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 NH₄⁺. Long thought to be the preferred form of N for phytoplankton uptake, NH₄⁺ may actually suppress overall growth when concentrations are sufficiently high. NH₄⁺ has been well known to be inhibitory or repressive for NO₃⁻ uptake and assimilation, but the concentrations of NH₄⁺ that promote vs. repress NO₃⁻ 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 NH₄⁺ and NO₃⁻ 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 NO₃⁻/NO₂⁻ reduction to NH₄⁺ and photorespiration that may lead to growth suppression are highlighted. While more is known about diatoms, taxon-specific differences in NH₄⁺ and NO₃⁻ 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 Pae rl 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).
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

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**Table 1.** Abbreviations used although this article.

| Abbreviation | Definition |
|--------------|------------|
| 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-biphosphatocarboxylase/oxygenase |

**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).
an increase in the availability of NH$_4^+$ in near surface oceanic waters (e.g., Huesemann 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 (Gilbert et al. 2014a,b,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 Nieuwenhuiize 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 have also been reported in tributaries of the Chesapeake Bay (Lomas et al. 2002; Gilbert et al. 2005), nearshore waters adjacent to the heavily fertilized Yaqui Valley, Mexico (Gilbert 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.

**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 Gilbert (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) |
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 $\mu$M NH$_4^+$ resulted in cessation of NO$_3^-$ uptake by diatom-dominated assemblages, while subsequent depletion of NH$_4^+$ concentrations to <$4$ $\mu$M 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 $\mu$M 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 $\mu$M. 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 a 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$_3^-$] is 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 Gilbert 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; Gilbert 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 Gilbert 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 Gilbert 1999a,b). Diatoms appear to be NO$_3^-$ opportunist. 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 Gilbert 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; Gilbert 1998; Gilbert 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 Gilbert and
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 Okeechobee, 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
to latter successional species (Britto and Kronzucker 2002 and references therein).

**From NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+}, typically exceeding several tens to hundreds of\(\mu\)M, both the total N taken up and overall growth with NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} than on NO\textsubscript{3}\textsuperscript{−} (e.g., Raven et al. 1992; Britto and Kronzucker 2002 and references therein). Examples of growth suppression by NH\textsubscript{4}\textsuperscript{+} enrichment are numerous. Total N productivity was found to decrease in the Chesapeake Bay as the proportion of NH\textsubscript{4}\textsuperscript{+} increased when all samples received the identical total N enrichment, 30\(\mu\)M (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\textsubscript{4}\textsuperscript{+} is the preferred N form and that total N uptake and growth on NH\textsubscript{4}\textsuperscript{+} is the same or exceeds that on NO\textsubscript{3}\textsuperscript{−} 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\textsubscript{4}\textsuperscript{+} concentration (most of the change occurring at < 10\(\mu\)M NH\textsubscript{4}\textsuperscript{+}) 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\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} syndrome” (Gerend\textasciitilde/C19 as et al. 1997; Britto and Kronzucker 2013). At very elevated concentrations, normally exceeding several hundred\(\mu\)M, NH\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} 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\textsubscript{4}\textsuperscript{+} have been mostly associated with increased NH\textsubscript{4}\textsuperscript{+} at levels not normally considered to be toxic for phytoplankton growth, i.e., at levels in the tens of\(\mu\)M 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\textsubscript{4}\textsuperscript{+}
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\textsubscript{3} repression by NH\textsubscript{4} can be alleviated is through the addition of NO\textsubscript{3} (Goyal et al. 1982; Below and Gentry 1987; Britto and Kronzucker 2002, among others), implying that NH\textsubscript{4} repression of NO\textsubscript{3} uptake and reduction is not necessarily or always absolute. It is also known that co-provision of both NO\textsubscript{3} and NH\textsubscript{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\textsubscript{4} is in excess (Escobar et al. 2006; Podgórskas and Szal 2015). Importantly, sensitivity to NH\textsubscript{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\textsubscript{3} therein will be revisited later in this article.

