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Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions

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Abstract

Anthropogenic activities are altering total nutrient loads to many estuaries and freshwaters, resulting in high loads not only of total nitrogen (N), but in some cases, of chemically reduced forms, notably NH_4^+. Long thought to be the preferred form of N for phytoplankton uptake, NH_4^+ may actually suppress overall growth when concentrations are sufficiently high. NH_4^+ has been well known to be inhibitory or repressive for NO_3^- uptake and assimilation, but the concentrations of NH_4^+ that promote vs. repress NO_3^- uptake, assimilation, and growth in different phytoplankton groups and under different growth conditions are not well understood. Here, we review N metabolism first in a "generic" eukaryotic cell, and the contrasting metabolic pathways and regulation of NH_4^+ and NO_3^- when these substrates are provided individually under equivalent growth conditions. Then the metabolic interactions of these substrates are described when both are provided together, emphasizing the cellular challenge of balancing nutrient acquisition with photosynthetic energy balance in dynamic environments. Conditions under which dissipatory pathways such as dissimilatory NO_3^-/NO_2^- reduction to NH_4^+ and photorespiration that may lead to growth suppression are highlighted. While more is known about diatoms, taxon-specific differences in NH_4^+ and NO_3^- metabolism that may contribute to changes in phytoplankton community composition when the composition of the N pool changes are presented. These relationships have important implications for harmful algal blooms, development of nutrient criteria for management, and modeling of nutrient uptake by phytoplankton, particularly in conditions where eutrophication is increasing and the redox state of N loads is changing.

Increasing nutrient loads are among the most significant drivers of our "ever changing world" and their adverse effects on aquatic biodiversity and ecosystem health, including eutrophication, are well documented (Cloern 2001; Anderson et al. 2002; Howarth et al. 2002; Heisler et al. 2008; Glibert et al. 2014a). Emphasis has been placed on resolving whether systems are "limited" by nitrogen (N), phosphorus (P), or both (e.g., Howarth and Paerl 2008; Schindler and Hecky 2008; Schindler et al. 2008; see Table 1 for a list of abbreviations), to inform management and support recommendations for nutrient (N or P) control and to reduce eutrophication impacts. In contrast, there has been little research and discussion on whether nutrients at non-limiting concentrations influence primary producers differentially and how these metabolic responses may vary with different chemical forms of N. Given the global patterns of greater N than P fertilizer use, the overall increasing trend is for N to be the nutrient in excess relative to P and relative to phytoplankton stoichiometric needs (e.g., Childers et al. 2011; Glibert et al. 2013, 2014a).

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Table 1. Abbreviations used although this article.

AMT	Ammonium transporter
C	Carbon
CCM	Carbon concentrating mechanism
DON	Dissolved organic nitrogen
Fd	Ferredoxin
GDCT	T-protein subunit glycine decarboxylase
Gln	Glutamine
Glu	Glutamate
GS-GOGAT	Glutamine synthetase-glutamate synthase (also known as glutamine-2-oxoglutarate amidotransferase)
HAB	Harmful algal bloom
HAT	High affinity transporter
HNLG	High-nutrient, low-growth system, typically in reference to an estuary
LAT	Low affinity transporter
NH_4^+	Ammonium
N	Nitrogen
NiR	Nitrite reductase
NO	Nitric oxide
NO_3^-	Nitrate
NO_2^-	Nitrite
NPQ	Non-photochemical quenching mechanism
NR	Nitrate reductase
NRT	Nitrate transporter
2-OG	Oxoglutarate
P	Phosphorus
PEPCase	Phosphoenolpyruvate carboxylase
PGP	Phosphoglycolate phosphatase
ROS	Reactive oxygen species
Rubisco	D-Ribulose-1,5-biphosphatecarboxylase/oxygenase

Anthropogenic activities are altering both total nutrient loads, and they are changing the dominant form of N nutrient delivered to many coastal marine and freshwater systems. While the major oxidized form of N, nitrate (NO_3^-), is the dominant N form contributing to eutrophication in many aquatic ecosystems, there are several reasons why high loads of chemically reduced forms of N, such as ammonium (NH_4^+), urea, and dissolved organic nitrogen (DON) are on the increase. In the U.S., many regions converted from primary to secondary sewage treatment in the late 1970s to mid-1980s after the passage of the Federal Water Pollution Control Act (later renamed as the Clean Water Act). As a result, many large wastewater treatment plants were constructed that discharge large quantities of N as NH_4^+ (NRC 2000). Also, global fertilizer use has generally shifted from oxidized to reduced forms of N, with urea use now > 50% of global N fertilizer, surpassing NO_3^- as the most common N fertilizer worldwide (Glibert et al. 2006, 2014a). The development of industrialized animal agriculture in coastal areas has also resulted in significant sustained increases in NH_4^+ availability both via direct runoff and atmospheric deposition (Burkholder et al. 2006). Aquaculture operations are a rapidly increasing source of NH_4^+ and urea, especially fish cage aquaculture in coastal lagoons, quiet embayments and in inland waters, due to direct excretion and decomposition of undigested feed (Bouwman et al. 2013). Coastal and estuarine waters are not the only systems experiencing increases in NH_4^+, however. Increases in atmospheric deposition of NH_4^+ have also been significant in many nearshore and offshore waters (Aneja et al. 2003; Duce et al. 2008). It has also been predicted, and shown, that with increasing ocean acidification and climate change, NH_4^+ oxidation may be inhibited and stratification may increase, with resulting reduction in the injection of NO_3^- into surface waters, together leading to

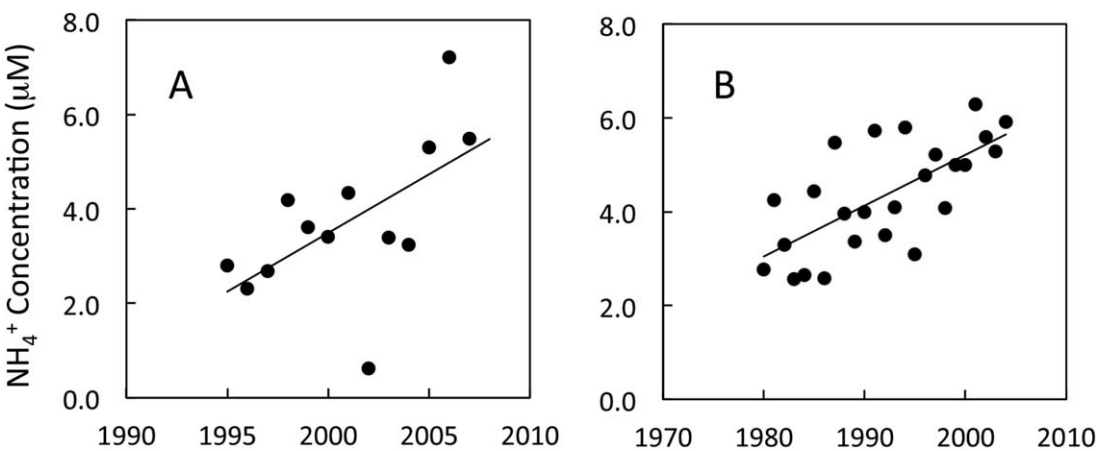


Fig. 1. Two examples of estuaries showing increasing concentrations of NH_4^+ in the water column over time. (A) northern Coastal Bays, Maryland; (B) San Francisco Bay Delta; Replotted from Glibert et al. (2011, 2014b).

Table 2. Summary of some of the major aspects of interaction between NH_4^+ and NO_3^- use by phytoplankton, their effects on growth and productivity, and representative associated key investigations of these interactions. Table modified and updated from Flynn et al. (1999).

Aspect	Example references
NH_4^+ is assimilated first, and only when it is depleted is NO_3^- utilized	Ludwig (1938), Harvey (1953)
Cells using NO_3^- must expend significant amounts of energy on reduction through to NH_4^+; this may have adverse effects on NO_3^- assimilation in the dark and on CO_2 fixation	Syrett (1956, 1981) and references therein
Above a threshold concentration of NH_4^+, NO_3^- use is inhibited	Syrett and Morris (1963), Conway et al. (1976)
NH_4^+ repression of NO_3^- assimilation is not due to NH_4^+ per se but a product of its assimilation	Syrett and Morris (1963)
Internal NO_3^- may continue to be reduced in vivo after uptake has been inhibited following the uptake of NH_4^+	Cresswell and Syrett (1979)
N replete cells using NH_4^+ cannot immediately use NO_3^-	Syrett (1981)
Enhanced ability to take up and assimilate NH_4^+ develops under N stress	McCarthy and Goldman (1979), Glibert and Goldman (1981), Syrett et al. (1986)
The enzymes of the NO_3^- assimilation pathway are inducible	Syrett (1981)
NO_3^- and free amino acids readily accumulate, but NH_4^+ accumulates to a much lower concentration within cells	Dortch (1982), Dortch et al. (1984)
NO_3^- and NH_4^+ uptake do not share the same transporter	Raven (1980), Syrett (1981)
Affinity of transporters do not appear to alter with N stress	Eppley et al. (1969), Dortch (1990)
Cells using NO_3^- can immediately use NH_4^+ at high rates	Horrigan and McCarthy (1982)
Despite above, there may be little if any improvement in growth rates when using NH_4^+; differences in growth on NO_3^- vs. NH_4^+ highly variable dependent on species and other aspects of growth conditions (e.g., light)	Solomon et al. (2010), Collos and Harrison (2014)
Diatoms may preferentially use NO_3^- and may use it both assimilatively and in non-assimilative photoprotection or energy balance	Lomas and Glibert (1999a,b)
Growth suppression of productivity in estuaries by elevated NH_4^+ loading suggested	Yoshiyama and Sharp (2006), Dugdale et al. (2007)
Photorespiratory gene expression increased in diatoms under NH_4^+ compared with NO_3^- growth	Parker and Ambrust (2005), Shi et al. (2015)

an increase in the availability of NH_4^+ in near surface oceanic waters (e.g., Hueseman et al. 2002; Doney 2006; Beman et al. 2011).

In fact, increasingly sustained, elevated concentrations (> 5 μM) of NH_4^+ and/or urea are now common in many estuaries worldwide. For example, concentrations of NH_4^+ in the San Francisco Bay Delta and in the Coastal Bays of Maryland now average 5–10 μM, with some months averaging > 30 μM, representing a several-fold increase over the past decades for both of these systems (Glibert et al. 2014b,c,d; Fig. 1). Similarly concentrations of NH_4^+ in the urban Delaware River, the Neuse River and Cape Fear Estuaries in the U.S. have increased substantially (Burkholder et al. 2006; Yoshiyama and Sharp 2006), as is the case in many European estuaries (e.g., Middleburg and Nieuwenhuize 2000), and along the China coast (e.g., Chen et al. 2010; Xu et al. 2012). Concentrations of urea as high as 25–50 μM-N have also been reported in tributaries of the Chesapeake Bay (Lomas et al. 2002; Glibert et al. 2005), nearshore waters adjacent to the heavily fertilized Yaqui Valley, Mexico (Glibert et al. 2006), and in the northern Great Plains of Canada (Bogard et al. 2012), among other regions. Based on known rates of urea hydrolysis to NH_4^+ (Solomon et al. 2010), such enrichment of urea can rapidly become a source of NH_4^+ enrichment.

The primary purpose of this review is to address the consequences of enrichment by N in different forms on phytoplankton metabolism and ultimately phytoplankton growth and community composition. This review sits at the intersection of physiology and ecology; this article highlights the major contrasts in metabolism among N forms and how those differences may affect productivity and community composition especially when N is supplied in excess as NH_4^+. We emphasize diatoms because they represent ca. 40% of marine C productivity (Nelson et al. 1995) and are ubiquitous in freshwaters, but more importantly their productivity appears to be disproportionately affected by increased concentrations of NH_4^+. We additionally contrast the major relevant aspects of diatom metabolism with those of cyanobacteria, dinoflagellates, and chlorophytes where data are available. We recognize that our broad taxonomic comparisons do not permit elucidation of the often-important,

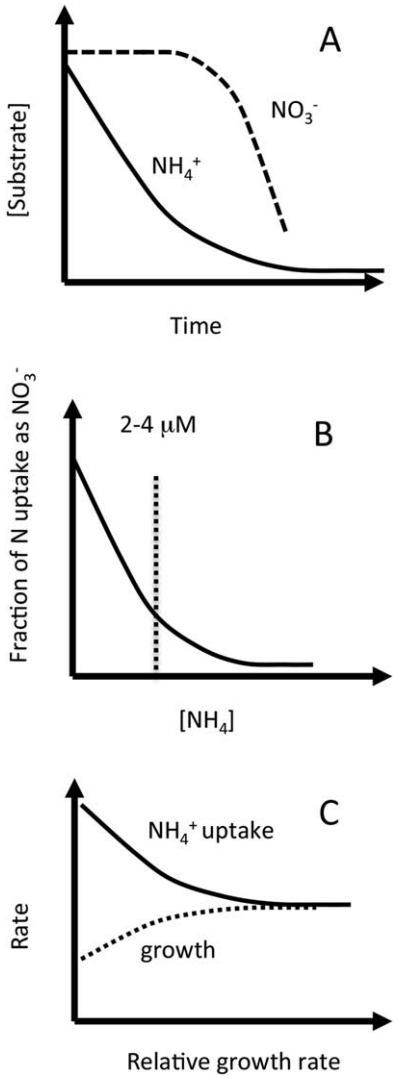


Fig. 2. (A) Conceptual relationship of the time course of NH_4^+ and NO_3^- depletion due to phytoplankton uptake in culture or in field assemblages [modified from McCarthy (1981)]. (B) Conceptual relationship between the fraction of uptake of N as NO_3^- relative to total N uptake as a function of increasing availability of NH_4^+ in the water column. The pattern of the relationship is presumably due to a combination of preferential uptake of NH_4^+ relative to NO_3^- and inhibition of NO_3^- uptake by NH_4^+ (or its assimilation products). (C) Conceptual relationship between the transient uptake of NH_4^+ and the growth rate; only at near-maximal growth rates are uptake and growth rates equal.

species-specific differences that are beginning to come to light with new gene transcriptional data (e.g., Bender et al. 2014). For the purpose of this article, we focus on NH_4^+ and NO_3^-, acknowledging that these are not the only forms of N used by phytoplankton, nor is NH_4^+ the only form of chemically reduced N that is increasing, as dissolved organic nitrogen (DON) is recognized to be increasingly important and a dynamic N form in phytoplankton nutrition (Berman and Bronk 2003; Glibert et al. 2006; Solomon et al. 2010). Nevertheless, NO_3^- and NH_4^+ are the dominant inorganic N forms in both freshwater and marine systems.

