Aquatic Botany 118 (2014) 45–61

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Nitrogen and sulfur assimilation in plants and algae

Mario Giordano [a,b,*], John A. Raven [c]

[a] Laboratorio di Fisiologia delle Alghe e delle Piante, Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy
[b] Institute of Microbiology ASCR, Algatech, Trebon 37981, Czech Republic
[c] Division of Plant Sciences, University of Dundee at TJHI, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 18 June 2014
Accepted 22 June 2014

Keywords:
Nutrient
Nitrogen
Sulfur
Uptake
Assimilation
Signaling

ABSTRACT

Nitrogen and sulfur are abundant constituents of plant and algal cells that are assimilated at the lowest oxidation number, as NH_4^+ and S^{2-}, although they can (in the case of sulfur, usually must) be acquired with their highest oxidation number, as NO_3^- and SO_4^{2-}. Some occasional differences and variants exists for transport and assimilation systems; the greatest differences in the way vascular plants and algae use N and S, however, most probably resides in regulation. For instance, nitrate assimilation in plants is strongly regulated by phosphorylation. In algae, redox regulation appears to be more important. Similarly, sulfate reduction has its main control step at the level of APS reductase in higher plants, whereas in algae a redox regulation has been recently been hypothesized for ATP sulfurylase, the first step in sulfate assimilation. Unfortunately, the information on the regulation of N and S acquisition and assimilation is limited to very few species (e.g. *Chlamydomonas reinhardtii*, *Arabidopsis thaliana*) this is especially true in the case of sulfur. This review attempts to highlight the points of divergence in N and S utilization by plants and algae, leaving aside the biochemical details and the features that do not show any obvious difference.

1. Objectives

In this review we attempt to provide an overall assessment of N and S assimilation in algae and plants. The task is not a simple one, given the patchiness of the information and the fact that most studies refer to few model organisms. Numerous reviews exist for nitrogen and sulfur metabolism in higher plants. Much less was published for algae (possibly with the exception of nitrogen metabolism in *Chlamydomonas reinhardtii*, e.g. Fernandez et al., 2009). For this reason we decided to put somewhat more emphasis on algae, while however always comparing them with the embryophytes. We integrated the biochemical and molecular information with ecological and evolutionary concepts; the latter aspects have often been disregarded in previous reviews, yet they are inextricably intertwined with phylogeny, gene expression and metabolic regulation and decisively concur to similarities and differences in the way photosynthetic organisms deal with N and S.

2. Sulfur and Nitrogen in plants and algae

Average stoichiometries of plants and algae show that N and S are among the most abundant component of photosynthetic cells (Giordano, 2013). The cost of the assimilation of N (especially) and S estimated on these stoichiometries is not trivial (Table 1). Under energy limitation, competition may occur among the intricately interconnected C, N and S assimilation pathways (Ruan, 2013; Fig. 1) and the extent of such competition may depend on the flexibility of cell stoichiometry, on the availability of these elements in the environment and on the degree by which their acquisition and assimilation can be modulated. The cost of the assimilation process increases for assimilation in shoots of vascular land plants, where nitrate and – especially – sulfate are commonly assimilated. The assimilation of nitrate and sulfate generates OH^- (Raven and Smith, 1976; Raven, 1986; Andrews et al., 2013). Although the OH^- generated in the roots is mostly released into the soil, most of the OH^- that is produced as a consequence of shoot nitrate and sulfate assimilation is retained within the plants and must be neutralized by the production of organic acids (Raven and Smith, 1976; Andrews et al., 2009, 2013). The energy cost of producing the OH^--neutralizing organic acids outweighs energy saving from the more direct use of photosynthetically generated reductants with oxyanion reduction restricted to the photoperiod. Getting rid of excess acid produced as a consequence of ammonium and

* Corresponding author at: Corresponding author at: Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Laboratorio di Fisiologia delle Alghe e delle Piante, Via Brecce Bianche, 60131 Ancona, Italy.
Tel.: +39 071 220 4652; fax: +39 071 220 4650.
E-mail address: m.giordano@univpm.it (M. Giordano).

http://dx.doi.org/10.1016/j.aquabot.2014.06.012
0304-3770/

Table 1
Estimate of costs for the assimilation of CO_2 into triose-P, nitrate into glutamate, sulfate into cysteine, for phytoplankton and for the shoot of herbaceous flowering terrestrial plants (herbaceous plants). The costs were estimated based on the ATP and electrons used in the most common assimilation pathway. The hydrolysis of 1 ATP was assumed to yield 55 kJ mol^{-1} and the transfer of $2e^-$ was assumed to correspond to 4 ATP equivalents. The average stoichiometries used for the calculations are those in Giordano (2013).

	Cost of the assimilation of 1 mole of CO_2, NO_3^- or SO_4^{2-}		Cost of CO_2, NO_3^- or SO_4^{2} assimilation in kJ according to average elemental stoichiometry, assuming $P=1$	
	ATP equivalents	kJ mol^{-1}	Marine phytoplankton	Herbaceous plants
$CO_2 \rightarrow CH_2OP$	11	605	75,020	87,120
$NO_3^- \rightarrow$ Glu	41	2255	36,080 (48% of C assimilation)	38,335 (44% of C assimilation)
$SO_4^{2-} \rightarrow$ Cys	33	1815	2360 (3% of C assimilation)	1561 (1.8% of C assimilation)

dinitrogen assimilation is much more difficult and it is restricted to roots, where ammonium or N_2 can be assimilated with excretion of protons to the rooting medium (Raven and Smith, 1976; Raven, 1986; Andrews et al., 2009, 2013).

N and S are both essential components of catalysts and intermediates of primary metabolism; both are found in amino-acids and hence proteins, in nucletotides (including ADP/ATP and $NAD(P)^+$/NAD(P)H) and hence in nucleic acids, in vitamins (N and S) and, in phototrophs, chlorophylls and phycobilin chromophores (N). Growth substances such as indoleacetic acid and cytokinins (N) are also essential to development in embryophytes and some algae. Both N and S are also involved in response mechanisms to changes in environmental conditions, both abiotic and biotic. These responses include qualitative and quantitative changes in the transcriptome (N) and proteome (N and S), in osmolites such us glycine betaine (N) and dimethylsulfoniopropionate (S), in growth substances such as auxin and cytokinins (N), in compounds like glutathione (N and S) involved in the defence against oxidative stress, in heavy metals chelators such as metallothioneins and phytochelatins (N and S), and in compounds defending

