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Full Length Research Paper

Growth response of natural phytoplankton to enrichment of urea and other forms of dissolved nitrogen

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The Broadwater of Myall Lakes, NWS Australia is brackish water which has a high variation in water quality in particular salinity and nutrient concentration. In these experiments, we determined the growth and species assemblages of natural phytoplankton community exposed to nutrient enrichment. Laboratory incubation condition was used to measure 10 days biomass and group-specific response of phytoplankton community. Four experimental occasions were conducted in autumn (April and May, 2005) and early summer (November and December, 2005) with two experiments each season. Biomass of phytoplankton was determined based on chlorophyll fluorescence, extracted chlorophyll a and cell abundance of phytoplankton, and community structure/species assemblages was based on manual identification until genus level using upright light microscope. During four experiment events, chlorophyll growth response was significantly higher in N+P treatment than control and N treatment only, with slow growth rate occurring 24 to 48 h following nutrient addition. There was an inconsistent trend of biomass in terms of cell abundance in respond to nutrient enrichment between experiment occasions, except for May experiment. Generally, our study found that the greatest difference of phytoplankton growth/biomass at the Broadwater of Myall Lakes was at site level instead of nutrient treatments. Our study also revealed that urea and other forms of dissolved N stimulated growth of group specific of phytoplankton, with P addition contributed considerably to changing in community structure of phytoplankton. The result of this study suggest that urea was not a factor for Cyanobacteria bloom as compared to the other dissolved N forms, consequently urea does not give a further enhance for Cyanobacteria bloom formation in The Broadwater of Myall Lakes system. This study revealed that enrichment of different forms of dissolved nitrogen stimulated the growth of phytoplankton taxa in ways that resulted in significant differences in species assemblages among treatments for most sites. Extra addition of P can initiate bloom conditions for cyanobacteria. In order to reduce this possibility, P flow from catchment areas has to be prevented.

Key words: Phytoplankton, community structure, nutrient, urea.

INTRODUCTION

One of the most serious management issues for coastal waters within the past few decades has been anthropogenic loading of nutrients and sediment, with subsequent eutrophication-enhanced phytoplankton growth (D'Elia et al., 1986; Janse, 1997; Ornolfsdottir et al., 2004). Excessive biomass associated with algal blooms is problematic for many native plant and animal species inhabiting polluted waters and has impacts on both the recreational and commercial use of waterways. Nuisance blooms may be characterized by objectionable taste and odour (Dzialowski et al., 2005) and the

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production of toxins (Codd, 2000; Kanoshima et al., 2003). Understanding factors controlling undesirable algal growth in coastal areas is an important step towards developing effective management strategies for sustaining the "health" of sensitive coastal waste bodies (Elser et al., 1990; Dodds et al., 1993).

In general, phytoplankton tends to be limited by N in marine waters and by P in freshwater systems (Elser et al., 1990), however the limiting nutrient for phytoplankton in estuarine/brackish waters may vary seasonally (D'Elia et al., 1986) and also depend on nutrient input ratio (Graneli, 1987; Zou et al., 2001).

In aquatic systems, available nitrogen is present primarily as dissolved nitrogen gas (N_2), and in ionic form as ammonium (NH^{4+}), nitrite (NO_{2-}), nitrate (NO_{3-}) and urea ($CO(NH_2)^2$) (Graham and Wilcox, 2000). Nitrogen may enter in to the aquatic systems by precipitation, fixation of atmospheric N or by input from surface and ground water drainage (Bronmark and Hansson, 1998). Nutrient cycling in sediments contributes significantly to water column concentrations of dissolved inorganic N as a result of nitrification and ammonification processes (Scheffer, 2001). As the top half centimetre of sediments is usually well oxygenated, N in the form of NO_{3-} is commonly released to overlying water (Holmboe et al., 2001). In anoxic sediments, ammonification of organic matter leads to the release of NH^{4+} (Koike and Sorensen, 1988), an N form readily utilised by algae and macrophytes (Scheffer, 2001). The most common dissolved organic source of N is urea, an excretory waste product of some metazoans and a product of regeneration by microheterotrophs (protozoans and bacteria) (L'Helguen et al., 2005).

Phytoplankton do not uptake all dissolved N forms at the same rate (McCarthy, 1977; Syrett, 1981; Richardson et al., 2001; Fan et al., 2003; Twomey et al., 2005). Richardson et al. (2001) found that the phytoplankton community in the Neuse River estuary showed preference for ammonium over nitrate, or possibly inhibition of nitrate uptake when ammonium is present. Studies by Fan et al. (2003) also demonstrated that phytoplankton tend to prefer reduced forms of N (NH_4^+ and urea) over oxidized forms (NO_{3-} and NO_{2-}), even when oxidized forms are available in higher concentration. But, when availability of NH_4 is less than the demand by phytoplankton, alternate forms of N (nitrate or urea) are used McCarthy (1977).

Numerous studies, mostly marine, have shown that urea is an important source of N for phytoplankton (Rees and Syrett, 1979; Kristiansen, 1983; Lund, 1987; Mitamura et al., 2000), often ranking equal or higher in importance than nitrate (Kudela and Dugdale, 2000). Generally, urea contributes only a small fraction of the total dissolved organic N pool in coastal and estuarine waters (McCarthy, 1972, 1977; Gilbert, 1998). Urea ($CO(NH_2)^2$) serves not only as a N source but also as a readily available source of carbon for photosynthesis (Berman and Chava, 1999). Glibert et al. (2005) suggest that urea could potentially trigger the development of harmful algae blooms. They found that in the Chesapeake Bay and Coastal Bays of Maryland, USA, urea was positively correlated with the outbreaks of several harmful species, which cause algal bloom. Studies of the effects of urea on phytoplankton growth have largely been conducted using phytoplankton in single species experiments (Eppley et al., 1971; Rees and Syrett, 1979; Horrigan and McCarthy, 1981; Harrison, 1988; Price and Harrison, 1988; Mitamura et al., 2000; Fan et al., 2003). Few systematic studies have examined the effects of urea on natural phytoplankton communities (Eppley et al., 1971; Turley, 1986). Of these, Turley (1986) conducted ship-based experiments on rates of N uptake which showed that urea was an important N source for phytoplankton in the stratified offshore waters of the European Shelf during summer. Eppley et al. (1971) also investigated phytoplankton uptake of nutrients from the coastal surface waters off southern California and found no dramatic difference in the chemical composition of phytoplankton cells grown on nitrate, ammonia and urea. To date, there have been no known reports of the effects of various N forms on the structure of phytoplankton assemblages.

Understanding phytoplankton growth response to nutrient loading is important since phytoplankton biomass and species dominance can be used as bio-indicators for aquatic ecosystem health (Bianchi et al., 2003; Lepisto et al., 2004; Verlecar et al., 2006).

The study was conducted using phytoplankton collected from the Myall Lakes, a near pristine but nutrient-sensitive brackish water body on the coast of New South Wales, Australia. We asked the following questions:

1) Do different forms of Nitrogen (NH^{4+}, NOx, urea) lead to similar growth patterns of individual taxon and to the development of similar phytoplankton assemblages in the lower Myall Lakes region?
2) Over what time frame do phytoplankton taxa respond to nutrient enhancement?
3) How does the phytoplankton response to urea and other N sources vary spatially within the lake?

METHODS

Field sampling and experimental design

The nutrient response study was conducted during autumn and early summer in 2005, with two experiments in each season. Phytoplankton samples were collected from surface waters (0-1 m) at three sites in BombahBroadwater, Myall Lakes System, New South Wales Australia (Figure 1). The study utilized phytoplankton from a site near the mouth of the upper Myall River, a site in the middle section of BombahBroadwater and a site in the northeast area of BombahBroadwater, near Bombah Point. During April and May (autumn), and November and December (early summer),

