrated (Carpenter,

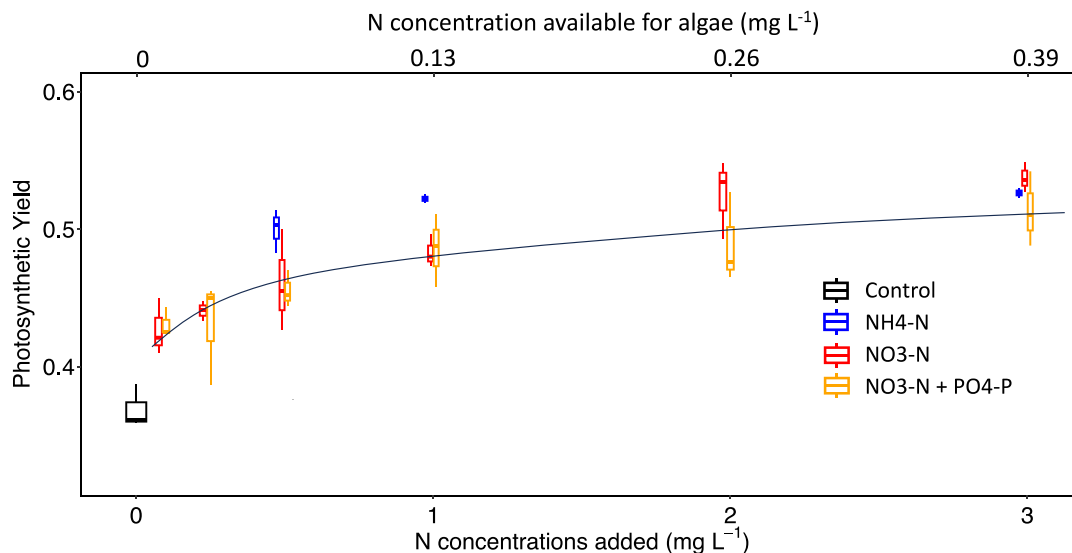


Fig. 5. The photosynthetic yield of the green algal species, *Monoraphidium*, in response to different forms and concentrations of nutrient standards (nitrate, ammonium, and nitrate + phosphate). A natural logarithm model fit for the NO₃-N and NO₃-N + PO₄-P treatments was shown on the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

