Evaluation of uptake kinetics during a wastewater diversion into nearshore coastal waters in southern California.

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Abstract

The global eutrophication of coastal ecosystems from anthropogenic nutrients is one of the most significant issues affecting changes to coastal oceans today. A three-week diversion of wastewater effluent from the normal offshore discharge pipe (7 km offshore, 56 m depth) to a shorter outfall located in 16 m water (2.2 km offshore) as part of the 2012 Orange County Sanitation District Diversion provided an opportunity to evaluate the impacts of anthropogenic nitrogen on phytoplankton community response. Nitrogen uptake kinetic parameters were used to evaluate the short-term physiological response of the phytoplankton community to the diverted wastewater and to determine if potential ammonium suppression of nitrate uptake was observed. Despite expectations, there was a muted response to the diversion in terms of biomass accumulation and ambient nutrients remained low. At ambient nitrogen concentrations, calculated uptake rates strongly favored ammonium. During the diversion based on the kinetic parameters determined during short-term experiments, the phytoplankton community was using all three N substrates at low concentrations, and had the capacity to use urea, then ammonium, and then nitrate at high concentrations. Ammonium suppression of nitrate uptake was evident throughout the experiment, with increasing suppression through time. Despite this interaction, there was evidence for simultaneous utilization of nitrate, ammonium, and urea during the experiment. The general lack of phytoplankton response as evidenced by low biomass during the diversion was therefore not obviously linked to changes in uptake rates, physiological capacity, or ammonium suppression of nitrate uptake.

Key words: wastewater effluent, nitrogen, phytoplankton, nitrogen isotopes, ammonium compounds, nitrogen uptake

1. Introduction

The contributions of anthropogenic nitrogen loads to the eutrophication of coastal systems has been well documented (see reviews, Howarth 2008; Paerl and Piehler 2008) and is considered one of the most globally important human-accelerated changes to coastal oceans (Howarth and Marino 2006; Scavia and Bricker 2006). Anthropogenic nutrient inputs have been linked to increased primary production and algal blooms (Lapointe et al. 2004; Beman et al. 2005), and are considered the most significant factor contributing to the increased frequency of harmful algal blooms (HABs) (Anderson et al. 2002; Hallegraeff 2004; Glibert et al. 2005; Heisler et al. 2008). Most coastal eutrophication studies have focused on nitrogen (N), since it is the primary macronutrient that limits the growth of phytoplankton in coastal waters (Ryther and Dunstan 1971; Eppley et al. 1979). The form of N is also important in the stimulation of some algal species responsible for HABs (Glibert et al. 2006), including in California (Kudela et al. 2010). Upwelling dominated systems have generally been perceived to be less affected by anthropogenic nutrients due to the sheer magnitude of natural (upwelled) nutrients as well as the highly dynamic conditions making these systems potentially more resilient. However, a growing number of studies have suggested that our perception of the resilience of these systems may be flawed (c.f. Capone and Hutchins 2013). The large quantities of anthropogenic nutrient sources in the Southern California Bight (SCB), mainly from wastewater treatment plants and agricultural activities, have sparked a series of studies focused on the impacts and effects of anthropogenic inputs on coastal ecosystems. Anthropogenic N sources, mainly as wastewater effluent, were shown to provide an equivalent contribution of N when compared to natural (upwelled) sources, thus essentially doubling the N loading to nearshore coastal waters in the

66 urbanized areas of the SCB (Howard et al. 2014). This highlights not only the magnitude of N in
67 the coastal environment, but also implies potentially altered composition of N forms as well,
68 since wastewater is typically comprised of ammonium and upwelling is dominated by nitrate
69 (Howard et al. 2014).

70 A historic analysis of satellite imagery documented chronic algal bloom hotspots co-
71 located with major anthropogenic sources of nutrients and determined algal blooms have
72 increased in geographic extent in the SCB beyond what could be supported by increased
73 upwelling (Nezlin et al. 2012). Consistent with increased anthropogenic loading, temporal trends
74 in dissolved oxygen concentrations in the SCB show that the rate of decline is four times higher
75 in the nearshore where anthropogenic nutrient discharge is substantial, compared with offshore
76 locations (Booth et al. 2014). Additional studies, focused on more refined spatial scales, have
77 documented the stimulatory effects of terrestrial and wastewater effluent discharges, resulting in
78 increased phytoplankton biomass and productivity as well as altered community composition
79 (Corcoran et al. 2010; Reifel et al. 2013). The inhibitory impacts of wastewater effluent,
80 specifically due to ammonium inhibition of nitrate uptake by phytoplankton, have also been
81 linked to decreased primary production and significantly altered phytoplankton community
82 composition (c.f. Dortch 1990; Dugdale et al. 2012; Glibert et al. 2015).

83 The Orange County Sanitation District (OCSD) conducted a planned diversion of treated
84 wastewater effluent from the primary outfall pipe located 7 km offshore (56 m water depth) off
85 Huntington Beach, California to a short outfall pipe, located only 2.2 km offshore in 16 m of
86 water, in order to inspect and rehabilitate the primary outfall pipe. This planned diversion of
87 treated wastewater effluent discharge into the shallow nearshore environment provided what
88 should have been an ideal opportunity to evaluate the impacts of anthropogenic N on

phytoplankton. The diversion of wastewater had the potential to impact both the quantity of N biologically available, as well as the form of N, both of which can affect phytoplankton uptake rates of N, community composition, growth and biomass.

The goals of this study were to use N uptake kinetics as a short-term metric of physiological capacity, to evaluate the response of phytoplankton to the diverted wastewater, to determine if ammonium suppression of nitrate uptake was observed, and to document any changes in N uptake rates before, during and after the effluent diversion. The experimental design assumed that elevated ammonium concentrations would be evident at station 2203 near the outfall, and that a strong biological response to the wastewater diversion would be observed, based on previous studies (Reifel et al. 2013). The lack of high levels of ammonium and the lack of biological response (Caron et al. this issue, Kudela et al., this issue) resulted in adjustment of the experimental design midway through the experiment, and introduced methodological issues that complicated interpretation of the results. Nonetheless, the data presented here provides useful information about the physiological status and response to nutrient enrichment by the ambient phytoplankton community. Specifically, these data can be used to address two questions: first, is there evidence for physiological inhibition of the phytoplankton assemblage that could explain the modest biological response observed, and second, is there evidence for a physiological response to the availability of anthropogenic nutrients?

2. Materials and Methods

2.1 Study Area

The Orange County Sanitation District (OCSD) discharges treated wastewater effluent through an ocean outfall that terminates 7 km offshore of Huntington Beach in 56 m water depth

112 at the shelf break (Figure 1). There is also a secondary, shorter outfall, located 2.2 km offshore at
113 a depth of 16m, for which only emergency discharges are permitted under the National Pollutant
114 Discharge Elimination System (NPDES). In order to inspect, assess and rehabilitate the 7 km
115 outfall pipe, OCSD diverted wastewater to the short, nearshore outfall from 11 September 2012
116 until 3 October 2012. There were 6 cruises from 6 September 2012 through 17 October 2012
117 during which CTD measurements, ambient nutrient concentrations and biomass measurements
118 were collected in the vicinity of both outfalls, capturing the pre-diversion, diversion, and post-
119 diversion periods. While we focus on station 2203, data for all stations are provided as context
120 for the environmental conditions during the study.
121
122 *2.2 Kinetics methods and experimental procedures*
123 Whole water was collected from station 2203 (Figure 1; maximum depth 33 m) for all
124 experiment dates to determine the N uptake kinetics of three N substrates (nitrate, ammonium
125 and urea) and to evaluate ammonium suppression of nitrate uptake. The overall chlorophyll *a*
126 (chl *a*) concentrations throughout the study area were low (see Kudela et al., this issue and Caron
127 et al. this issue), therefore, experiment water was collected from the chlorophyll maximum in
128 order to maximize the concentration of algal biomass in the incubation experiments (Figure 2).
129 Experiments were conducted during 4 different timepoints: prior to the start of the diversion on 6
130 September 2012 (experiment water collected from 15 m depth), during the diversion on 20
131 September 2012 (experiment water collected from 7 m depth), hours after the diversion ended on
132 3 October 2012 (experiment water collect from 12 m depth), and 2 weeks after the diversion on
133 17 October 2012 (experiment water collected from 15 m depth). The sampling depth was

consistently in the upper part of the chlorophyll maximum, with subsurface photosynthetic available radiation (PAR) of 100-200 µmol photons m^{-2} s^{-1}.

Samples were collected on cruises aboard the R/V *Yellowfin* in September and the M/V *Nerissa* in October from twelve-liter and five-liter (respectively) PVC Niskin bottles mounted on an instrumented rosette. Seawater was collected in 20-liter acid-cleaned polycarbonate (Nalgene) carboys and kept in dark coolers during transportation back to the laboratory. In the laboratory, water was dispensed into acid-cleaned 250 ml polycarbonate bottles and discrete samples of chl *a* and nutrients were collected, all within 24 hours of collection. Nutrients and chl *a* samples were also collected directly from the sample bottles (see Caron et al. this issue), and were not significantly different from the values obtained from the kinetics experiments (Table 1). For 6 September 2012 the nutrients were lost during storage, and nutrient concentrations from 5 m depth were substituted (both 5 m and 15 m depths were above the pycnocline). The uptake kinetics incubation bottles were inoculated with either ^{15}N-ammonium chloride (99 atom%; Cambridge Isotopes), ^{15}N-sodium nitrate (98 atom%), or ^{15}N-urea (98 atom%) at 12 substrate concentrations ranging from 0-38 µM N to duplicate sample bottles. To avoid confusion, all N values are reported as µM N, accounting for the molar difference in N between urea versus nitrate and ammonium. The total number of samples used for curve-fitting is noted in Table 3, accounting for some samples lost during processing or analysis. The ammonium suppression experiments were inoculated with 12 substrate concentrations of ammonium chloride ranging from 0-38 µM N and 10 µM ^{15}N-sodium nitrate to duplicate sample bottles.