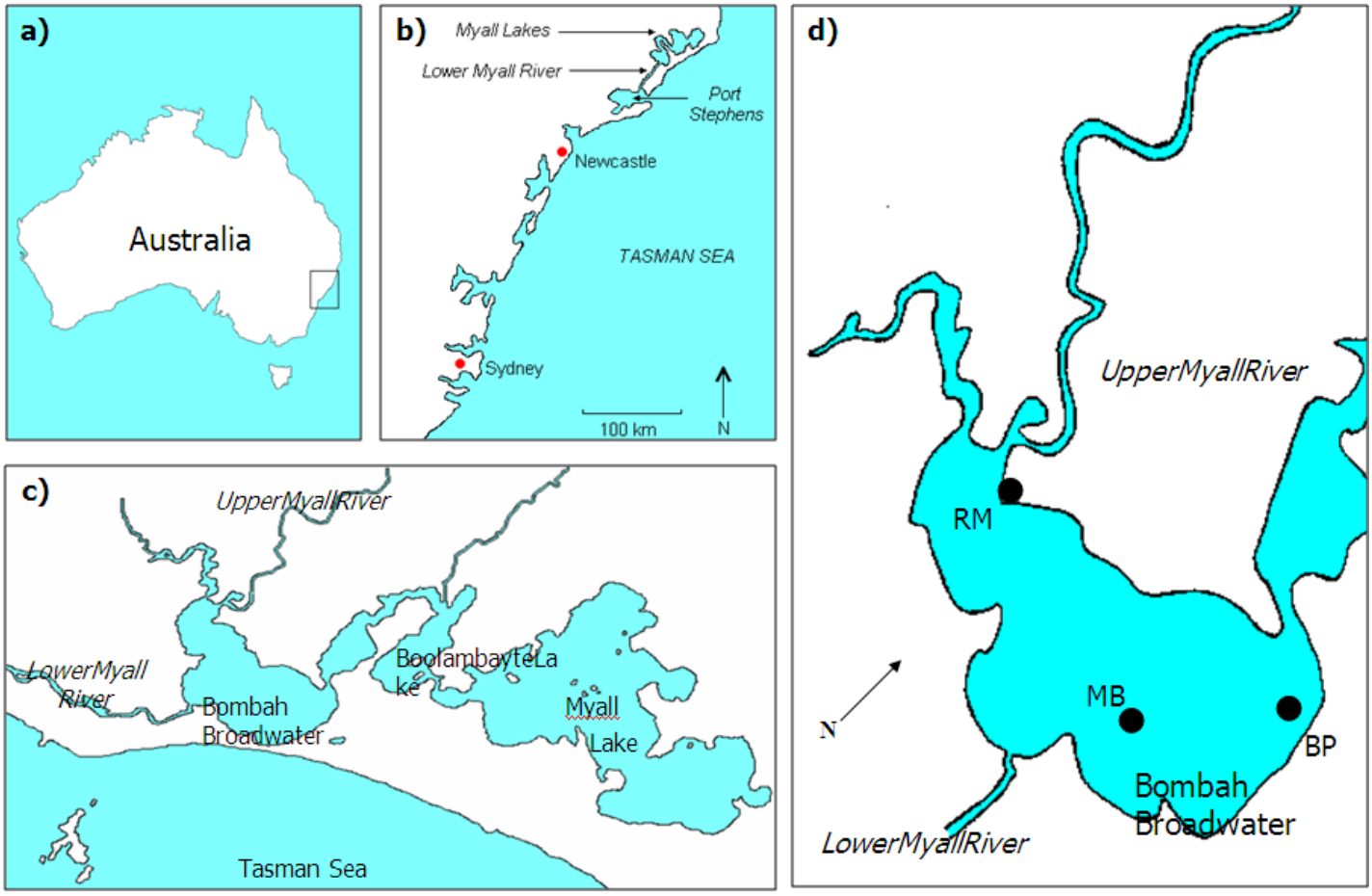

Figure 1. Map of Myall Lakes. Sampling location of water collected for nutrient enrichment study is indicated by filled round.

surface water samples (0 to 1 m, depth integrated), from the three sites (River Mouth, RM; Mid Broadwater, MB and Bombah Point, BP) were collected in 22 L carboys (two for each site). The carboys were transported under dim light to the laboratory at the Ourimbah Campus and held overnight at room temperature (about 20°C). The following morning, the contents of the carboys for each site were gently mixed and transferred to 12 × 2 L incubation containers. Nutrient treatments differed slightly between autumn and early summer experiments. In autumn, nutrient treatments included a control group (no additional nutrients), and treatments enhanced with nutrients: NH_4+P, NO_3+P and Urea+P. In the early summer, studies examined the phytoplankton response to just two nitrogen forms (NO3 and urea), with and without addition of P.

Nutrients were added once, just prior to incubation. Concentrations at the start of the incubations were 0.4 mg/L-N for nitrate, ammonia and urea; phosphate additions either met or exceeded the requirement for phytoplankton growth, based on the red field weight ratio. The analysis of the nutrient samples was conducted at the NSW EPA nutrient laboratory in Lidcombe. Water quality parameters (temperature, conductivity, salinity, dissolved oxygen, pH and turbidity) were recorded at all sites in the field and at 2 day intervals over each 10 day incubation period, using a calibrated Yeo-Kal water quality analyser.

All treatment replicates were incubated for 10 days in a large box fitted with a set of 10 Osram 58W Biolux light tubes and a sheet of fly screen to simulate sunlight at approximately 250 µmol photons m-2 s-1 (or 250 PAR, Photosynthetically Active Radiation). Daily photoperiod was set at 10 hours light and 14 h darkness and the temperature in the incubator was maintained within 2°C of field temperatures.

In vivo chlorophyll a fluorescence was measured daily in all samples using a Turner designs SCUFA® (self-contained underwater fluorescence apparatus) submersible fluorometer, calibrated for chlorophyll a concentration. Samples for analysis of acetone-extracted chlorophyll a (50 ml) and phytoplankton counts (110 ml) were removed prior to incubation and at 2 day intervals during incubation.

Phytoplankton biomass

50 ml water samples were filtered through 0.22 µm polycarbonate filters (Osmonics) and the filters stored in 6 ml vials at –20°C in a freezer for 24 h. Chlorophyll samples were then extracted with 5 ml of 90% acetone and frozen for over 48 hours. Extracted chlorophyll a (chl a) was then measured spectrofluorometrically (Hitachi F-3000). The chlorophyll fluorescence was measured daily and prior to incubation using SCUFA submersible fluoro meter.

Water samples (110 ml) from each treatment replicate were preserved using approximate 1 mL Lugol's Iodine solution for later identification and enumeration. Similar with another variable (chlorophyll a), cell abundance and dominant taxa were determined

prior to incubation and for 2 days interval. The upright light microscope was used to count phytoplankton following the methods described by Lund and Le Cren (1958), and Hotzel and Croome (1999). Identification were made to genus level in most cases as identification of all individuals to species was often difficult and/or excessively time consuming (Prescott, 1978; Entwisle, 1997; Sonneman et al., 2000).

Data analysis

All variables (chlorophyll fluorescence, extracted chl a, cell abundance and total dominant groups) were tested by two-way ANOVA to determine the effect of nutrient treatment to phytoplankton growth. When Levene's tests revealed variance were heterogeneous, raw data for all variables were transformed to log (n+1) (Underwood, 1997). To differentiate means, a least significant difference (LSD) multiple comparison procedure was performed. Overall means from LSD results were plotted to determine the pattern of variable (Chl fluorescence, chlorophyll a, and total cells and taxa abundance) between nutrient treatments among sites for each experimental event,. All analyses were performed using the SPSS version 11.0 software program.

To examine nutrient treatment effects on phytoplankton assemblages, multivariate statistical analyses were performed on log (x+1) transformed data using PRIMER V 5.2 non-metric multidimensional scaling (nMDS) software. Points that are close together represent samples that are very similar in species composition; points that are very far apart correspond to the very different communities (Clarke, 1993).

RESULTS

Water quality

Temperature varied between sampling events. The range of laboratory incubation temperatures varied between 19.5 and 24.7°C. All sampling occasions have various salinity levels between sites with the lowest salinity of 0.6 ppt recorded in April 2005 at Rivermouth. In contrast, December 2005 has higher salinity level, with the range between 3.9 and 6.5 ppt. The average pH value varied between 6.2 and 7.5. Turbidity varied considerably between sites. River mouth has a higher turbidity for autumn's sampling, with the highest turbidity of 18.5 NTU recorded in May 2005. In contrast, early summer (December 2005) turbidity is low for all sites with a range between 0.1 and 0.9 NTU.

Phytoplankton biomass (chl fluorescence, extracted chla and cell abundance)

Chlorophyll fluorescence and extracted chl a concentration initially were low but increased greatly (bloom conditions within in 2 to 6 days) in all nutrient treatments, except for control (no nutrients) and some case with N treatment only (Figures 2 to 5, a to f). Peak chl was reached on Days 6 to 8 in autumn experiment (April and May 2005) and on Day 5 to 7 in early summer experiment (November and December, 2005) (Figures 2 to 5a to f). In all occasions, the peak values of chlorophyll fluorescence and chl a concentration in N+P treatments for all sites were greater than 15 µg/L, with most experiments exhibiting peaks >40 µg/L. The range of peak values for chl fluorescence and chl a concentration were between 45 to 210 µg/L and 25 to 350 µg/L, respectively. This result indicated that urea and the others dissolved N forms (with P in excess) in our experiment stimulated bloom condition (Figures 2, 3, 4, 5a to f). However, chl fluorescence and chl a concentration for most cases of the experiment decrease gradually after about 5 to 8 days until the incubation of samples was terminated (10 days).

Results of two-way ANOVA analysis showed that chlorophyll fluorescence varied significantly between nutrient treatments only in May 2005, with LSD tests confirming that the treatment with Urea+P addition exhibited higher chlorophyll fluorescence than the other nutrient treatments (Figure 7b and Table 1). Extracted chl a concentration differed significantly with N form added during both autumn months, with chl a being significantly higher with Nitrate+P than Urea+P and NH4+P in April 2005 (Figure 7e and Table 1); in May Urea+P showed a significantly greater in chl a concentration than the other dissolved N forms (Figure 7f and Table 1).

Chlorophyll fluorescence and chla levels were higher in N+P treatments than those in nitrate or urea-enriched treatments (Figures 4 and 5). However, comparing between two forms of dissolved N (Nitrate and Urea without P), phytoplankton biomass (chlorophyll fluorescence and chl a) were significantly higher in urea than nitrate addition trials (Figures 4 and 5, Table 3). Significant interaction of chl a concentration was observed between nutrient treatments (N+P addition) and sites in April experiment only (Figure 7e, Table 1). The other significant interaction of chl a concentration between nutrient treatments (N addition only) and site was recorded in early summer experiments (Figure 7g and h; Table 2). This result indicated that there was no consistency in response to N form with sampling site. One-way ANOVA analysis showed that chl a concentration differed significantly between N+P treatments at BP and MB.