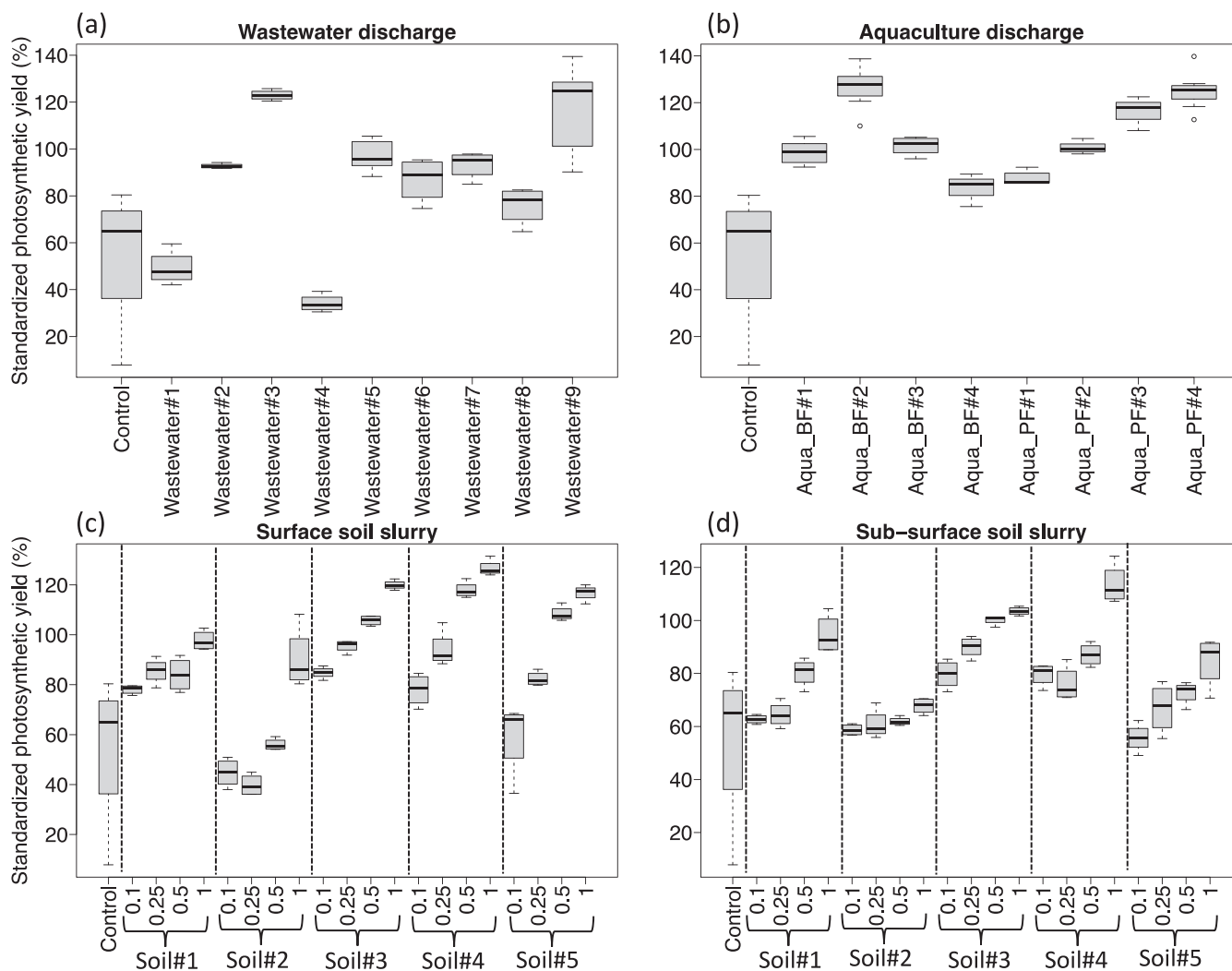


Fig. 6. The standardized photosynthetic yield of *Monoraphidium* (% relative to the photosynthetic yield response of *Monoraphidium* to 1 mg L⁻¹ of NO₃-N) to three different nutrient sources with a range of dilutions. 0.1, 0.25, 0.5, and 1 refer to 10 %, 25 %, 50 %, and 100 % of each soil slurry diluted with DI water. Aqua_BF: aquaculture barramundi farms. Aqua_PF: aquaculture prawn farms. Control: treatment of the N-starved negative control.

Table 1

Summary of the multivariate analysis results demonstrating the nutrient and organic carbon parameters that were most highly correlated (cells shaded) with the photosynthetic yield responses of *Monoraphidium* to three nutrient sources. Values in the TN, TDN, and DIN columns are the adjusted R² for the correlation between individual nutrient parameters from different nutrient sources and the photosynthetic yield responses. The shaded nutrient and organic carbon parameters are the group of parameters that result in the highest adjusted and predictive R² (see final two columns) for the photosynthetic yield response.

Nutrient sources	TN	TDN	DIN	DON	PN	TOC	DOC	POC	TP	PO ₄ -P	DOP	PP	TOC:TN	POC:PN	DOC:TDN	Adjusted R ²	Predictive R ²	log(TDN) R ²
	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²	R ²				
Soil slurries	0.20	0.58	0.42	0.55												0.75	0.66	0.73
Aquaculture farms	0.66	0.78	0.75	-0.03												0.87	0.80	0.75
Wastewaters	0.75	0.78	0.74	0.04												0.78	0.73	0.79