All bottles were incubated in a laboratory incubator at ambient temperature (16-19^{o} C) under 65-80 µmol photons m^{-2} s^{-1} irradiance using standard cool-white fluorescence illumination. The incubator irradiance was lower than ambient mid-day values at the time of collection (200,

175, 500, and 180 µmol photons m^{-2} s^{-1} respectively) and was also lower than half-saturation values (except for 17 October) based on Pulsed Amplitude Modulation Electron Transport Rate measurements (E_k; µmol photons m^{-2} s^{-1}; see Kudela et al. this issue), which were 479, 619, 371, and 49 µmol photons m^{-2} s^{-1} for each date respectively. Incubations were maintained for 1 hour and filtered onto precombusted GF/F filters (<100 mm Hg), frozen immediately, and subsequently dried at 50° C. Samples were analyzed for total particulate N and isotopic enrichment using a Finnigan Delta XP isotope ratio mass spectrometer at the University of California, Santa Cruz, using acetanilide as the reference standard.

Nitrogen specific uptake rates were calculated as described by Dugdale and Wilkerson (1986) from accumulation of ^{15}N into the particulate material at the end of the incubation and were not corrected for the effects of isotopic dilution. Ammonium uptake rates are therefore underestimates of *in situ* rates. For determination of kinetic constants, raw data was first inspected visually for biphasic, multiphasic, or linear behavior. For biphasic/multiphasic kinetics, only data points corresponding to the first phase were selected to compute affinity (α) and the maximum uptake rate (V_{max}), following Collos et al. (2007). In the case of a linear uptake relationship, the slope of the regression was considered comparable to the initial slope of a hyperbolic curve (e.g. Cochlan et al. 2008; Lee et al. 2015). In two cases, an inhibitory relationship was observed for nitrate uptake (in the absence of ammonium) and ammonium uptake; there is no obvious physiological explanation for suppression of nitrate uptake at moderate N concentration, and it was therefore assumed that this was an experimental artifact. Suppression of ammonium uptake has occasionally been reported and is likely the result of a more complex physiological response involving interaction of nutrient uptake with other cellular processes (Glibert et al. 2015).

An iterative non-linear least squares technique was used for the curve fitting (Kaleidagraph; Abelbeck Software) that utilizes the Levenberg-Marquardt algorithm (Press et al. 1992) to determine the half-saturation (K_s, µM N) and maximum uptake (V_{max}, h^{-1}) parameters of a Michaelis-Menten curve for N kinetics, using the following equation:

$$V = \frac{V_{max} \bullet S}{K_s + S} \quad (1)$$

Where V_{max} values were calculated as V_{chl}, equivalent to ρ divided by the chlorophyll concentration (Dickson and Wheeler, 1995) and S (µM N) is the initial substrate concentration, accounting for both ambient and added nutrients. For 6 September, the nutrient concentration from the closest available depth (30 m) was used; 0.06 µM NO_3, 0.15 µM NH_4, and 0.68 µM N-urea. For ammonium suppression experiments, ambient nitrate concentrations were not known at the time of incubation, so 10 µM NO_3 was added. Ambient nitrate and ammonium concentrations were included in the calculated substrate concentrations. For comparison to previous publications we also provide V_{max} normalized to PN in Table 3, for comparison with historical estimates. The substrate affinity (α) was calculated as $\alpha = V_{max}/K_s$ and determined from the initial slope of the Michaelis-Menten plot at sub-saturating concentrations ($<K_s$) or from the slope of the regression for those kinetics curves exhibiting a non-saturating, but not multiphasic, response (Healey, 1980). For standardization purposes and for comparison to the literature, a Michaelis-Menten equation was fit where possible, and non-standard kinetics fits are noted where appropriate in Table 3 and 3-5. For calculation of ammonium inhibition, the variation of the Michaelis-Menten equation proposed by Varela and Harrison (1999) was used:

$$\rho = \rho_{max} \bullet \left[1 - \frac{I_{max} \bullet [NH_4}{K_i + [NH_4}\right] \quad (2)$$

where ρ = the nitrate uptake rate (μM N h^{-1}), ρ_{max} is the maximum rate in the absence of ammonium, I_{max} is the maximum inhibition (ranging from 0 to 1), K_i is the concentration of ammonium where ρ is half-maximum, and [NH_4 is the ambient ammonium concentration (μM N).

2.3 Analytical methods

Chl *a* samples were collected in duplicate, filtered onto Whatman GF/F filters, extracted in 7 mL of 90% acetone for 24h at -20^{o} C and analyzed using a model 10AU fluorometer (Turner Designs, CA) using the acidification method (Parsons et al. 1984) with pure chlorophyll as the calibration standard. Dissolved inorganic nutrients were filtered through 0.45 μm Whatman syringe filters into 50 mL low-density polyethylene tubes and stored frozen (-20° C) before analysis. Nitrate plus nitrite (hereafter referred to as nitrate) were analyzed with a LaChat Quick Chem 8000 Flow Injection Analysis system using standard colometric techniques (Smith and Bogren 2001). Ammonium samples were manually analyzed using the fluorometric method of Holmes et al. (1999) and urea samples were also analyzed manually using the diacetyl monoxime thiosemicarbizide technique (Goeyens et al. 1998) with a 10 cm pathlength cuvette. Particulate nitrogen (PN) and particulate organic carbon (POC) samples were filtered onto precombusted GF/F filters (<100 mm Hg), frozen immediately, and subsequently dried at 50^{o} C. These samples were acidified and analyzed at the Marine Sciences Institute Analytical Laboratory at the University of California, Santa Barbara on an Exeter Analytical Elemental Analyzer.

3. Results

3.1 Ambient nutrient concentrations

All three forms of N (nitrate, ammonium and urea) were consistently low throughout the study period. The ambient nutrient concentrations collected simultaneously with experiment water are provided in Table 1. Nitrate was below 0.5 µM N, ammonium below 0.25 µM N and urea below 0.7 µM N. The chl *a* concentrations collected from the experiment water were low pre-diversion (1.0 µg L^{-1}), and increased to 3.5 µg L^{-1} during the diversion, which is relatively low for the San Pedro area (Seubert et al. 2013; Seegers et al. 2015; Caron et al., this issue). The PN concentration remained relatively unchanged throughout the study period while POC doubled. The C:N ratio increased during the diversion to 8.1 from a pre-diversion and 2 weeks post-diversion value of 6.4.

Throughout the study period, diatoms dominated the phytoplankton community composition, as reported in Caron et al. (this issue). In the 6 September and 20 September experiments, community composition was analyzed from the experiment water and diatoms comprised >96% of the community composition (E. Seubert and D. Caron, unpublished data). The specific genera that were present in the water collected for the experiment conducted on 6 September 2012 included *Cylindrotheca* spp., *Guinardia* spp., *Pseudo-nitzschia* spp., (both size classes) and *Rhizosolenia* spp. Of those, *Rhizosolenia* spp. and *Pseudo-nitzschia* comprised 90% of the diatom assemblage (E. Seubert and D. Caron, unpublished data). The genera identified in the water collected for the experiment on 20 September 2012 included *Chaetoceros* spp., *Cylindrotheca* spp., *Lauderia* spp., *Leptocylindrus* spp., *Navicula* spp., *Pseudo-nitzschia* spp. (both size classes), *Rhizosolenia* spp., *Skeletonema* spp., and *Thalassiosira* spp. *Pseudo-nitzschia* comprised 60% of the diatom composition and was the dominant genera observed (D. Caron and E. Seubert, unpublished data). The floral composition was not analyzed in the experiment water

246 from the October experiments but Caron et al. (this issue) reports on overall assemblage
247 throughout the study.
248
249 *3.2 N Uptake Kinetics*

250 The relative preference and affinity of different N substrates in low and high nutrient
251 environments can be assessed using nutrient uptake kinetic parameters. At low ambient nutrient
252 concentrations, ($S<K_s$), the initial slope (α) is a more robust indicator of affinity whereas at high
253 ambient nutrient concentrations, the maximum uptake rate, V_{max}, can be used to assess
254 preference (Healey, 1980). Phytoplankton uptake kinetics can take many different forms, with
255 saturation-uptake (Michaelis-Menten) kinetics often referred to as "classic" kinetics. Following
256 Collos et al. 1997, we refer to induced kinetics where the response is approximately linear, and
257 biphasic kinetics where a plateau at lower substrate concentrations is observed followed by either
258 a second saturation-uptake response or linear response to increasing substrate concentrations. We
259 note that these observed responses are further complicated for experiments using natural
260 assemblages rather than monospecific cultures, since the observed kinetics are some weighted
261 function of the response of each individual species, each of which may exhibit classic, biphasic,
262 or linear kinetics (e.g. Flynn 1999).