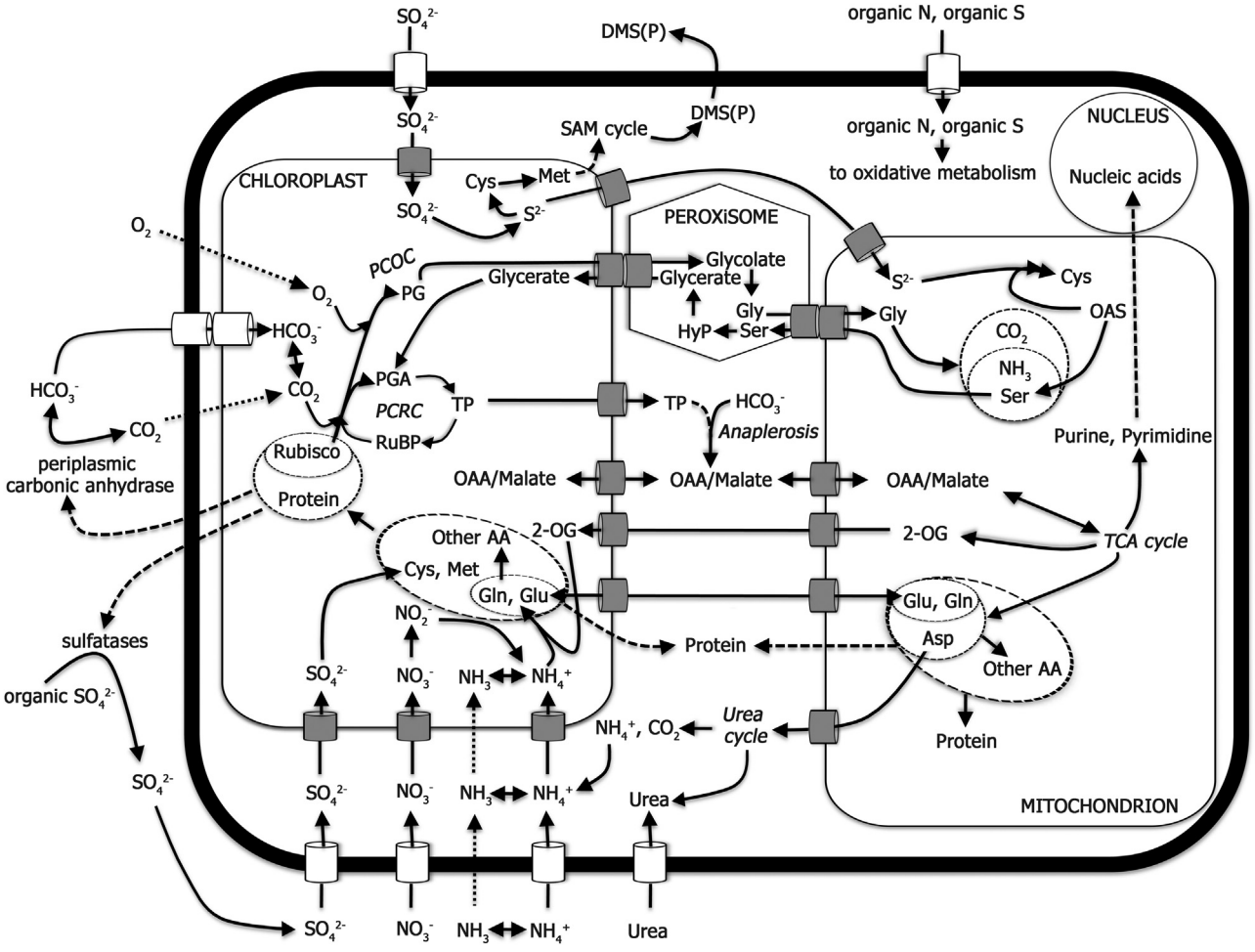

Fig. 1. Main interaction among C, N and S metabolism in algae and plants. Solid lines indicate known pathways. Dashed lines indicate pathways that are either not entirely known or are not shown in details in this figure. The dotted lines show the diffusive path of CO_2 and NH_3. Metabolic processes (e.g. anaplerosis, PCRC, PCOC) are in italics. Abbreviations: 2-OG, 2-oxoglutarate; AA, amino acids; Asp, aspartate; Cys, cysteine; DMS(P), dimethylsulfide (dimethylsufoniopropionate); Met, methionine; Gln, glutamine; Glu, glutamate; Gly, glycine; HyP, hydroxypyruvate; OAA, oxalacetate; OAS, *O*-acetylserine; PCOC, photosyntehic carbon oxidative cycle (photorespiration); PCRC, photosynthetic carbon reductive cycle (photosynthesis); PG, 2-phosphoglycolate; PGA, 3-phosphoglycerate; Rubisco, ribulose bisphosphate carboxylase/oxygenase; RuBP, Ribulose 1,5-bisphosphate; SAM. *S*-adenosilmethionine; Ser, serine; TP, triosephosphate.

the organisms against biophages, both parasites and grazers (N and S) (Wium-Andersen et al., 1982; Berger and Schager, 2004; Cooper and Williams, 2004; Rausch and Wachter, 2005; Gonzalez-Ballester and Grossman, 2009; Andrews et al., 2013 and references there in). The requirement for these acclimation responses to changes in the environment may bear on the demand for N and S.

Nitrogen is often the proximate limiting nutrient on land (Masclaux-Daubresse et al., 2010) as it is in the sea (Falkowski, 1997), whereas it is more rarely controlling growth in freshwaters (Maberly et al., 2002; Giordano et al., 2005b,c; Elser et al., 2007). Sulfur deficiency occasionally restricts growth of terrestrial plants (Andrew et al., 1952), especially in intensive cultivation of higher yield crops where N-fertilizers are used, and after SO_2 emission have been reduced by the 1979 Oslo protocol (Hell and Hillebrand, 2001 and references therein). In today's aquatic environments, S limitation is rare (Giordano et al., 2005c; Norici et al., 2005), although this has most likely not always been the case, since S concentration have been increasing (although not monotonically) in the ocean over the long term to reach a maximum after the Mesozoic era (Ratti et al., 2011). However, the Oslo protocol caused a decrease of sulfate also in freshwaters of Europe and, to a minor extent, of North America (Giordano et al., 2005c); hence, hints of S being limiting or nearly limiting have emerged in some Cumbrian (Maberly and Giordano, unpublished) and African lakes (Moss, 1969). Evidence of codon bias sparing S, but not N use in the highly expressed ribosomal proteins of bacteria, including freshwater and marine cyanobacteria (Bragg et al., 2012), is consistent with S rather than N deficiency having been more significant in the evolution of these organisms, but is also consistent with explanations related to ribosome function rather than elemental availability. The environmental absolute and relative (to other nutrients) availability of N and S is rather different in freshwater, marine and terrestrial environment (Elser and Hassett, 1994; Giordano et al., 2005c). This, in combination with an overall similar elemental stoichiometry of photosynthetic organisms (Giordano, 2013), may be at the origin of differences in the N and S metabolism of terrestrial, marine and freshwater organisms. The greater availability of S in the ocean, where S is usually one of two orders of magnitude more abundant than in freshwater (Giordano et al., 2005c), for instance, decreases the need for extracellular sulfatases (yet arylsulfatase are present in the genomes of many marine algae; http://www.ncbi.nlm.nih.gov/gene/?term=arylsulfatase+algae) and for high affinity transporters (which are expressed in *Chlamydomonas*, but apparently not in the halotolerant volvocalean *Dunaliella salina*; Gonzalez-Ballester and Grossman, 2009; Weiss et al., 2001). The ability of different species to deal with low sulfate, at least to some extent, appears to be linked to the evolutionary history of clades, so that species that emerged at a time when S was less abundant in the ocean and that now are mostly represented in freshwaters are better equipped to adjust to growth at low S than clades that rose to dominance in an already sulfate rich ocean, which instead are often unable to grow at sulfate concentrations below a few mmol L^{-1} (Giordano et al., 2000; Ratti et al., 2011, 2013). Frequent combined N limitations of primary production in the ocean may be a result of Fe or P limitation of diazotrophy (Raven, 2012, 2013a,b).

In plants, NO_3^- is also used as an osmoticum and it can be accumulated in vacuoles up to 100 mM for the generation and control of turgor (Andrews et al., 2005; Waraich et al., 2011). Nitrate vacuolar accumulation has also been proposed for some intertidal macroalgae; in some *Laminaria* and *Fucus* species, for instance, a nitrate internal concentration more than 10-fold higher than in the environment can be found in the winter, when growth is slower; this stored nitrate is then depleted in spring/summer (Young et al., 2007).

Sulfate accumulation and storage is also known to occur in the vacuoles of vascular plants, with efflux systems, located on the tonoplast, that control the mobilization of this resource (Buchner et al., 2004 and references therein). In algal cells, S compounds may be used for osmotic control (Keller et al., 1999; Giordano et al., 2005c). This role (among many others) has been proposed for DMSP, which in some algae constitutes the largest S pool in the cell (Stefels, 2000; Giordano et al., 2005c; Ratti and Giordano, 2008). The production of DMSP is stimulated by N limitation (Stefels, 2000 and references therein); this led to the hypothesis that, when oxygenation of surface waters made sulfate abundant and, concomitantly, N assimilation more difficult (see below), DMSP constituted a good alternative to N-based osmolites (Keller et al., 1999; Giordano et al., 2005c). The ability to produce DMSP may have thus represented an adaptive advantage, in N-limited oceans. DMSP may also act as storage for S and C in the cell, to ensure that an appropriate stoichiometry with N is maintained, when N is limiting (Stefels, 2000). Terrestrial plants have mostly lost the ability to produce DMSP; only some angiosperms belonging to the unrelated genera *Saccharum*, *Spartina* and *Wollastonia* re-acquired it, but with biosynthetic pathways that are different from those of algae (Stefels, 2000 and references therein). Some marine macroalgae have sulfate as the main anion (at least in terms of negative charge) in the vacuole, invariably in combination with protons as the main cations. This vacuolar composition is found in some species of the Desmarestiales and Dictyotales (Phaeophyceae: Fucophyceae) and the Plocamiales (Rhodophyta: Florideophyceae) (McClintock et al., 1982; Sasaki et al., 2002; Yano et al., 2004). The reason for these very acidic vacuoles with their very high sulfate concentration is not clear. Defence against grazers as a result of the very low pH is a distinct possibility (Pelletreau and Muller-Parker, 2002; Molis et al., 2009), though this provides no explanation of why sulfate is accumulated in addition to the more usually accumulated chloride. The inhabitant of high-sulfate, high-magnesium lakes, *Chara buckelii*, has magnesium and sulfate as the major charged components in the vacuole (Hoffmann and Bisson, 1990).

The cell walls of most marine red and brown algae contain abundant sulfated galactan polymers (carrageeenans, agar, fucoidans; e.g. Arad and Levy-Ontman, 2010). Sulfated mannans are common in diatoms, where they are often associated with the frustules. Terrestrial plants, instead, lack sulfated polysaccharides in their cell wall (e.g. Popper and Tuohy, 2010; Pomin, 2010), although they were secondarily regained by the seagrass *Ruppia maritima* (Aquino et al., 2005). The lack of these compounds may be a feature that terrestrial plants inherited from their freshwater algal ancestors (freshwater charophycean green algae, typically do not contain these compounds in their walls). However, it should be noticed that sulfated cell wall polysaccharides are present in some marine green macroalgae of the genus *Ulva* (Robic et al., 2009) and *Codium* (Farias et al., 2008). It is therefore likely that the occurrence of sulfated galactans is not related to phylogeny, but it is rather an adaptation to the environment (Aquino et al., 2005). Vascular plants also synthesize large numbers of sulfated metabolites, which are often involved in biotic interactions (Kopriva et al., 2012).