**The need for a reassessment of N preference, with a focus on NH\textsubscript{4} -enriched conditions**

With N in many environments tending toward increasing enrichment, together with the observations of dichotomous phytoplankton communities typically developing on NH\textsubscript{4} vs. NO\textsubscript{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\textsubscript{4} vs. NO\textsubscript{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\textsubscript{4}, we start by briefly reviewing N metabolism in a “generic” eukaryotic cell. We contrast the metabolic pathways and regulation of NH\textsubscript{4} and NO\textsubscript{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\textsubscript{3} and NH\textsubscript{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\textsubscript{4} and NO\textsubscript{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\textsubscript{4} is transported into the cell, a cation is transported, whereas for NO\textsubscript{3} transport, an anion is transported. Assuming all other processes equal, this will lead to the cytosol being more acidic following NH\textsubscript{4} assimilation and more basic after NO\textsubscript{3} assimilation, with resulting effects on redox reactions within the cell (Raven 2013 and references therein). With a redox state of −3 for NH\textsubscript{4} and +5 for NO\textsubscript{3}, it takes eight electrons to...
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 Gilbert 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., Gilbert 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 Zyvyaginskaia 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 synthetase (GS) and glutamate synthase (GOGAT; also known as glutamine-2-oxoglutarate aminotransferase, Fig. 6). This pathway yields Glu, the product of Glu 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 chloroplastic 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 chloroplastic N metabolism is the important pathway of reduction of oxidized N (Takabayashi et al. 2005). In fact, GSII in the diatom *Skeletomena 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\textsubscript{3} transport and assimilation are generally repressed by NH\textsubscript{4} (or its assimilation products), NH\textsubscript{4} transport and assimilation are usually unaffected by the presence of NO\textsubscript{3} (Clarkson and Luttgte 1991; Navarro et al. 1996; Flynn and Fasham 1997; Flynn et al. 1997; Crawford and Glass 1998; Daniel-Vedele et al. 1998). NH\textsubscript{4} affects NO\textsubscript{3} metabolism by down-regulation of transport of NO\textsubscript{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\textsubscript{3} reducing capacity in the cell; when Gln levels are low, and when NO\textsubscript{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\textsubscript{3} unless their internal N status is sufficiently low. As the supply of NH\textsubscript{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\textsubscript{3} is up-regulated. AMTs in some species are up-regulated by the depletion of NO\textsubscript{3}, but the inverse relationship does not appear to be the case; that is they are not down-regulated by the prevalence of NO\textsubscript{3} (e.g., Hockin et al. 2012).

The availability of NH\textsubscript{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\textsubscript{4} and the metabolite 2-OH (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\textsubscript{4} (Post et al. 2012). It is of note that NH\textsubscript{4} generally does not accumulate in the cell to the same extent as NO\textsubscript{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\textsubscript{4}. Thus, the degree of phytoplankton uptake and growth repression and suppression conditions by a given amount of NH\textsubscript{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\textsubscript{3} or NH\textsubscript{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\textsubscript{4} on NO\textsubscript{3} reduction. At least for the diatom Thalassiosira weissflogii, the membrane-bound form of the enzyme is seemingly not repressible by NH\textsubscript{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\textsubscript{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\textsubscript{4} and NO\textsubscript{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\textsubscript{4} to NO\textsubscript{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\textsubscript{4} and NO\textsubscript{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
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\textsubscript{3} vs. NH\textsubscript{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 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 H2O2 as a result (e.g., Collién et al. 1995). The presence of a chloroplastic 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 NH4+ growth compared with NO3−-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 (Kana 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 NH4+ (tens of μM) compared with similarly treated algae but with NO3− 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 NH4+ as a stress. Because N assimilation is an important sink for reducing power (through reduced Fd and NAD(P)H), the addition of NO3 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 NO3 and NO2 via NR and NiR in a nonassimilatory mode that complements such reduction in N assimilation (e.g., Lomas and Glibert 1999a,b; Parker and Armbust 2005; Kamp et al. 2011; Rosenwasser et al. 2014; Fig. 8). The reduction of NO3 to NH4 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 NO2 by diatoms has been commonly observed during NO3− uptake (e.g., Anderson and Roels 1981; Collos 1982), and there are also numerous reports of release of NH4+, as well as release of DON from both field and laboratory cultures using NO3− (Lomas et al. 2000 and references therein). Dissimilatory NO3 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 NO3− or NO2 in the cell. Without these substrates the options for redox homeostasis are limited, and that via dissimilatory NO3− reduction is absent.