In Comparing sites, when phytoplankton biomass differed significantly, chlorophyll fluorescence and chla were greater at MB in the autumn experiments than at the other sites. However, RM had higher chlorophyll fluorescence and chla than the other sites in early summer (LSD tests, $P<0.05$) (Figure 7a to h, Table 2).

Cell abundance was peaked at Day 6 or 8 in autumn and at Day 6 or 10 in early summer months (Figure 3g, h and i). The average peak value of cell abundance in early summer is higher than in autumn experiments, with value for autumn and early summer account for 132,000 and 221,000 cell/ml, respectively. Furthermore, cell abundance in N treatment only tends to be lower than for N+P treatments for all sites (Figures 3 and 4), with cell

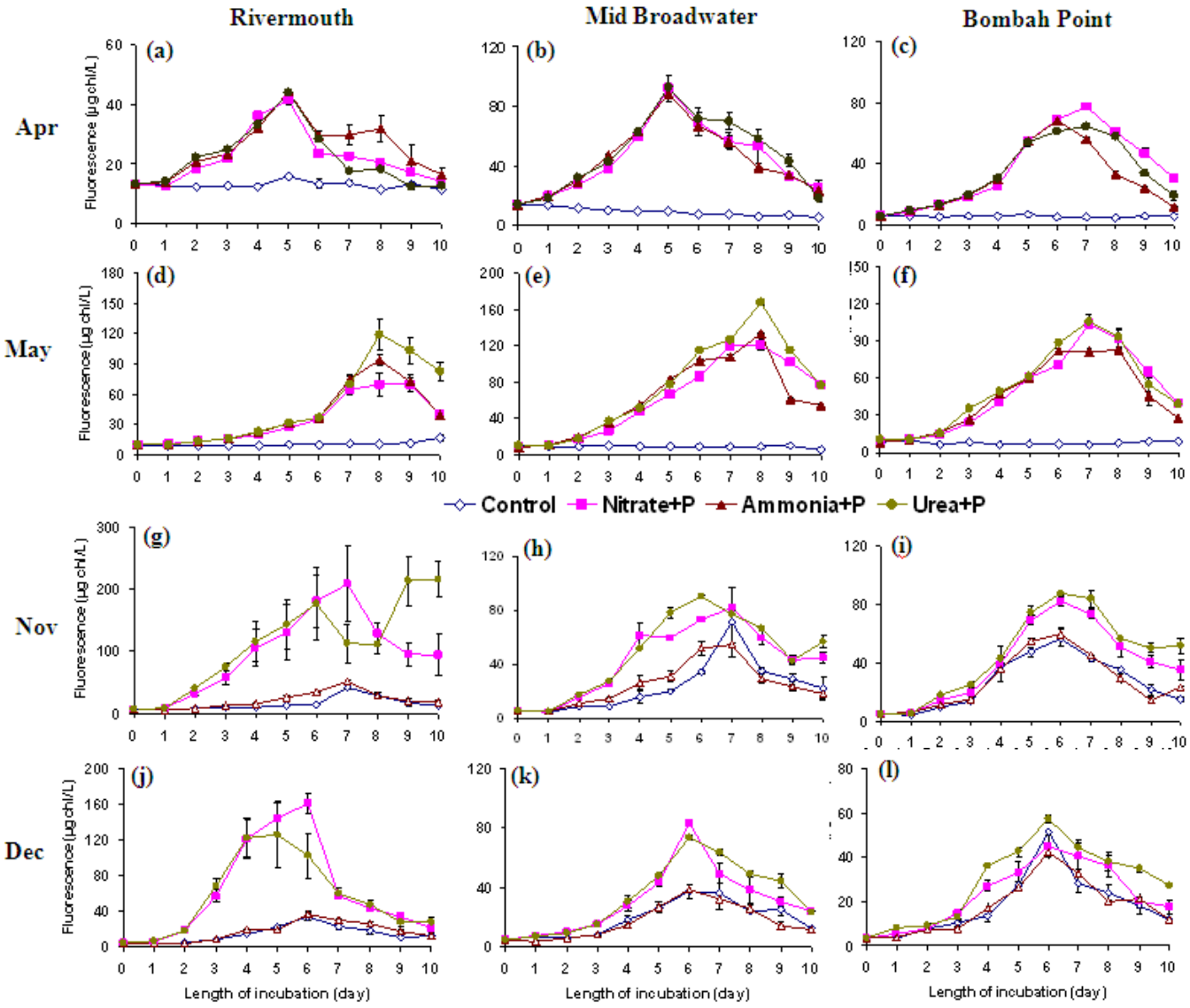

Figure 2. Chlorophyll fluorescence (Mean±SEE, n=3) between nutrient treatments over period of incubation at all sites (notes thatY-axis has a different scale).

abundance remain low over the period of incubation for most cases of the experiment. However, addition of different dissolved N forms did not show any significant differences in cell abundance for most experimental events, except for May experiment (Table 2). The dominant taxa in the May experiment was Microspora.

The variability in cell abundance within site was large. Nutrient enrichment caused a significant difference of cell abundance between sites and treatments for both autumn and early summer experiments Figure 7 (i, j, k, l) and Tables 2 and 3 showed that BP has consistently higher cell abundance than the other sites. However, there was no significant interaction of cell abundance between nutrient enriched treatments (either N+P or N addition only) and sites for all events (Tables 2 and 3).

Phytoplankton abundance

The main classes of phytoplankton showed varying responses to nutrient treatment (Figure 6). Cyanophyceae, Chlorophyceae and Bacillariophyceae were the most represented classes for all experimental events. Significant changes in phytoplankton population were detected between nutrient enriched treatments in the May experiment (Figure 6, Table 2). Urea+P addition significantly enhanced the abundance of Cyanophyceae compared to NO3+P and NH4+P in May (ANOVA, $P<0.05$). Furthermore, Cyanophyceae was the most abundant class at BP for all set of experiments (Figure 8 a, d; Table 2). However, there was no significant interaction between nutrient treatments and sites for

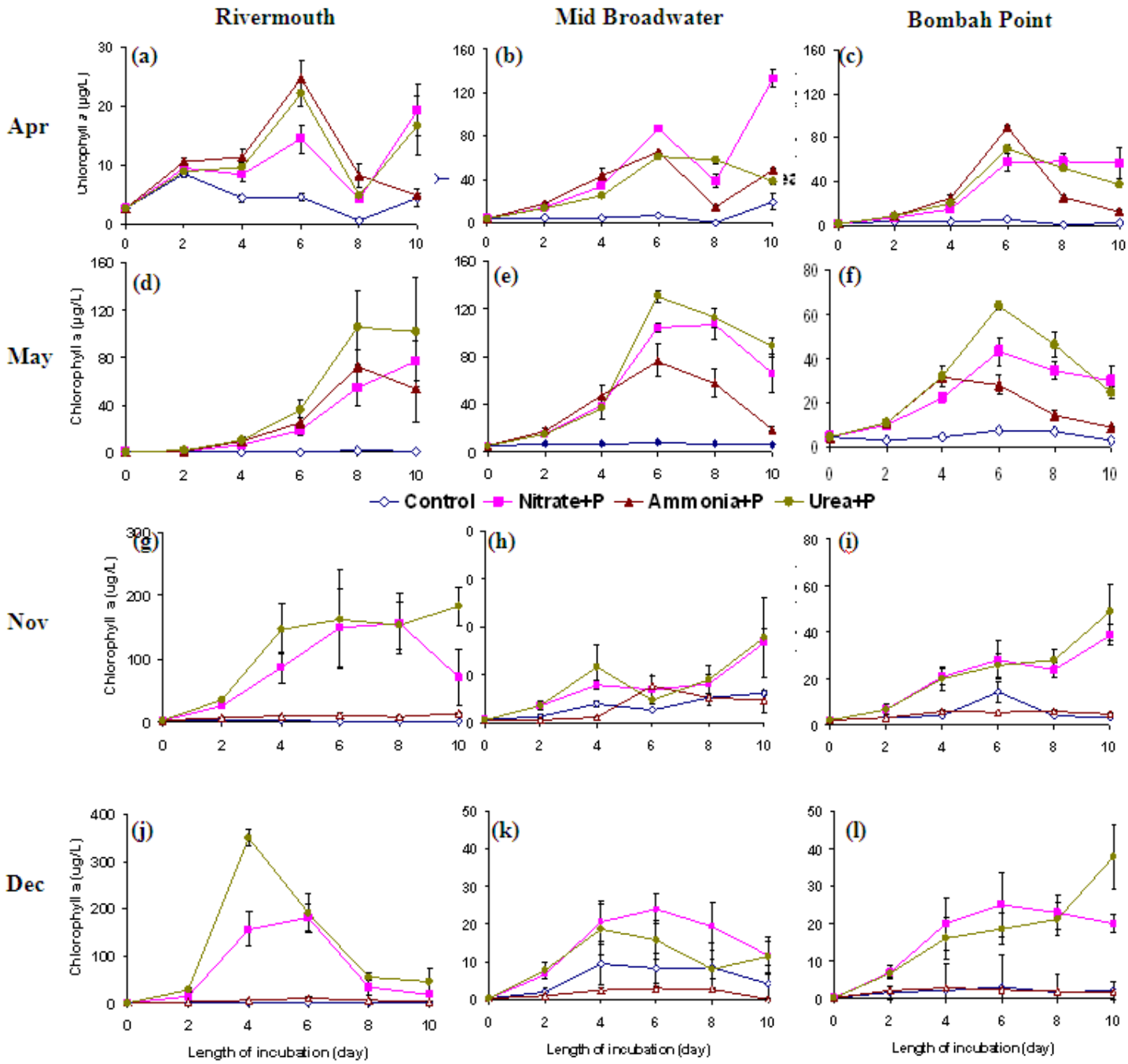

Figure 3. Chlorophyll *a* concentration (Mean±SEE, n=3) between nutrient treatments over period of incubation at all sites (notes thatY- axis has a different scale).