The preference for NH_4^+: through the historic lens

Many aspects of the complex and differential effects of NO_3^- and NH_4^+ on phytoplankton metabolism have long been known, but there are some important new discoveries (Table 2). A central tenet of the relationships and interactions between these substrates is that NH_4^+ is considered to be the preferred form of N for phytoplankton uptake (e.g., McCarthy 1981; Raven et al. 1992 and references therein). Preferential uptake or use is defined variously in the literature, generally involving a comparison of (1) rates of drawdown of one substrate relative to another, (2) uptake affinity, (3) maximal or in situ rate of uptake, or (4) an index of relative preference (RPI) for different N forms. Although there is some debate as to the value of each of these indices, the general finding of most reports is the same: typically NH_4^+ is preferentially used, especially when N is limiting.

The preference for NH_4^+ is considered to be due, at least in part, to lower energy requirements for the cell, and NH_4^+ is more easily transported across the cell membrane than NO_3^- under balanced growth and N limited conditions. The lower energetic costs of uptake and assimilation of NH_4^+ leads to the common assumption—and the common observation—that NH_4^+ is generally taken up by algae first, and only after its near depletion is NO_3^- taken up (Ludwig 1938; Harvey 1953; Fig. 2A). Evidence for NH_4^+ preference is grounded in the classical physiological literature, and, importantly, in studies where N was the limiting nutrient. Preferential use of NH_4^+ was originally documented in batch culture experiments in the 1930s–1950s (reviewed in Syrett 1981), and field observations of this phenomenon have been made since at least the 1960s (MacIsaac and Dugdale 1969, 1972; McCarthy et al. 1975, 1977). Delayed uptake of NO_3^- relative to that of NH_4^+ has been observed in enclosure studies in which 10 μM NH_4^+ resulted in cessation of NO_3^- uptake by diatom-dominated assemblages, while subsequent depletion of NH_4^+ concentrations to < 4 μM allowed resumption of NO_3^- uptake and diatom growth (Wilkerson et al. 2006; Parker et al. 2012). Preferential uptake of NH_4^+ over NO_3^- at NH_4^+ concentrations exceeding a few μM has also been documented through the declining proportion of NO_3^- uptake in relation to total N uptake as the concentration of NH_4^+ in the water column increases (Fig. 2B). For example, McCarthy et al. (1975) illustrated that the uptake of oxidized forms of N in Chesapeake Bay never exceeded more than a few percent of the total N ration when NH_4^+ concentrations exceeded 1–2 μM. Berman et al. (1984) reported a similar finding in Lake Kinneret, as did Dugdale et al. (2007) for San Francisco Bay estuarine phytoplankton. Moreover, numerous studies, including early work by Syrett (1955, 1956), and culture studies by McCarthy and Goldman (1979) demonstrated

that uptake rates of NH_4^+ by N-limited cells can far exceed the amount of N required for growth, further underscoring favorable uptake of this N form (Fig. 2C). It is of note, also, that many macroalgae also appear to prefer NH_4^+ and have a capacity for excess uptake over growth demands (Rees 2007 and references therein).

Generalizing from these as well as a wealth of other studies, it has been interpreted that preferential use of NH_4^+ is expected when NH_4^+ is available at only a few μM. It has thus been commonly argued that phytoplankton growth on NH_4^+ should be higher than that on NO_3^-, or at least growth on both substrates should be equal. Raven et al. (1992, p. 20) summarized the logic of this argument, "If the use of the resource needing more manipulation [e.g., NO_3^-]..., in order to achieve the same product formation [moles] product per second), then the cell doubling time will be significantly increased since more [moles] of the product of the resource manipulation will be required to double cell mass..." Consistent with this hypothesis, several studies have shown that some phytoplankton species grown on NH_4^+ or urea have higher growth rates than on NO_3^- (e.g., Herndon and Cochlan 2007; Solomon et al. 2010 and references therein), although this is far from a universal observation.

The seeming favorability for NH_4^+ by phytoplankton is actually a function of both the preferential use of NH_4^+ and its favorable energetics, and the repressive effect (often referred to as inhibition) of NH_4^+ on NO_3^- uptake and assimilation (Dortch 1990 and references therein). Repression of NO_3^- uptake or assimilation by NH_4^+ has been well studied for many decades (e.g., Morris and Syrett 1963; Dortch 1990 and references therein; Lomas and Glibert 1999a,b; Table 2). The repression of NO_3^- uptake by NH_4^+ occurs at NH_4^+ concentrations as low as a few μM (e.g., Eppley et al. 1969; Dortch and Conway 1984; Lund 1987; Cochlan and Harrison 1991; L'Helguen et al. 2008 among others). From work in the subarctic Pacific, Wheeler and Kokkinakis (1990) even suggested that concentrations of NH_4^+ between 0.1 μM and 0.3 μM caused complete repression of NO_3^- assimilation, and L'Helguen et al. (2008) reported that similar concentrations of NH_4^+ caused repression of NO_3^- uptake in the oligotrophic Atlantic. In the San Francisco Bay Delta, much higher concentrations of NH_4^+, 4–10 μM, have been associated with repression of NO_3^- uptake by NH_4^+ based on direct measurements (e.g., Dugdale et al. 2007; Glibert et al. 2014c), and similar concentrations were found to repress NO_3^- uptake in laboratory cultures of diatoms and dinoflagellates (Lomas et al. 2000). The extent and threshold concentrations of repression by NH_4^+ on NO_3^- metabolism have been shown to depend on the algal species present, their physiological status (Dortch and Conway 1984; Dortch et al. 1991; Maguer et al. 2007) and the environmental conditions to which they have been exposed (e.g., Harrison et al. 1996; Lomas and Glibert 1999a,b; Xu et al. 2012). Cells growing on highly elevated NO_3^- concentrations, as in the case of a nutrient-rich environment may require considerably more NH_4^+ to repress cellular NO_3^- activity than is the case for a cell with a low cellular NO_3^- content, as in oligotrophic environments.

Diversity in N metabolism and consequences for community composition and productivity

The preference for NH_4^+ is not universal. In contrast to reports of repression of NO_3^- uptake by NH_4^+, a substantial body of literature has shown that in cool, nutrient-rich environments, large diatoms use a disproportionate fraction of total N as NO_3^- even when NH_4^+ is available at levels in excess of 10 μM (e.g., Maestrini et al. 1982; Probyn and Painting 1985; Lomas and Glibert 1999a,b). Diatoms appear to be NO_3^- opportunists. For example, in river-dominated estuaries and upwelling systems, the occurrence of many rapidly growing diatom species has been highly correlated with the large and/or frequent additions of NO_3^- (e.g., Goldman 1993; Lomas and Glibert 1999a). Diatoms are the dominant protist in NO_3^--rich water columns during spring blooms. Marine pelagic ecosystems with predominantly NO_3^- sources are often dominated by diatoms (e.g., Kudela and Dugdale 2000; Wilkerson et al. 2000) and typically have short, efficient food webs at the base of major natural fisheries (e.g., coastal Peru) and high rates of export of organic matter from the photic zone (e.g., Eppley and Peterson 1979). Interestingly, the brown intertidal macroalgae, *Fucus* sp. and *Laminaria* sp. also appear to have very high rates of NO_3^- assimilation, especially in winter, suggesting they too may be NO_3^- opportunists (e.g., Young et al. 2007).

The different patterns of uptake of NO_3^- and NH_4^+ are embodied in the classic oceanographic paradigm of new and regenerated production (Dugdale and Goering 1967). This paradigm recognizes the distinction between production resulting from those reduced N forms, primarily NH_4^+ and urea, that are regenerated in situ (from zooplankton excretion or bacterial remineralization in the water column or sediment) and production resulting from the use of oxidized N forms, primarily NO_3^-, resulting from allochthonous ("new") inputs to a system (Fig. 3). Greater flows of organic material through the microbial loop generally occur when systems are more enriched with chemically reduced N forms, NH_4^+ and urea, and the resulting communities are often dominated by mixotrophic dinoflagellates or (pico)cyanobacteria as well as bacteria (Eppley and Peterson 1979; Legendre and Rassoulzadegan 1995; Berg et al. 1997, 2003; LaRoche et al. 1997; Glibert 1998; Glibert et al. 2001). Empirically similar patterns occur in lakes, with spring assemblages of diatoms seguing to cyanobacteria when chemically reduced N is abundant in late summer (e.g., Donald et al. 2011).

Large-scale nutrient manipulation experiments suggest dichotomous communities develop in response to comparable NH_4^+ and NO_3^- enrichment. In mesocosm studies Glibert and

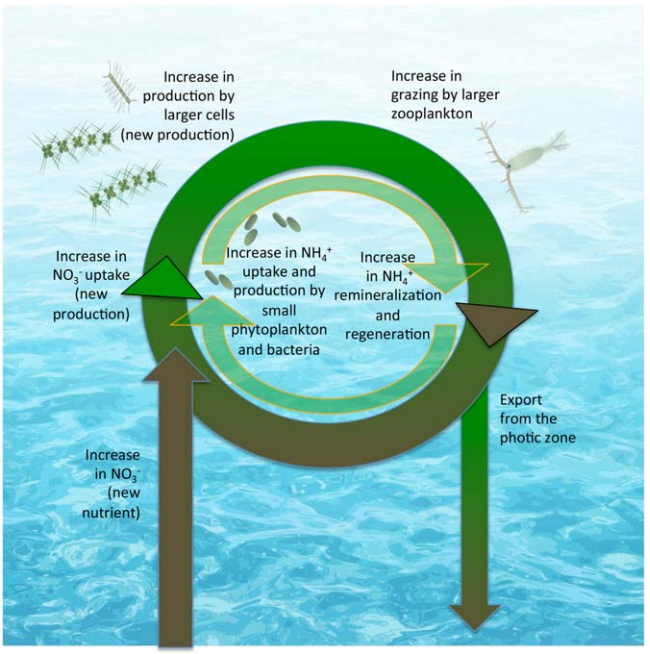


Fig. 3. Conceptual food web relationships resulting from changes in the ratios of NH_4^+: NO_3^-; regenerated vs. new production (*sensu* Dugdale and Goering 1967). In this conceptual framework, an increase in NO_3^- (via upwelling or other source) increases production by larger phytoplankton cells, typically diatoms, in turn supporting larger grazers. The recycling of NH_4^+ from excretion and remineralization pathways (inner cycle) supports growth of smaller cells, typically non-diatomaceous, as well as bacteria.

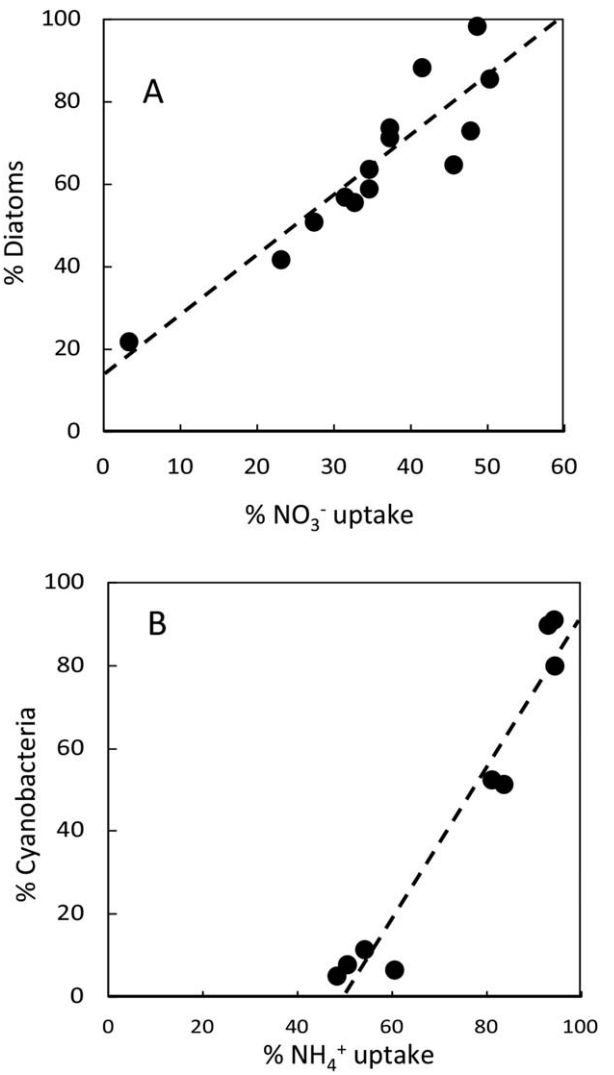


Fig. 4. Results of measurements of the uptake of NO_3^- and NH_4^+ and the resulting relationship with the phytoplankton assemblage in mesocosm experiments. (A) The relationship between the proportion of diatoms in the assemblage and the percent NO_3^- uptake. (B) The relationship between the proportion cyanobacteria in the community and the percent uptake of NH_4^+. Replotted from Glibert and Berg (2009).