263 Based on the isotopic enrichment and concentration of ^{15}N-substrate added, and the atom
264 % excess and PN at the termination of the experiments, an average (+/- 1 SD) 1.67-4.74 +/- 2.71-
265 6.68%, 1.64-6.61 +/- 2.72-8.71%, and 0.80-3.82 +/- 1.34-6.24% of the ^{15}N-substrate was
266 incorporated for nitrate, ammonium, and urea uptake kinetics respectively. Substrate exhaustion
267 was therefore not considered an issue, and was not an obvious source of error in defining the
268 uptake-kinetics responses.

269 The pre-diversion experiment exhibited the full range of possible kinetics responses (Fig.
270 2, Table 3). For ammonium, reasonable saturation-uptake behavior was observed. Nitrate and
271 urea results were unsaturated (biphasic or linear), and therefore, preclude calculation of V_{max} as
272 described above using the full dataset. Nitrate and urea both exhibited saturation-uptake below
273 approximately 6 µM N, with a second (biphasic) phase above that concentration. We therefore
274 truncated the data between 6-10 µM N for those N species, based on visual examination of the
275 data, calculating the kinetics parameters using the reduced range, and also fit a linear response
276 curve (induced kinetics) to the urea data (Figure 3, Table 3). V_{max} is reported as the highest
277 measured value for urea uptake and α was determined from linear regression for the initial slope,
278 while the truncated kinetics are provided for both nitrate and urea. Given the non-classic uptake
279 response, these data from the Michaelis-Menten equation should be interpreted cautiously. K_s
280 was generally comparable to or higher than ambient concentrations (see Table 1) suggesting that
281 α is the most appropriate metric for comparison of N utilization; mean ambient concentrations
282 were also lower than 6 µM N, suggesting that the truncated kinetics parameters generally
283 represent uptake responses for pre-diversion waters when ambient N concentrations were low.
284 Based on α, preference at low (ambient) concentrations was urea>nitrate>ammonium, while urea
285 > ammonium >> nitrate using V_{max} values.

286 The experiment results during the diversion (20 September 2012) exhibited saturation-
287 uptake responses for all three substrates (Figure 3, Table 3) but with some indication of
288 suppression at elevated ammonium concentrations above ~12 µM N, and with considerable
289 variance at the highest nitrate concentration. The α values were similar across substrates, but
290 ambient concentrations were at (ammonium) or greater than (urea) K_s values. Ammonium and
291 nitrate K_s values were comparable (0.58 and 0.33 µM N, respectively), while the K_s for urea was

considerably higher (4.79 µM N), and V_{max} values showed a strong gradient with urea > ammonium > nitrate. Taken together the results indicated that the community was likely utilizing all three substrates at low nutrient concentrations (α, urea ≈ ammonium ≈ nitrate), with comparable uptake for nitrate and ammonium at moderate (ambient) nutrient concentrations. The V_{max} results indicated a greater potential for urea uptake at high nutrient concentrations.

The first post-diversion experiment (3 October 2012) was conducted several hours after the end of the diversion and ammonium exhibited a classic Michaelis-Menten response (Figure 4, Table 3), with urea possibly exhibiting biphasic kinetics but with a statistically significant fit using a saturation-uptake response. The nitrate results were unsaturated, therefore, V_{max} and α are reported but K_s was not calculated. Ambient concentrations for all stations were similar to values during the diversion and K_s values for ammonium and urea were again similar to or greater than ambient concentrations. At low nutrient concentrations urea and nitrate exhibited similar affinity and lower affinity for ammonium (α, urea ≈ nitrate >> ammonium) while nitrate was preferred over urea and ammonium (V_{max}, nitrate > urea > ammonium) at high nutrient concentrations.

The final experiment conducted on 17 October 2012, 2 weeks after the diversion ended, exhibited the highest V_{max} and α results for ammonium throughout the study period (Figure 5, Table 3). While all three substrates could be fit to a saturation-uptake response, urea exhibited a biphasic response (increasingly linear) above ~1 µM N and did not saturate at the highest nutrient addition, while nitrate exhibited a potential inhibitory response at intermediate (~5-20 µM N) additions. With the increase in V_{max} for ammonium, at high concentrations V_{max} followed the pattern ammonium > urea > nitrate, while affinity was highest for urea (urea > nitrate > ammonium). At moderate concentrations, K_s values followed the same pattern as for α.

Using the ambient nutrient concentrations for station 2203 where the incubation water was collected, it is possible to calculate the relative utilization of nitrate, ammonium, and urea for each experiment from Table 4 using saturation-uptake kinetic parameters. The relative percent use of nitrate, ammonium, and urea are presented in Table 4. Given the caveat that a saturation-uptake response was used for (truncated) data and the majority of uptake kinetics responses exhibited biphasic or linear kinetics, the percentages provide an approximation of what N sources were being used by the phytoplankton assemblage before, during, and after the diversion. Before and after the diversion, ammonium dominated N-uptake. During the diversion (20 September) urea was dominant, while at the end of the diversion (3 October) nitrate strongly dominated. There was no consistent pattern across all time periods, other than utilization of all N-substrates.

3.3 Ammonium Inhibition Experiments

All of the experiments exhibited reasonable uptake-inhibition responses (using equation 2) throughout the study period, shown in Figure 6 and summarized in Table 5. Since ambient nitrate concentrations were not known at the time of the experiments, 10 µM NO_3 concentrations were used, complicating interpretation of the results given that ambient concentrations did not exceed 1 µM NO_3, and inhibition parameters are therefore only directly relevant to ambient conditions if suppression of nitrate uptake by ammonium is not sensitive to nitrate concentration. The estimated concentration of ammonium required to completely suppress nitrate uptake (calculated as 9X K_I; Cochlan and Bronk, 2003) was high relative to ambient concentrations (Table 2), and ranged from 3.8-32.4 µM N. The maximum uptake of nitrate at zero ammonium (ρ_{max}) was low throughout the study period and ranged from 0.001 to 0.03 µM N h^{-1}, but

generally consistent with the kinetics results. The K_I values ranged from 0.4 to 3.6 µM NH_4 L^{-1}, higher than ambient ammonium concentrations at the time of experiment water collection (Table 1), but within the ambient concentration range observed in the pre- and during diversion timepoints for the overall study (Table 2).

4. Discussion

While it was anticipated that the diversion of treated wastewater effluent into the shallow, nearshore zone would provide a unique opportunity to evaluate how changes in anthropogenic N inputs affect nearshore coastal ecosystems, the realized conditions were more consistent with low ambient nutrient concentrations, despite the considerable discharge of effluent. As the primary limiting macronutrient in coastal waters, changes in N inputs or N forms can have significant effects on phytoplankton growth, community composition and biomass. The diversion of wastewater effluent into the nearshore had the potential to impact both the quantity and forms of N present on local spatial scales. The unanticipated lack of biological response during the experiment, the methodological issues such as mismatch between *in situ* irradiance and incubation conditions, the small number of kinetics experiments, and the lack of highly elevated ammonium concentrations resulted in a limited, but still meaningful, dataset. Specifically, these data can be used to address two questions: first, is there evidence for physiological inhibition of the phytoplankton assemblage that could explain the modest biological response observed, and second, is there evidence for a physiological response to the availability of anthropogenic nutrients?

4.1 Overall field observations of ambient nutrient concentrations and biological response

360 The results of a previous diversion event in Santa Monica Bay, California occurring from
361 28-30 November 2006 (Reifel et al. 2013) were used to estimate the phytoplankton response to
362 the OCSD diversion as a persistent patchy bloom with predicted chl *a* concentrations up to 40-50
363 $\mu g\ L^{-1}$ (OCSD, 2011). This was based on the expected ambient concentration of 42 $\mu M\ NH_4\ L^{-1}$
364 after accounting for dilution, and the formation of a shallow plume with a thickness of 4-5 m.
365 The resulting biological response was anticipated to be 4 times higher than the historical mean
366 high value. Given the prevalence of diatoms and dinoflagellates in the region, it was expected
367 that HAB organisms such as the diatom genus *Pseudo-nitzschia* or several dinoflagellate genera
368 including *Alexandrium*, *Dinophysis*, and *Cochlodinium* could become a significant component of
369 the bloom response.
370 Throughout the study period, the observed chl *a* concentrations during the OCSD
371 diversion were low, $<5\ \mu g\ L^{-1}$ (Caron et al., this issue) with some evidence of increased chl *a*
372 towards the end of the diversion and in subsurface observations (Lucas and Kudela, this issue;
373 Seegers et al., this issue), but overall, much lower than expected based on the predicted response
374 documented in the Environmental Impact Report (OCSD, 2011). Grow-out experiments revealed
375 the physiological capacity of the phytoplankton community to utilize the effluent for growth
376 (Seubert et al., this issue).
377 As described in Kudela et al. (this issue), the Santa Monica Bay diversion was similar in
378 terms of ambient physical conditions, pre-diversion nutrient concentrations, discharge depth, and
379 time of year; primary differences were the diversion durations and the amount of chlorination.
380 OCSD employed enhanced chlorination (approximately doubling the chlorine concentration to 5-
381 6 $\mu g\ L^{-1}$) followed by dechlorination (neutralization with sodium bisulfite) of the effluent
382 discharge in order to minimize the impact of discharge on microbial populations. This enhanced