Cyanophyceae during the experiment (Tables 2 and 3).

There was no consistent trend in Chlorophyceae abundance observed between nutrient treatments among sites. A two-way ANOVA analysis showed that Chlorophyceae abundance differed significantly between nutrient treatments only in May and December, while Urea+P and NH_4+P exhibited a higher chlorophyceae abundance than NO_3+P in autumn). On the other hand, NO_3+P addition showed a significantly higher Chlorophyceae cell concentration than Urea+P in December 2005 (Figure 6, Table 3). In May, a significant interaction in chlorophyceae between nutrient treatments and sites was found (Figure 7b and e). The abundance of bacillariophyceae was relatively low in all experiments (Figure 6g to i) and differed significantly between nutrient treatments in the December 2005 experiment only, with Urea+P treatments being significantly higher than NO_3+P. There was significant interaction between nutrient

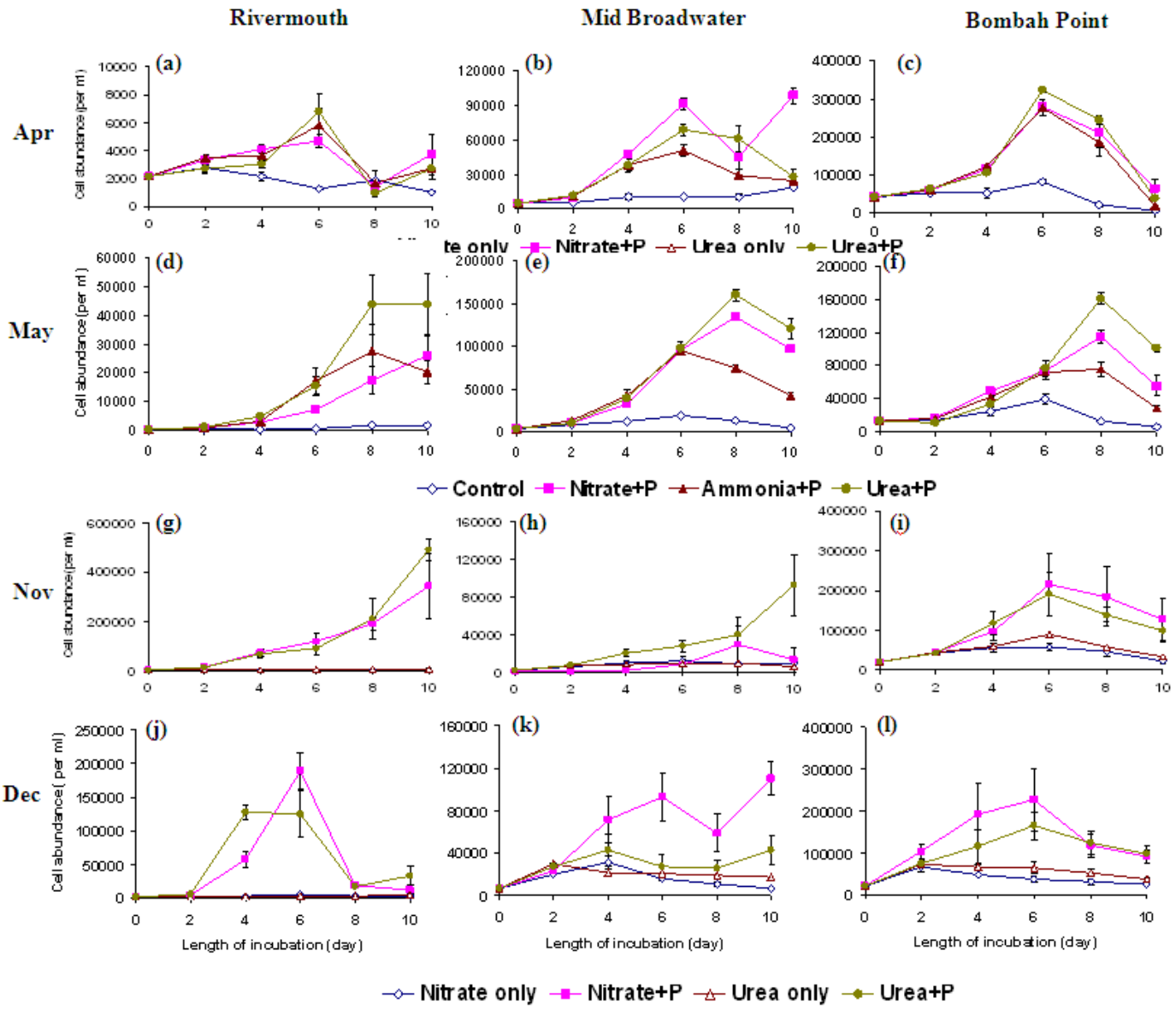

Figure 4. Total cell abundance (Mean±SEE, n=3) between nutrient treatments over period of incubation at all sites (notes thatY- axis has a different scale).

treatments and sites, with MB being the only site showing a significant difference in Bacillariophyceae between nutrient treatments (Figure 7e and f).

Phytoplankton community structure

The nMDS ordinations can be seen in Figure 8. The ordination shows that there is a clear separation of species assemblages between nutrient treatments for most months sampled and sites, except for BP in May and RM in April.

For RM in May, November and December, there was a distinct clustering of species assemblages; with clear separation between nutrient treatments (Figure 8b to d). ANOSIM result gave a global R value ranged between 0.7 and 0.8 indicating a highly significant difference ($P<0.05$). In contrast, there was no clear clustering of species assemblages between nutrient treatments for BP in autumn experiment (May only). The global R value of 0.4 indicated no significant difference of species assemblages between nutrient treatments ($P>0.05$). Plot for early summer experiment (Novrmber and December) showed a clear clustering of species assemblages between N+P and N treatment only (Figure 8c, d, g, h, k and l) with a global R value ranged between 0.6 and 0.9 indicating a highly significant difference of species assemblages between with and without P treatments.