Berg (2009) showed that NO_3^- uptake was directly related to the fraction of the community as diatoms, while the proportion of NH_4^+ uptake was directly proportional to the fraction of the community as cyanobacteria (Fig. 4). In mesocosm experiments conducted in hypereutrophic Wascana Lake, Saskatchewan, Canada, NO_3^- enrichment led to a proportionately greater increase in chlorophyll *a* (Chl *a*) (relative to total wet-weight algal biomass) and a greater initial response by diatoms, while NH_4^+ enrichment led to a proportionately greater increase in cyanobacteria (Donald et al. 2011, 2013). Similarly, in experiments conducted in the San Francisco Bay Delta, proportionately more Chl *a* and fucoxanthin (generally indicative of diatoms) were produced in enclosures enriched with NO_3^- than in treatments with the same total N enrichment as NH_4^+. In the latter case, proportionately more chlorophyll *b* (Chl *b*) (generally indicative of chlorophytes, i.e., green algae) and zeaxanthin (generally indicative of cyanobacteria) were produced (Glibert et al. 2014c). Domingues et al. (2011) also showed that enrichment by NH_4^+ in a freshwater tidal estuary favored chlorophytes and cyanobacteria, whereas diatoms were favored under NO_3^- enrichment. Toxic cyanobacterial species also appear to predominate over diatoms when N is supplied in chemically reduced relative to oxidized forms in the hypereutrophic Lakes Taihu, China, and Okechobee, Florida (McCarthy et al. 2009). Additionally, there are also similar reports from field studies showing that dinoflagellates, many of which form harmful algal blooms (HABs), are also associated with increased dominance of N in reduced rather than oxidized form (e.g., Berg et al. 2003; Glibert et al. 2006; Heil et al. 2007; Rothenberger et al. 2009). Interestingly, in terrestrial systems, a similar pattern of selection of species and growth is observed when soils are enriched with NO_3^- compared with NH_4^+. Soil enrichment with NO_3^- often leads to early successional species, while enrichment with NH_4^+ leads

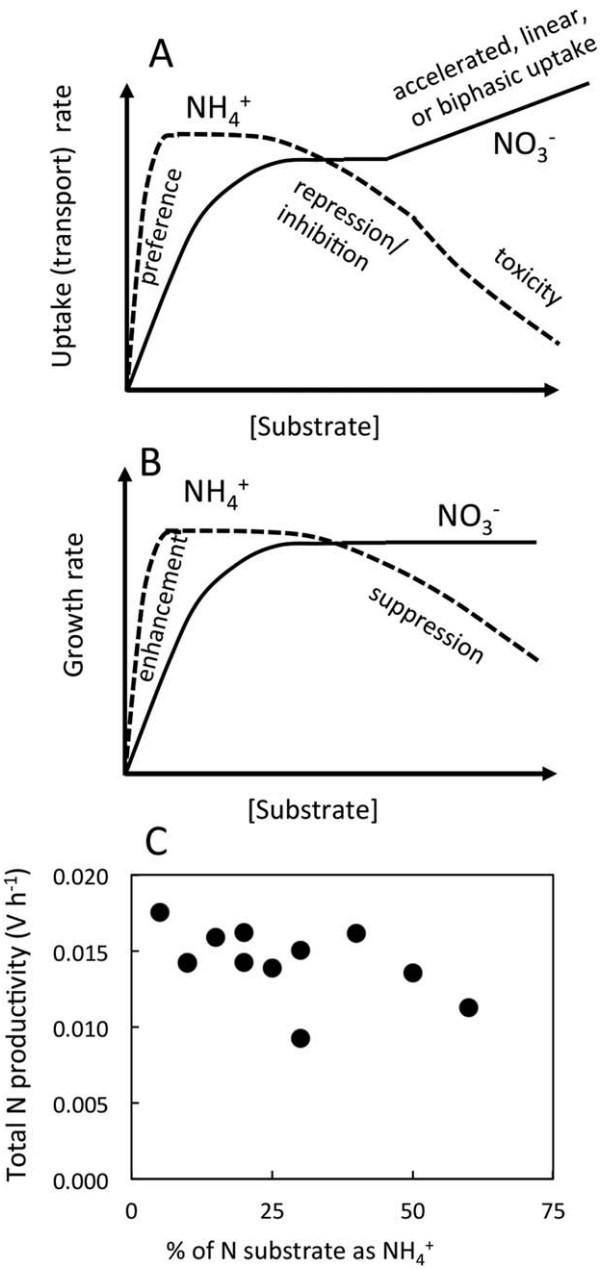


Fig. 5. (A) Conceptual relationships with respect to uptake or transport kinetics of NH_4^+ (dashed line) relative to NO_3^- (solid line) illustrating the tendency toward preference, repression/inhibition of uptake of NH_4^+ uptake and toxicity in contrast to the potential for accelerated, linear or biphasic uptake kinetics of NO_3^-. Note that at low concentrations of substrate, uptake of NH_4^+ may display not only a lower K_s, but may also have a higher V_{max}. The result is the paradoxical behavior of NH_4^+ kinetics relative to those of NO_3^-. (B) Comparable relationship except for growth, illustrating the potential for growth enhancement and suppression with NH_4^+ as the substrate in comparison to NO_3^- as the growth substrate. (C) Example of the effect of NH_4^+ (solid line) and NO_3^- (dashed red line) on the rate of total N uptake when both are provided to a natural assemblage in varying relative proportions. These data are from an experiment conducted on water from the Chesapeake Bay, enriched with a total of 30 μM-N, but in varying ratios of NO_3^-: NH_4^+ and incubated for a 1 h period. The resulting relationship suggested a decline in total N uptake as the percent of NH_4^+ increased (Glibert unpubl. data).

to latter successional species (Britto and Kronzucker 2002 and references therein).

From NH_4^+ preference to growth suppression

While differential community composition has been associated with different forms of N, it has also been documented that under conditions of highly elevated NH_4^+, typically exceeding several tens to hundreds of μM, both the total N taken up and overall growth with NH_4^+ enrichment can be suppressed rather than enhanced (e.g., Dagenais-Bellefeuille and Morse 2013 and references therein, Glibert et al. 2014c; Fig. 5A,B). In fact, many algae and higher plants have lower rates of growth on NH_4^+ than on NO_3^- (e.g., Raven et al. 1992; Britto and Kronzucker 2002 and references therein). Examples of growth suppression by NH_4^+ enrichment are numerous. Total N productivity was found to decrease in the Chesapeake Bay as the proportion of NH_4^+ increased when all samples received the identical total N enrichment, 30 μM (Fig. 5C), and similar observations have been made in experiments conducted in the San Francisco Bay Delta (Glibert et al. 2014c). Thus, the conceptual model that NH_4^+ is the preferred N form and that total N uptake and growth on NH_4^+ is the same or exceeds that on NO_3^- is not borne out in all cases.

Yoshiyama and Sharp (2006) summarized decades of data from the Delaware Bay and observed that the primary productivity rate per unit Chl *a* declined exponentially with increasing NH_4^+ concentration (most of the change occurring at < 10 μM NH_4^+) and classified these systems as High-Nutrient, Low-Growth (HNLG). In the San Francisco Bay Delta it has been suggested that a similar phenomenon of growth suppression is responsible for the lack of spring blooms ever since NH_4^+ loading from sewage effluent increased several decades ago (Wilkerson et al. 2006; Dugdale et al. 2007) and observational and experimental evidence are confirmatory (e.g., Wilkerson et al. 2006; Parker et al. 2012). In the higher plant literature this is known as the "NH_4^+ syndrome" (Gerendás et al. 1997; Britto and Kronzucker 2013). At very elevated concentrations, normally exceeding several hundred μM, NH_4^+ can be toxic for growth (e.g., Britto and Kronzucker 2002 and references therein), a condition from which the cell does not easily recover. The environmental relevance of direct toxicity by NH_4^+ in estuaries and freshwaters is limited, however, to those sites receiving such excessive loads of this N form. In most estuarine and freshwaters, reports of growth suppression by NH_4^+ have been mostly associated with increased NH_4^+ at levels not normally considered to be toxic for phytoplankton growth, i.e., at levels in the tens of μM range. Collectively, the observations of preferential use at the low end of the substrate availability spectrum, together with repression and/or toxicity at the high end of the substrate spectrum, have led to NH_4^+

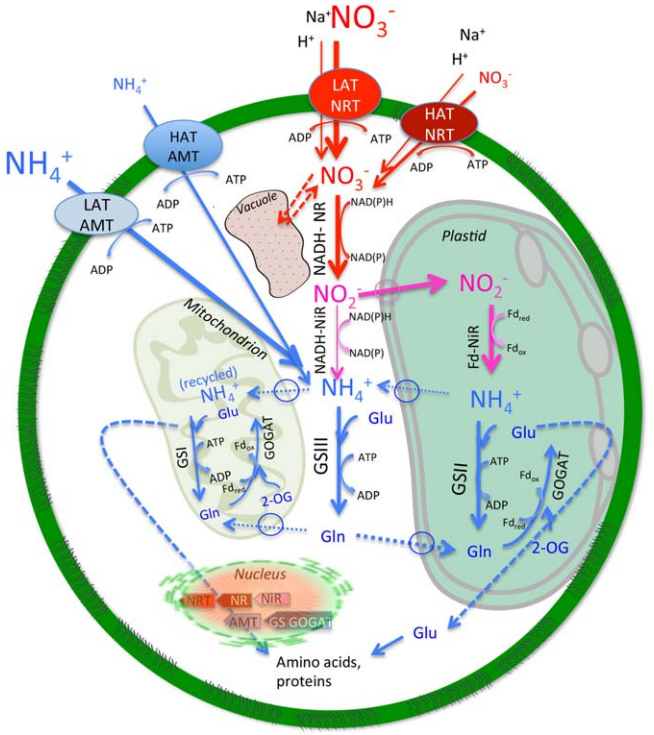


Fig. 6. Simplified conceptual relationship of a generic diatom cell and the major metabolic pathways of uptake and assimilation of NO_3^- and NH_4^+. Note that assimilative NO_3^- reduction typically occurs in the cytosol, while NO_2^- reduction occurs in the chloroplast. NH_4^+ assimilation can occur in the cytosol, in the chloroplast and in the mitochondrion. The nucleus controls enzyme and transporter production. See text and Table 1 for details.

being characterized as a "paradoxical" nutrient (Britto and Kronzucker 2002; Dugdale et al. 2012, Fig. 5).

Interestingly, in higher plants it is well documented that one of the means by which NO_3^- repression by NH_4^+ can be alleviated is through the addition of NO_3^- (Goyal et al. 1982; Below and Gentry 1987; Britto and Kronzucker 2002, among others), implying that NH_4^+ repression of NO_3^- uptake and reduction is not necessarily or always absolute. It is also known that co-provision of both NO_3^- and NH_4^+ can induce synergistic growth compared with growth on either substrate alone (e.g., Weissman 1964; Britto and Kronzucker 2002). In fact, total N uptake in higher plants can be up to 75% higher when the two substrates are co-provided relative to when either substrate is provided alone (Kronzucker et al. 1999).

Relatively new data, also based on higher plants studies, suggest that when growth suppression occurs, it may be due, at least in part, to redox imbalances and a surplus of reductant when NH_4^+ is in excess (Escobar et al. 2006; Podgórska and Szal 2015). Importantly, sensitivity to NH_4^+ stress varies widely in both higher plants and in the eukaryotic phytoplankton. This concept of redox balance and energy balance and the important role of NO_3^- therein will be revisited later in this article.

The need for a reassessment of N preference, with a focus on NH_4^+-enriched conditions

With N in many environments tending toward increasing enrichment, together with the observations of dichotomous phytoplankton communities typically developing on NH_4^+ vs. NO_3^-, a seemingly simple, but ultimately complex, set of questions pertaining to cellular regulation arise: How does the cell metabolize N in excess of levels normally considered sufficient for nutrition, and how do metabolic pathways differ when the N is in the form of NH_4^+ vs. NO_3^-? Is there a difference in primary productivity or growth of phytoplankton if N is in oxidized vs. reduced form? If so, does the apparent selection of specific taxa in environments with higher concentrations of reduced N have a physiological basis? Similarly, how do environmental factors such as temperature and light affect these putative physiological relationships?

To begin to understand the metabolic, physiological and ecological consequences of life in "reduced" vs. "oxidized" N environments, why some taxa appear to be favored under one condition relative to the other, and why growth may be suppressed at elevated levels of NH_4^+, we start by briefly reviewing N metabolism in a "generic" eukaryotic cell. We contrast the metabolic pathways and regulation of NH_4^+ and NO_3^- when these substrates are provided individually under equivalent growth conditions and then when both are provided to the cell, with emphasis on the complexity of metabolic regulations under non-steady-state conditions with N in excess. Then, we contrast some of the known taxon-specific differences in metabolism of different N forms. We provide a synthesis of these relationships in terms of consequences for dominant taxa and rates of primary production as would be observed in a natural, N-enriched environment. Finally, we conclude with implications of these insights for HABs, relevance of N form in nutrient criteria development and modeling of nutrient uptake by phytoplankton, particularly in a world where eutrophication is increasing and the redox state of N loads in many regions is changing in favor of chemically reduced N forms.

NO_3^- and NH_4^+ transport and assimilation

An idealized eukaryotic algal cell is capable of taking up and assimilating a range of N substrates into materials for growth (Fig. 6, simplified only for NH_4^+ and NO_3^- uptake and assimilation pathways). From the cell metabolism perspective, an obvious but important distinction in the transport of these two N forms is that when NH_4^+ is transported into the cell, a cation is transported, whereas for NO_3^- transport, an anion is transported. Assuming all other processes equal, this will lead to the cytosol being more acidic following NH_4^+ assimilation and more basic after NO_3^- assimilation, with resulting effects on redox reactions within the cell (Raven 2013 and references therein). With a redox state of -3 for NH_4^+ and $+5$ for NO_3^-, it takes eight electrons to

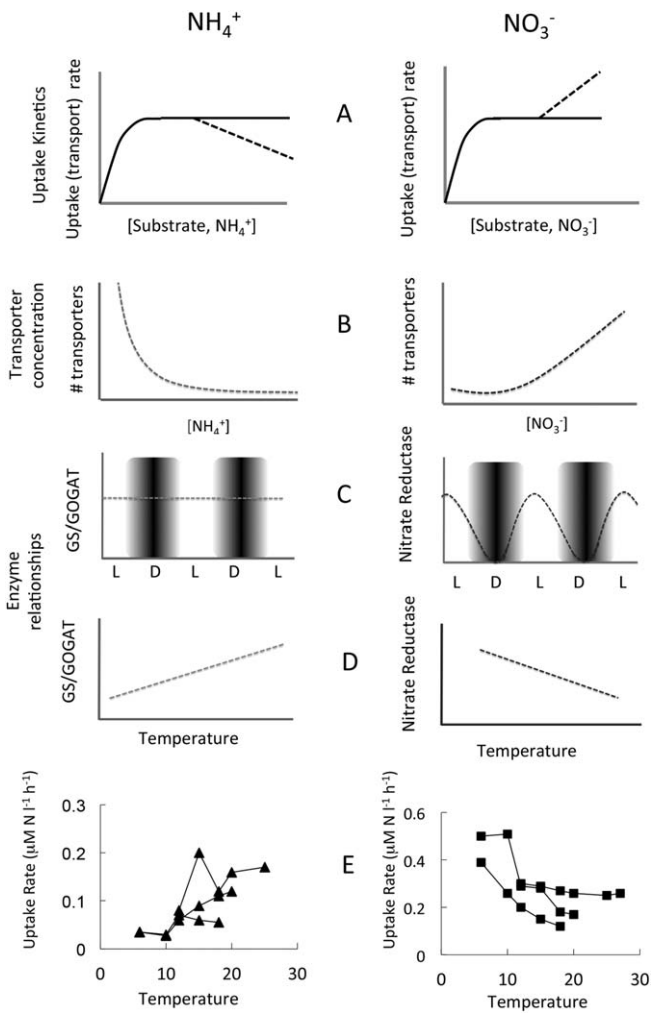


Fig. 7. Comparison of the regulation of N transport and their assimilative enzymes for "generic" phytoplankton cells growing exclusively on NH_4^+ (left-hand panels) or NO_3^- (right-hand panels). (A) Generic relationships of NH_4^+ and NO_3^- uptake kinetics. Note the potential for biphasic uptake of NO_3^- and suppression of uptake of NH_4^+. (B) Generalized relationship of the numbers of transporters in relation to the availability of substrate for that transporter. (C) Generalized relationship between enzyme activity of GS-GOGAT and NR as a function of time of day. (D) Generalized relationships of activity of GS-GOGAT and NR as a function of temperature. (E) Example relationships of NH_4^+ and NO_3^- uptake as a function of temperature (replotted from Lomas and Glibert 1999a).

reduce NO_3^- to NH_4^+ in the cell. This difference suggests that redox regulation is at the heart of the differences in NH_4^+ and NO_3^- metabolism, a theme that will be reinforced throughout this review.