383 chlorination process produced disinfection byproducts that strongly inhibited phytoplankton
384 photophysiology and growth lasting for 24 hours and 3 days respectively (Kudela et al., this
385 issue). It is unknown to what extent the disinfection byproducts influenced results of these
386 reported kinetics experiments. Given the impact on photosynthesis, it is possible that nitrate
387 uptake would be more sensitive than either ammonium or urea uptake, given the energetics
388 associated with both uptake and assimilation (reviewed by Glibert et al. 2015).
389 Caron et al. (this issue) observed a strong response of bacterial biomass (by an order of
390 magnitude) and suggested nutrient immobilization within the bacterial food web as an
391 explanation for the low ambient dissolved N concentrations that were observed by both Caron et
392 al. (this issue) and McLaughlin et al. (this issue). The ambient N concentrations described in
393 Caron et al. and McLaughlin et al. (this issue) were well below the estimated concentrations of
394 total inorganic N of up to 40 µM N in the EIR report based on expected plume dilution (OCSD,
395 2011). While the plume dilution was estimated at 1:30 based on the diffuser design, Rogowski et
396 al. (2014) estimated dilution of greater than 1:100 within 1 km of the outfall pipe using
397 Lagrangian drifter data. The observed discrete nutrient data were consistent with this estimate
398 (Caron et al., this issue), but note that Lucas and Kudela (this issue) present evidence suggesting
399 that dilution was not uniform in space and time. McLaughlin et al. (this issue) measured
400 nitrification rates throughout the study period and concluded that rapid oxidation of effluent
401 ammonium proximal to the outfalls contributed significantly to the pool of ‘new’ nitrate.
402 Additionally, the observed low $\delta^{15}N$ and $\delta^{13}C$ from suspended particulate organic matter
403 suggests the “nitrified” effluent ammonium was incorporated into the biomass. Taken together,
404 these results suggest that temporary suppression of phytoplankton, accompanied by rapid

405 bacterial transformation of N, resulted in both low autotrophic biomass and low ambient nutrient
406 concentrations during the diversion.
407 The current study used N uptake kinetics and ammonium inhibition experiments as a
408 metric to evaluate the potential and realized response of phytoplankton to the diverted
409 wastewater so as to determine whether there were inhibitory affects to N uptake rates, which
410 would ultimately affect growth and biomass. From these results, it appears that the
411 phytoplankton were fully capable of utilizing nitrate, ammonium, and urea. Relative utilization
412 of the three N species depends in part on ambient concentrations. At low N concentrations ($<K_s$),
413 the α values suggest equivalent affinity for all three substrates (Table 3). At moderate
414 concentrations ($\sim K_s$) nitrate and ammonium would be preferred relative to urea. At high
415 concentrations, generally greater than observed during the experiment, there was physiological
416 capacity, particularly post-diversion, to increase uptake for all three N species but especially
417 ammonium. Based on the kinetics and average ambient concentrations, all three N species were
418 dominant at varying times (Table 4) and regardless of timepoint there was physiological capacity
419 to utilize both low and high ambient concentrations of all three N substrates.

420

421 *4.2 Ammonium inhibition of nitrate uptake was not a driver of low biological response*

422 The inhibitory effects of high ammonium concentrations on nitrate uptake have been
423 documented in both laboratory and field studies (see reviews by Dortch, 1990; Mulholland and
424 Lomas 2008; Collos and Harrison, 2014). Elevated ammonium concentrations in southern
425 California have mainly been attributed to wastewater discharges (e.g. MacIsaac et al. 1979;
426 Thomas and Carsola, 1980; Howard et al. 2014), making ammonium inhibition a particularly
427 relevant aspect of focus for the OCSD wastewater diversion study.

The ammonium concentrations before the diversion began (prior to 11 September 2012) were the highest observed, but still relatively low (Table 2), and the range of ammonium concentrations decreased during and post-diversion (Tables 1 and 2), with an average of less than 0.65 µM N. The concentration of ammonium required to completely inhibit saturated nitrate uptake ($9xK_I$; Table 5) was 3.8 – 32.4 µM N, well above ambient concentrations observed during the diversion (Tables 1 and 2). Comparing ρ_{max} (Table 5) to ρ_{max} for the kinetics experiments (V_{max} from Table 3 multiplied by chlorophyll from Table 1), values are similar except for 3 October, where the maximum nitrate uptake estimated from the ammonium inhibition experiment was an order of magnitude lower (0.001 versus 0.023 µM N h^{-1}). That date also exhibited induced or biphasic kinetics for nitrate uptake, and 10 µM NO_3 was not saturating. The similarity between values from the two sets of experiments suggests that ammonium was not strongly suppressing nitrate uptake at ambient concentrations, but given that ammonium was always present at some baseline level and that I_{max} values were also less than 1 µM N, there was undoubtedly some suppression of nitrate uptake, even at those low ambient concentrations. Based on the comparable ranges of α and K_s for nitrate and ammonium, and the generally higher V_{max} values for ammonium (Table 3), the kinetics data suggest that equivalent or higher growth rates would be attained when utilizing a combination of both nitrate and ammonium. Thus while there was clear evidence for suppression of nitrate uptake by ammonium it was not likely the primary cause of the suppressed biological response observed during the experiment.

These results are consistent with other studies that performed uptake-inhibition experiments using similar methods. Cochlan and Bronk (2003) summarized results from the Southern Ocean as well as other field studies, and reported suppression of nitrate uptake at low to moderate ammonium concentrations, with an average I_{max} of 0.63 and K_I of 0.10 µM N, while

Kokkinakis and Wheeler (1987, as reported in Cochlan and Bronk 2003) reported an I_{max} of ~0.50 for Oregon upwelling waters, and Dugdale and MacIsaac (1971, as reported in Cochlan and Bronk 2003) reported I_{max} = ~0.80 for Peru upwelling waters. L'Helguen et al. (2008) emphasized the importance of cell size, and reported reduced suppression of nitrate uptake for large-sized (> 2µm) cells, with I_{max} = 0.67 and K_I=0.13 µM N. Those authors emphasized that larger cell-sized phytoplankton assemblages, such as found in this study, were less likely to exhibit suppression compared to smaller cell-size populations in the open ocean, consistent with the summarized data for coastal versus open ocean waters presented in Cochlan and Bronk (2003).

Several caveats exist in comparing our results to these previous studies. First, we used saturating nitrate concentrations, and it is unknown whether the suppression response would be similar at ambient nitrate concentrations. Second, as summarized by Dortch (1990), results are dependent on phytoplankton species composition and nutritional history, while Reay et al. (1999) reported reduced affinity for nitrate but not ammonium at suboptimal temperatures. Third, in at least one study (Yin et al. 1998), suppression of nitrate uptake by ammonium was eliminated under severe light-limiting conditions. In that study I_{max} went from 0.30 in saturating light to 0.38 in moderate light-limitation, but dropped to zero with severe light-limitation. The authors suggested that this was an adaptive response to increasing cellular N-quotas with increasing light limitation. Given that our experiments were conducted at irradiance levels less than E_k, it is possible that the observed I_{max} values (0.29-0.83) were lower than would be observed under full irradiance.

Despite these caveats several similarities emerge between the OCSD diversion and previous studies. As observed by others, particularly in coastal waters, I_{max} never reached 1.0 in

474 this study. Our K_I values ranged from 0.42-3.61 µM N, higher than those reported by Cochlan
475 and Bronk (2003) and L'Helguen et al. (2008), perhaps due to the experimental design which
476 included low ambient irradiance and saturating nitrate concentrations. Despite evidence for
477 simultaneous utilization of all three N species, suppression of nitrate uptake by ammonium
478 increased with time as evidenced by increasing I_{max} and decreasing K_I values (Table 5), while the
479 relative proportion of ammonium used increased to nearly the same percentage as the beginning
480 of the experiment (Table 4), suggesting that the phytoplankton assemblage did respond to
481 changes in the form and concentration of N present, albeit subtly.
482
483 *4.3 Uptake kinetics and preference of N form*
484 The determination of both the half-saturation constant, one indicator of affinity in low
485 nutrient conditions, as well as V_{max}, an indicator of preference in high nutrient conditions, allows
486 for the calculation of the slope (α), a more robust indicator of nutrient affinity at sub-saturating
487 conditions ($<K_s$), relevant for the nutrient concentrations observed in this study (Healey, 1980).
488 These parameters should not be confused with the relative preference index (RPI, McCarthy et
489 al. 1977) which is not ecologically relevant (Dortch, 1990), and is more relevant as a measure of
490 N sufficiency, rather than physiological preference (McCarthy, 1981).
491 The full range of possible kinetics responses were observed during this study, including
492 saturation-uptake (Michaelis-Menten), biphasic, linear, and potentially, inhibitory kinetics. Of
493 the 12 curves generated, ammonium most often followed saturation-uptake kinetics while nitrate
494 and urea were more often biphasic, exhibiting a saturation-uptake response at low (less than ~6
495 µM N) concentrations. On 20 September and 17 October, ammonium and nitrate (respectively)
496 appeared to exhibit inhibitory kinetics. At the physiologically low concentrations used during the

experiments this inhibitory response is unexpected. However, since the same tracer was used for all experiments and the inhibitory response was not consistently observed, there is no obvious explanation for the observed suppression of nitrate uptake. We have therefore chosen to fit those data to an uptake-saturation response (equation 1 above), but note the discrepancy. Suppression of ammonium at elevated concentrations has been observed (Glibert et al. 2015) but given the variability in uptake at high ammonium concentrations, we elected to fit a saturation-uptake curve to these data as well, since fitting an uptake-inhibition relationship would not change the initial slope and would have a negligible effect on V_{max} (not shown).