The presence of sulfolipids in thylakoid membranes has often been linked to P-limitation (e.g. Van Mooy et al., 2006, 2009). Regardless of the growth conditions, however, the concentration of sulfolipids is typically higher in the thylakoids of green algae than in vascular plants, and it is even higher in cyanobacteria and diatoms (Vieler et al., 2007; Goss and Wilhelm, 2010). It has been proposed that the abundance of sulfolipids in diatoms is involved in the definition of domains within non granal thylakoidal membranes determining some sort of structural and functional heterogeneity (Goss et al., 2009).

3. N and S sources

3.1. Inorganic N and S sources

Algae and plants are capable of using molecular N_2 when associated with symbiotic diazotrophyc bacteria (Lambers et al., 2008; Thompson et al., 2012; Rai et al., 2003; Schmidt et al., 2013) or directly in the case of some cyanobacteria (e.g. Sohm et al., 2011 and references therein; Zehr, 2011 and references therein). No biological mechanisms for the assimilation of elemental S are known, although algae and plants can produce, directly or indirectly, elemental S which has antibiophage properties (Wium-Andersen et al., 1982; Berger and Schager, 2004; Cooper and Williams, 2004; Rausch and Wachter, 2005).

The most common forms of inorganic N utilized by photosynthetic organisms are combined N compounds, mostly NO_3^- and NH_4^+, occasionally as NO_2^-; (Kotur et al., 2013). In oxygenated soils, NO_3^- (+V) is the main source of combined N for plants; in the ocean, the production based on nitrate is high in upwelling zones, but lower in the rest of the ocean, where most of the productivity involves recycled N (Falkowski and Raven, 2007; Raven et al., 1992a,b). Photosynthetic primary productivity in oceanic waters is often limited by the availability of combined N, with inorganic combined N concentration frequently well below 10 μM (Falkowski and Raven, 2007). In freshwater, although algal elemental stoichiometry is rather similar to that of their marine counterpart, the ambient N:P ratio is much higher than in the ocean and primary production is typically controlled by P. This is mostly due to the higher abundance of diazotrophic organisms and to the higher precipitation of P in insoluble complexes with particulate matter, whose concentration is orders of magnitude more abundant in freshwater than in the ocean (Falkowski and Raven, 2007). In cultivated soils and in some desert soils, NO_3^- concentrations are often several orders of magnitude higher (1–20 mM; Andrews et al., 2013 and references therein) than those typically measured in the ocean.

External ammonium is chemically stable in oxygenated as well as in anoxic environments. In oxygenated environments chemolithotrophs (nitrifiers) convert ammonium to nitrite and then nitrate; in anoxic environments, ammonium, with nitrite as co-substrate, is converted to water and dinitrogen in the anammox reaction. However, these reactions still leave a very significant flux from dinitrogen fixation and from animal, parasite and decomposer metabolism that is used in photosynthetic growth.

Ammonium (from soil and natural waters: Raven et al., 1992a,b) and, to a much smaller extent ammonia (by dry deposition to vegetation: Raven et al., 1992a,b; Jones et al., 2007a,b) are important sources of nitrogen for photosynthetic organisms. The analysis by Raven et al. (1992a,b) shows that, globally, more N is assimilated by primary producers as N_2, organic N, NH_4^+ and NH_3 than as NO_2^- and NO_3^-, in both the aquatic and the terrestrial environments.

All algae and plants are able to take up and assimilate ammonium and one or more forms of organic nitrogen. While most algae and plants can also take up and assimilate nitrate, there are significant exceptions, such as some strains of the very abundant (in terms of cell numbers) warm-water marine phytoplanktonic cyanobacterium *Prochlorococcus* (Raven, 1984; Raven et al., 1992b, 2013). The natural environment typically has a mixture of sources of combined N. Reduced N forms predominate in the surface oligotrophic ocean, where recycling of N derived from living organisms into phytoplankton is rapid and there is little scope for nitrifiers. In terrestrial habitats with high levels of nitrification inhibitors, algae and plants living there grow more rapidly on reduced nitrogen than on equivalent concentrations of nitrate. At the other extreme, disturbance of soils with low levels of nitrification inhibitors leads to mineralization and nitrification, and the ruderal plants that typically inhabit such environments often grow more rapidly on nitrate than on ammonium (Raven, 1984; Raven et al., 1992b). However, for some algae and plants a mixture of oxidized and reduced N sources gives faster growth, when other resources are present in abundance; this could be in part the result of lower energy costs of acid–base regulation in the assimilation when both the proton-producing ammonium and the hydroxyl-producing nitrate are concomitantly taken up (Raven and Smith, 1976; Raven et al., 1992b).

For S, the bias is very much towards SO_4^{2-} with very small contributions from S^{2-}.

Even with NH_4^+ as the dominant N source in a waterlogged substrate when nitrate has been removed by denitrification, there is still the possibility of nitrate uptake by the roots of vascular waterplants as result of nitrification in an oxygenated rhizosphere. This is especially the case in plants of the isoetid life form, where essentially all of the CO_2 enters and all the photoproduced O_2 comes out, through the root system rather than the photosynthetically active leaves, which have a very thick and impermeable cuticle (an extreme example is *Lobelia dortmana*). The oxygenated rhizosphere permits nitrification, and hence nitrate uptake by roots (Risgaard-Petersen and Jensen, 1997; Ottosen et al., 1999). This has been demonstrated for the freshwater *Litorella uniflora* and *L. dortmana*, and the marine non-isoetid *Zostera marina*.

High concentrations of ammonium may inhibit growth of photosynthetic organisms; this has been attributed to various causes (Allen and Smith, 1986). One possibility (which requires growth) is the excretion of protons produced in ammonium assimilation into organic matter; this ratio is at least one proton per ammonium. If these protons are excreted into a spatially limited or poorly buffered medium, then acidity damage to the organism could occur. This is more likely to occur to vascular plants in soil or a small hydroponic volume than in phytoplankton, where photosynthesis will offset the ammonium effect on external pH, at least for ammonium assimilation in the light (Flynn et al., 2012). An alternative mechanism of ammonium toxicity is 'futile cycling' of ammonium in the low-affinity transport system (LATS; see below) concentration range, involving aquaporin-like NH_3 channels, discussed below (Kronzucker et al., 2001; Coskum et al., 2013).

Sulfur is mostly acquired as sulfate (+VI). Sulfate represents the main S sources in oxygenated soils and it is highly abundant in the ocean, with concentrations around 28 mol m^{-3} (Giordano et al., 2005c) and it is the form in which S is often excreted by animals. It is noteworthy that, in spite of the constant high availability of sulfate, some non-green marine microalgae have retained the ability to respond to sulfate deficiency, although some phytoplankters appear to be unable to grow at sulfate concentrations lower than 5 or 1 mol m^{-3} (Ratti et al., 2011, 2013; Bochenek et al., 2013). In freshwater (Giordano et al., 2005c) and in pristine land soils (Johnson, 1984), the availability of sulfate may be substantially lower.

In oxygenated environments, sulfide is subject to chemical oxidation and also to biological oxidation by chemolithotrophic bacteria. Furthermore, sulfide is toxic even at low concentrations (see the comprehensive review by Lamers et al., 2013), the obvious target being cytochrome oxidase. These considerations limit the global extent to which exogenous sulfide is used by oxygenic photolithotrophs. Sulfide is produced in the photic zone largely by sulfate reduction by chemoorganotrophic growth of bacteria in waterlogged soil or sediment where oxygen diffusion from the water body is insufficient to maintain aerobic respiration. In low ionic strength freshwater sediments, the low sulfate availability means that much of the sulfide produced is sequestered as ferrous sulfide. In marine environments, and those inland water bodies with high sulfate, iron supply in the sediment is usually not sufficient to sequester all of the sulfide, which builds up as S^{2-}, HS^- and H_2S in quantities which vary with pH. In marine sediments,