Transport of both NH_4^+ and NO_3^- across the cell membrane is performed by NH_4^+ and NO_3^- transporters, AMTs, and NRTs, respectively, that perform proton (H^+)-coupled transport (e.g., Navarro et al. 1996; Galván and Fernandez 2001; Rogato et al. 2015), although there is evidence of Na^+ rather than H^+ symport with NO_3^- in marine diatoms, (Rees et al. 1980; Boyd and Gradmann 1999; Fig. 6). Net diffusion is generally limited to very high concentration of substrate (> many tens of μM). Diffusive influx of NH_4^+ (AMT-dependent uniport) depends on an electrogenic pump (H^+ or Na^+) to maintain the inside-negative electrical potential difference (Raven 1980). Most marine eukaryotic phytoplankton appear to be able to take up NO_3^-, and all of the genomes sequenced to date contain genes encoding for NO_3^- transporters. Knowledge is rapidly advancing on the regulation of both NRT and AMTs in different algal taxa (Hildebrand 2005; Bender et al. 2014; Rogato et al. 2015).

There are both high affinity (HATs, operating at low concentrations of substrate) and low affinity transporters (LATs, operating at relatively high concentrations of substrate) at the cell surface for each substrate. HATs are saturable and are typically expressed under N-limiting conditions, while LATs may be non-saturable and generally expressed only when the substrate is abundant (Howitt and Udvardi 2000; Rogato et al. 2015 and references therein). The presence of non-saturable LATs may lead to uptake kinetics that appear linear or biphasic generally at concentrations much higher than a natural cell would normally encounter (Fig. 7A). Biphasic kinetics are much more commonly reported for NO_3^- uptake than for NH_4^+ uptake. Non-saturable or biphasic kinetic relationships have been reported for both cultures and natural algal communities at concentrations up to 300 μM NO_3^-, suggesting that NO_3^- uptake capability can be greater than that of NH_4^+ at very high N concentrations (e.g., Collos et al. 1997; Lomas and Glibert 1999a). In contrast, under N-limited conditions, transport rates of NH_4^+ are often higher than those of NO_3^- (Flynn et al. 1999), while at high NH_4^+ concentrations, a suppression of uptake is sometimes seen in NH_4^+ uptake kinetics (e.g., Glibert et al. 2013; Fig. 7A).

There are important differences in the regulation of NRTs and AMTs in phytoplankton, as well as in virtually all plants (Rogato et al. 2015). In general, NO_3^- transporters are induced by the presence of their substrate (NO_3^-), whereas NH_4^+ transporters are induced by the absence or deficiency of their substrates, or repressed by increased availability of their substrate, NH_4^+ (Clarkson and Luttge 1991; Navarro et al. 1996; Crawford and Glass 1998; Daniel-Vedele et al. 1998; Fig. 7B). Thus, increasing concentrations of NO_3^- yield more NRTs, whereas increasing concentrations of NH_4^+ yield fewer AMTs. NO_3^- can act as a positive signaling molecule, its presence an inducer of both NO_3^- uptake and reduction (Coruzzi and Bush 2001). This phenomenon of acceleration ("shift-up") of NO_3^- uptake in the presence of NO_3^- has been well described in phytoplankton in both the classical physiological literature and more recently in molecular studies (e.g., Dugdale et al. 1981 and references therein; Allen et al. 2011 as an example). In contrast, NH_4^+ and its assimilation products are negative signaling molecules that act as repressors of NH_4^+ transport and its assimilation, down-regulating these processes when availability of NH_4^+ increases within

the cell (Flynn and Fasham 1997; Flynn et al. 1997; Post et al. 2012). However, it is not just the internal availability of N that regulates uptake, it is also the quota of N relative to C that is important (Rogato et al. 2015 and references therein); the importance of the C and N relationship will be further emphasized in following sections.

The first step of intracellular NO_3^- metabolism is reduction to NO_2^- in the cytosol through the activity of nitrate reductase (NR; Fig. 6). Large intracellular accumulations of NO_3^- may occur, but the genes associated with the specific transport of NO_3^- to and from vacuoles have not been identified (Raven 1987; Allen et al. 2006; Bender et al. 2014). There are several types of NR; they are structurally different, they vary with different algal functional types, and may be regulated quite differently (e.g., Berges 1997; Morozkina and Zvyagilskaya 2007). Traditionally it was thought that NR is localized in the cytosol of the cell; however, there is considerable evidence that a form of NR also exists in the plasmalemma and other cellular membranes (Jones and Morel 1988), particularly in diatoms, chlorophytes, and cyanobacteria (Jones and Morel 1988; Tischner et al. 1989; Stöhr et al. 1993; Berges 1997; Dagenais-Bellefeuille and Morse 2013 and references therein). In dinoflagellates most of the NO_3^- reduction appears to occur in the chloroplast (Berges and Mulholland 2008), and in chlorophytes there is substantial NR associated with pyrenoids, the bodies within chloroplasts that have high concentrations of carbon (C)-assimilating enzymes (Lopez-Ruiz et al. 1985; Fischer and Klein 1988).

Once reduced in the cytosol, the resulting NO_2^- is rapidly transported into the chloroplast (typically via localized transporters) where it is further reduced to NH_4^+ by the activity of Fd-dependent nitrite reductase (NiR) (Galván et al. 2002, Fig. 6). This Fd-dependent NiR is localized in the chloroplast, but, based on a putative targeting sequence, it is hypothesized that there is also a cytosolic form of NiR that is NADPH-dependent, at least in diatoms (Armbrust et al. 2004; Allen et al. 2006).

Assimilation of NH_4^+, either derived from direct uptake or from reduction of NO_2^-, occurs via a series of reactions involving (for most algal species) the enzymes glutamine (Gln) synthetase (GS) and glutamate (Glu) synthase (GOGAT; also known as glutamine-2-oxoglutarate amidotransferase, Fig. 6). This pathway yields Glu, the product of Gln and oxoglutarate (2-OG) (Scanlan and Post 2008, Fig. 6). Both Glu and Gln are essential for amino acid metabolism and cellular N regulation, as they are both N acceptors and N donors (Dagenais-Bellefeuille and Morse 2013). Most cyanobacteria assimilate N through the GS-GOGAT pathway, but some also have Glu dehydrogenase (GDH) which may present an advantage for those species in that NH_4^+ assimilation through GDH is not ATP-requiring (Muro-Pastor et al. 2005).

There are several forms of nuclear-encoded GS, one localized to the chloroplast (form GSII), and one or two forms localized in the cytosol or the mitochondrion (GS I or III), at least in diatoms (Robertson and Alberte 1996; Takabayashi et al. 2005; Siaut et al. 2007). The plastid form is Fd-dependent, while the mitochondrial form is hypothesized to be NAD(P)H-dependent (Alipanah et al. 2015 and references therein). The chloroplastic form is inducible by external N but the cytosolic form more constitutively expressed. This localization is important in the metabolism of different N forms because, for many plants and at least in diatoms, the gene encoding for the chloroplastidic GSII, as well as total GS activity, appears to be up-regulated in cells assimilating NO_3^- but not in cells assimilating NH_4^+. These findings support the premise that chloroplastidic N metabolism is the important pathway of reduction of oxidized N (Takabayashi et al. 2005). In fact, GSII in the diatom *Skeletonema costatum* has been shown to be a genetic marker for NO_3^- assimilation (Allen et al. 2006), while GSIII, the mitochondrial form, is expressed more in the assimilation of NH_4^+ derived from deamination and hydrolysis of organic N (Siaut et al. 2007).

Transcription and activity of enzymes involved in NO_3^- and NH_4^+ metabolism are also regulated by various external cues, as well as cellular fluxes of various C and N substrates (Takabayashi et al. 2005 and references therein). NR abundance and activity typically vary on a diel basis (e.g., Packard et al. 1971; Berges et al. 1995; Brown et al. 2009) although some diatoms may continue to assimilate NO_3^- in darkness, while GS-GOGAT activity is generally maintained throughout the day (Clark et al. 2002; Fig. 7C). However, based on a study of the diurnal expression of the genes encoding N assimilation, Brown et al. (2009) found that NR abundances and activity are not under circadian control, regulated instead by changes in the metabolic pools of NO_3^- and NH_4^+ that, in turn, regulate the N assimilating enzymes.

Temperature also affects enzymes associated with NO_3^- and NH_4^+ metabolism differently. NR activity has an inverse relationship with temperature (generally between 12°C and 25°C; Gao et al. 1983; Kristiansen 1983; Lomas and Glibert 1999a,b), while GS-GOGAT activity is positively related to temperature across the same range (e.g., Clayton and Ahmed 1986; Fig. 7D). The assimilation of NO_3^- would thus be expected to be higher at lower temperatures (10–15°C), and indeed, over the temperature range 5–25°C, NO_3^- uptake by both diatom-dominated and dinoflagellate-dominated natural communities show an inverse relationship with temperature while NH_4^+ uptake shows a positive relationship (Lomas and Glibert 1999a,b; Fan et al. 2003; Fig. 7E). Taken together, the regulation of NH_4^+ and NO_3^- uptake and assimilation is quite different with respect to environmental cues, a feature that can influence phytoplankton assemblage dynamics in N-enriched systems.

N uptake and assimilation when both N forms are supplied together

Except in culture studies, rarely are phytoplankton in an environment in which only one form of N is available. More typically the cell is exposed to both oxidized and reduced forms of N. While NO_3^- transport and assimilation are generally repressed by NH_4^+ (or its assimilation products), NH_4^+ transport and assimilation are usually unaffected by the presence of NO_3^- (Clarkson and Luttge 1991; Navarro et al. 1996; Flynn and Fasham 1997; Flynn et al. 1997; Crawford and Glass 1998; Daniel-Vedele et al. 1998). NH_4^+ affects NO_3^- metabolism by down-regulation of transport of NO_3^- across the cell membrane and repression of NR abundance and activity (Vergera et al. 1998). In most algae, the regulation is via the size of the Gln pool (e.g., Flynn et al. 1994), although in cyanobacteria and some other taxa, the metabolite 2-oxoglutarate (2-OG) also serves this regulatory function (Muro-Pastor et al. 2001, 2005; Post et al. 2012). The availability of Gln and the Gln/Glu ratio govern the NO_3^- reducing capacity in the cell; when Gln levels are low, and when NO_3^- is available, NR is up-regulated. Alternatively, when Gln levels are high, NR activity levels are "throttled" back (Flynn et al. 1994; Campbell 1999). In essence, cells generally do not de-repress (express) an ability to transport NO_3^- unless their internal N status is sufficiently low. As the supply of NH_4^+ becomes insufficient to maintain a high internal N-status, indicated by a decline in internal Gln: Glu ratios (Flynn et al 1989, 1994), then the ability to transport and use NO_3^- is up-regulated. AMTs in some species are up-regulated by the depletion of NO_3^-, but the inverse relationship does not appear to be the case; that is they are not down-regulated by the prevalence of NO_3^- (e.g., Hockin et al. 2012).

The availability of NH_4^+ may also serve to decrease further uptake of itself. For example, in many cyanobacteria, the transcriptional activator of N assimilation genes, NtcA, and therefore of AMT1 expression, is negatively controlled by NH_4^+ and the metabolite 2-OG (Coruzzi and Bush 2001; Lindell and Post 2001; Muro-Pastor et al. 2001, 2005). When this metabolite accumulates, it represses further N assimilation of NH_4^+ (Post et al. 2012). It is of note that NH_4^+ generally does not accumulate in the cell to the same extent as NO_3^- (e.g., Dortch 1982).

At the enzyme level, the degree of repression of NR is a function of the relative balance of NR and its repressor, NH_4^+. Thus, the degree of phytoplankton uptake and growth repression and suppression conditions by a given amount of NH_4^+ varies with cell status and the environmental conditions (see, for example, Thompson et al. 1989; Dortch et al. 1991; Yin et al. 1998 and references therein). Any environmental factor that affects the availability of substrate, the nutritional state of the cell, or the rate of enzyme activity will affect the rate at which NO_3^- or NH_4^+ is transported and assimilated (Dortch 1990). In addition to the potential environmental controls, N uptake may also depend on the presence of multiple forms of NR that alter the effects of NH_4^+ on NO_3^- reduction. At least for the diatom *Thalassiosira weisflogii*, the membrane-bound form of the enzyme is seemingly not repressible by NH_4^+, whereas the cytosolic form is repressible (Jones and Morel 1988). As noted by Berges (1997), it may be of significance that the NR of dinoflagellates appears to be localized in the chloroplast, as lack of repression of NR by NH_4^+ in some dinoflagellates has been found.

Coupling/uncoupling of uptake and growth; C and N homeostasis

Only under the condition of steady state, a condition rarely achieved in natural environments, is the rate of nutrient uptake equivalent to the rate of growth (Goldman and Glibert 1983). In steady or quasi-steady-state conditions, homeostatic mechanisms keep the acquisition of materials and energy in balance with the cellular growth demands. Consequently, when cells are grown under balanced growth conditions and comparable environmental conditions but under different N substrates, the differential—but acclimated—metabolism of NH_4^+ and NO_3^- generally leads to the same result: equivalent or nearly equivalent growth rates (e.g., Solomon et al. 2010; Collier et al. 2012; Collos and Harrison 2014 and references therein). If sufficient substrate is provided to saturate the growth demand (but not so high as to be growth suppressing), maximal growth rate is then defined by the ambient environmental conditions of growth (light, temperature, pH, etc.), and cells should just balance their N uptake to balance their growth demand. In N-enriched environments, where the form of N may change from NH_4^+ to NO_3^- or vice versa, balancing redox potential is an especially important process. The effect of changes in external supplies critically depends on the supply-demand of C, N, and other elements within the cells (e.g., Flynn et al. 2010).

Under non-steady-state conditions relating to variable nutrient or energy availability, cellular adjustments in acquisition efficiency and capacity decouple these "simple" relationships and the underlying kinetic relationships are always "chasing," rather than anticipating, the environmental change. Cells in dynamically fluctuating environments are also more likely to experience superimposed stresses, including fluctuating availability of different N compounds, variable light, temperature, and other conditions, all of which can create conditions affecting the interactions of NH_4^+ and NO_3^- and the degree of balance or imbalance in the coupling of C and N assimilation.