Biphasic uptake kinetics (operating at low and high nutrient concentrations) and non-saturating kinetics have been described as adaptations to pulsed high nutrient inputs, particularly in coastal assemblages (Lomas and Glibert 1999). Collos et al. (1997), Lomas and Glibert (1999), and Fan et al. (2003) considered the involvement of diffusion of nitrate at high extracellular nitrate concentrations, while Serra et al. (1978) proposed diffusive and mediated transfer. Regardless of the mechanism, as discussed by Flynn (1999), biphasic or linear kinetics cannot be explained by diffusion of nitrate because it is incompatible with our understanding of algal physiology. Collos et al. (2005) suggested that for nitrate specifically, multiphasic kinetics may be the norm rather than the exception in coastal waters, which would be consistent with the results of this study (but does not explain the apparent inhibition observed for 17 October). A recent review (Glibert et al. 2015) also advocates for interaction of diffusive transport, low- and high-affinity transporters, leading to biphasic or multiphasic and induced kinetics, but also emphasizes that uptake is ultimately modulated by other downstream cellular processes.

The variability exhibited from a small number of experiments is a reminder that nutrient uptake and assimilation, particularly for mixed natural assemblages, can be complex and does

not necessarily follow saturation-type enzyme kinetics (c.f. Glibert et al. 2013; Glibert et al. 2015). In these experiments the overall conclusions do not change significantly as a result of the mathematical formulation for the uptake response, at least at the ambient nutrient concentrations observed, but it is important to remember that uptake is only one facet of nutrient utilization.

In the pre-diversion experiments, there was a higher affinity for ammonium; however, during the diversion, there was no obvious preference amongst substrates and the phytoplankton assemblage was likely utilizing all three species of N, a change from pre-diversion results. This was surprising as the expectations prior to the study were that the phytoplankton community would adapt to prefer ammonium due to the large load of ammonium discharged from the outfall pipe into shallow coastal waters (~2,000 µM N during the diversion event, Caron et al. this issue). At the end of the diversion (3 October) nitrate uptake accounted for the largest percentage (Table 4), while two weeks later ammonium was again dominant.

The indication of biphasic and induced kinetics at elevated nutrient concentrations does suggest that, if effluent discharge were to result in substantially higher nutrient concentrations, there could be a more dramatic response to N form and concentration than observed. In particular, urea uptake was low at ambient concentrations but exhibited a strong linear response with little evidence of saturation, while both the V_{max} and I_{max} for ammonium increased while K_I decreased through the experiment.

The overall community composition was dominated by diatoms before and during the diversion (Caron et al. this issue), therefore, changes in N preference cannot be attributed to broad changes in community composition. The ambient N concentrations, especially ammonium, were relatively low throughout the study (Tables 1 and 2; and Caron et al. and McLaughlin et al. this issue), which likely contributed to the observed uptake kinetics. The low ambient N

543 concentrations can be attributed to immobilization of nutrients due to the large bacterial response
544 (Caron et al. this issue), and the low ammonium concentrations are likely due to the high rates of
545 nitrification (McLaughlin et al. this issue). Given those circumstances it is perhaps not surprising
546 that all three N substrates were utilized and that there was not a dramatic shift towards preference
547 for any one substrate based solely on the kinetics results.

548 The values for α, the most relevant parameter for the observed ambient nutrient
549 concentrations during this study, are low compared with similar diatom-dominated assemblages
550 on the US West Coast (MacIssac and Dugdale, 1969, Dortch and Postel, 1989, Dickson and
551 Wheeler, 1995, Kudela and Peterson, 2009). The K_s values for nitrate and ammonium were also
552 low compared with other natural assemblages, including both dinoflagellate- and diatom-
553 dominated communities from upwelling dominated areas (Dickson and Wheeler, 1995; Kudela et
554 al. 2008a; Kudela and Peterson, 2009), whereas the urea K_s values were similar to previous
555 studies (Dortch and Postel 1989; Kudela and Cochlan 2000; Kudela et al. 2008a, 2008b).

556 Xu et al. (2012) and Yuan et al. (2012) reported N uptake rates in regions of Hong Kong
557 coastal waters that are influenced by sewage as well as river discharge. The maximal uptake rates
558 ranged from 0.05-0.08 µM N µg Chl a^{-1} h^{-1} (Xu et al., 2012) and 0.01-0.07 µM N µg Chl a^{-1} h^{-1}
559 (Yuen et al., 2012). These rates were much higher than the pre- and during-diversion rates
560 reported in the current study, but similar to the post-diversion rates. The rates at the sewage
561 influenced station were comparatively lower than the other stations, <10 µM N µg Chl a^{-1} h^{-1}(Xu
562 et al., 2012), which is closer to the rates measured in this study pre- and during diversion, but
563 lower than post-diversion rates. Thus the kinetics results from this study are consistent with
564 adaptation to low nutrient concentrations, with potential uptake rates that are comparable to other
565 coastal regions.

In summary, there was no obvious physiological impairment related solely to N uptake kinetics that would explain the low observed biological response to the diversion. Ammonium suppression of nitrate uptake was not sufficient to account for the lack of biological response, and there was physiological capacity to respond to ambient N substrates. Kudela et al. (this issue) documented photophysiological inhibition from the disinfection byproducts and Caron et al. (this issue) observed the large bacterial response and potential competition for nutrients. Low concentrations of ammonium observed throughout the study were likely due to high nitrification rates (McLaughlin et al. this issue) and immobilization of nutrients due to bacterial response (Caron et al. this issue) rather than advection of the plume out of the study area (Lucas and Kudela, this issue).

There was a clear change in the N uptake kinetics during the diversion of wastewater into the nearshore shallow waters, but the phytoplankton community was likely utilizing all three substrates (nitrate, ammonium and urea) for growth. At ambient concentrations, receiving waters exhibited a strong preference for ammonium pre-diversion and post-diversion. This is consistent with simultaneous presence of all three N species and rapid biochemical transformations driven by the microbial assemblage, leading to presence and simultaneous uptake of multiple N forms during the diversion. The increase in V_{max} for ammonium following the diversion, in combination with decreasing I_{max} and K_I values, suggests that a more pronounced bloom response, such as documented in Santa Monica (Reifel et al. 2013), would likely occur if not for the unusual circumstances (suppressed photophysiology of the phytoplankton assemblage; rapid nitrification driven by heterotrophic bacteria) that occurred during the diversion. Our results serve as a reminder that the response of natural phytoplankton assemblages to human perturbations such as wastewater discharge are complex and difficult to predict (c.f. Anderson et

589 al., 2002; Hallegraeff 2004; Glibert et al. 2005; Howarth 2008; Paerl and Piehler 2008), and
590 eutrophication does not always lead to massive algal blooms, even when the physiological
591 capacity to utilize the nutrients exists.
592
593
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801 Tables

802 Table 1 Nutrient concentrations and other ancillary data collected simultaneously with
803 experiment water from station 2203 (see Figure 1) on the 4 cruises listed in the table. Units are in
804 parentheses. Average particulate nitrogen (PN) values were calculated from the kinetics
805 experiments; the standard deviation [SD] of the individual samples for kinetics curves from each
806 experiment is provided.

	6 September 2012 Pre-diversion	20 September 2012 During Diversion	3 October 2012 Post-diversion	17 October 2012 Post-diversion
Temperature (^{o}C)	16.1	18.0	19.6	17.3
Salinity	33.38	33.36	33.55	33.40
Chlorophyll (µg L^{-1})	1.02	3.54	0.4	0.97
NO_3 (µM N)	0.06*	0.11	0.21	0.48
NH_4 (µM N)	0.15*	0.17	0.01	0.22
Urea (µM N)	0.68*	0.66	0.06	0.22
PO_4 (µM P)	0.47*	0.30	0.48	0.45
C:N Ratio (molar)	6.74	8.11	7.61	6.44
PN (µM N) [SD]	5.95 [0.96]	12.8 [1.34]	3.75 [1.51]	7.97 [1.55]

807 *Water from 5 m depth was used because the samples from incubation depth leaked during
808 storage.

809

Table 2 Average ambient nutrients concentrations throughout the study period collected on weekly cruises at all stations shown in Figure 1. Units of measurements are in parenthesis and under the timepoint columns, the range of concentrations observed are in parenthesis.

	Pre-diversion	During Diversion	Post-diversion
Chl *a* ($\mu g\ L^{-1}$)	0.6 (0.2-1.3)	1.2 (0.05-5.1)	1.1 (0.2-4.8)
NO_3 (μM N)	5.4 (0.1-19.9)	2.1 (0.01-14.4)	2.1 (0.2-13.9)
NH_4 (μM N)	0.9 (0.1-4.0)	0.6 (0.07-1.1)	0.5 (b.d.-2.6)
Urea (μM N)	1.4 (0.4-5.0)	0.6 (0.2-1.8)	0.2 (0.02-1.6)
C:N	7.3 (4.3-9.9)	6.6 (5.9-7.5)	7.0 (4.1-12.4)
PN (μM)	1.1 (0.2-2.7)	2.4 (0.4-5.9)	2.0 (0.4-2.9)
POC (μM)	8.6 (0.9-23.5)	16.4 (2.6-44.8)	14.3 (2.3-25.5)

b.d. = below the limit of detection.