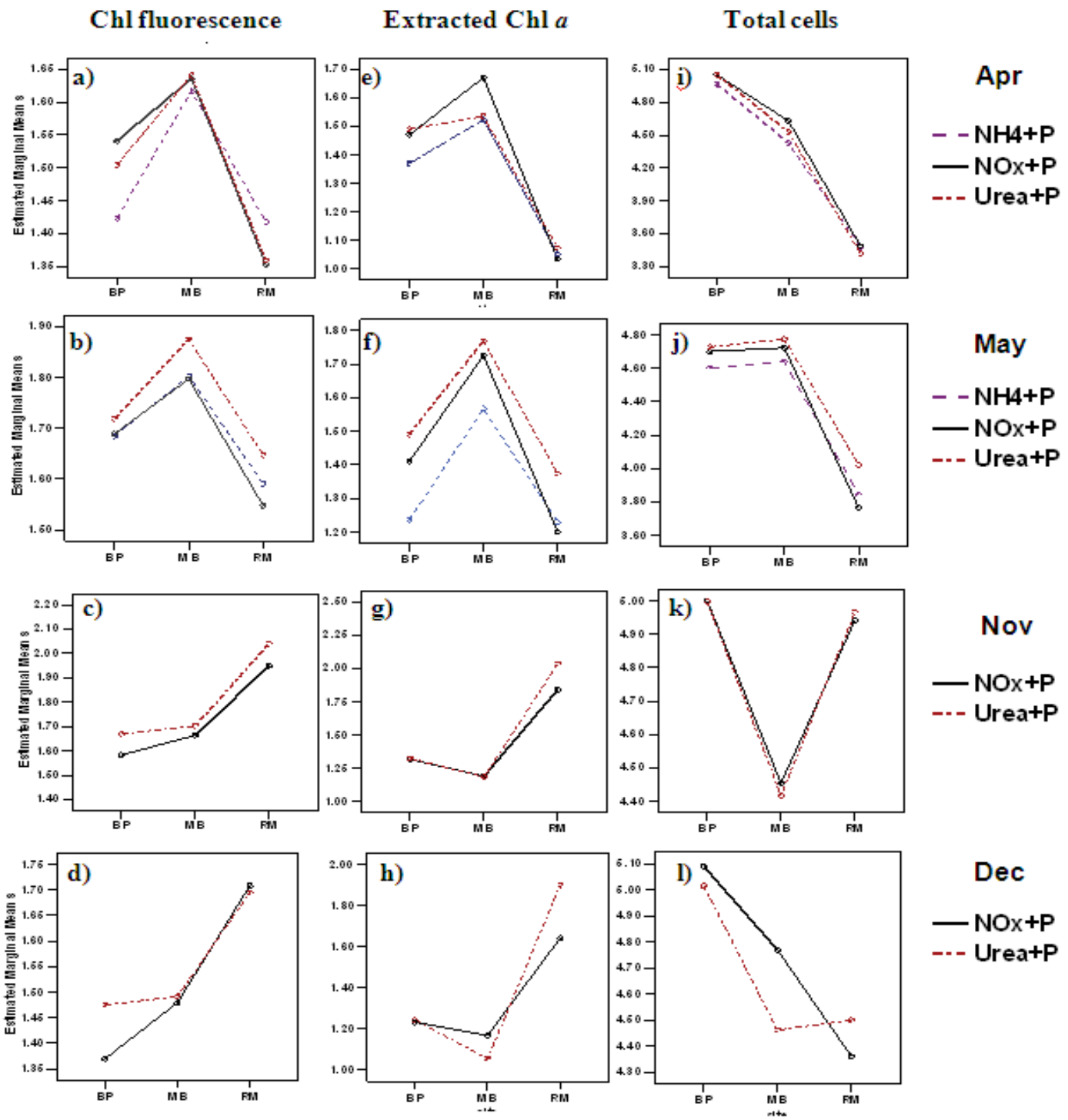

Figure 5. Mean of LSD result plots for chl fluorescence, chlorophyll *a* and total cell abundance (Log (n+1) transformed) between nutrient treatments within sites for each month of experiment. Notes that there is a different treatments between April, May 2005 and November, December 2005.

Dissimilarity percentage of species assemblages varied between pair of nutrient treatment. However, SIMPER result showed that nutrient treatment in autumn had less effect on dissimilarity of species (Table 4). In contrast, in early summer experiment, dissimilarity percentage of species assemblages between nutrient treatments was higher than those in autumn experiment. (Table 4). SIMPER result showed that each site and experiment

Table 1. Mean square and significant value derived from ANOVA repeated measured of chl fluorescence, chlorophyll a conc total cell, Cyanophyceae, Chlorophyceae and Bacillariophyceae abundance (log (n+1) transformed) for autumn experiment (Apr and May'05). NB. df degree of freedom: * = P < 0.05;** = P < 0.01; *** = P < 0.001. RM for Rivermouth, MB for MidBroadwater and BP for Bombah Point.

Date	Source of variation	df	Chl fluor	Chlorophyll a	Total cell	Cyanophyceae	Chlorophyceae	Bacillario-phyceae
	Treatment	2	0.006^{ns}	0.07** NO_3+P>U+P>NH_4+P	0.11^{ns}	0.12^{ns}	0.24^{ns}	0.09^{ns}
Apr	Site	2	0.73*** MB>BP>RM	3.32*** MB>BP>RM	28.74*** BP>MB>RM	72.47*** BP>MB>RM	15.35*** MB>BP>RM	5.54*** RM,BP>MB
	Treatment*Site	4	0.034^{ns}	0.05***	0.05^{ns}	0.06^{ns}	0.07^{ns}	0.57^{ns}
	Residual	18	0.01	0.01	0.04	0.14	0.08	0.54
	Treatment	2	0.11*** U+P>NO_3+P, NH4+P	0.45*** U+P>NO_3+P,NH4+P	0.27** U+P>NO_3+P,NH4+P	0.26** U+P>NO_3+P,NH4+P	0.36** U+P, NH4+P>NO3+P	0.51ns
May	Site	2	0.59*** MB>BP>RM	2.13*** MB>BP>RM	10.46*** BP>MB>RM	27.09*** BP,MB>RM	2.06** RM>MB,BP	4.29*** MB>RM>BP
	Treatment*Site	4	0.005^{ns}	0.05ns	0.17^{ns}	0.01^{ns}	0.36^{ns}	0.46^{ns}
	Residul	18	0.002	0.03	0.09	0.05	0.19	0.25

event tends to have different dominant taxa, for example palmella and coelastrum (chlorophyceae) were dominant taxa for RM, while ulothrix and gloeocystis (chlorophyceae) were abundant at MB. Furthermore, Merismopedia (cyanophyceae) was predominant taxa in BP (Table 4). In May 2005, microspora (chlorophyceae) was the most abundance taxa and present at all nutrient treatments and all sites. Chroococcus (cyanophyceae) was the predominant taxa for early summer (November and December 2005). On the other hand, gloeothece (cyanophyceae) was the taxa that only present in nutrient treatment when P was not in excess at RM in Nov 2005 experiment (Table 4).

DISCUSSION

It was assumed that micronutrients (for example vitamins, Si, Mn, Fe, Mg, Ca) in the Myall Lakes system were not a limiting factor for phytoplankton growth, at least during the first week of incubation. This approach is comparable to numerous other phytoplankton growth studies; in these, micronutrients were not supplied (D'Elia et al., 1986; Dodds et al., 1993; Ayukai, 1996; Bernhard and Peele, 1997; Ault et al., 2000; Blomqvsit, 2001; Cadee, 2003; Ornolfsdottir et al., 2004a; Carter et al., 2005; Dzialowski et al., 2005).

Water quality and chlorophyll growth response

Bombah broadwater exhibits highly variable water quality parameters, in particular salinity and nutrient concentrations, due to the influence of salt-water inflow and nutrient loading from the catchment area. The phytoplankton of bombah Broadwater showed a significant response in biomass (chlorophyll fluorescence and chlorophyll a) to the addition of urea and other dissolved nitrogen forms, when phosphorous was in excess. During all experimental occasions, phytoplankton biomass was significantly higher in dissolved N+P treatments than in control and N-only treatments. Ornolfsdottir et al. (2004a) observed that phytoplankton growth rate with N+P addition was double and triple than that of control treatments (without N or P) following nutrient addition.

Slow response of phytoplankton growth to nutrient enrichment in this study was possibly due to the dominance of small-celled cyanobacteria. The current study indicates that when the phytoplankton community is dominated by large-celled phytoplankton (for examole microspora in May experiment), the growth response of phytoplankton to nutrient enrichment is faster. This finding supports previous studies which

Table 2. Mean square and significant value derived from ANOVA repeated measured of chl fluorescence, chlorophyll *a* conc total cell, Cyanophyceae, Chlorophyceae and Bacillariophyceae abundance (log (n+1) transformed) between nutrient treatments (with and without P) for early summer experiment (Nov and Dec, 2005). NB. df degree of freedom: * = P < 0.05;** = P < 0.01; *** = P < 0.001. RM for Rivermouth, MB for MidBroadwater and BP for Bombah Point.

Date	Source of variation	df	Chl fluor	Chlorophyll a	Total cell	Cyanophyceae	Chlorophyceae	Bacillariophyceae
Nov (N+P)	Treatment	1	0.12^{ns}	0.01^{ns}	0.0004^{ns}	0.01^{ns}	0.00^{ns}	1.19^{ns}
	Site	2	1.18*** RM>BP,MB	4.81*** RM>BP,MB	2.93*** BP,RM>MB	7.41*** BP>MB>RM	17.99*** BP,MB>RM	9.24*** RM>BP>MB
	Treatment*Site	2	0.01^{ns}	0.09^{ns}	0.01^{ns}	0.05^{ns}	0.05^{ns}	0.08^{ns}
	Residual	12	0.03	0.09	0.13	0.26	0.09	0.59
Nov (N only)	Treatment	1	0.16*** U>NO_3	0.77*** U>NO_3	0.10ns	0.06ns	0.01ns	0.08^{ns}
	Site	2	0.31*** BP>MB>RM	0.06^{ns}	10.46*** BP>MB>RM	38.87*** BP>MB>RM	2.06** RM>MB,BP	0.24^{ns}
	Treatment*Site	2	0.02*	0.84***	0.17^{ns}	0.53^{ns}	0.36^{ns}	2.89^{ns}
	Residul	12	0.01	0.04	0.09	0.35	0.19	0.25
Dec (N+P)	Treatment	1	0.03^{ns}	0.06^{ns}	0.15^{ns}	0.73^{ns}	0.61* NO_3+P>U+P	1.73** U+P> NO_3+P
	Site	2	0.64*** RM>MB>BP	3.69*** RM>BP,MB	3.07*** BP>MB>RM	8.60*** BP>MB>RM	0.37ns	2.01*** RM>BP,MB
	Treatment*Site	2	0.03^{ns}	0.27^{ns}	0.37ns	0.06^{ns}	0.50^{ns}	1.56***
	Residul	12	0.01	0.14	0.15	0.27	0.14	0.20
Dec (N only)	Treatment	1	0.002^{ns}	0.31* U>NO_3	0.13^{ns}	0.01^{ns}	0.04^{ns}	0.05^{ns}
	Site	2	0.02^{ns}	0.63** MB>BP>RM	13.46*** BP>MB>RM	25.03*** BP>MB>RM	0.29ns	2.84** BP>RM>MB
	Treatment*Site	2	0.01^{ns}	2.52***	0.14^{ns}	0.77*	0.16^{ns}	0.11^{ns}
	Residul	12	0.01	0.07	0.04	0.16	0.09	0.34

report strong correlations between size and growth rate of algae, with large uni-cellular algae (for example diatoms), showing a greater growth rate per unit volume than small-celled taxa (Irwin et al., 2006; Nielsen, 2006), in particular when nutrients are in surplus (Ornolfsdottir et al., 2004a). The current study demonstrated that urea-only and nitrate-only enrichments in the early summer experiments yielded significantly different responses for chlorophyll fluorescence and chlorophyll a concentration, but not for cell abundance. Graziano et al. (1996) noted that the main response of cyanobacteria to nitrogen addition was an increase in chlorophyll a while cell numbers remained relatively low. The Broadwater study also showed that cyanophyceae chlorophyll responded positively to N-only treatments, compared to other phytoplankton groups. Enrichment with both urea and P resulted in greater phytoplankton growth than with N-only treatments. This was not surprising as it is well known that simultaneous N and P enrichment in lakes always produces greater growth responses of phytoplankton than with single nutrient (N or P) enhancement (Elser et al., 1990; Dzialowski et al., 2005).