Fundamentally there are two mechanisms to adjust imbalances: up-regulate the pathways for acquisition of the constituent that is in least supply, or down-regulate the cellular

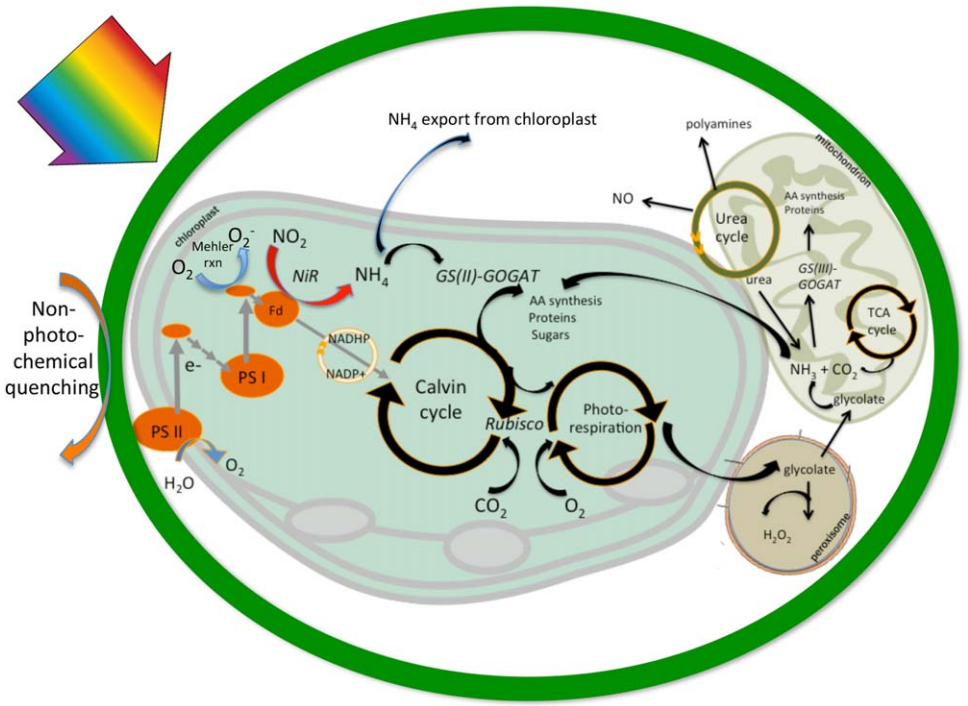


Fig. 8. Conceptual schematic illustrating, for a generic algal cell, the electron transport of photosynthesis, the coupling to the Calvin cycle and N assimilation pathways, and the various mechanisms for energy and excess reductant dissipation. The dissipatory mechanisms shown include non-photochemical quenching, Mehler activity, dissimilatory NO_3^-/NO_2^- reduction to NH_4^+, and photorespiration. Also shown are the relationships between the Calvin cycle and N assimilation pathways, photorespiration, and the urea cycle in the chloroplast, peroxisome and the mitochondrion.

constituent that is in over-supply. Cells have various signaling molecules that facilitate this regulation, and they have various "release valves" to rebalance the flow of energy or materials when imbalances develop. The conceptual model developed herein is that of a cell depending on different mechanisms to rebalance their cellular redox status under growth on NO_3^- vs. NH_4^+. Chloroplasts are of particular importance in this regard as they "... have the potential to act as delicate environmental sensors, since they harbor numerous metabolic pathways that are readily unbalanced by environmental fluctuations" (Kangasjärvi et al. 2012, p. 1). Through signaling pathways that sense a change in cellular metabolites, internal N or C pools, or redox state of the cell, a change in the regulation of N uptake or metabolism occurs through changes in gene and hence enzyme expression and activity. Such signaling pathways and metabolic feedbacks may be disrupted or overwhelmed when the cell is subjected to stress, including a change in the redox state of the N compound on which they are growing.

Energy pressure valves and C and N assimilation in diatoms—cycles, cross-talk, and feedback

Cellular energy balance has been termed the "broker" of coordinated regulation between N and C interactions (Foyer et al. 2011). Balancing energy-generating and energy-using processes with those of N- and C-acquisition and N- and C-assimilation is delicate. The assimilation of N and that of C are linked in multiple biochemical pathways and thus C and N metabolites have various "cross-talk" in the cell and mechanisms to regulate the flux of metabolites into the cell and cellular redox status (e.g., Turpin and Harrison 1979; Turpin 1991; Coruzzi and Bush 2001; Wang et al. 2014). Redox regulation is a function of not only the flow of reductant through photosynthesis, but also the demands for energy via metabolism. The "energy pressure" can be thought of as a measure of the reductant state or availability for a cell. During photosynthesis, energy pressure can be related to the ratio of light absorption to assimilation (Kana et al. 1997).

Some of the well-documented processes which aid in the regulation of cellular redox and energy balance that often arise from imbalances in photochemistry and C assimilation include non-photochemical quenching operating around the thylakoid pH gradient and xanthophyll cycle, cyclic electron flow around photosystem II, export of excess NAD(P)H through the malate shuttle, and the Mehler reaction (the water-water cycle; e.g., Lavaud et al. 2003; Ruban et al. 2004; Scheibe 2004; Wilhelm et al. 2006; Fig. 8). These mechanisms help to dissipate excess electron energy that may result from light stress, where electron transport through the light reactions and associated thermochemical reactions in the thylakoids in photosynthesis is in excess of C

assimilation capacity, i.e., when the light reactions or electron transport chain of photosynthesis go into "overdrive" (Kana et al. 1997; Chung et al. 2008). These mechanisms are expressed differentially in different phytoplankton groups. There appears to be some inconsistency in the literature with respect to whether, and to what extent, diatoms have Mehler activity (Lomas and Glibert 1999a; but *see* Wilhelm et al. 2006 and references therein), but chlorophytes are well recognized to have such a pathway and to release H_2O_2 as a result (e.g., Collén et al. 1995). The presence of a chloroplastidic malate pathway in diatoms also appears to be a matter of some debate. Whereas Ocheretina et al. (2000) suggest there is no such malate pathway, Allen et al. (2009) suggested such a pathway based on transcriptome data which will require further verification via genetics and biochemistry. In higher plants, enhanced activity of malate shuttles and of Mehler activity under NH_4^+-growth compared with NO_3^--growth has been shown (e.g., Gerendás et al. 1997; Scheibe 2004; Guo et al. 2007a,b), as has enhanced xanthophyll cycling, one of the important non-photochemical quenching (NPQ) mechanisms available to most non-cyanobacteria phytoplankton. There is good evidence that cryptophytes lack xanthophyll cycling yet still maintain effective NPQ (Kaňa et al. 2012). Enhanced xanthophyll cycling has been shown to be the case in the diatom *Thalassiosira pseudonana* in culture and in diatom-dominated phytoplankton assemblages enriched with NH_4^+ (tens of μM) compared with similarly treated algae but with NO_3^- enrichment (Shi et al. 2015; Glibert et al. unpubl. data).

Recent physiological and molecular studies especially of diatoms have revealed much insight into feedback mechanisms of N and C assimilation and the role of pathways that serve as release pressure values when metabolism is stressed, including elevated concentrations of NH_4^+ as a stress. Because N assimilation is an important sink for reducing power (through reduced Fd and NAD(P)H), the addition of NO_3^- can divert the distribution of electrons among different chloroplastic and mitochondrial pathways (Rosenwasser et al. 2014). An important pathway regulating overall cellular energy balance in diatoms is the reduction of NO_3^- and NO_2^- via NR and NiR in a nonassimilatory mode that complements such reduction in N assimilation (e.g., Lomas and Glibert 1999a,b; Parker and Armbrust 2005; Kamp et al. 2011; Rosenwasser et al. 2014; Fig. 8). The reduction of NO_2^- to NH_4^+ in the chloroplast uses the reducing power of the Fd system, and it can serve as a sink for excess reductant, derived from the splitting of water, that may develop when photochemistry exceeds assimilatory capacity (e.g., conditions of high light and cool temperatures). Such reactions can protect the chloroplast electron transport chain from over-reduction. Cool temperatures may enhance a condition of excess reductant because the biophysical light reactions of photosynthesis are relatively temperature-insensitive, but the biochemical reactions (e.g., Calvin Cycle reactions and non-photochemical reactions in the thylakoid) are temperature-sensitive leading to slower rates of C assimilation than of the light reactions. In order for such a dissimilatory pathway to function, release of N in a more reduced state should be observed. In fact, release of NO_2^- by diatoms has been commonly observed during NO_3^- uptake (e.g., Anderson and Roels 1981; Collos 1982), and there are also numerous reports of release of NH_4^+, as well as release of DON from both field and laboratory cultures using NO_3^- (Lomas et al. 2000 and references therein). Dissimilatory NO_3^- reduction in diatoms has also been suggested as an energy-providing mechanism under conditions of extended darkness or hypoxia (Kamp et al. 2011). Clearly an important criterion for such pathways to function is the availability of NO_3^- or NO_2^- in the cell. Without these substrates the options for redox homeostasis are limited, and that via dissimilatory NO_3^- reduction is absent.

Arguably one the most important reactions in cellular redox homeostasis is photorespiration, initiated by O_2 consumption via the oxygenase reaction of the enzyme that also catalyzes the fixation of CO_2 via the carboxylase reaction, D-ribulose-1,5-bisphosphatecarboxylase/oxygenase, Rubisco (Fig. 8). Rubisco is an enigmatic enzyme, as it has dual catalytic reactions with both CO_2 and O_2 (Fig. 8); it has been characterized as "hamstrung by slow catalysis and confusion between CO_2 and O_2 as substrates, an 'abominably perplexing' puzzle" (Tcherkez et al. 2006, p. 7246). Photorespiration has been considered as an evolutionary holdover from a time when CO_2 concentrations were far higher than today, and as an unproductive, energy-expensive, wasteful pathway. While costly, the importance of photorespiration appears to be its role as an important redox-balancing pathway by exporting reduced equivalents to the peroxisome and mitochondria. Thus, photorespiration may be "an important pathway that makes the best of a bad situation caused by Rubisco's seemingly inevitable oxygenase activity" (Peterhensel et al. 2010, p. 10).

Photorespiration shares many metabolic products with those of N assimilation using C skeletons synthesized in the TCA cycle (Fig. 8). Photorespiration provides no net gain in C or energy for the cell (i.e., no net growth) and it imposes other cellular costs in terms of the repair, quenching, and other functions impeded by increased oxygenase activity (Raven 2011; Voss et al. 2013; Raven et al. 2014). Photorespiration increases under conditions of high light, high O_2, and high temperature—all factors that favor the oxygenation: carboxylation ratio of Rubisco (Kangasjärvi et al. 2012). Importantly, when growth is on NH_4^+ rather than NO_3^-, photorespiratory responses to other stress, such as high light and cold temperatures, can increase significantly. When NO_3^- is comparatively unavailable to the cell, the sink for NADPH consumption via NR-catalysed NO_3^- reduction is not available, and photorespiration becomes the alternative electron sink (Keys and Leegood 2004; Guo et al. 2007a,b; Nunes-Nesi

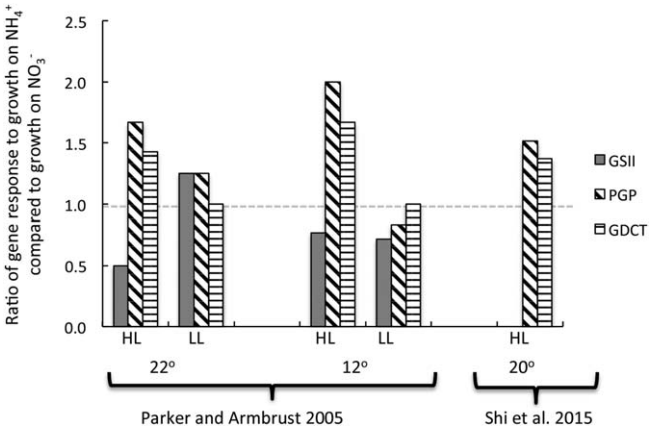


Fig. 9. Comparison of response of different genes to growth on NH_4^+ vs. NO_3^- under different temperature and light conditions in the diatom *T. pseudonana* from two independent studies, Parker and Armbrust (2005) and Shi et al. (2015). Note that the number of copies of glutamine synthetase II (GSII) was lower on NH_4^+ growth (ratio < 1.0) in all cases except 22°C and low light (LL), and that the number of copies of the two genes involved in photorespiration, phosphoglycolate phosphatase (PGP) and T-protein subunit glycine decarboxylase (GDCT), were higher (ratio > 1.0) in all cases under NH_4^+ growth, except low light, especially at 12°C. Data were replotted from Parker and Armbrust (2005) and Shi et al. (2015).

et al. 2010). This has been nicely shown in the diatom *T. pseudonana*, in which genes associated with photorespiration, phosphoglycolate phosphatase (PGP) and T-protein subunit glycine decarboxylase (GDCT), were up-regulated when NH_4^+, rather than NO_3^-, was the N growth substrate and when cells were shifted to higher light (Parker et al. 2004; Parker and Armbrust 2005; Shi et al. 2015; Fig. 9).

It must be emphasized that much of the work on the interactions of photorespiration with N metabolism has been carried out on C_3 flowering plants that rely on diffusive CO_2 entry. CO_2 concentrating mechanisms (CCMs) are necessary in algae because pCO_2 concentrations in natural waters, especially seawater, are too low to saturate Rubisco carboxylase with diffusion alone; they serve to increase the concentration of CO_2 at the site of Rubisco (Roberts et al. 2007a,b; Wu et al. 2014a and references therein). Thus, CCMs increase the rate of carboxylation relative to oxygenation, and as a consequence there should be less photorespiration under a given set of environmental conditions.

Diatoms and most other algae are well documented to have CCMs (Raven and Beardall 2003; Armbrust et al. 2004; Granum et al. 2005; Wilhelm et al. 2006; Roberts et al. 2007a,b; Raven et al. 2008; Hopkinson et al. 2011). In fact, large diatoms have greater CCM capacity than small diatoms to overcome their smaller surface/volume ratio and the associated additional diffusional constraints (Wu et al. 2014a,b). The presence of CCMs, which should decrease the photorespiratory flux, creates a seeming paradox for these cells: why have both CCMs and significant rates of photorespiration? Both are costly, and CCMs should reduce photorespiration, yet both appear to be essential to the success of diatoms among other algae. The presence of CCMs may, in fact, provide an explanation for why enhancement of the downstream pathways of photorespiration with NH_4^+ availability contributes to a change in the carboxylase and oxygenase activity at the site of Rubisco. For at least some diatoms (and C_4-like or C_3–C_4 intermediate-like plants), CCMs involve, among other enzymes, the enzyme phosphoenolpyruvate carboxylase (PEPCase; Reinfelder et al. 2000). The activity of this enzyme changes with exposure to different N forms, and is generally higher under NO_3^- availability compared with NH_4^+ availability (Guo et al. 2007a,b). Therefore, at a constant inorganic C supply, the affinity for CO_2 increases when NO_3^- is the growth N substrate, resulting in a higher C assimilation per unit N when a CCM is operating (Raven 1991; Raven et al. 2005). Inversely, with NH_4^+, the affinity of PEPCase for CO_2 is less, resulting in lower CO_2 at the site of Rubisco, and photorespiration correspondingly may increase. Quite simply, the various balances between the enzymes PEPCase, other CCM enzymes, NH_4^+ and NO_3^- transporters, NR and GS/GOGAT all may change under NO_3^- vs. NH_4^+ availability, resulting in a change in the balance of carboxylase/oxygenase activity for Rubisco. Therefore, cellular localization of the different isoforms of the enzymes and their respective roles in primary assimilation of N or in N (re)-assimilation become important in regulating substrate availability at the site of these enzymes (e.g., Granum et al. 2005 and references therein).