dissimilatory sulfate reduction permits free sulfide accumulation; because in these environments there is limited supply of ferrous iron relative to the supply of sulfate and hence of sulfide, little ferrous sulfide is produced, and the amount of free dissolved sulfide is higher than in freshwater. The natural abundance of the stable isotopes of sulfur, ^{34}S and the predominant ^{32}S, is a useful tool to assess whether sulfide or sulfate is the source of the S entering the plant. The uptake and assimilation of sulfate results in little fractionation of the S isotopes relative the external sulfate, and the same seems to be true of sulfide (Fry et al., 1982; Raven and Scrimgeour, 1997; Cambridge et al., 2012, and references therein). If sulfate supply is not limiting the rate of dissimilatory sulfide production, the sulfide has a significantly lower $^{34}S/^{32}S$ ratio than the source sulfate. Since there is little S isotope discrimination in the uptake and assimilation of sulfate or sulfide, the low $^{34}S/^{32}S$ that has been found in rhizophytic plants from the marine benthic zone has been interpreted as the use of sulfide as a S-source (Fry et al., 1982; Raven and Scrimgeour, 1997; Cambridge et al., 2012, and references therein). Salt-marsh plants and mangroves have low (similar to sulfide) S isotope ratios throughout the plants, while in seagrasses rhizomes generally have a low S isotope ratio, the green shoots have a ratio closer to that of seawater, and roots have an intermediate value (Raven and Scrimgeour, 1997; Cambridge et al., 2012, and references therein). The occurrence of a plant S isotope value indicating a contribution of exogenous sulfide to plant S does not necessarily mean entry of sulfide (Raven and Scrimgeour, 1997). If there is an oxygenated biosphere, chemolithotrophic oxidation of sulfide could generate sulfate with little further S isotope fractionation. This sulfate would not be mixed with seawater sulfate if there is a sufficient anoxic barrier between seawater and the rhizosphere, although the initial production of sulfide with a low $^{34}S/^{32}S$ requires, as indicated above, relatively unrestricted access to seawater sulfate. However, an uncatalyzed entry of undissociated H_2S through the plasmalemma would provide a parallel (to sulfate active influx) pathway for S entry, if the steady-state cytosolic H_2S is less than that in the rhizosphere. Raven and Scrimgeour (1997) suggested a H_2S permeability coefficient of the plasmalemma of 10^{-5} m s^{-1}, based on water uncatalyzed permeability through lipid bilayers made from lipids found in the plasmalemma and the observed trend of permeability with molecular mass of direct proportionality to the square root of relative molecular mass. Such a flux would support the whole-plant S requirement, granted acceptable assumptions about the composition of the rhizosphere (Raven and Scrimgeour, 1997). Subsequent research failed to find a role for an aquaporin from a sulfide-oxidizing Archaean, and measured a H_2S permeability of at least 5×10^{-5} m s^{-1} of the lipid part of the membrane. Cuevasanta et al. (2012) found a plasmalemma-like lipid bilayer permeability for H_2S of 5×10^{-4} m s^{-1}. While there is a 50-fold range in these estimates (all for 25 °C), even the lowest value could explain plants S nutrition in terms of H_2S permeation (Raven and Scrimgeour, 1997). The highest of these values could lead to very high internal concentrations when growth rate is low. This could in part be offset by a HS^- channel (Czyzewski and Wang, 2012); with the inside-negative potential difference across the plasmalemma and the predominance of HS- among the species of sulfide at cytosol pH of about 7.4, this would lead to a futile cycle of sulfide species across the plasmalemma, powered by the energy source that maintains the inside-negative electrical potential.

Assuming that the use of sulfide as S source by plants, either directly or indirectly, as discussed above, is limited to salt-marsh plants and mangroves and to the below-sediment parts of seagrasses, and that all S obtained by these plants or plant parts come from sulfide, it is possible to estimate the global fraction of S assimilated by oxygenic photolithotrophs which is as sulfide rather than sulfate. Starting with the global net primary productivity of these organisms, Duarte and Chiscano (1999) give a value of 13 Tmol C per year for seagrasses, while Gattuso et al. (1998) give values of 12 Tmol C per year for salt marsh plants and 13 Tmol per year for mangroves (using values given for the gross primary productivity and for the ratio between gross primary productivity and respiration). Assuming that the atomic C:S ratio in coastal marine vascular plants is the same as that (145) for leaves of herbaceous vascular land plants (Broadley et al., 2004; White et al., 2007), that all of the S in salt marsh plants and mangroves comes from sulfide and half of the S in seagrasses (what enters through roots) comes from sulfide, the total global sulfide assimilation is 0.212 Tmol S per year. For terrestrial and inland water plants, the net primary productivity in terms of C from Field et al. (1998), i.e. 4700 Tmol C per year and the C:S ratio of 144 translate to 32.6 Tmol of sulfate S assimilated per year. Field et al. (1998) give a net primary productivity value for marine macrophytes (presumably seaweeds plus seagrasses plus salt marshes) of 83 Tmol C per year; if the C:S ratio is 144, the calculation yields a yearly production of 0.576 Tmol total S. Subtracting the sulfide S value of 0.212 Tmol S per year gives a value of 0.364 Tmol sulfate per year. For marine phytoplankton, the net C assimilation per year is of 3960 Tmol C. With an atomic C:S of 167 (Quigg et al., 2011), the sulfate-S assimilation per year is 23.7 Tmol S. The total sulfate assimilation is then 56.9 Tmol S per year, while the total assimilation of sulfide-S by oxygenic photolithotrophs is 0.212 Tmol per year. These approximate calculations suggest that not more than 0.37% of total S assimilated by photolithotrophs is as sulfide, while Raven et al. (1992a,b) suggested that over 50% of the N assimilated globally by photolithotrophs is at or above the reduction level of N_2.

3.2. Organic N and S sources

A variety of organic N sources are employed by algae, such as amino acids, urea, peptides, and proteins (Näsholm et al., 2009; Schmidt et al., 2013; Fernandez et al., 2009). Although inorganic N is traditionally considered by far the main source of N for plants, there are soils where the N requirement by plants is in large excess of the supply of inorganic N, on an annual basis (Näsholm et al., 2009). Conspicuous amounts of humic substances, proteins and amino acids (also as a consequence of microbial, fungal and root proteolytic activities) are present in some areas, although the proportion of this N that is actually available to plants is probably much lower. Amino acids seem to be the main source of organic N for plants. Genomic data have shown that a very large number of amino acids transporters exist (Rentsch et al., 2007).

In algae, the large phylogenetic heterogeneity and the relatively small number of species investigated make it difficult to make generalization on the ability use organic N. In most cases, organic N utilization seems to be inhibited by the presence of NH_4^+ (Fernandez et al., 2009).

The use of amino acid as the N source as been demonstrated for the model species *C. reinhardtii*, which deaminates a large number of L-amino acids outside the cells (Muñoz-Blanco et al., 1990), via catalysis by a periplasmic amino acid oxidase induced by N starvation (Callon et al., 1993). Ammonium is then acquired and fixed, while the oxoacid derived from the deamination is not used (Muñoz-Blanco et al., 1990). An operon-like cluster of genes involved in urea/arginine uptake and assimilation has however been identified in *Ostreococcus tauri* (Derelle et al., 2006) and *C. reinhardtii* (Fernandez et al., 2009) genomes. Only in the case of asparagine has the presence of a specific amino acid transporter been demonstrated, in *Chlamydomonas* (Kirk and Kirk, 1978). Use of external organic N as a N source for vascular plants is very widespread (Schmidt et al., 2013).

Urea is the predominant global form in which N is applied in fertilizers (Liu et al., 2003). A large number of algal and plant species are able to grow on urea, both in the case of exogenous

urea and in the case of recycling of internal organic N (e.g. from catabolism of arginine or purines) (Solomon et al., 2010; Witte, 2011; Werner et al., 2013). In *C. reinhardtii*, urea is taken up by an energy-dependent system (Williams and Hodson, 1977); it is then stored or used in a two step process to generate NH_4^+ by deamination with the catalysis of ATP-urea amidolyase (UALase; Dagestad et al., 1981): an initial ATP-dependent carboxylation of urea produces allophanate; a subsequent hydrolytic reaction generates $2NH_3$ and $2HCO_3^-$ from allophanate. Differently from yeasts, in which UALase is a single enzyme derived by the fusion of a urea carboxylase and an allophanate hydrolase, in *C. reinhardtii* the two activities are allocated on distinct proteins (Solomon et al., 2010). UALase, among the algae tested, was only found in green algae of the Chlorophyceae and the Trebouxiophyceae (Leftley and Syrett, 1973; Bekheet and Syrett, 1977). Urea can also be broken down by urease (urea amidohydrolase); this cytosolic enzyme catalyzes the hydrolytic cleavage of urea to ammonia and carbamate; carbamate then spontaneously gives origin to another molecule of NH_3 and carbonic acid (Solomon et al., 2010). Urease occurs in green algae of the Charophyceae (and their descendants, the embryophytic plants), Prasinophyceae and Ulvophyceae, as a well as cyanobacteria, Rhodophyta, Dinophyta, Haptophyta and Ochrophyta of the classes Bacillariohyceae, Fucophyceae, Pelagophyceae and Tribophyceae (Leftley and Syrett, 1973; Bekheet and Syrett, 1977; Dyhrman and Anderson (2003); Solomon et al., 2010; Agostoni and Erdner, 2011). Urea uptake by the marine diatom *Phaeodactylum tricornutum* requires external sodium (Rees et al., 1980), a finding that is consistent with the occurrence of sodium-coupled urea active influx. Active transport of urea into cells of the freshwater green alga *Chara australis* (=*Chara corallina*) involves $2Na^+$:1 urea co-transport (Walker et al., 1993); this alga also has plasmalemma symport of Na^+ with K^+ and with phosphate (see Reid et al., 2000). Urea influx at the plasmalemma of *A. thaliana* involves, as is the case of ammonium and nitrate, high-affinity and low-affinity systems. The high-affinity active influx system involves the proton:urea cotransporter AtDUR3 (Liu et al., 2003; Kojima et al., 2007a,b; Wang et al., 2008; Witte, 2011). The low-affinity passive (non-energized) system involves a specific major intrinsic protein (aquaporin; Kojima et al., 2007a,b; Wang et al., 2008; Witte, 2011). The role of uncatalyzed urea permeation through the lipid bilayer component of membranes seems to be minimal (Raven, 1984).