Although urea treatments exhibited higher abundances of some taxa, at some instances, there was no consistent phytoplankton growth response to urea compared to other dissolved nitrogen forms. This finding indicates that phytoplankton in the Myall Lakes are able to use all DIN (dissolved inorganic nitrogen) forms efficiently. Other factors may be responsible for site-to-site differences observed in the response to the different treatments. Ornolfsdottir et al. (2004b) report that the variability in phytoplankton community structure and biomass in Galvestone Bay, USA, was in response to nutrient pulses, which are modified by the physical (temperature and light), chemical and biological (grazing and phytoplankton community) characteristics of the Bay.

Cell response

The only significant differences observed in cell abundance, among the various nutrient treatments, occurred in May, when Urea+P addition mediated a

Table 3. Three taxa mostly common for all nutrient treatments at Day 6 for all sites and all experiment events. *** = > 20,000 cells/ml, ** = 10,000-20,000 cells/ml, * = < 10,000 cells/ml. Bolded genera has cell abundance more than 50,000 cells/ml. RM for Rivermouth, MB for MidBroadwater and BP for Bombah Point.

Season/month	Nutrient treatments	Dominant taxa		
		RM	**MB**	**BP**
Autumn (Apr)	NO_3+P	*Oocystis**	*Ulothrix****	*Merismopedia****
		*Coelastrum**	*Microspora***	*Chroococcus****
		*Palmella**	*Chroococcus**	*Aphanopcapsa****
	NH_4+P	*Palmella**	*Ulothrix****	*Merismopedia****
		*Cryptomonas**	*Chroococcus****	*Chroococcus****
		*Oocystis**	*Microspora**	*Coelasphaerium****
	Urea+P	*Coelastrum**	*Ulothrix****	*Merismopedia****
		*Botryococcus**	*Microspora***	*Chroomonas****
		*Palmella**	*Chroococcus***	*Chroococcus****
Autumn (May)	NO_3+P	*Microspora**	*Microspora****	*Microspora***
		*Ulothrix**	*Ulothrix***	*Ulothrix***
		*Gloeocapsa**	*Gloeothece***	*Merismopedia***
	NH_4+P	*Microspora***	*Microspora****	*Merismopedia****
		*Gloeocapsa***	*Gloeocapsa***	*Microspora***
		*Ulothrix**	*Gloeothece***	*Chroococcus**
	Urea+P	*Microsopora***	*Microspora****	*Microsopora****
		*Ulothrix**	*Gloeothece***	*Merismopedia***
		*Gloeocapsa**	*Gloeocapsa**	*Gloeocapsa**
Early summer (Nov)	NO_3 only	*Microspora**	*Chroococcus***	*Chroococcus****
		*Gloeothece**	*Merismopedia**	*Merismopedia***
		*Chroococcus**	*Aphanocapsa**	*Coelasphaerium***
	NO_3+P	*Chroococcus**	*Ulothrix****	*Merismopedia****
		*Coelastrum**	*Euglena**	*Gloocystis****
		Gonium	*Chroococcus**	*Chroococcus****
	Urea only	*Microspora**	*Chroococcus**	*Chroococcus****
		*Gloeothece**	*Merismopedia**	*Merismopedia****
		*Ankistrodesmus**	*Microspora**	*Coelasphaerium****
	Urea+P	*Gonium**	*Ulothrix****	*Merismopedia****
		*Palmella**	*Chroococcus***	*Coelasphaerium****
		*Chroococcus**	*Scenedesmus**	*Chroococcus****
Early summer (Dec)	NO_3 only	*Merismopedia**	*Chroococcus**	*Merismopedia****
		*Microspora**	*Merismopedia**	*Chroococcus****
		*Palmella**	*Microspora**	*Aphanocapsa****
	NO_3+P	*Gloeothece****	*Gloeocystis****	*Chroococcus****
		*Chroococcus****	*Merismopedia***	*Merismopedia****
		*Palmella**	*Chroococcus***	*Coelasphaerium***
	Urea only	*Microspora**	*Merismopedia***	*Chroococcus****
		*Chroococcus**	*Chroococcus**	*Merismopedia****
		*Merismopedia**	*Coelasphaerium**	*Aphanocapsa***
	Urea+P	*Gloeothece****	*Chroococcus***	*Merismopedia****
		*Chroococcus****	*Merismopedia**	*Chroococcus****
		*Euglena**	*Chroomonas**	*Aphanocapsa****

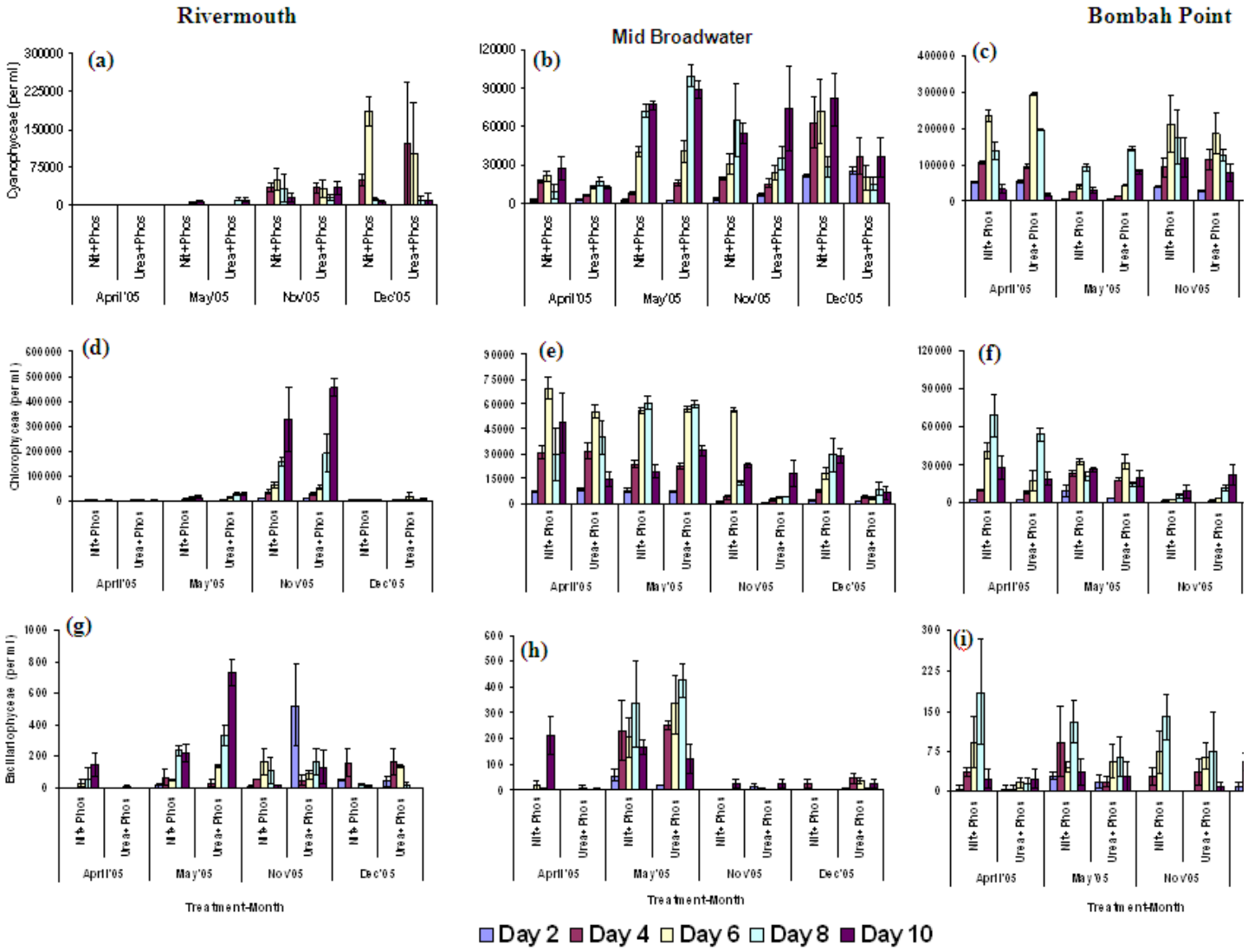

Figure 6. Cyanophyceae, chlorophyceae and bacillariophyceae abundance (mean±SEE, n=3) between two nutrients treatments within months for three sites (notes that Y-axis is at different scale).

greater response in phytoplankton growth than NO3+P or NH4+P. During this time, the phytoplankton assemblages at all three sites were dominated by cyanophyceae (merismopedia and gloeocapsa) and chlorophyceae (microspora and ulothrix). This finding is in line with Kristiansen (1983) who found that urea stimulated the highest uptake rate by marine phytoplankton at Oslofjord, and that algae showed better growth on urea than on NH4. The current study showed significantly higher cell abundance with urea than with nitrate, and may suggest that the N in urea is assimilated at a faster rate than the N in nitrate. Ammonia might be preferentially taken up over nitrate because less metabolic energy is required to assimilate the reduced ammonium form (Richardson, 2001).