While often overlooked in photosynthetic organisms, the mitochondria also play critical roles in energy balance. Two N-related pathways are relevant and they may also change under NH_4^+ nutrition compared with NO_3^- nutrition. First, in diatoms, and likely some other algae, there is a urea cycle (Armbrust et al. 2004; Allen et al. 2011; Weyman et al. 2015; Fig. 10). The long-known function of the urea cycle in animals is to excrete excess N produced by amino acid catabolism; like photorespiration, the urea cycle had long been considered a waste pathway. However, in diatoms the urea cycle appears to play a role in exchange of nutrients between the mitochondria and the cytoplasm, and potentially the plastid (Bender et al. 2012) and may help to regulate NH_4^+ metabolism (Armbrust et al. 2004; Allen et al. 2011). Because of this cycle, marine diatoms, in contrast to chlorophytes, also have acquired a mitochondrial urea transporter and, in fact, based on bioinformatics, a complete mitochondrial GS-GOGAT cycle has been hypothesized (Allen et al. 2011; Fig. 10). Caution must be expressed here and throughout this review regarding localization of this process; subcellular compartment-defined metabolic models are a common product of genome-sequencing publications, and these need to be treated with some skepticism.

The urea cycle appears to function differentially under NO_3^- vs. NH_4^+ growth. It has been shown that a supplement

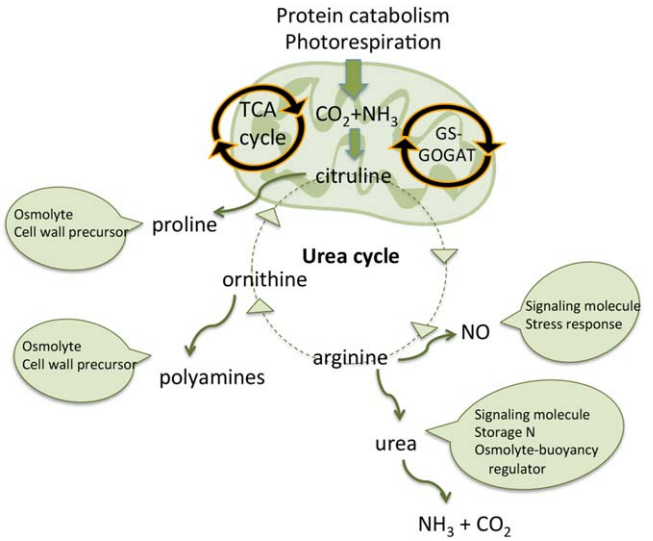


Fig. 10. Conceptual schematic of the mitochondrial urea cycle in a generic diatom cell and the potential fates of urea cycle intermediates.

of NH_4^+ to N-depleted diatom cells stimulates the urea cycle (Allen et al. 2011; Hockin et al. 2012; Bender et al. 2014; Rosenwasser et al. 2014). There are some key intermediates that are variably affected by N form or availability (Fig. 10). For example, increases in NH_4^+ have been related to increased polyamine synthesis (Moschou et al. 2012 and references therein). Polyamines such as spermidine and putrecine are key precursors for the siliceous cell walls of diatoms, and, in some cases, diatom toxins (Allen et al. 2006). When polyamine synthesis is overexpressed, typically as a result of lack of reducing power, thickened cell walls may be produced, leading to enhanced cell sinking (Nunn et al. 2013).

The second mitochondrial pathway potentially affected by the form of N nutrition is mitochondrial respiration. It has been suggested, at least for higher plants, experiencing solely NH_4^+ nutrition, excess redox equivalents may be oxidized on the mitochondrial electron transport chain, which may lead to elevated electron "leakage" and increased production of reactive oxygen species (ROS, Podgórska and Szal 2015 and references therein). ROS include not only hydroxyl radical $HO^{\bullet}$, superoxide anion O_2^-, H_2O_2, and singlet oxygen $^1O_2$, but also NO. The urea cycle is one of many processes that can generate intermediates that can be a substrate for NO synthesis (e.g., Allen et al. 2006; Hockin et al. 2012; Sharma et al. 2012); both arginine and NO_3^- are substrates for NO synthase, for example. In at least the green alga *Clamydomonas*, NO, acting as a signaling molecule, may inhibit, at the transcriptional level, the expression of genes involved in both NH_4^+ and NO_3^- transport (AMT and NRT), and at the post-translational level, NO also rapidly (and reversibly) represses the HAT transporters of both N forms, and may also inhibit NR activity, but not that of NiR or GS in intact cells (Sanz-Luque et al. 2013). All ROS forms can be damaging to cells if sufficiently high, leading to a peroxidation of lipids, oxidation of proteins, enzyme inhibition, and various other responses (Sharma et al. 2012; Rosenwasser et al. 2014). In many higher plants, NH_4^+ nutrition has been associated with enhanced ROS production, and even though many also plants have developed antioxidant defense systems (e.g., superoxide dismutase) that counteract the effect of enhanced ROS, it is often insufficient to counteract this oxidative stress (Podgórska and Szal 2015 and references therein).

Thus, different mechanisms for rebalancing cellular redox under conditions of non-steady-state growth may function under different environmental conditions (Fig. 11). Particularly in diatoms, under cool, NO_3^--rich conditions, dissimilatory NO_3^-/NO_2^- reduction to NH_4^+ serves as a major sink for excess reductant. Under warm conditions, but with NO_3^- as the dominant N substrate, the activity of NR is reduced (but dissimilatory NO_3^- and NO_2^- reduction to NH_4^+ remains important), but activity of Rubisco also increases, with the result that overall rates of C fixation are proportionately higher. Under cool temperatures with NH_4^+ as the primary N substrate, the cell is more likely to balance its redox state mainly through photorespiration, as the NO_3^- and NO_2^- reduction pathways are not available. Finally, under warm conditions with NH_4^+ as the dominant N substrate, C assimilation increases due to the higher temperature optima of Rubisco, but photorespiratory rates remain high. These are, indeed the mechanisms or "strategies" that have been shown in, or suggested by, both laboratory and field experiments (e.g., Lomas and Glibert 1999a,b, 2000; Parker and Armbrust 2005). However, these pathways also have negative feedbacks and consequences on cell metabolism when homeostasis is not attained. Thus, in addition to transporter and enzyme repression by NH_4^+ (or its assimilation products), overexpression of photorespiration or mitochondrial respiration, which can occur under NH_4^+ nutrition, can result in enhanced ROS activity, which may further N uptake repression and growth suppression.

Bioactive compound and toxin production under nutrient imbalanced conditions

In addition to the enhanced production of ROS and polyamine synthesis in diatoms under NH_4^+ nutrition discussed above, there is other evidence that production of some algal toxins may be different under nutrition on different forms of N. For the toxigenic dinoflagellate *Alexandrium tamarense* grown on NO_3^-, NH_4^+ or urea, then pulsed with increases in each of the N forms, the highest cellular toxin content was found to be for cells grown on NH_4^+. Leong et al. (2004) found, in general, that NH_4^+ (and urea) induced production of the N-containing gonyautoxins (GTX), while oxidized

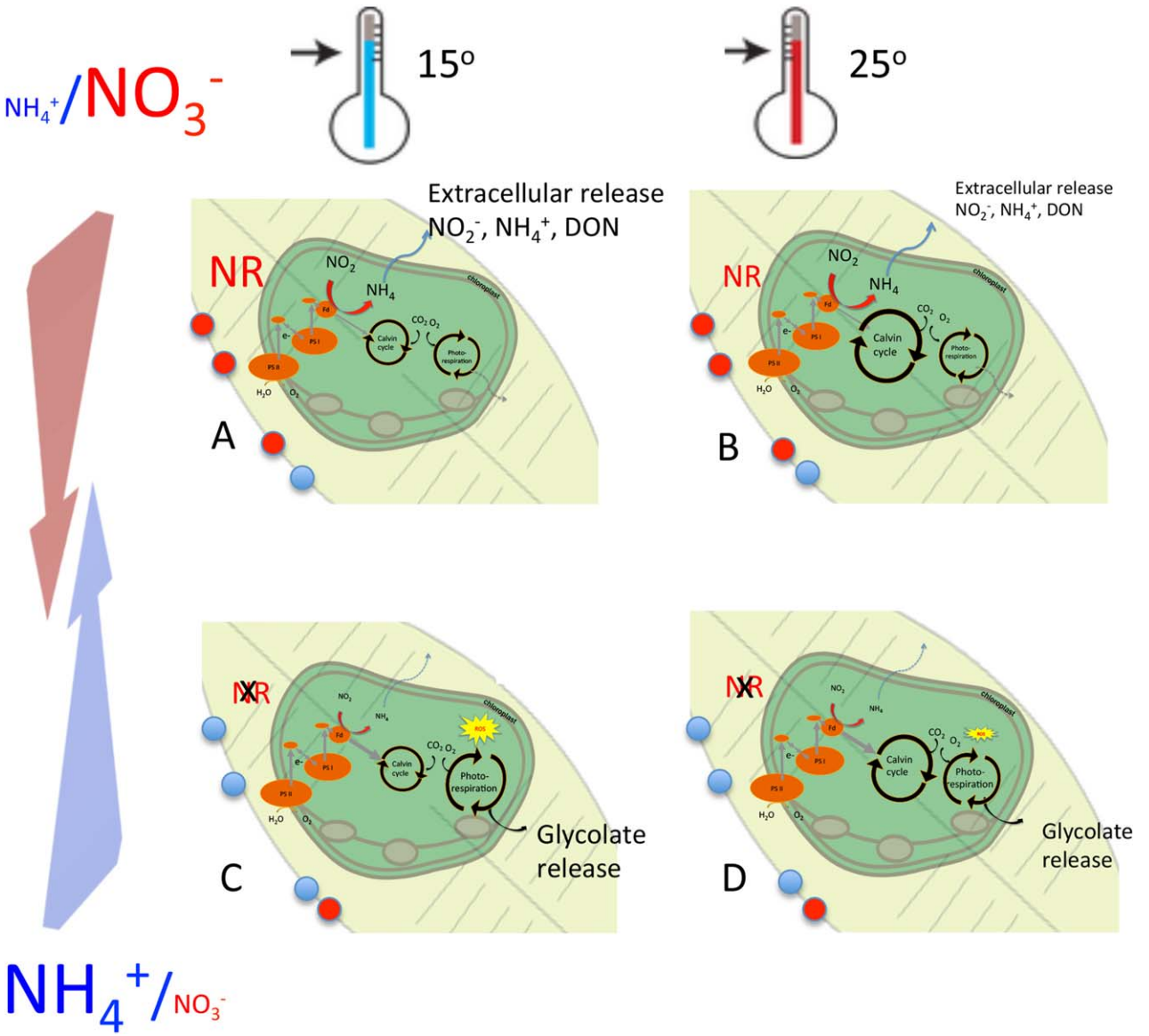


Fig. 11. Conceptual diagram illustrating the relative abundance of different N transporters (red—NO_3^- transporters; blue—NH_4^+ transporters), and importance of the pathways of NO_3^- reduction (including dissimilatory NO_3^-/NO_2^- reduction to NH_4^+), C assimilation and photorespiration for diatoms growing under a range of NH_4^+/NO_3^- ratios and temperatures. The various pathways are exaggerated or minimized under each condition to illustrate their relative importance as well as how these proportions change under the different conditions. Note that under cool temperature and elevated NO_3^- availability, NO_3^- and NO_2^- reduction to NH_4^+ is important both in assimilatory and dissimilatory pathways, leading to cellular release of NO_2^-, NH_4^+ and DON. Photorespiration increases under elevated NH_4^+ availability, leading to glycolate release, as does the potential for over-reduction leading to reactive oxygen (ROS) production. Under warmer conditions, extracellular release of N is less than under cool conditions because of higher C assimilation rates, and glycolate release is comparatively less due to higher rates of re-assimilation of the photorespiratory products. Carbon assimilation increases as temperatures warm in all cases. See text for details.

forms of N induced higher relative abundance of C toxin (C2) and that overall highest intracellular concentrations were found when cell were grown on NH_4^+, followed by urea and then NO_3^-. These findings are consistent with the notion that competition for N in metabolic pathways differs for the different N sources. A similar finding was reported from nutrient amendment experiments conducted on a field population of the related species *Alexandrium fundyense* (Hattenrath et al. 2010). The importance of the chloroplast in N and C metabolism and toxin production again comes into play, but is poorly understood. It is known that many phototrophic dinoflagellates, such as *Karlodinium veneficum*, only make toxin—and only eat—during the light period (Adolf et al. 2008), and thus toxin production must be linked to photosynthesis, but the exact mechanism is not known.

In toxin-producing cyanobacteria such as *Microcystis*, numerous studies have shown positive, direct relationships between N availability and toxin production (e.g., Lee et al. 2000; Vézie et al. 2002; Downing et al. 2005; Van de Waal et al. 2009). Microcystins are small molecules synthesized by nonribosomal peptide synthases, but among the microcystins there is considerable variability in structure and C : N composition (Van de Waal et al. 2009 and references therein). It has specifically been suggested that microcystin may play an important role in redox control and in the detection of redox changes in the cell; it can affect proteins related to C and N metabolism (Neilan et al. 2013). The C-nutrient balance hypothesis suggests that enhanced N loading will favor production of metabolites such as alkaloids, while limitation by N may favor production of C-rich compounds (e.g., Bryant et al. 1983; Van de Waal et al. 2009). Although studies with respect to N form and microcystin production are comparatively few, it has been shown that additions of N do enhance microcystin production when sufficient P is available for growth, and at least in one study, addition of NH_4^+ compared with NO_3^- resulted in elevated microcystin concentrations well above guidelines and sustained the bloom for a substantially longer period of time (Donald et al. 2011). Accordingly also, under P limitation, N-rich toxins are favored as N can accumulate in excess (e.g., Granéli and Flynn 2006; Van de Waal et al. 2014 and references therein). Collectively these results are consistent with the notion put forward that toxin production may be associated with pathways of energy balance or cellular stoichiometric rebalancing (e.g., Glibert and Burkholder 2011; Van de Waal et al. 2014 and references therein).