A complete urea cycle has been discovered in diatoms (Bowler et al., 2008, 2010). This is suggestive of the fact that these algae can utilize this form of inorganic N, whether obtained from the environment or generated internally; in spite of various hypothesis (e.g. anaplerotic N and C mobilization, cycling N in photorespiration; Allen et al., 2006; Hockin et al., 2012), the exact role of this pathway, which was previously only known for metazoan, in diatom remains elusive. The utilization of urea is also well described in plants.

In some freshwater basins (Giordano et al., 2005c) and in soil (Kertesz, 2000), a large proportion of S can be contained in organic molecules. However, there is no evidence for the direct use of such molecule as a source of S for photosynthetic organisms. Many microorganisms, including microalgae, are capable of releasing enzymes that can cleave inorganic S from organic compounds and make the S available for plants and algae (Gonzalez-Ballester and Grossman, 2009; Kertesz, 2000). It has been proposed that the release of sulfate and sulfonates from organic compound is one of the mechanisms through which rhizobacteria facilitate plant growth (e.g. Schmalenberger and Noll, 2014). Sulfate esters can be cleaved by the predominantly eukaryotic arylsulfatases (ARS), which produce SO_4^{2-} and the residual phenol (Gonzalez-Ballester and Grossman, 2009 and references therein), and by alkylsulfatases, whose catalysis generates SO_4^{2-} and an aldehyde (Kahnert and Kertesz, 2000; Hagelueken et al., 2006). Arylsulfatases are by far the most common and best studied of these enzymes, and 18 putative ARS genes were identified in *C. reinhardtii* genome (Gonzalez-Ballester and Grossman, 2009). Two of these genes (and their products) were characterized (ARS1 and ARS2); the transcription of both genes is stimulated by S starvation and the proteins are released outside of the cell (de Hostos et al., 1989; Ravina et al., 2002).

3.3. Changes in the availability of S and N over time

Long-term changes of S availability in the oceans have been convincingly reconstructed (Ratti et al., 2011 and references therein). In the Archaean oceans, sulfate concentration is believed to have been lower than 200 μM (Habicht et al., 2002), to increase to 1–5 mM in the Proterozoic, when however, most of the S in the ocean was in the form of sulfide (Shen et al., 2002; Canfield, 2004; Kah et al., 2004), following a temporary increase to about 10 mM in the Lomargundi–Jatuli excursion (2.3–2.06 Ga ago), following the Global Oxygenation Event 2.4–2.3 Ga ago (Planavsky et al., 2012; Martin et al., 2013; Scott et al., 2014). A temporary increase of marine sulfate concentration (to approx. 15 mM) probably occurred in later Ediacaran oceans, followed by a progressive decline into the Cambrian (Horita et al., 2002; Petrychenko et al., 2005), when, based on the analysis of inclusions in salt crystals, it was in the range of 3–12 mM (Gill et al., 2011). It was only in the Carboniferous that sulfate concentration became higher than 15 mM (Gill et al., 2007). Although transient variations of these concentrations may have occurred thereafter (Luo et al., 2010; Newton et al., 2011), a sulfate concentration in the order of 13–27 mM most likely characterized the late Paleozoic to early Mesozoic oceans. It has been proposed that such changes have had a strong influence on the radiation of phytoplankton groups, with algae with chloroplasts derived from red algae (Chl *a* + *c* algae) being favoured over the algae of the green lineage, in the high sulfate mesozoic oceans (Giordano et al., 2005c; Ratti et al., 2011, 2013). The extant oceanic sulfate concentrations are probably the highest ever reached, with an average value of 28 mM, although, of course spatial and short-term temporal variations do occur, especially in coastal waters. In today's freshwater habitats, sulfate availability is usually much lower than in the ocean and variability is much greater; values between 0.01 and 1 mM sulfate characterize most freshwaters environments (Holmer and Storkholm, 2001; Giordano et al., 2005c; Norici et al., 2005). This also fits with the greater contribution of green algae to primary productivity in these environments as compared to the oceans. There are rare lakes with high sulfate concentration; for instance, high sulfate concentrations have been determined in lakes in Saskatchwan, and the Characean *C. buckelii* from these lakes has a high magnesium and sulfate concentration in the vacuole (Hoffmann and Bisson, 1990).

In the anoxic Archaean, combined N is believed to have been mostly present in the form of NH_3/NH_4^+ with a rather minor contribution of nitrification and denitrification; limited oxidized N inputs were due to volcanism and lightning acting on atmospheric N_2, CO_2 and H_2O (Navarro-González et al., 2001). At this time, biological N_2 fixation was facilitated by the availability of Fe, but not of Mo, with the further benefit of the absence of O_2 in lack of damage to nitrogenase. Nitrogen fixation has a greater demand for phosphorus than production based on combined nitrogen (Raven, 2012, 2013a,b). The availability of phosphorus in the ocean would have been increased by the absence of silicifying organisms; the lack of silicifying organisms would have also determined a higher concentration of silicic acid, which may have competed with phosphate for binding to iron species (Planavsky et al., 2010; Raven, 2013a,b), thus making Fe more available to diazotrophs. It is however possible that phosphorus input from land decreased, if there was little elevation of continents (Anbar and Knoll, 2002 and references therein).

The global oxygenation event, (2.5–)2.4–2.3 billion years ago (Bekker et al. (2004); Anbar et al., 2007; Busigny et al., 2013) was followed by the Lomogundi–Jatuli excursion (2.2–2.06 Ga) of C and S isotopes associated with a glaciation (Bekker et al., 2003; Papineau, 2010; Bekker and Holland, 2012; Planavsky et al., 2012; Partin et al., 2013; Reinhard et al., 2013). The isotope excursion indicates increased organic C burial, increased oceanic sulfate concentration and increased oceanic and atmospheric O_2. There were also the first substantial (commercially exploitable) phosphorite deposits indicating increased P concentration in the ocean. This P increase was probably related to increased terrestrial weathering (Bekker et al., 2003; Papineau, 2010; Bekker and Holland, 2012; Planavsky et al., 2012; Partin et al., 2013; Reinhard et al., 2013). The natural abundance ^{15}N:^{14}N ratio has been used as proxy for the N cycle, although the numerous possible reactions with their kinetic and equilibrium fractionations (Handley and Raven, 1992) make the interpretation for the latest Archaean (Busigny et al., 2013) and the Lomagundi–Jatuli (Papineau et al., 2013) uncertain; consequently, also the interpretations in terms of (e.g.) diazotrophy and denitrification are subject to reservations. It is likely that, at this time, the surface ocean were subject to similar constraints as that in the Phanerozoic ocean and the Cryogenian–Ediacaran, with the same combination of glaciation, organic C burial, O_2 and sulfate increase, and increased P input as shown by phosphorite deposits (Papineau, 2010; Planavsky et al., 2010; Raven, 2013a,b). Nitrification–denitrification (in anoxic zones) cycles and sedimentation removed combined N, while nitrogen fixation would be favoured by the availability of P and Mo, but constrained by the limited supply of Fe and the presence of O_2. There are also significant differences between the Lomagundi–Jatuli and the Cryogenian–Ediacaran. One example is the relatively late (Neoproterozoic) appearance of planktonic diazotrophic cyanobacteria (Sanchez-Baracaldo et al., 2014), implying that any open ocean nitrogen fixation in Lomagundi-Jatuli times would have been by non-cyanobacterial diazotrophs (see Sohm et al., 2011).