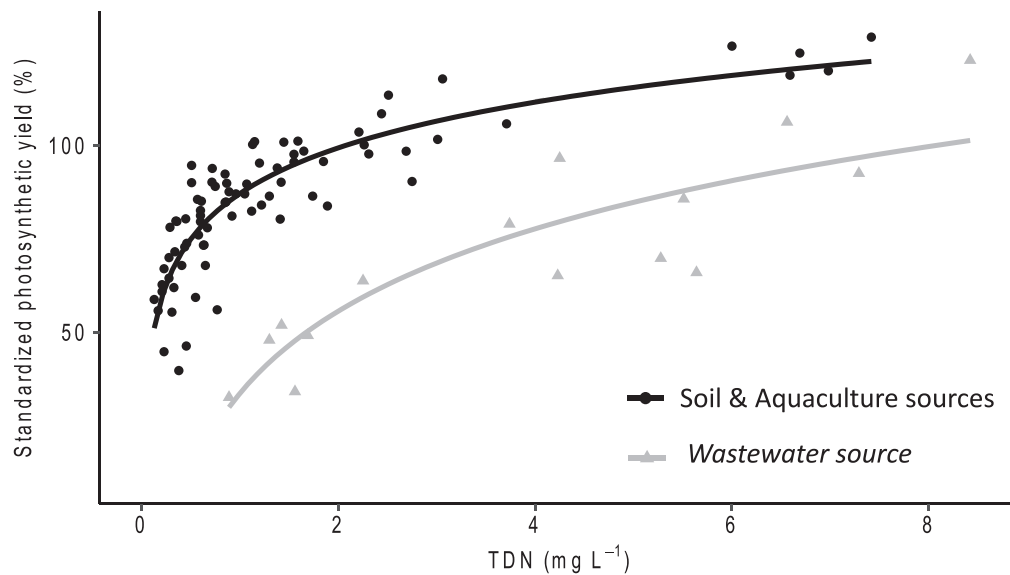


Fig. 7. Logarithm curve fitting for standardized photosynthetic yield of *Monoraphidium* (% relative to the photosynthetic yield response of *Monoraphidium* to 1 mg L⁻¹ of NO₃-N) data at increasing TDN concentrations (mg L⁻¹) in the source. Wastewater treatment plant discharges were compared with the combined data of soil slurry and aquaculture.

1996). Secondly, the rapid bioassay is less labour-intensive than longer-term bioassays. Finally, this method is suitable for testing many treatments and replicates simultaneously.

This study also identified the nutrient parameters from three different nutrient sources (aquaculture, soil slurry, wastewater) which were most highly correlated with algal responses. Across the three nutrient source types, TDN (DIN + DON) was the single most highly correlated parameter of those measured in this study, with TDN, or log-transformed TDN concentrations explaining over 72 % of the algal photosynthetic response variation to the addition of each nutrient source. Additionally, the same TDN concentration from soil slurry and aquaculture samples showed significantly higher algal photosynthetic yield response than the wastewater samples. This suggests that soil slurry and aquaculture samples may contain other nutrients and organic carbon that stimulate photosynthesis more effectively than wastewater.

The ability of multiple parameters to increase the correlation was examined, and 75 % to 87 % of the variation in the algal photosynthetic response to soil slurry and aquaculture samples was found to be explained by the following parameters: TDN, POC, PP, and TOC:TN ratio for aquaculture samples, and DIN, DOC, PO₄-P, TP, DOP, and ratios of POC:PN and DOC:TDN for soil slurries. These results suggest that organic nutrients from soil slurry and aquaculture samples affected algal responses, possibly via transformation of organic forms of nutrients into more bioavailable forms by microbes during the 3-day incubation. This highlights the importance of investigating the environmental impacts of nutrient sources, such as soil erosion-derived nutrients and aquaculture farm discharges, that had a higher proportion of bioavailable organic

nutrients.

5.1. Testing the response of natural algal communities, in algal bioassays, to nutrient addition

Our study showed that algal communities along the Brisbane River-Moreton Bay and Logan River-Moreton Bay transects were inconsistent in their response to nutrient additions, with algal communities at some sites responding to nutrients whilst others did not. This is likely due to whether algae were nutrient saturated, and therefore capable of responding. This is consistent with previous studies showing that nutrient-saturated algae do not increase photosynthetic yields, or nutrient uptake rates in response to nutrient inputs (e.g., Morris and Lewis, 1988; Saeck et al., 2016; Tank et al., 2017).

A previous multi-year study in Moreton Bay by Saeck et al (2016) identified a DIN threshold of 2 μmol/L (0.028 mg L⁻¹), above which 82 % of the algal community at Moreton Bay sites did not increase their photosynthetic yield in response to nitrogen addition. In contrast, four of the six Moreton Bay sites in the present study (BM4, BM5, LM3 and LM4) with increased photosynthetic yields had DIN concentrations ranging between 0.032 and 0.048 mg L⁻¹, somewhat higher than the DIN threshold from Saeck et al. (2016) of 0.028 mg L⁻¹, although it is acknowledged that the number of sites examined in our study was low, so it is not directly comparable. Therefore, determining thresholds for algal responses to nutrients in waters with nutrients that may reach saturating concentrations temporally and spatially is challenging. This limits the ability to predict the degree to which algal communities will

respond to source inputs, and points to the benefits of studies using standardized bioassays. Our study of the N-starved algal species, *Monoraphidium*, repeatedly and reliability showed a gradient of photosynthetic yield responses to nitrogen additions ranging from 0.1 to 2 mg L⁻¹.