Functional group differences: unique N strategies

In addition to the unique features of different algal functional groups described above, there are other metabolic characteristic that suggest the diatoms are indeed NO_3^- specialists, while cyanobacteria, especially picocyanobacteria, and many chlorophytes and dinoflagellates, may be better adapted to use of NH_4^+ (Table 3; Fig. 12). Molecular phylogenies of NRT transporters confirm that there are clear differences between those of diatoms and of other major algal groups (Song and Ward 2007; Chan et al. 2011; Kang et al. 2011; Kang and Chang 2014; Fig. 13). There are also differences between centric and pennate diatoms in the regulation of transporters (Bender et al. 2014). In general, diatoms tend to have more copies of HAT-NRT transporters (e.g., Armbrust et al. 2004). Additionally, Lomas and Glibert (2000) reported that diatoms had significantly higher cell-specific rates of NR activity than did the flagellates they tested. A broad survey of algae grown in culture suggested differences in NH_4^+ tolerance, with chlorophytes being most tolerant, and cyanobacteria and dinoflagellates being more tolerant than diatoms or raphidophytes (Collos and Harrison 2014). Such a spectrum of responses is consistent with emerging understanding of the differences in C and N transport and assimilation in different functional groups relative to diatoms (Wilhelm et al. 2006 and references therein), including constitutive expression of HAT-AMTs in cyanobacteria, lack of LAT-AMTs in chlorophytes but more copies of NRTs in diatoms, and differences in downstream cycles such as photorespiration and the urea cycle.

In cyanobacteria, different abilities to take up and assimilate NO_3^- and NH_4^+ have been reported for cells that fix N_2 vs. those that do not (e.g., Flores and Herrero 1994). Even within the picoplankton cyanobacteria that do not include N_2-fixers, there is wide diversity in ability to use NO_3^- or NH_4^+ (Scanlan and Post 2008 and references therein). Some picocyanobacteria cannot take up NO_3^- at all (Moore et al. 2002; Rocap et al. 2003), while some can take up both NO_3^- and NO_2^- (Martiny et al. 2009). The NO_3^- transporters in the cyanobacteria are structurally and evolutionarily different from the NRTs of diatoms. Repression of NR and NiR by NH_4^+ in picocyanobacteria has been shown to be quite variable, being near complete in *Synechococcus elongatus*, but being comparatively insensitive in *Synechocystis* (Kobayashi et al. 2005).

Picoplankton may have another advantage over large eukaryotes in avoidance of effects of excess NH_4^+ accumulation in the cell: their small size. Cell size sets biophysical constraints on many aspects of physiology, including nutrient transport (e.g., Finkel et al. 2010 and references therein). Small cells have a greater rate of C assimilation per unit Rubisco, while a higher allocation of cell N to Rubisco in larger cells leads to a higher burden on N metabolism (Wu et al. 2014a). Thus, the metabolic cost of maintaining more Rubisco leads to a higher N requirement associated with light absorption and photosynthesis in larger cells (Wu et al. 2014a).

Such differences between diatoms and cyanobacteria are consistent with the evolutionary lineage of these two groups. It is generally accepted that Rubisco evolved when the CO_2/O_2 ratio in the atmosphere was higher than present proportions, and thus mechanisms to discriminate between the substrates was not necessary. Diatoms evolved over a period when O_2 levels were increasing (Young et al. 2012). In contrast, many cyanobacteria (including the freshwater N_2 fixers) evolved during a period when the Earth had little or no O_2, and conversely much higher CO_2 (e.g., Tabita et al. 2007) and therefore metabolism of oxidized forms of N would not have been required. However, both marine N_2-fixing and non N_2-fixing picocyanobacteria, including *Synechococcus* and *Prochlorococcus* evolved and diversified rather later among cyanobacteria when significant O_2 had been established (Sanchez-Baracaldo et al. 2014).

Diatoms have a complex endosymbiotic lineage and thus are a "melting pot of biochemical characteristics" (Rosenwasser et al. 2014, p. 2740). They "appear to have red

Table 3. General summary of the differences between major functional groups in terms of many mechanisms for N and C acquisition, metabolism, and energy and reductant dissipation. These characteristics are meant as generalities and general conditions under which such mechanisms may be observed and thus the known species-specific differences therein are not captured here. The size of the font indicates hypothesized relative importance of the process. See text and table of abbreviations for details.

N or C acquisition or fixation, energy and reductant dissipatory strategies	Diatoms	Cyanobacteria (focus on non-N_2 fixing picocyanobacteria)	Dinoflagellates	Chlorophytes
N transporters and enzymes; and metabolism	Proportionately more NRTs than AMTs and more than other functional groups and higher cell-specific rates of NR; fully functional urea cycle	Generally constitutive expression of HAT- AMTs; Structurally different NRTs if present; cell size constraints in picocyanobacteria	Variable; Generally mixotrophic (phagotrophic)	Generally lack LAT-AMTs
Form and activity of Rubisco	Form IB	Form IA&B; greater rate of C assimilation per Rubisco	Form II; less favorable for C fixation	Form IB
Mixotrophy (phagotrophy)	**NO**	**NO**	**YES**	**No**, primarily
Xanthophyll cycling	**YES** (diadoxanthin-diadinoxanthin)	no xanthophyll cycling, but zeaxanthin is the most common pigment	**YES** (diadoxanthin-diadinoxanthin)	**Yes** (alloxanthin-violaxanthin-zeaxanthin); cycle present but presumed to be less significant than in other algae
Mehler reaction (water-water cycling)	? conflicting reports	**YES**	**YES**	**YES** and associated high rates of H_2O_2 production
Dissipatory NO_3^-/NO_2^- reduction	**YES** largely under cool temperatures and high NO_3^- conditions	? likely to be minimal, but dependent on extent to which cells have NO_3^- pathways	**Yes**	? no known direct studies to date
Photorespiration	**YES** largely under warm temperatures and high NH_4^+ conditions	**Yes**	**YES**	**YES**

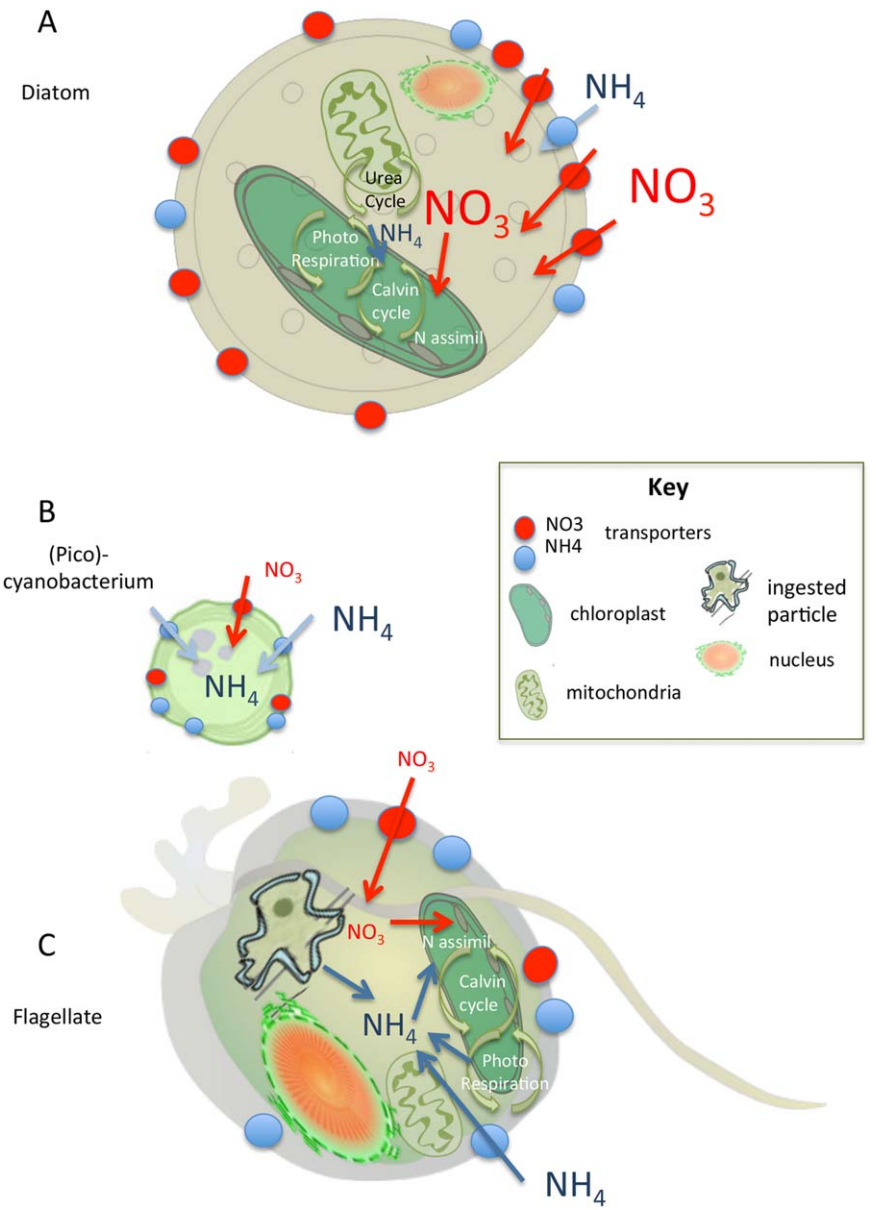
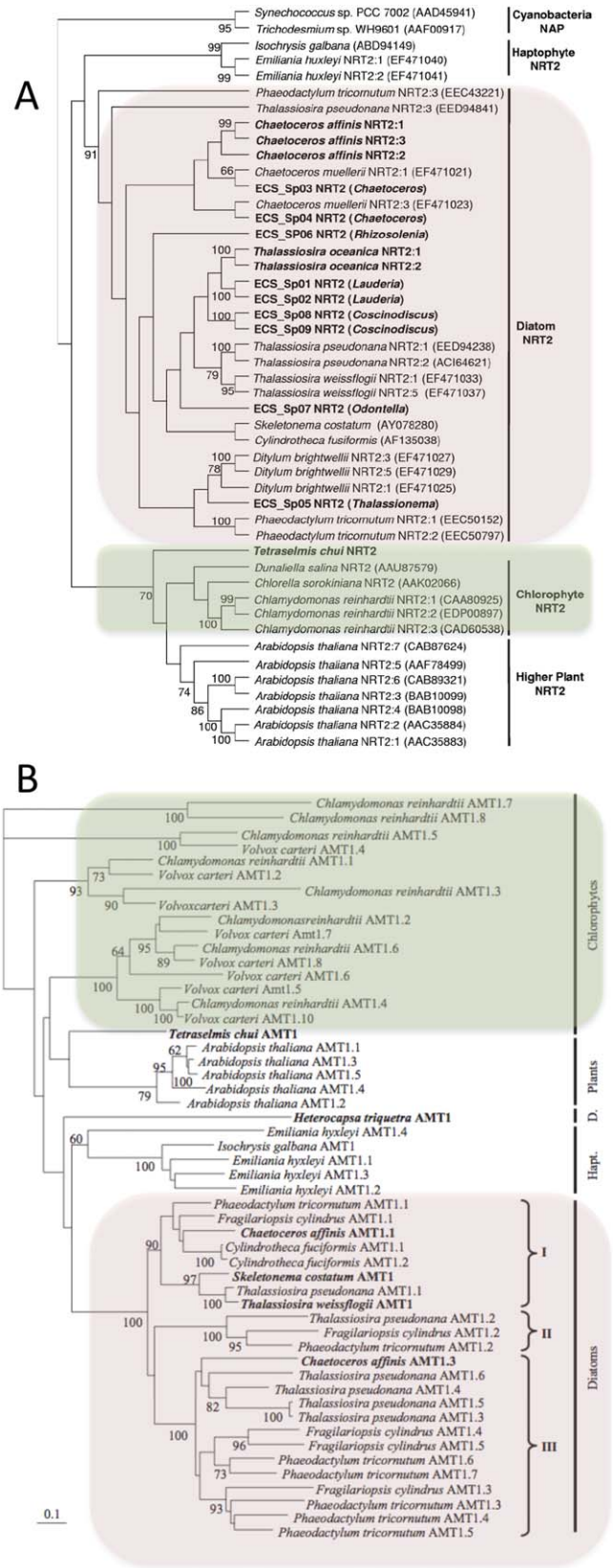


Fig. 12. Conceptual diagrams comparing N uptake and assimilation in a (A) diatom, (B) picocyanobacterium, and (C) dinoflagellate. Note that the diatom has proportionately more NO_3^- transporters than the other functional groups and has larger internal NO_3^- pools, the dinoflagellate may also consume particles through mixotrophic feeding providing another source of NH_4^+ for this type of cell via digestion. Arrows illustrating the flux of N with their respective transporters are only shown for a few transporters for clarity.

algal-derived chloroplasts empowered largely by green algal proteins, working alongside mitochondria derived from the non-photosynthetic symbiont" (Prihoda et al. 2012, p. 1543). These "red-algal line" specialists only emerged after the evolution of an oxidizing environment. The ability to use NO_3^- would have required not only the presence in the genome, and control of the expression of NO_3^- and NR, but that of synthesis of NRT in the plasma membrane. However, when this all occurred in evolutionary time is not known (Raven 1996).

Photosynthetic dinoflagellates represent an interesting contrast to diatoms (Table 3). While N metabolism in diatoms may be "unorthodox" (Prihoda et al. 2012, p. 1543), dinoflagellates have been termed "bizarre products of evolution" (Medlin and Fensome 2013, p. 263). One of the unique features of all dinoflagellates is their disproportionately large and unusual genome structure, and consequently there are a number of potentially novel regulatory mechanisms and processes (e.g., Hackett et al. 2004 and references therein). The plastids of the basal, peridinin-containing



dinoflagellates were derived by secondary endosymbiosis of red algal cells (Delwiche 1999; Hackett et al. 2004). Subsequent loss of the peridinin-containing plastids was followed, in some cases, by acquisition of replacements by tertiary endosymbiosis from a variety of oxygenic photosynthetic organisms from the green and the red lines of evolution (Hackett et al. 2004), leading to a large array of different types of plastids in dinoflagellates (Delwiche 1999).

Peridinin-containing dinoflagellates also have a distinctly different form of Rubisco from that found in other oxygenic organisms. Basal, peridinin-containing dinoflagellates have an apparent disadvantage in that their Rubisco is the so-called Form II (Morse et al. 1995). This form is novel among eukaryotic algae and was likely acquired from anaerobic proteobacteria via horizontal gene transfer. The kinetic properties of Form II Rubisco are less favorable for carboxylase activity than Forms IA and B Rubisco (cyanobacteria), Form IB (Chl *b* containing algae and higher plants) and Form ID (Whitney and Andrews 1998; Tcherkez et al. 2006; Marin et al. 2007) and this low CO_2: O_2 selectivity of the Form II Rubisco should be more favorable for photorespiration at the direct expense of photosynthesis. In fact, high rates of photorespiration have been measured or inferred at least in some dinoflagellate species (e.g., Burris 1977; Suggett et al. 2009). Higher rates of photorespiration may relate to the seeming preference of dinoflagellates for reduced relative to oxidized N forms.