In the mid Proterozoic, in the context of a moderately oxidizing atmosphere, weathering is believed to have been a source of sulfate for the anoxic deep waters. This process in combination with an active biological C pump, would have fed sulfate reducing bacteria determining the onset of about one billion years of sulfidic oceans (Canfield, 1998; Anbar and Knoll, 2002). At this time, nitrate became the main form of combined N in the oxygenated surface water because O_2 allows nitrification. Fe would have been less available than previously due to conversion to insoluble Fe^{3+} in the surface ocean, while Mo would have been more available as MoO_4^{2-} (Anbar and Knoll, 2002, but see Asael et al., 2013; Gilleaudeau and Kah, 2013). However, in the deeper sulfidic ocean the situation was reversed, with Fe more available than Mo (Anbar and Knoll, 2002). The scarcity of Fe in the surface ocean profoundly affected the N biogeochemical cycle, due to its impact on N_2 fixation and combined N assimilation. In the late Proterozoic, the increased weathering caused by the breaking up of Rodinia supercontinent made metals more available, although in oxygenated waters Fe would tend to precipitate as Fe^{3+}. The higher availability of Mo, however, presumably somewhat increased N_2 fixation and the inventory of combined N in the water column, because of the higher specificity of MoFe-nitrogenases compared to VFe- and FeFe-nitrogenases (with their limited phylogenetic occurrence) and because of its role in NO_3^- reduction (Anbar and Knoll, 2002), ameliorating primary productivity. However, due to the increasing oxygenation (which hampered N_2 fixation) and the higher P influx from the continents, this did not cause a shift of limitation from N to P (Anbar and Knoll, 2002; see also Rowell et al., 1998 for data on how the metal use in nitrogenase can alter ^{15}N discrimination). The Cryogenian–Ediacaran transient O_2 increase, and its probable impact on the N cycle, has been mentioned above in the context of the Lomagundi excursion.

On land, such large variation in the chemical form and availability of N and S are unlikely to have occurred (if not locally and in the short-term, although often within very broad ranges; Crawford and Glass, 1998). The presence of sulfate and possibly nitrate on land is presumably coeval with atmosphere oxygenation, which likely affected land appreciably earlier than oceans. The radiation of terrestrial plants in the Phanerozoic may therefore have happened in a much more stable nutritional context, even considering that nutrient soil content is significantly dependent on the age of the soil (Lambers et al., 2008). The terrestrial N cycle, which presumably became an important component of global N biogeochemistry in the Phanerozoic and was not as tightly constrained by metal availability (Anbar and Knoll, 2002), possibly also affected primary production in the ocean by appreciably contributing to the overall N budget (Anbar and Knoll, 2002). Recent events such as the use of fertilizers and the impact of anthropogenic S emission (and their subsequent, equally anthropogenic, decline) have probably induced physiological response on terrestrial plants whose selective pressure is however difficult to determine and possibly rather modest.

4. Acquisition

4.1. Nitrogen

In both plants and algae, a variety of high (HANT) and low (LANT) affinity transport systems have been identified. These transport systems are energized by proton co-transport ($2H^+$:$1NO_3^-$) or, in the marine flowering plant *Z. marina*, sodium co-transport ($2Na^+$:$1NO_3^-$) (Gárcia-Sánchez et al., 2000; Rubio et al., 2005); less definitive evidence of sodium co-transport of nitrate (and ammonium, urea and certain amino acids) in eukaryotic marine algae is summarized in Table 7.6 of Raven (1984). Cyanobacteria use an ABC transporter for nitrate that is directly energized by ATP and does not involve co-transport (Kobayashi et al., 1997). NRT1 transporters are present in plants and have also been identified in the genomes of *Chlamydomonas*. The NRT1 gene family comprises a large number of genes some of which are also involved in the transport of peptides and possibly a variety of other metabolites such as auxin, abscissic acid, histidine and glucosinolates (Leran et al., 2014). In *Arabidopsis*, atNRT1.1 may act as a high or low affinity transporter depending on phosphorylation; the phosphorylated form has a high affinity for nitrate and is expressed when ambient nitrate is limiting (Wang et al., 1998; Liu and Tsay, 2003). AtNRT1 is also involved in other processes, such as stomatal opening (but see Raven, 2003 for stomatal function in plants growing without access to nitrate). Other NRT1 have been characterized as low affinity transporters (Huang et al., 1999; Zhou et al., 1998).

NRT2 transporters are widely distributed among prokaryotes and eukaryotes (Fernandez et al., 2009 and references therein). As it is common for anion transporters, NRT2s contain 12 transmembrane helices with a loop after domain 3 and cytosolic regions at both C and N termini (Forde, 2000). In plants and in *Chlamydomonas*, the C terminus cytosolic domain of NRT2 is especially long (Fernandez et al., 2009) and NRT2 is sometimes flanked by an additional protein named NAR2 (nitrate assimilation-related protein 2). The presence of such protein is believed to be necessary for high affinity transport, at least in *Arabidopsis* and *Chlamydomonas* (Fernandez et al., 2009 and references therein).

In plants, NRT2 transporters with both high and low affinity for nitrate exist. The high affinity transporters can be inducible (iHATS), usually by nitrate, or constitutive (cHATS; Orsel et al., 2006). Also in *Chlamydomonas* a variety of high (HANT) and low (LANT)

affinity nitrate transporters are present (Fernandez et al., 2009 and references therein). In this green alga, at least four different systems have been characterized: three of these systems (I, II, III) are induced by nitrate and are under the regulatory control of NIT2 protein, are inhibited by NH_4^+ but not by chloride and are maximally active at high CO_2 (see Fernandez et al., 2009 and references therein for details). System IV shows a rather different behaviour from the others: it is capable of transporting both NO_3^- and NO_2^- with a Km in the order of 40–30 μmol L^{-1} for both. Differing from the other three *Chlamydomonas* systems, system IV does not require nitrate for its expression, it is not inhibited by ammonium but is sensitive to chloride, and it is maximally active at low CO_2 (Galván et al., 1996; Rexach et al., 1999). Although it is probably a NRT2 member, the gene that encodes for system IV has not been identified (Rexach et al., 1999; Navarro et al., 2000).

A third group of nitrate/nitrite transporters has been found and characterized in *Chlamydomonas*, the NAR1 (nitrate assimilation related component 1) transporters. The NAR1.1 (and possibly the NAR1.2) gene product is in fact a chloroplast nitrite transporter, regulated by carbon availability. The NAR1.1 gene is located in the nitrate cluster, linked to NIA1 (the gene encoding nitrate reductase) and it is co-regulated with the other genes in the cluster, with which it shares the inhibition by ammonium under NIT2 control. The NAR1.1 gene belongs to the FNT (formate nitrite transporters) family, which seems to be absent in plants and animals (Mariscal et al., 2006). In *Chlamydomonas*, not all NAR1 proteins are plastidial: NAR1.5 is thought to be mitochondrial and NAR1.3, NAR1.4 and NAR1.6 are believed to be plasmalemma transporters (Fernandez et al., 2009 and references therein).

NAR1 genes have been found in *Ostreococcus* (single copy) and other algae, fungi and protozoa (Fernandez et al., 2009). The NAR1 gene products are also present in *Saccharomyces* and *Plasmodium*, although these organisms do not assimilate nitrate/nitrite; it is therefore possible that, also in algae, these proteins are involved in the transport of other monovalent ion besides nitrite (e.g. bicarbonate and formate). The fact that *Chlamydomonas* NAR1.2 gene responds to inorganic carbon availability and is under the control of CCM1 gene, which superintends to the activation of CO_2 concentrating mechanisms (Giordano et al., 2005a), reinforces the idea of a role for some NAR1 gene in inorganic carbon transport. In *Xenopus* oocytes, NAR1.2 protein indeed transports bicarbonate (together with nitrite; Mariscal et al., 2006). NAR1.1 and NAR1.6 are inhibited by NIT2 and respond to N sources; they therefore are most likely genuine nitrite transporters. Other products of NAR1 genes are controlled by neither NIT2 nor CCM1 and are thus putatively involved in transport processes not directly related to N and C acquisition and trafficking.

It is important to note that there are nitrite-specific transporters in the plasmalemma of both *C. reinhardtii* (Galván et al., 1996) and *A. thaliana* (Kotur et al., 2013), with half-saturation values of 3.5 mmol m^{-3} and 185 mmol m^{-3} respectively. Neither of these transporters seem to have been completely characterized genetically.

The acquisition of NH_4^+, both in plants and in algae, mostly occurs through transporters of the AMT/MEP family (Andrews et al., 2013; Fernandez et al., 2009). These transporters appear to conduct a symport of ammonium N and H^+ with a 1:1 stoichiometry. It seems that NH_3, rather than NH_4^+, is the species transported and that the stoichiometry of the transport is $1NH_3$ for $2H^+$; this, inside the cell, after equilibration of NH_3 with NH_4^+, which produces the $1NH_4^+$:$1H^+$ stoichiometry that is typically attributed to this transporters (see Andrews et al., 2013 for a thorough discussion of this topic). The transport of NH_3 by an AMT transporter also ensures that these channels are highly selective for N and do not transport also K^+, which has a hydrated radius similar to that of NH_4^+. It is also possible that low affinity NH_4^+ transport takes place via K^+ channels. A similar situation may occur in the case of the low affinity ammonium transporter system (LATS) of *Chlamydomonas* (Ullrich et al., 1984). The AMT1 family of *Chlamydomonas* comprises a large number of genes, with members distributed throughout the alga genome. Some of these genes (AMT1.1–6) are closely related to the AMT transporters of plants, whereas AMT1.7–8 are more distantly related. Such a large number of genes encoding for AMT1 transporters is possibly just the result of functional redundancy, but it may also reflect the need to adjust to changing N availability (as it is suggested by the various modes of regulation of the AMT transporters in the presence of different N source and amounts) and to regulate exchanges between cell comportments and with the environment (Fernandez et al., 2009 and references therein). The involvement of *Chlamydomonas* ammonium transporters with chemotactic response to NH_4^+ in solution has also been proposed (Ermilova et al., 2004). Ammonium transport in other algae is not as well characterized. It is worthwhile mentioning that the green alga (prasinohyte) *Micromonas*, in contrast to *Chlamydomonas* and *Chlorella* and the chromoalveolates (at least those studied in this respect so far), contain AMT2 genes of bacterial origin, in addition to the AMT1 genes (McDonald et al., 2010).