Table 3 The kinetic parameters for N uptake determined from natural assemblages, reported as μM N μg Chl^{-1} h^{-1} for V_{max}, μM N for K_s, and V_{max}/K_s for α. For comparison with previous studies the average PN and corresponding chlorophyll values for each date are provided in Table 1. For curve fits exhibiting non-saturating or biphasic kinetics, a subset of substrate concentrations was used and the kinetic parameters are reported separately by date (italicized values). For induced (linear) kinetics, V_{max} is the highest observed value, K_s is not calculated, and α is the initial slope of the data. Values in parentheses are Standard Errors. The coefficient of determination (r^2) and number of samples (n), excluding zero-values, used in the curve fits are reported. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Survey Date	NO_3				NH_4				Urea			
	V_{max}	K_s	α	r^2 (n)	V_{max}	K_s	α	r^2 (n)	V_{max}	K_s	α	r^2 (n)
6 Sept. 2012	0.012	--	$5E^{-4}$	0.80 (14)	0.018 ($5E^{-4}$)	0.55 (0.08)	0.03	0.97 (22)	0.02	-	0.001	0.59 (7)
6 Sept. 2012	*0.005 ($3E^{-4}$)*	*0.22 (0.22)*	*0.02*	*0.97 (14)*	-	-	-	-	*0.004 ($1E^{-4}$)*	*0.86 (0.1)*	*0.005*	*0.96 (7)*
20 Sept. 2012	0.008 ($5E^{-4}$)	0.33 (0.11)	0.025	0.87 (22)	0.016 ($9E^{-4}$)	0.58 (0.16)	0.02	0.91 (22)	0.062 (0.004)	4.79 (1.4)	0.03	0.90 (11)
3 Oct. 2012	0.058	-	0.002	-	0.025 ($7E^{-4}$)	0.33 (0.05)	0.07	0.96 (22)	0.053	-	0.001	0.88 (12)
3 Oct. 2012	*0.003*	*0.44*	*0.007*	*0.75 (7)*	-	-	-	-	*0.01 (0.001)*	*0.73 (0.33)*	*0.01*	*0.96 (14)*
17 Oct. 2012	0.016 (0.001)	0.26 (0.11)	0.06	0.83 (22)	0.034 (0.001)	0.23 (0.07)	0.15	0.76 (22)	0.021	-	0.012	0.88 (22)
17 Oct. 2012	-	-	-	-	-	-	-	-	*0.017 ($7E^{-4}$)*	*0.35 (0.06)*	*0.048*	*0.98 (14)*

Table 4 Calculated N uptake as a percentage of total N uptake for each experimental time point, using kinetics parameters from Table 3 and ambient nutrient concentrations from Table 1 (for 6 September the mean values from Table 2 were used). Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Experiment Date	*Percent Nitrate*	*Percent Ammonium*	*Percent Urea*
6 Sept. 2012	20.4	61.41	18.19
20 Sept. 2012	10.47	18.99	70.53
3 Oct. 2012	93.85	3.01	3.14
17 Oct. 2012	29.69	47.55	22.76

Table 5 Ammonium inhibition experiment results for each incubation experiment, conducted with 10 µM NO_3 L^{-1} concentrations. Parameters shown include the theoretical N uptake at zero ammonium concentration (ρ_{max}), maximal realized inhibition (I_{max}), the substrate (ammonium) concentration at which nitrate uptake is reduced to 50% of maximal value (K_I), the estimated concentration of ammonium required to completely inhibit nitrate uptake (9 x K_I). Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Experiment Date	(ρ_{max} (µM N h^{-1})	I_{max} (%)	K_I (µM N)	9 x K_I (µM N)	r^2
6 Sept. 2012	0.007	0.29	2.76	24.84	0.81
20 Sept. 2012	0.03	0.64	3.61	32.49	0.81
3 Oct. 2012	0.001	0.68	1.24	11.16	0.94
17 Oct. 2012	0.01	0.83	0.42	3.78	0.89

Figures

Figure 1 Map of study area. The two outfalls are indicated by the solid black lines, the primary outfall terminates at station 2205 and the secondary, shorter, outfall terminates at station 2202. All experiment water was collected at station 2203 and discrete samples (Table 2) were collected at all stations shown.

Figure 2 Vertical profiles of Temperature (solid line), Salinity (dashed line), Fluorescence converted to chlorophyll units (gray circles) and PAR (black diamonds) from Station 2203 for 6 September 2012 (A), 20 September 2012 (B), 3 October 2012 (C), and 17 October 2012 (D). The horizontal dashed line in each panel denotes the depth of water collection for kinetics experiments.

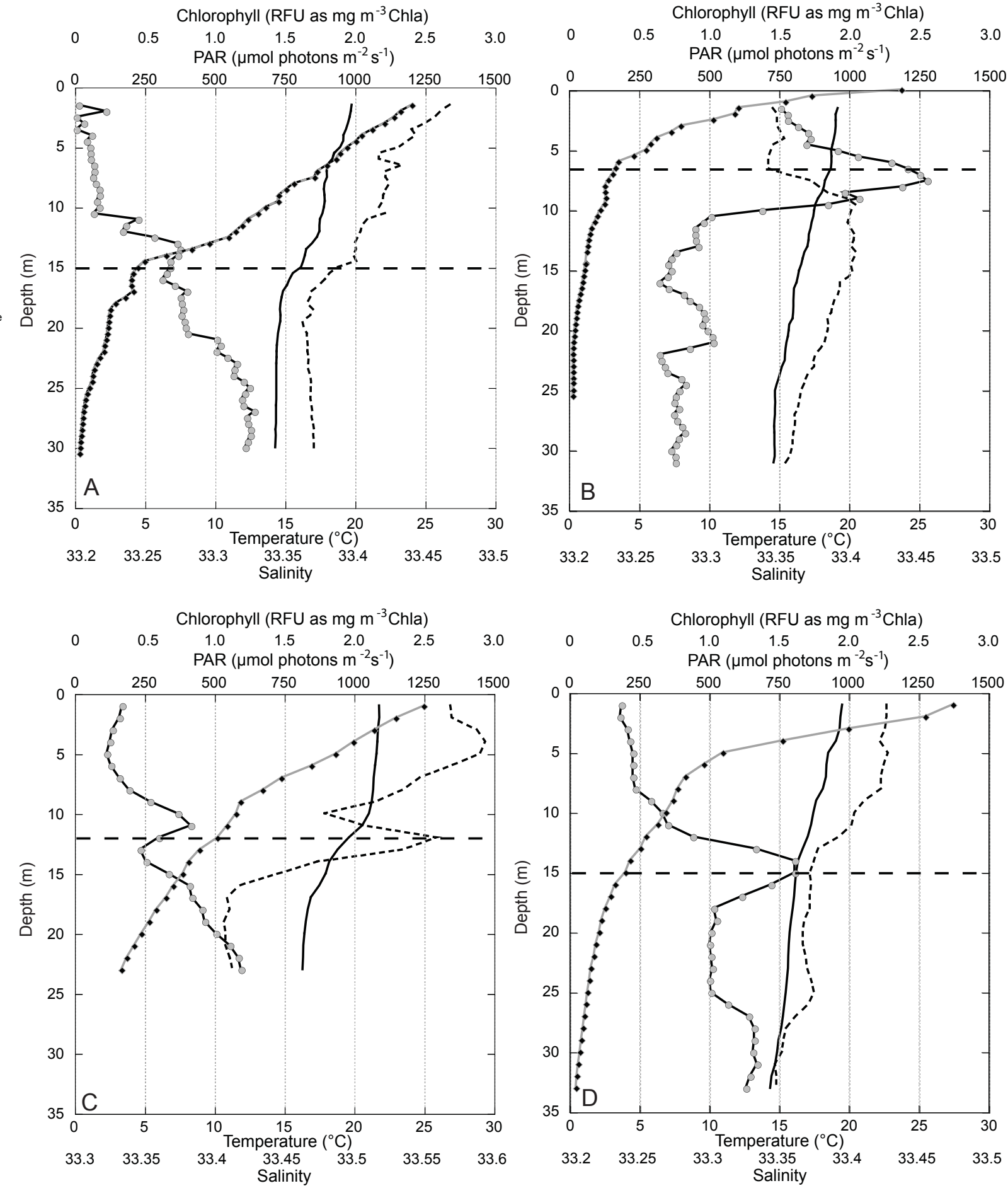
Figure 3 Chlorophyll-specific uptake rates (V_{max}; µM N µg Chl^{-1} h^{-1}) for nitrate (A), ammonium (B) and urea (C) for natural assemblages plotted versus substrate concentrations (µM N) calculated as ambient plus added N before the diversion began on 6 September 2012 (black circles) and during the diversion on 20 September 2012 (black squares). Grey shading indicates the subset of data used to fit kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