The lower rate of C fixation in mixotrophic dinoflagellates may be more than compensated for by the gain of C and other metabolites through grazing. It is now recognized that virtually all photosynthetic algae (except diatoms and cyanobacteria) are mixotrophs, with the capability of digesting prey, whether or not they maintain the ability to

Fig. 13. (A) Phylogenetic tree of NO_3^- transporter (NRT2) sequences in cyanobacteria, eukaryotic phytoplankton, and higher plants. Results indicated that NRT2 sequences belonging to cyanobacteria, haptophytes, chlorophytes, and diatoms formed four distinctive clades at the phylum level. (B) Phylogenetic tree of NH_4^+ transporter (AMT1) sequences in eukaryotic phytoplankton (diatoms, Hapt-haptophytes, D-dinoflagellates and chlorophytes) and higher plants. Results show that AMT1 sequences of higher plants were most closely related to those in chlorophytes and that haptophyte and diatom AMT1s formed distinct monophyletic clades. Diatom AMT1s were further divided into three orthologous subclasses. In both trees the diatoms are highlighted in a pink box and the chlorophytes in a green box to accentuate the differences. Note that only the lower tree includes dinoflagellates. In both figures, numbers at the nodes are bootstrap values based on 1000 resamplings, and only values of $>60\%$ are shown and bold font represents the sequences obtained in the studies from which these figures were reproduced. The scale bar represents an estimated number of amino acid substitutions per position. GenBank accession numbers for panel A are provided in parentheses and in panel B are provided in the original paper. Reproduced from Kang et al. (2011-panel A), and from Kang and Chang (2014-panel B) with permission of the American Society for Microbiology (A) and the J. Mar. Sci. Technol. (Taiwan).

photosynthesize (e.g., Burkholder et al. 2008; Flynn et al. 2013). In fact, the net growth rate of many dinoflagellates is higher when they are growing as mixotrophs than when growing as strict phototrophs (e.g., Adolf et al. 2008; Burkholder et al. 2008; Glibert et al. 2009; Jeong et al. 2010). Nutrition in mixotrophs is far more complex than assimilation of the major inorganic N ions (e.g., Mitra and Flynn 2010; Flynn et al. 2013). The dynamics of feeding and digestion can be likened to that in a consumer with a gut, and that includes considerable cycling of metabolites, including NH_4^+, produced during digestion. Digestion is a complex process involving many N-assimilating enzymes, including ureases, hydrolases, peptidases, and amino-transferases (Dagenais-Bellefueille and Morse 2013). Regulation of NH_4^+ transporters is thus not only a function of substrate availability resulting from external supply, but also the extent of internal metabolic pathways that are NH_4^+-generating, namely photorespiration and the extent of mixotrophic nutrition.

Proposed mechanisms of growth suppression in HNLG systems

The mechanisms of cellular energy and redox balance described herein suggest a suite of potential responses by phytoplankton to NH_4^+ and NO_3^-, depending on whether the cells are N-deficient or N-sufficient, the amount of each substrate provided, ambient environmental conditions, the taxonomic group, and specific metabolic adaptations. While NH_4^+ may be preferentially taken up at the low end of the substrate availability spectrum when cells are N deficient, and may even provide a growth advantage, as NH_4^+ availability increases, and as its availability increases in proportion to NO_3^-, the potential for growth suppression, and its "paradoxical" impact increase (e.g., Fig. 5).

With increasing NH_4^+ loads, declines in productivity, especially of diatoms as seen for HNLG estuaries (Yoshiyama and Sharp 2006), appear to be related to the failure of alternate electron pathways to balance C and N metabolism and redox (again, particularly in diatoms), leading to growth suppression. As described herein, there are several ways in which elevated NH_4^+ can lead to growth suppression. In addition to NH_4^+ repression of NO_3^- uptake and assimilation, NH_4^+ may differentially affect oxidation and reduction of the chloroplastic metabolic pathways, leading to enhanced photorespiration or increased ROS. Importantly, suppression of growth can occur without direct or lethal NH_4^+ toxicity. The wide array of redox sensitive pathways, proteins, and enzymes can affect processes such as photosynthesis, biosynthesis, antioxidant activity, and signaling pathway translation among other metabolic activities (Rosenwasser et al. 2014). Reduced growth by phytoplankton, especially that of diatoms, in cool waters increasingly enriched with NH_4^+ may arise because of a failure of NR- and NiR-related dissipatory pathways, and enhanced photorespiration at the expense of other pathways of excess energy dissipation. Photorespiration, while serving an important function in cellular energy balance and in reduction of photoinhibitory damage, is enhanced with excess NH_4^+ supply leading to negative feedbacks that can ultimately lead to suppression of growth. The very metabolic dynamics that make diatoms highly productive in turbulent and NO_3^--enriched environments (upwelling, spring blooms) may make them uniquely susceptible to growth suppression when the interwoven, and normally fine-tuned, regulation of light harvesting, C fixation, N assimilation, and photorespiration become imbalanced and uncoupled.

There is thus a dichotomy in use of oxidized vs. reduced N substrates and in sensitivity or tolerance to excess NH_4^+ by different phytoplankton functional groups (Table 3; Fig. 14) as is the case in terrestrial plants (Britto and Kronzucker 2013; Podgórska and Szal 2015 and references therein). Phytoplankton community structure is mirrored by a suite of interconnected ratios of transporters, enzymes, regulator proteins, and synthesis and dissipatory pathways inside the cell. The extent to which various constituents and pathways are expressed by different species may impart advantages depending on the availability of substrate, from limiting to supersaturating in conjunction with other regulating environmental factors, especially temperature and light. In turn, these different phytoplankton groups may ultimately support different food webs (e.g., Eppley and Peterson 1979; Legendre and Rassoulzadegan 1995; Glibert 1998). It is the fundamental differences between functional groups or species—in active transport mechanisms, cell N assimilation genes, and pathways—that provide the mechanisms for ecological competition and for the underpinnings of the concept of "new" and "regenerated" production (Dugdale and Goering 1967). These relationships not only hold at the limiting end of the substrate spectrum but also at the substrate saturated to super-saturated end.

Implications and conclusions

There clearly is much to be learned about the physiological response by different functional groups to excess nutrient supply, especially reduced forms of N. More work is needed at the "excess scale," i.e., substrate saturation as a "stress" (Glibert et al. 2013), and under conditions in which both nutrient and energy stresses are superimposed. With the expansion of eutrophication, many coastal, estuarine and inland waters now have nutrient loads and concentrations that exceed those of "saturation" and can be thought of as "super-saturating." And, with eutrophication and climate change, the proportion of N nutrient forms is changing in many marine and freshwater systems. Culture studies that go beyond steady state (and growth on NO_3^- as the sole medium) and that expose cells to potentially stressful light and temperature conditions, or other conditions more

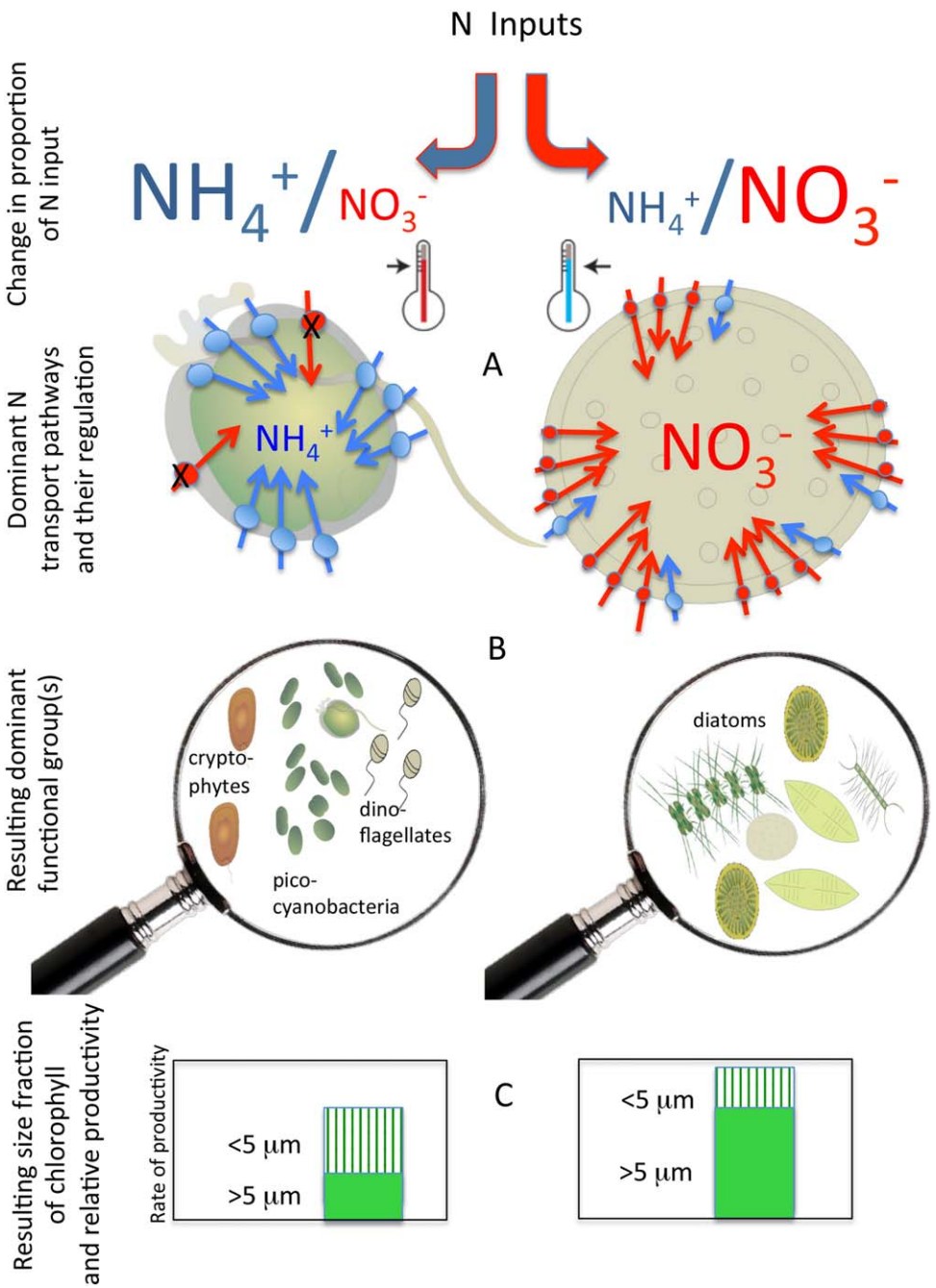


Fig. 14. Summary conceptual schematic illustrating the effect of changes in the proportion of NH_4^+ and NO_3^- in the loads of N provided to a natural system. When NH_4^+ is the dominant form, and when waters are warmer, flagellates, cyanobacteria, and chlorophytes among other classes may proliferate, leading to overall productivity dominated by the small size class of algae (e.g., < 5 μm). In contrast, when NO_3^- is the dominant form provided, especially under cooler water conditions, diatoms more likely dominate, and the overall production will be more likely dominated by cells of a larger size class (e.g., > 5 μm). Moreover, Chl *a* yield and total production may be higher than under the NH_4^+ enrichment condition.

representative of the dynamic and changing conditions of natural, N-enriched waters are needed to fully disentangle the complexities of effects of N form at all growth conditions. Relatedly, care must be taken in applying appropriate methods for understanding physiological regulation of N nutrition. If the pathways of inhibition or repression are downstream of the light reactions of photosynthesis, then metabolic down-regulation may occur even in the absence of measured differences in electron or nutrient transport. Progress has been significant, especially with molecular approaches, but much ecophysiological work remains. Genome sequencing and localization predictions based on

targeting sequences have provided much needed information about the putative protein complements in different marine microalgae, yet the subcellular localization of many of the key proteins are yet to be fully described, and the resulting conceptual subcellular metabolic models need further elucidation and confirmation.

Knowing that many HAB species (or, in some cases, suitable food for mixotrophic HABs)—and their metabolic products (including toxins) are disproportionately favored when N is in excess or when chemically reduced forms of N are available should be further motivation to accelerate this line of inquiry and to incorporate consideration of the redox form of N in management considerations. As anthropogenic N supply continues to trend in the direction of increasing concentrations of chemically reduced forms of N, an understanding of the adaptations of different functional groups of algae to varying N forms becomes ever more important.

The ecological effects of NH_4^+ loading and the importance of changes in NO_3^-: NH_4^+ in phytoplankton succession also have important implications for nutrient criteria development, as criteria are largely based on total N or P and total biomass measures such as Chl *a* (e.g., Bricker et al. 2007; Harding et al. 2014). Such an un-nuanced view fails to recognize that the excess of N loading, its redox state, and stoichiometric imbalances of C, N, and P have consequences for not just the quantity, but also the quality, of primary producers and ultimately for higher trophic levels—and such relationships are modified by the interplay of multiple growth factors.

The importance of N form has begun to be more systematically incorporated in ecosystem models, but as nutrient (especially NH_4^+) environmental loads increase, the need for both appropriate physiological data and model parameterization increase accordingly. Direct inhibitory terms relating external or internal nutrient concentrations to differential transport rates have been applied in various models for some time (e.g., Collos 1989; Parker 1993; Flynn et al. 1997). Recently, Follows et al. (2007), among others, have incorporated terms describing NH_4^+ repression of NO_3^- uptake in marine ecosystem models that simulate global phytoplankton community structure. Dugdale et al. (2013), in an estuarine model, were able to correctly predict whether spring phytoplankton blooms develop in Suisun Bay, California, based on only a minimum number of parameters and processes including inhibition/repression kinetics of NO_3^- by NH_4^+. Model approaches from the cellular to global scale have thus shown the importance of inclusion of inhibitory, not just assimilatory terms for N, but more efforts to include these terms in ecosystem models must be made.

In answering the seemingly simple question posed at the beginning of this review, yes, differences in productivity and ultimately species composition should result when the form of N changes; NH_4^+ at environmentally relevant concentrations, for increasingly N-enriched systems, has profound effects on metabolism and growth, effects which lie at the metabolic level and that are not necessarily a function of direct toxicity. Yes, there is a physiological basis for our understanding of "new" and "regenerated" production and the differing phytoplankton communities they support. And, yes, such differences may be more pronounced under natural, dynamic, and otherwise stressful conditions than under conditions of balanced, acclimated and steady-state growth.

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