In all the experiments, the variability in phytoplankton growth shown by N form was less than that shown for the different sites, and was reflected by the significant differences among sites in both biomass and assemblage structure. Spatial variability is partly affected by rainfall events and site-specific differences in water chemistry, including light availability and salinity. Nutrient availability at each site would naturally vary due to relative distances of sites from the upper Myall River, and their proximity to saline intrusion (Lower Myall River). In addition, it is known that phytoplankton growth response to nutrient enrichment varies with season, river flow regimes and the prior nutritional status of phytoplankton (D'Elia et al., 1986, Richardson et al., 2001). Salinity also contributes significantly to phytoplankton biomass and assemblage structure for Mid Broadwater samples (Redden and Rukminasari, 2008). Flamer et al. (1998) showed that in

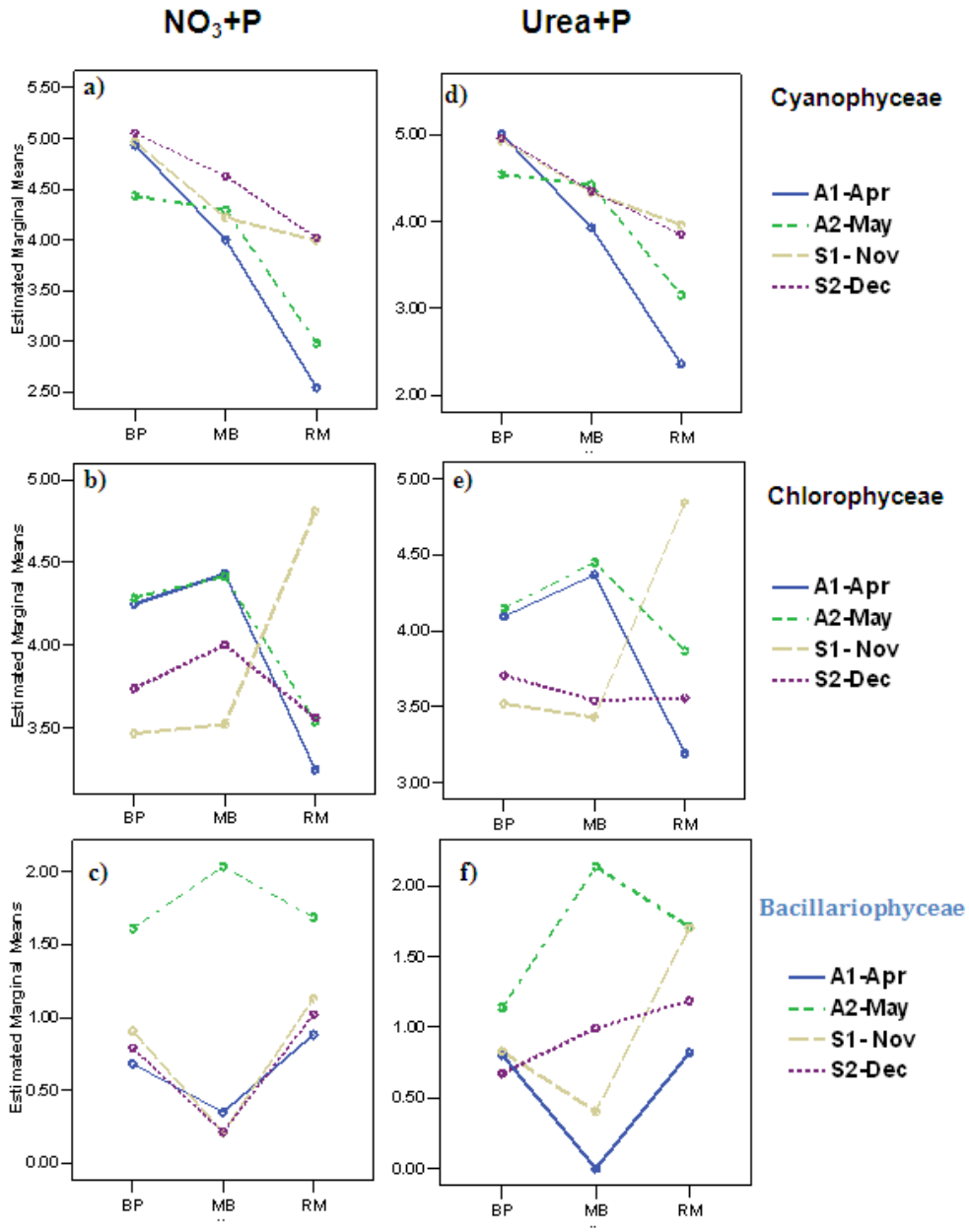

Figure 7. Mean of LSD result plots for cyanophyceae, chlorophyceae and bacillariophyceae (Log (n+1) transformed) between two nutrient treatments (NOx+P and Urea+P) among months and sites.

Perdido Bay, Florida, the response of phytoplankton to experimental N and P addition was correlated with both season and salinity gradient.

Phytoplankton assemblages

Tilman et al. (1982) and Sommer (1989) demonstrated that increases in nutrients in natural waters results in changes of the community structure of phytoplankton. The current study revealed that enrichment of different forms of dissolved nitrogen stimulated the growth of phytoplankton taxa in ways that resulted in significant differences in species assemblages among treatments for most sites. For the six phytoplankton classes those were most common, cell abundance was higher with urea and ammonia enrichment than with nitrate addition. This result indicates that there may be group–specific preferences for uptake of urea and ammonia. While the mechanism by which algae assimilate urea is poorly understood (Siuda and Chrost, 2006), the current study indicated that most, if not all, of the phytoplankton groups

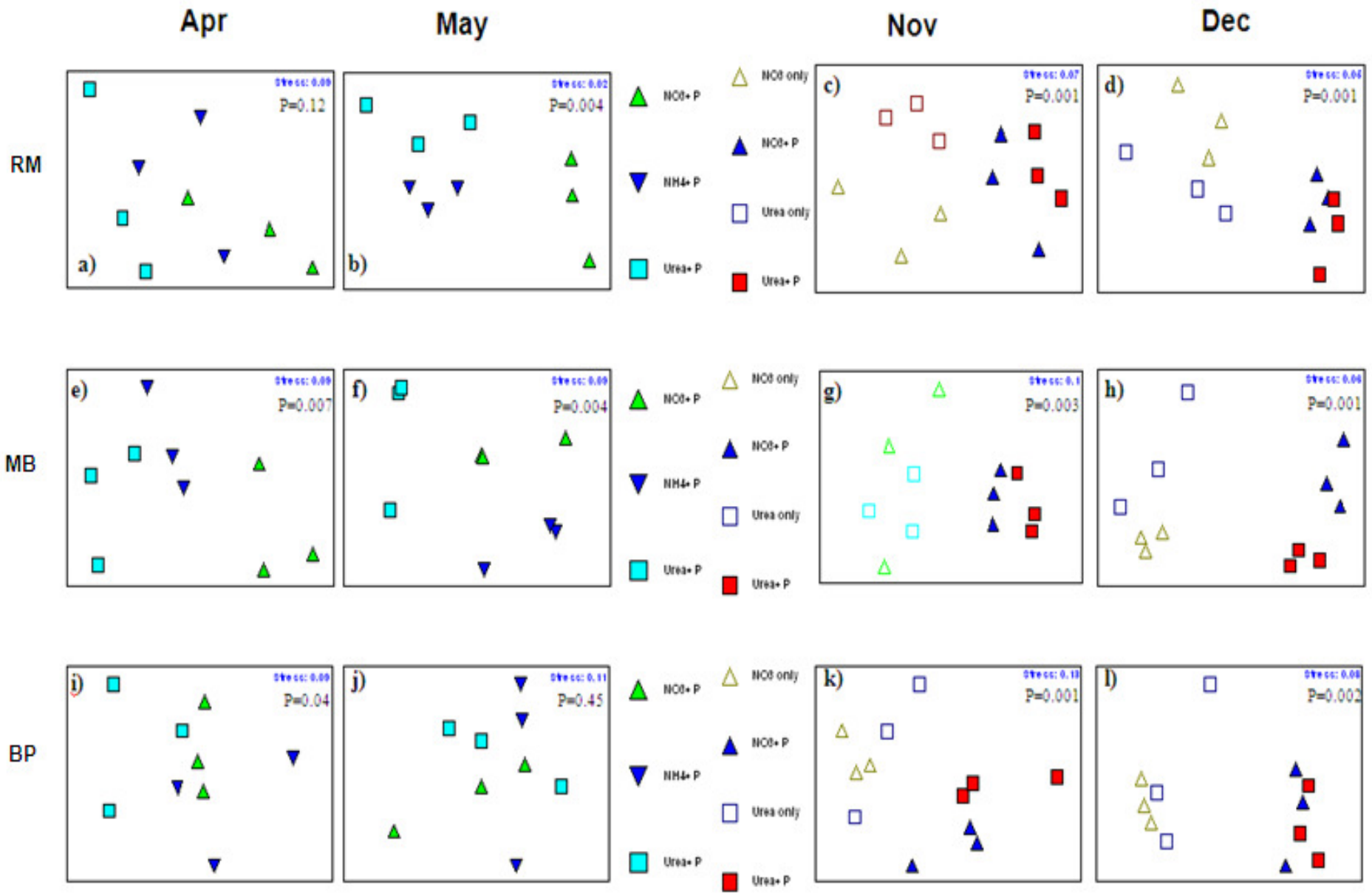

Figure 8. nMDS ordination plots for pseceis of phytoplankton between nutrient treatments for each site at Day 6 of incubation. Control points omitted for Apr and May'05 as a very low abundance of cells. Notes that ther is a different treatment between Apr, May'05 and Nov,Dec'05. RM=Rivermouth (a-d), MB=MidBroadwater (e-h) and BP=Bombah Point (i-l).