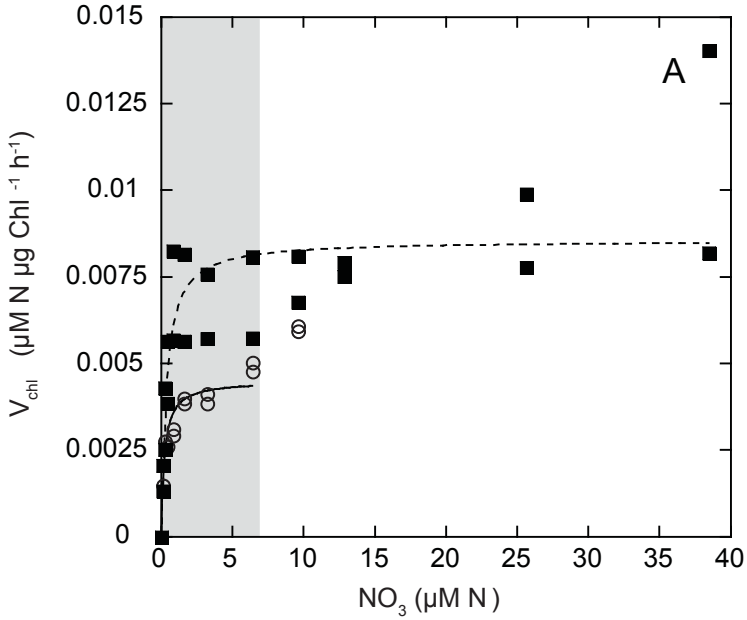
Figure 4 Chlorophyll-specific uptake rates (V_{max}; µM N µg Chl^{-1} h^{-1}) (black circles) for nitrate (A), ammonium (B) and urea (C) plotted versus substrate concentrations (µM N) calculated as ambient plus added N for natural assemblages. Experiment water was collected on 3 October 2012 several hours after the diversion ended. Grey shading indicates the subset of data used to fit

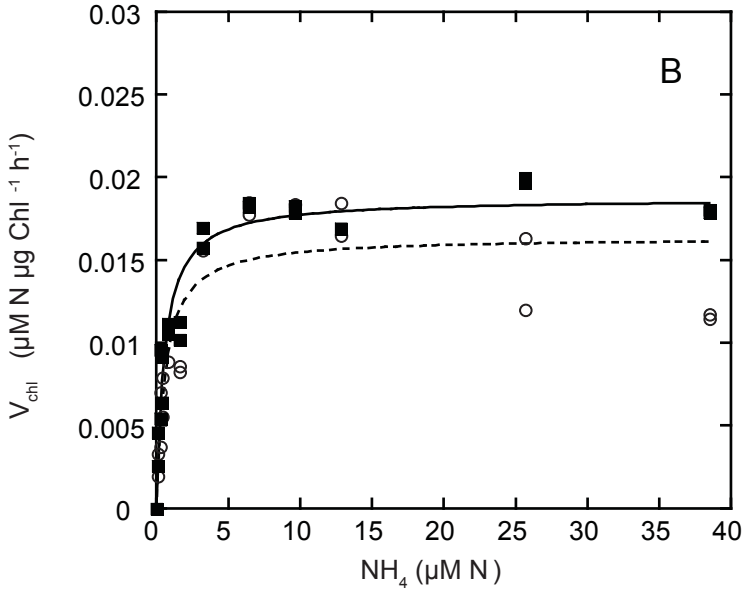
kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

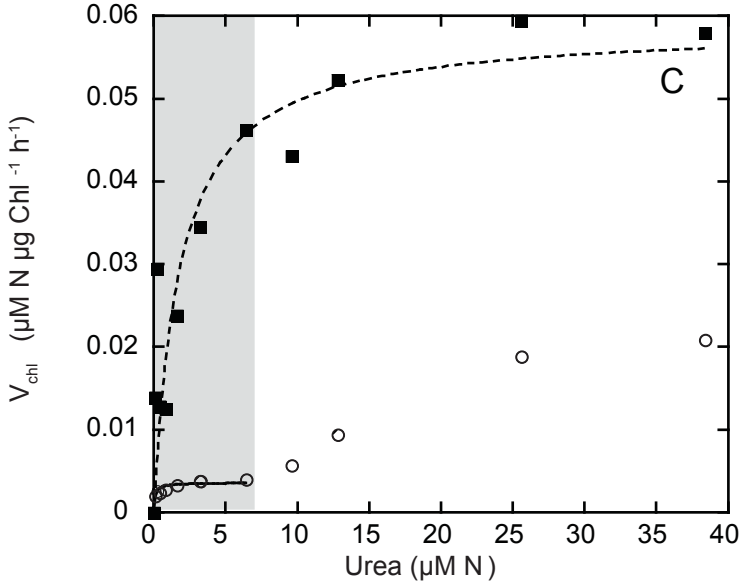
Figure 5 Chlorophyll-specific uptake rates (V_{max}; µM N µg Chl^{-1} h^{-1}) (black circles) for nitrate (A), ammonium (B) and urea (C) plotted versus substrate concentrations (µM N) calculated as ambient plus added N for natural assemblages. Experiment water was collected on 17 October 2012, 2 weeks after the diversion ended. Grey shading indicates the subset of data used to fit kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

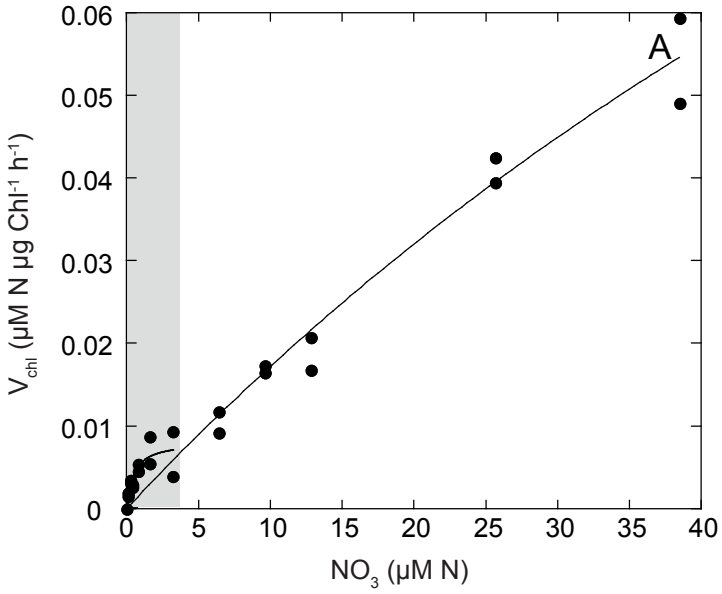
Figure 6 Ammonium inhibition results for experiments conducted on 6 September 2012 (A) and 20 September 2012 (B), 3 October 2012 (C) and 17 October 2012 (D). Substrate concentrations included ambient and added substrate, comprised of 10 µM ^{15}N-NO_3 and varying concentrations of ^{14}N-NH_4. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

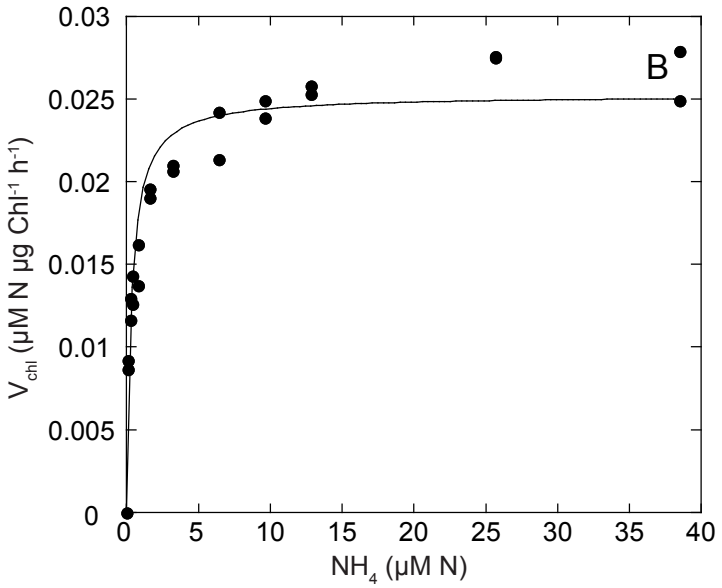

Chlorophyll (RFU as mg m-3 Chla)
PAR (µmol photons m-2 s-1)
Depth (m)
Temperature (°C)
Salinity
A
B
C
D


A
0.015
0.0125
0.01
0.0075
0.005
0.0025
0
V_{chl} (µM N µg Chl $^{-1}$ h^{-1})
0 5 10 15 20 25 30 35 40
NO_3 (µM N)


B
0.03
0.025
0.02
0.015
0.01
0.005
0
V_{chl} (µM N µg Chl $^{-1}$ h^{-1})
0 5 10 15 20 25 30 35 40
NH_4 (µM N)


C
0.06
0.05
0.04
0.03
0.02
0.01
0
V_{chl} (µM N µg Chl $^{-1}$ h^{-1})
0 5 10 15 20 25 30 35 40
Urea (µM N)


A
0.06
0.05
0.04
0.03
0.02
0.01
0
V_{chl} (µM N µg Chl^{-1} h^{-1})
0
5
10
15
20
25
30
35
40
NO_3 (µM N)


B
0.03
0.025
0.02
0.015
0.01
0.005
0
V_{chl} (µM N µg Chl^{-1} h^{-1})
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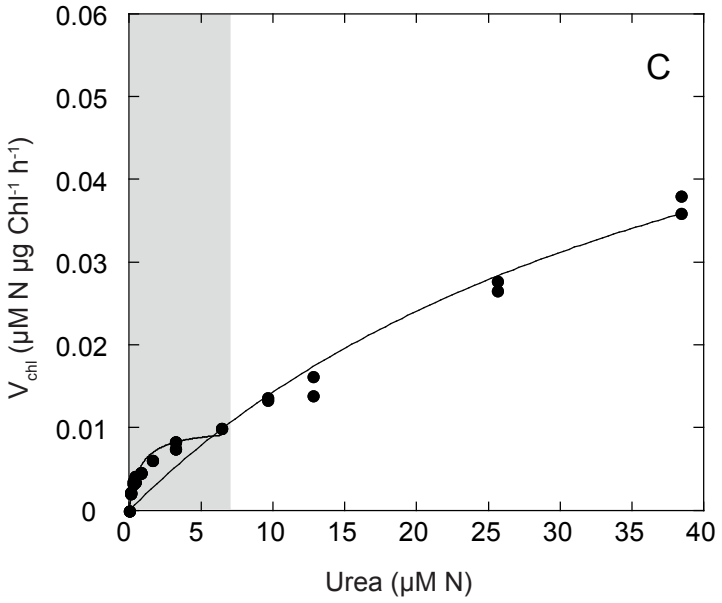
10
15
20
25
30
35
40
NH_4 (µM N)