5.2. Nutrient parameters correlating with algal bioassay responses

The single nutrient parameter that was most highly correlated with algal photosynthetic yield responses across all three nutrient sources was TDN concentration (DIN + DON). In contrast, the correlation with DIN concentrations was lower, with 16 % lower for the soil slurry treatment, and 3–4 % lower for wastewater and aquaculture treatments. These findings suggest that DON may be important in determining the algal response.

There are a number of potential explanations, including: many algal species can utilize DON, although the compounds used are only the chemically simpler forms, such as urea and dissolved free amino acids (e.g., Fiedler et al., 2015). However, these simple forms typically only make up a small percentage of DON in fresh and marine waters (e.g., Bogard et al., 2012; Bronk et al., 1994). Additionally, DON is only used by algae when DIN is not sufficient, as it is more energetically expensive to take up (Bronk et al., 2007; Reynolds, 2006). Soil slurries may have a higher proportion of simpler forms of DON, compared to wastewater and aquaculture discharges, although this was not measured in our study. Soil slurry DON is derived from leaf litter decay and fertilizer application, e.g., urea on farming lands. In contrast, wastewater and aquaculture-derived DON are likely to have more complicated origin, such as human waste, synthetic products like pesticides/herbicides, textile and pharmaceutical industries, farmed animal excretory products, and DON leached from algal blooms in ponds (e.g., Burford and Williams, 2001; Lester et al., 2013; Mallick et al., 2022).

The other reason is that microbes may have transformed complex DON molecules into more bioavailable forms, e.g., DIN, urea, and dissolved free amino acids over the three-day incubations (Berman and Bronk, 2003; Seitzinger and Sanders, 1997). Therefore, dissolved organic matter may be providing a source of both nitrogen and organic carbon for microbial growth. This is further supported by the finding from previous studies that the C:N ratios and organic carbon concentrations in soil slurries and agriculture discharges also correlated with algal responses (Garzon-Garcia et al., 2018; Geurts et al., 2010; Truong and Marschner, 2018). In contrast, it appears that the DON in wastewater was less bioavailable, as DIN was the dominant predictor of algal response. A previous study on wastewater-derived DON also showed that, in the absence of bacteria, DON from wastewater was not bioavailable to the cultured green algae species, *Selenastrum capricornutum* (Pehlivanoglu and Sedlak, 2004). Therefore, we propose that bacterially mediated nutrient transformation from organic forms to inorganic forms during a relatively short incubation period (e.g., 3-day) could be important to determine the response of algae to different nutrient sources.

The influence of organic carbon on the bioavailability of nutrients to bacterial and algal communities has been highlighted in previous studies. For example, changes in labile carbon-to-nutrient ratios have been found to change nutrient assimilation by heterotrophic bacteria, i. e., the maximum nitrogen uptake was found when C:N mass ratio was approx. > 114:9 and N:P ratio was < 9:1, and the maximum phosphorus uptake was found when C:N ratio was approx. > 170:21 and N:P ratio was < 21:1 (Graeber et al., 2021). Additionally, organic carbon addition changed algal biomass and community structure in a 20-day mesocosm experiment in a eutrophic lake (Fonseca et al., 2022). Conversely, organic matter from catchments can result in inhibitory impacts on algal growth, depending on the organic carbon concentrations (Burford et al., 2022; Neilen et al., 2020; Neilen et al., 2017). Therefore, the impacts of organic nutrients and carbon supply on nutrient bioavailability and the interaction between the two on algae warrant further exploration.