Table 4. ANOSIM Pairwise tests and SIMPER results for phytoplankton assemblages showing significant nutrient treatment effects (P<0.05) on Day 6. Number of permutations was too low to detect significant differences between pairs of samples. Bolded genera are more numerous in the 1st treatment of the listed pairs. Global R values > 0.800 are considered to show strong differences between treatment pairs.

Season	ANOSIM pairwise test		SIMPER results	
(Month)	Pair	Global R statistic	Diss (%)	Three taxa most dominant for dissimilarity in pairs
Autumn (Apr)	**Mid Broadwater**			
	NO_3+P vs NH_4+P	0.889	23.1	*Gloeothece 20%, Merismopedia 12%, Euglena 12%*
	NO_3+P vs U+P	0.963	28.5	*Gloeothece 17%, Coelasphaerium 11%, Euglena 10%*
	NH_4+P vs U+P	0.444	17.3	*Coelasphaerium 18%, Gloeocystis 12%, Gonium 12%*
	Bombah point			
	NO_3+P vs NH_4+P	0.222	12.1	*Anabaena 15%, Gonium 10%, Melosira 8%*
	NO_3+P vs U+P	0.444	12.4	*Strombomonas 11%, Gymnodinium 10%. Chlamydomoas 9%*
	NH_4+P vs U+P	0.148	13.7	*Anabaena 14%, Gonium 9%, Chlamydomonas 8%*
Autumn (May)	**Rivermouth**			
	NO_3+P vs NH_4+P	1.000	32.9	*Ulothrix 18%, Tetraspora 16%, Melosira 11%*
	NO_3+P vs U+P	1.000	36.2	*Ulothrix 17%, Tetraspora 14%, Melosira 19%*
	NH_4+P vs U+P	0.519	19.3	*Palmella 12%, Chroococcus 12%, Volvox 12%*
	Mid Broadwater			
	NO_3+P vs NH_4+P	0.555	16.6	*Aphanocapsa 24%, Coelasphaerium 12%, Anabaena 11%*
	NO_3+P vs U+P	1.000	23.1	*Ulothrix 22%, Strombomonas 21%, Coelasphaerium 9%*
	NH_4+P vs U+P	1.000	26.4	*Ulothrix 18%, Strombomonas 17%, Aphanocapsa 12%*
Early Summer (Nov)	**Rivermouth**			
	NO_3 vs NO_3+P	0.852	47.1	*Ankistrodesmus 10%, Gloeothece 10%, Chroococcus 8%*
	NO_3 vs Urea	0.667	36.4	*Palmella 11%, Chroomonas 11%, Cryptomonas 10%*
	NO_3+P vs U+P	0.296	27.5	*Gonium 15%, Gloeothece 12%, Actinium 10%*
	Urea vs U+P	1.000	48.8	*Gonium 10%, Kircheneriella 8%, Gloeothece 11%*
	Mid Broadwater			
	NO_3 vs NO_3+P	-0.222	32.4	*Aphanocapsa 13%, Coelasphaerium 7%, Scenedesmus 7%*
	NO_3 vs Urea	0.741	42.1	*Merismopedia 19%, Gloeothece 15%, Aphanocapsa 10%*
	NO_3+P vs U+P	1.000	47.1	*Chroomonas 14%, Ulothrix 11%, Franceia 8%*
	Urea vs U+P	1.000	59.5	*Merismopedia 12%, Chroomonas 11%, Gloeothece 11%*
	Bombah point			
	NO_3 vs NO_3+P	1.000	35.4	*Gloeothece 12%, Scenedesmus 10%, Palmella 7%*
	NO_3 vs Urea	0.185	25.4	*Palmella 10%. Scenedesmus 9%, Microspora 9%*
	NO_3+P vs U+P	0.370	27.4	*Gloeothece 13%, Aphanocapsa 8%, Coelasphaerium 8%*
	Urea vs U+P	0.741	35.9	*Gonium 10%, Chodatella 10%, Aphanocapsa 8%*
Early Summer (Nov)	**Rivermouth**			
	NO_3 vs NO_3+P	1.000	57.2	*Gloeothece 19%, Euglena 10%, Chroococcus 10%*
	NO_3 vs Urea	0.148	34.1	*Merismopedia 25%, Chroococcus 14%. Palmella 11%*
	NO_3+P vs U+P	0.111	23.2	*Ulothrix 13%, Palmella 10%, Carteria 10%*
	Urea vs U+P	0.963	61.7	*Gloeothece 19%, Microspora 13%, Euglena 12%*
	Mid Broadwater			
	NO_3 vs NO_3+P	1.000	48.2	*Gloeocystis 15%, Ulothrix 12%, Microspora 10%*
	NO_3 vs Urea	0.481	26.9	*Scenedesmus 20%. Strombomonas 12%, Coelasphaerium 11%*
	NO_3+P vs U+P	0.926	27.6	*Gloeocystis 23%, Carteria 9%, Gloeocapsa 8%*
	Urea vs U+P	1.000	43.3	*Scenedesmus 15%, Ulothrix 15%, Microspora 14%*
	Bombah point			
	NO_3 vs NO_3+P	1.000	45.8	*Oodinium 11%, Coelasphaerium 9%, Chlamydomonas 8%*
	NO_3 vs Urea	0.259	29.2	*Cosmarium 8%, Coelasphaerium 8%, Oodinium 7%*
	NO_3+P vs U+P	0.296	28.6	*Oodinium 13%, Coelasphaerium 9%, Chodatella 8%*
	Urea vs U+P	0.963	45.7	*Franceia 8%, Chlamydomonas 8%, Microsphora 8%*

assimilate and utilise urea. Siuda and Chrost (2006) report that cyanobacteria can utilize urea as NH_4-following hydrolization of urea via enzymatic processes, and that some algal species (especially green algae) produce ATP-dependent urea amidolyase; some diatoms and dinoflagellates produce intracellular urease. Although, we did not investigate the physiology of urea uptake and assimilation by phytoplankton, this would be an interesting direction for further research in coastal systems which receive large urea inputs from the catchment area.

Olden (2000) and Richardson et al. (2001) report that phytoplankton have different capabilities for nutrient uptake, which may result in species-specific or group-specific responses to different nitrogen forms and concentrations. Phytoplankton responses to different forms of nitrogen may be expressed as differential growth rates, that are manifested in the prevailing phytoplankton assemblage structure (Stolte et al., 1994; Pickney et al., 1998; Richardson et al., 2001).

Large-celled phytoplankton tend to be more abundant and to dominate under high nutrient conditions, in contrast to small cells, whose relative abundance tend to decrease with increasing nutrient supply (Irwin et al., 2006). The current study similarly found that nutrient enrichment leads to changes in community structure and to the dominance of large cells over small cells. The dominant taxa in the May experiment were large-celled microspora (chlorophyceae), which replaced small-celled taxa (for example merismopedia, chroococcus) abundance. chlorophyceae appeared to be more competitive than cyanophyceae when nutrients were in surplus. This finding is in agreement with Mitrovic et al. (2001), who reported that chlorophyceae under nutrient-rich conditions tend to replace Cyanophyceae abundance.

Conclusion

There were no consistent trends which would indicate that urea produced a greater phytoplankton growth response than other forms of dissolved N, with P in excess. However, BombahBroadwater phytoplankton did show significant site-to-site, as well as seasonal variation in growth response, which reflects temporal and spatial variability in the distribution and abundance of phytoplankton taxa.

While the enrichment of urea and all other dissolved nitrogen forms stimulated bloom production and a shift in assemblage structure, the availability of P was also a factor that contributed significantly to changes in assemblage structure of phytoplankton in Bombah Broad water. Phytoplankton from the Mid-Broadwater site were the most responsive to nutrient enrichment in autumn (April and May 2005) while those from the Rivermouth site were most responsive in early summer (Nov and Dec 2005). Pronounced site-to-site variability within BombahBroadwater indicates that any examination of phytoplankton responses to nutrient inputs from the catchment requires multi-site and multi-season observations.

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