C
0.06
0.05
0.04
0.03
0.02
0.01
0
V_{chl} (µM N µg Chl^{-1} h^{-1})
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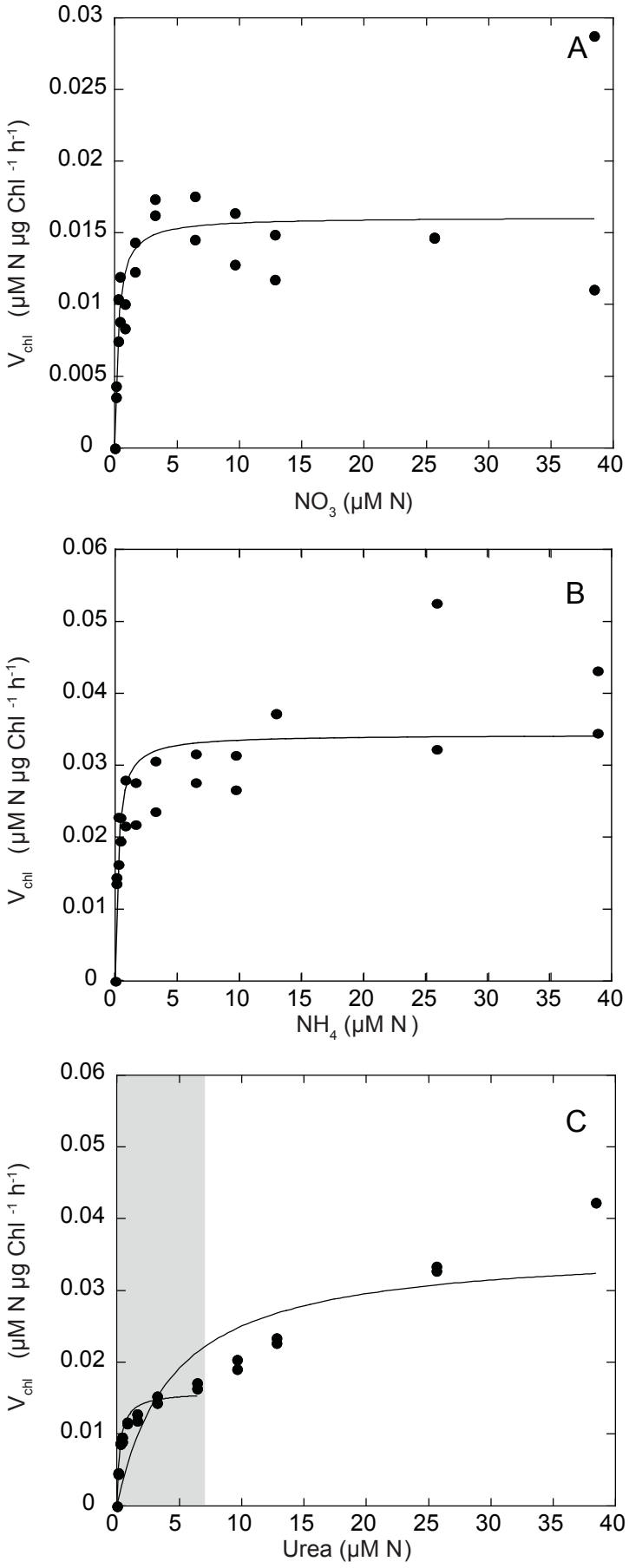
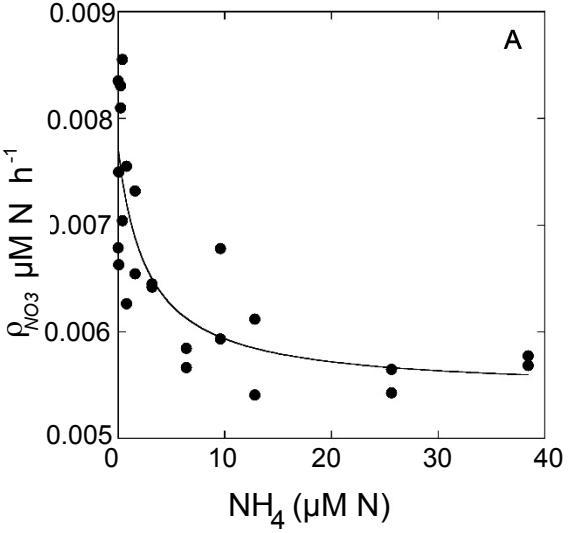
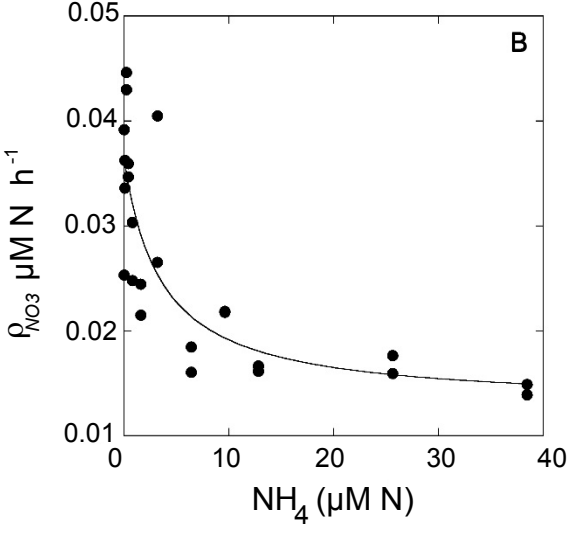
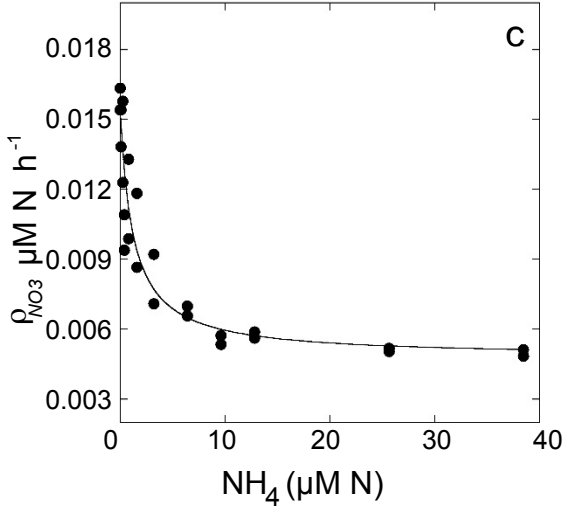
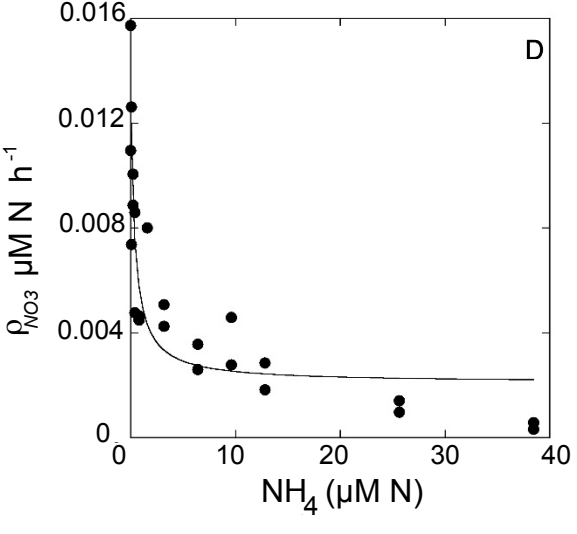
5
10
15
20
25
30
35
40
Urea (µM N)


A
B
C
V_{chl} (µM N µg Chl $^{-1}$ h^{-1})
NO_3 (µM N)
NH_4 (µM N)
Urea (µM N)
0
0.005
0.01
0.015
0.02
0.025
0.03
0.04
0.05
0.06
5
10
15
20
25
30
35
40


A
0.009
0.008
0.007
0.006
0.005
0
10
20
30
40
ρ_{NO3} µM N h^{-1}
NH_4 (µM N)


B
0.05
0.04
0.03
0.02
0.01
0
10
20
30
40
ρ_{NO3} µM N h^{-1}
NH_4 (µM N)


C
0.018
0.015
0.012
0.009
0.006
0.003
0
10
20
30
40
ρ_{NO3} µM N h^{-1}
NH_4 (µM N)


D
0.016
0.012
0.008
0.004
0
0
10
20
30
40
ρ_{NO3} µM N h^{-1}
NH_4 (µM N)

Highlights:

- Nitrogen uptake kinetics used to evaluate phytoplankton physiological response
- Ammonium inhibition was not sufficient to account for lack of biological response
- Simultaneous utilization of nitrate, ammonium and urea observed
- Physiological capacity to respond to ambient concentrations of all three N substrates