Diatoms respond in a species-specific manner to growth at high NH_4^+ concentrations; for instance, while *Cylindrotheca closterium* is unable to grow at 10 mM NH_4^+, *P. tricornutum* thrives in such condition (see Fig. 4 in Giordano, 2013). Experiments conducted in M.G.'s lab showed that the ammonium effect on *Cylindrotheca* can be substantially reduced if the concentration of K^+ in the growth medium is increased; the addition of K^+ in *Phaeodactylum*, instead, has no effect (A. Norici, M. Giordano, J.A. Raven, unpublished). This is suggestive of different NH_4^+ uptake systems in the two algae, with the low growth rate in *Cylindrotheca* at high NH_4^+ possibly due more to K^+ starvation than to NH_4^+ toxicity (because of competition for the same channels, at high NH_4^+ K^+ influx would be strongly limited). Also futile cycling of NH_4^+ through low affinity ammonium transporters (Britto and Kronzucker, 2006) may provide an explanation for some of these observations. Two very similar AMT transporters have been cloned from *Cylindrotheca fusiformis*, which are expressed mostly in N-starved cells, followed by cells grown in the presence of nitrate and then NH_4^+-grown cells (Hildebrand, 2005). Also in flowering plants NH_3/NH_4^+ toxicity is species-specific. The current view is that toxicity is the result of NH_3 movements at the plasmalemma and the tonoplast by an aquaporin-related channel combined with NH_4^+ transport by AMT, inwardly-rectifying K^+ channels and non-selective cation channels (Coskum et al., 2013).

Chlamydomonas is among the few organisms in which both AMT transporters and the related *Rhesus* (Rh) proteins have been identified (Fernandez et al., 2009). Although the Rh protein may act as a bidirectional NH_3 channel, there are evidences that in *Chlamydomonas* it is involved in CO_2 permeation at the plastid envelope (Soupene et al., 2002, 2004).

4.2. Sulfur

Sulfate is taken up by a variety of transporters (Garcia-Garcia et al., 2012; Takahashi et al., 2011, 2012 and reference therein). Transporters of the SULTR (sulfate transporters) family, performing a nH^+/SO_4^{2-} ($n \geq 3$) transport, have been identified in all photosynthetic organisms studied so far. The driving force of this transport is the proton gradient across membranes and the high affinity component of the transporters is activated when S is limiting (Takahashi et al., 2011 and references therein). These transporters typically have 12 transmembrane domains (Smith et al., 1995); regulatory (possibly) anti-sigma factor antagonist domains are located at the C terminus (Shibagaki and Grossman, 2010). In embryophytes, the SULTR gene have been classified in 4 families

(SULTR1 to 4), with often different location and function: SULTR1 are plasmalemma high affinity transporters; SULTR2 are low affinity transporter possibly involved in long distance sulfate transfer; SULTR3 are mostly foliar constitutive transporter, most likely located in the plastid envelope, of unclear physiological role; SULTR4 transporters are located on the tonoplasts and those identified and characterized so far transport from the tonoplast outward (Buchner et al., 2004; Takahashi et al., 2012). In *Chlamydomonas*, in addition of SULTR transporters, also transporters of the SLT (Sac1-like transporters) group have been identified based on sequence similarity; their expression and protein abundance was found to increase under sulfate deficiency (Pootakham et al., 2010). These transporters are usually involved in Na^+/SO_4^{2-} co-transport. Sequences of the SLT type have been identified in the genome of the marine haptophyte *Emiliania huxleyi*, although they have a rather low degree of similarity with *Chlamydomonas* SLT sequences. It is not at all sure that these proteins are sulfate transporters (Bochenek et al., 2013). *E. huxleyi* genome contains a number of other putative sulfate transporters, among which plant type SULTR; not all of these transporters have however been thoroughly investigated and may therefore be involved in function unrelated to sulfate uptake (see Takahashi et al., 2011 Bochenek et al., 2013 for details). Takahashi et al. (2012) showed that SULTR and SAC1/SLT phylogenies differ substantially: SULTR genes constitute a sister group of animal (SLC26), yeast (SUL) and their fungal homologous genes; but the SAC1/SLT transporters belong to a distinct gene "tribe". A completely different sulfate transport system has been described for the chloroplasts of green algae and some non vascular plants such as *Marchantia* (but not for the chloroplast of vascular plants, for which the mechanism of plastidial sulfate transport is still not known; Laudenbach and Grossman, 1991; Melis and Chen, 2005; Lindberg and Melis, 2008). The plastidial sulfate transporter of green algae comprises a sulfate binding protein exposed to the outside of the membrane, two intrinsic proteins and an ATP-binding and -hydrolyzing protein (ATP hydrolysis energize this transport) (Laudenbach and Grossman, 1991; Sirko et al., 1990). The inner plastid envelope membrane (the membrane at which active transport can occur) is derived from the plasma membrane of a cyanobacterium. A similar ABC-type sulfate transport is believed to be also present on the plasmalemma of cyanobacteria. The molecular genetic evidence for the occurrence of this transporter is not strong (Aguilar-Barajas et al., 2011); however, the elegant work of Ritchie (1996) on intact cells of *Synechococcus* R2 suggests ATP-dependent active sulfate transport rather than ion symport or antiport. Also passive efflux of sulfate is believed to occur; it could be important for the redistribution of sulfate through plant tissues and organs; in this case the sulfate motive force is the outside positive membrane potential at the plasmalemma (Takahashi, 2010). The inside positive membrane potential of vacuoles generated by H^+-ATPases or H^+-pyrophoshate probably supports the import of sulfate in vacuoles (Martinoia et al., 2007). However the tonoplast transporters that have been so far identified in *A. thaliana*, all belonging to the SULT4 gene family, only operate in the outward direction (Kataoka et al., 2004; also see Koprivova et al., 2013).

5. Assimilation

Regardless of the oxidation number of the chemical form in which N and S are acquired, they are incorporated into organic compounds as ammonium-ammonia(-III) and sulfide(-II), the most reduced forms of N and S in nature. Alternative ways to assimilate N presumably exist: for example, in *Viola*, nitro-compounds in plant defence are presumably formed by oxidation of amino-groups (thus, even in this case, initial assimilation is in ammoniacal form). The sulfonic acid group in sulfolipid is formed by incorporation of sulfite into an organic sulfonate, while the sulfate ester of cell wall polysaccharides of brown, green and red algae and seagrasses, and low compounds involved in biotic interactions in plants (Kopriva et al., 2012) involves non reducing sulfation pathways employing activated sulfate forms.

5.1. Nitrogen

The regulation of N assimilation in plants and algae differ in many aspects, among the most relevant is the regulatory mechanism of the cytosolic enzyme nitrate reductase (NR), which catalyses the initial reduction of NO_3^- to NO_2^-, in most cases using NADH as the electron donor. In both plants and algae, NR is regulated at the gene and protein level. Although both in algae and plants NO_3^- and light are among the main effectors of N metabolism, the regulation of NR gene expression and enzyme activity is rather different in these two groups of organisms.

The post-translational regulation of NR, in plants, is mostly effected by reversible phosphorylation in association with the interaction with 14-3-3-proteins (e.g. Lambeck et al., 2012). In *Arabidopsis*, the 14-3-3-binding occurs through the phosphorylation of Ser-534 (Kanamaru et al., 1999). It has been proposed that 14-3-3-mediated phosphorylation connects photosynthesis, ATP synthesis and nitrate reduction with protein turnover and diel sugar metabolism rhythms (MacKintosh (2004)). Investigations conducted on the lycophyte *Selaginella kraussiana* (which possesses a NADH-dependent cytosolic NR) and the moss *Physcomitrella patens* (whose NR is NADPH-dependent) suggest that the NR regulation by phosphorylation evolved early on in vascular plants, but was probably not present in non vascular photosynthetic organisms. It must be said, however, that NR was reported to be regulated by phosphorylation also in the red alga *Gracilaria chilensis* (Chow and Cabral de Oliveira, 2008), although the mechanisms of such regulation appears to be different from that of higher plant and, if confirmed, may have been acquired independently from the mode of regulation of terrestrial plants. In *Chlamydomonas*, and possibly in most other algae (Berges, 1997), the phosphorylation site through which 14-3-3 protein operates is not present in the NR protein. In *C. reinhardtii*, the enzyme is not subjected to phosphorylation–dephosphorylation and it appears to be under a redox control centred on the plastoquinone pool of the chloroplast, with a higher NR activity when the PQ pool is more reduced (Giordano et al., 2005b). It is noteworthy that in plants NR activity is stimulated when the electron transfer chain is more oxidized (Sherameti et al., 2002). The redox regulation of NR in *C. reinhardtii* is also associated with the presence of nitrate: NR becomes inactive in the absence of NO_3^- and can be reactivated by NO_3^- resupply and, in vitro, by ferricyanide oxidation (Fernandez et al., 2009 and references therein). It has been proposed that plastidial malic dehydrogenase is involved in the fine-tuning of reductants utilization for nitrate reduction, via its involvement in the malate/oxalacetate shuttle and thus the modulation of the redox state of the chloroplast and the cytosol (Quesada et al., 2000).