Arguably one the most important reactions in cellular redox homeostasis is photorespiration, initiated by O2 consumption via the oxygenase reaction of the enzyme that also catalyzes the fixation of CO2 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 CO2 and O2 (Fig. 8); it has been characterized as “hamstrung by slow catalysis and confusion between CO2 and O2 as substrates, an “abominably perplexing’ puzzle” (Tcherkez et al. 2006, p. 7246). Photorespiration has been considered as an evolutionary holdover from a time when CO2 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” (Peterhansel 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 O2, and high temperature—all factors that favor the oxygenation: carboxylation ratio of Rubisco (Kangasjärvi et al. 2012). Importantly, when growth is on NH4+ rather than NO3−, photorespiratory responses to other stress, such as high light and cold temperatures, can increase significantly. When NO3 is comparatively unavailable to the cell, the sink for NADPH consumption via NR-catalysed NO3 reduction is not available, and photorespiration becomes the alternative electron sink (Keys and Leegood 2004; Guo et al. 2007a,b; Nunes-Nesi
been carried out on C3 flowering plants that rely on diffusive interactions of photorespiration with N metabolism has (Parker and Armbrust 2005; Shi et al. 2015; Fig. 9). When cells were shifted to higher light (Parker et al. 2004; 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 NH4+, rather than NO3−, 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 C3 flowering plants that rely on diffusive CO2 entry. CO2 concentrating mechanisms (CCMs) are necessary in algae because pCO2 concentrations in natural waters, especially seawater, are too low to saturate Rubisco carboxylase with diffusion alone; they serve to increase the concentration of CO2 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 NH4+ availability contributes to a change in the carboxylase and oxygenase activity at the site of Rubisco. For at least some diatoms (and C4-like or C3–C4 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 NO3− availability compared with NH4+ availability (Guo et al. 2007a,b). Therefore, at a constant inorganic C supply, the affinity for CO2 increases when NO3− 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 NH4+, the affinity of PEPCase for CO2 is less, resulting in lower CO2 at the site of Rubisco, and photorespiration correspondingly may increase. Quite simply, the various balances between the enzymes PEPCase, other CCM enzymes, NH4+ and NO3− transporters, NR and GS/GOGAT all may change under NO3− vs. NH4+ 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 NH4+ nutrition compared with NO3− 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 NH4+ 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 NO3− vs. NH4+ growth. It has been shown that a supplement
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 putrescine 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, Podgorska and Szal 2015 and references therein). ROS include not only hydroxyl radical HO•, superoxide anion O$_2^-$, H$_2$O$_2$, and singlet oxygen $^1$O$_2$, 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 Cladophora, 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 (Podgorska 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 Armburst 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
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$_3^-$ and NO$_2^-$ . 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; Vézí 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 (Neiland 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., 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-N₂ 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 IAT-AMTs |
| Form and activity of Rubisco | Form IB | Form I & 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 H₂O₂ production |
| Dissipatory NO₃/NO₂ reduction | YES largely under cool temperatures and high NO₃ conditions | ? likely to be minimal, but dependent on extent to which cells have NO₃ pathways | Yes | ? no known direct studies to date |
| Photorespiration | YES largely under warm temperatures and high NH₄⁺ conditions | YES | YES | YES |
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 CO₂:O₂ 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
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; Podgór ska 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., Eppl ey 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
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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