5.3. Implications of the study

This study showed that the combination of dissolved inorganic and particulate nutrients and dissolved organic nutrients (e.g., DOC, POC, PP, and DOP) and C to N ratios (e.g., TOC:TN, POC:PN, and DOC:TDN) best explained the algal photosynthetic responses to different nutrient sources, although the cause-effect mechanisms are not well understood. Organic forms of nutrients and carbon are not typically regulated or monitored, with the focus being on TN and DIN monitoring (Branosky et al., 2011; King and Kuch, 2003; Zheng et al., 2021). Therefore, our study suggests that a broader suite of parameters should be measured if the aim is to determine environmental impacts, at least based on our algal assays using *Monoraphidium* spp. Our study also showed that the scale of the algal response to nitrogen sources are driven by both the concentrations of a range of nutrients and organic carbon, suggesting that the nutrient concentration-based monitoring and regulation from nutrient sources can be as importance as the load-based ones that has been typically used for different catchments, such as the total maximum daily load programs (Eheart et al., 2004). It is acknowledged that algal bioassays are culture based and conducted in the laboratory-, not field-based. Additionally, algal bioassays are only one, albeit important, measure of the ecosystem response to nutrient inputs. The nutrient characteristics of each point and non-point nutrient source can vary over time and space, e.g., level of treatment of wastewater, and as such has impacts on the broader implications of the results (Muralikrishna and Manickam, 2017; Romero et al., 2013). However, this study points to the need to further understand the impact of a wider range of nutrient and organic carbon parameters for assessing the algal responses of point and non-point sources. The findings from our study also have the potential to improve algorithms in water quality models to predict algal responses to nutrient inputs from different sources across catchments.

Consistent with previous studies (Franklin et al., 2018; Garzon-Garcia et al., 2018), the present study showed that soil slurries, i.e., simulated catchment erosion from streambanks and gullies, can significantly stimulate algal photosynthesis, and that both different soils affect the scale of the response. This means that the selection of sites to most cost-effectively reduce soil nutrient inputs and hence their environmental impacts is critical. A previous study from our research team demonstrated that the soil slurry nutrient concentrations can be used to rapidly map out the potential soil-derived critical nutrient source areas (Lu et al., 2023b). This type of rapid assessment, together with the standardized algal bioassays, could be beneficial in identifying and optimizing areas for mitigation of catchment nutrient export.

6. Conclusions

This study showed that using a standardized bioassay with cultured algae provides a consistent and reliable measure of algal responses to different nutrient sources of nitrogen (wastewater, aquaculture ponds, soil slurries). The nutrient characteristics of the three types of nutrient sources assessed in this study varied significantly, and these characteristics affected the response in the algal bioassay. The single parameter that best explained the algal photosynthetic responses to nutrient source addition was TDN (DIN + DON) or log-transformed TDN concentrations. The same concentration of TDN from soil slurry and aquaculture discharges had a significantly higher algal response than treated wastewater. A combination of organic carbon, along with organic and inorganic nutrients, resulted in the highest correlation (R^2 ranged from 0.75 to 0.87) with algal response, providing improved metrics to better evaluate the impacts of point and non-point sources on algal responses. This study has significantly improved our understanding and evaluation of the effect of point and non-point sources of nutrients on algal responses, via algal bioassays. However, future work is needed to scale up this work to examine the ecosystem level of responses to different nutrient sources. This study highlights the variable impacts that different nutrient sources might cause on the aquatic ecosystem due to

their different nutrient and organic carbon concentrations and the significance of prioritising the management of the most impactful nutrient source. This study is helpful for catchment nutrient load managers to prioritise and implement management actions to reduce nutrient export from the most impactful nutrient sources and provide more benefits to the waterways.

CRediT authorship contribution statement

Jing Lu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing, Visualization. **Alexandra Garzon-Garcia:** Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing, Visualization. **Ann Chuang:** Data curation, Investigation, Methodology, Writing – review & editing. **Joanne Burton:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Visualization. **Cameron Jackson:** Funding acquisition, Resources, Writing – review & editing. **Jenny Rogers:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Michael Newham:** Writing – review & editing, Conceptualization, Visualization. **Emily Saack:** Writing – review & editing. **Mark Allan:** Conceptualization, Writing – review & editing. **Michele A. Burford:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michele Burford reports financial support was provided by Australian Research Council. Michele Burford reports financial support was provided by Sydney Water. Michele Burford reports financial support was provided by Queensland Urban Utilities. Michele Burford reports financial support was provided by Healthy Waterways and Catchments. Michele Burford reports financial support was provided by Queensland Department of Environment and Science. Jing Lu reports financial support was provided by Griffith University Australian Rivers Institute. Jing Lu reports a relationship with Griffith University Australian Rivers Institute that includes: employment. No other interests were declared. 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2023.111425>.

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