In *Gracilaria tenuistipitata*, a circadian oscillation of NR abundance and activity was observed in diel light-dark cycles and, with the opposite rhythmicity, in constant light, suggesting the operation of an endogenous clock at the mRNA level (Falcão et al., 2010). However, in the congeneric *G. chilensis* such endogenous clock is absent (Chow et al., 2004). An internal clock has also been invoked to explain the periodicity of NR expression and activity in higher plants (e.g. Pilgrim et al., 1993; also see Lillo et al., 2001).

An unusual regulation was observed in the NR of *Heterosigma akashiwo* (Coyne, 2010). In this raphidophycean, the NAR1 gene encoding for NR is constitutively expressed, even in the absence of nitrate and in the presence of ammonium. An apparent biphasic expression pattern of NAR1 was observed upon addition of nitrate to N-starved cultures, although the addition of NO_3^- to

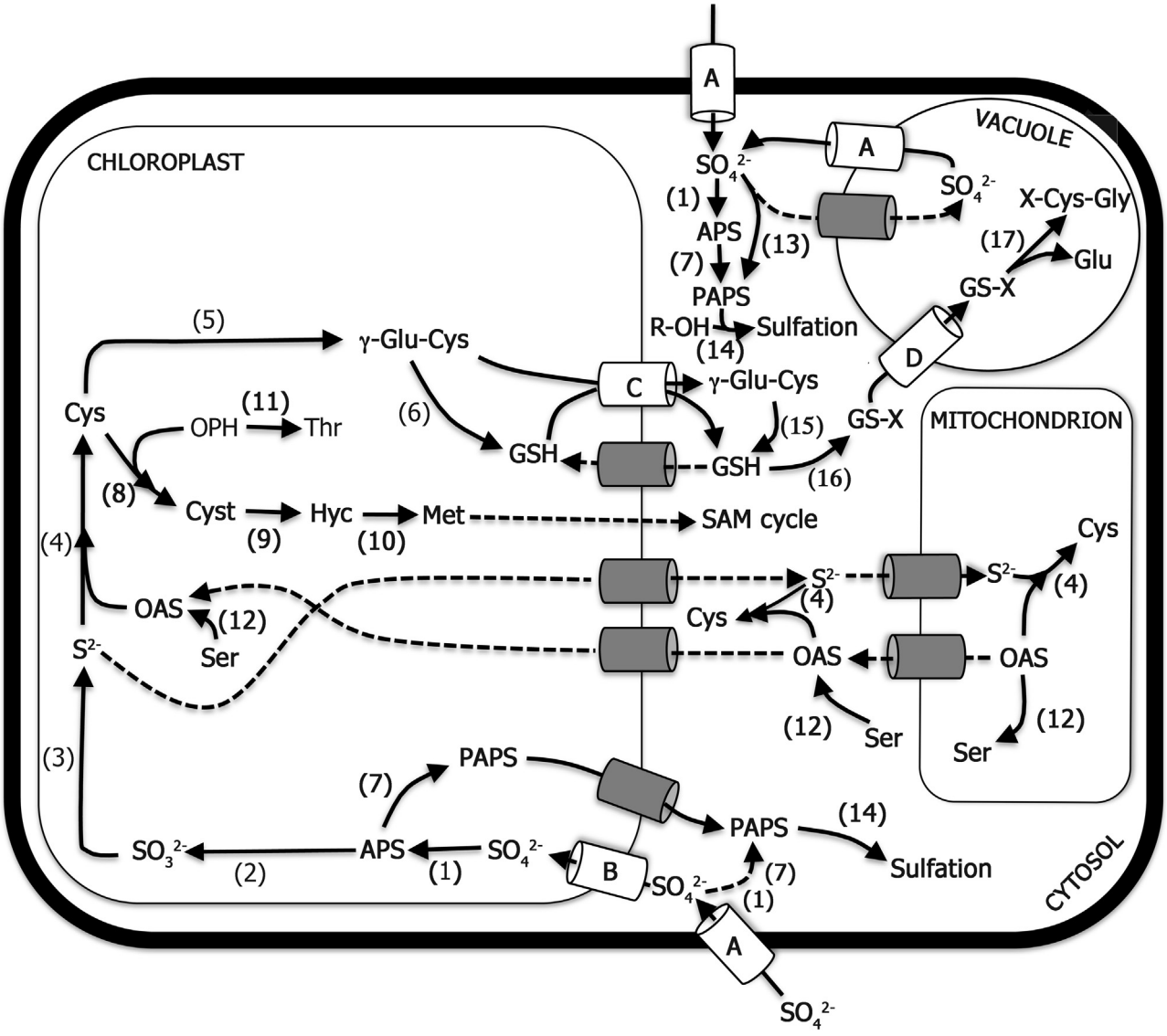

Fig. 2. Main pathways of sulfate metabolism in algae and plants. The letters identify the type of transporter (if no letter is indicated, no convincing identification of the transporter is available). The numbers indicate the enzymes. *Transporters*: A = nH$^+$/SO_4^{2-} (SULTR) or nNa$^+$/SO_4^{2-} (SLT) co-transports; B = ABC type transporters or unknown transporter for SO_4^{2-} uptake by the chloroplast; C = *CLT*, thiol transporter (chloroquine resistance transporter-like transporter); D = *MRP*, multidrug resistance-associated protein. *Enzymes*: (1) ATP sulfurylase; (2) APS kinase; (3) adenosine 5′-phosphosulfate (APS) reductase; (4) *O*-acetylserine (*thiol*)lyase; (5) γ-glutamylcysteine synthetase; (6) glutathione synthetase; (7) APS kinase; (8) cystathionine γ-synthase; (9) cystathionine β-lyase; (10) methionine synthase; (11) threonine synthase; (12) serine acetyltransferase; (13) fused ATP sulfurylase-APS kinase (present only in some chl *a* + *c* algae); (14) sulfotransferase; (15) glutathione synthetase; (16) glutathione-*S*-transferase; (17) γ-glutamyltransferase. *Metabolites*: Cys, cysteine; Cyst, cystathionine; Glu, glutamate; γ-Glu-Cys, γ-glutamylcysteine; GSH, glutathione; GS-X, glutathione conjugate; Hcy, homocysteine; Met, methionine; OPH, *O*-phosphohomoserine; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; R-OH, hydroxylated precursor; *SAM*, *S*-adenosylmethionine (the specific reactions of the SAM cycle are not shown); Ser, serine; *SiR*, sulfite reductase; Thr, threonine; X-Cys-Gly, cysteinylglycine conjugate. See Giordano and Prioretti, 2014 for details.

NO_3^--deplete cultures caused an increase in the abundance of NAR1 transcript. The author interpreted these data as a competitive response to changing NO_3^- availability. Nitrate reductase activity in the presence of NH_4^+ was also observed in the cyanobacterium *Synechococcus* sp. UTEX 2380, which also showed a biphasic kinetic of NR (Ruan, 2013). More recently, Stewart and Coyne (2011) observed that in the NR of *H. akashiwo* and *Chattonella subsalsa* a 2/2 hemoglobin (2/2Hb) domain exist in the hinge-2 region. The 2/2Hbs of *Heterosigma* and *Chattonella* have a rather high similarity to the mycobacterial 2/2Hbs, which are known to catalyze the conversion of NO to NO_3^-. These authors proposed a dual function for these NRs, which would be capable of converting NO to NO_3^- and subsequently operate a reductive NO_3^- assimilation.

Nitrate reductase seems to be absent in the culturable strains of the cyanobacterial genus *Prochlorococcus* (Lopez-Lozano et al., 2002; Moore et al., 2002; Martiny et al., 2009); only some low light ecotypes are able to reduce nitrate. Martiny et al. (2009) conducted a metagenomic analysis and discovered that a number of high light and low light adapted, non-culturable *Prochlorochoccus* lineages do possess the ability for NO_3^- and NO_2^- assimilation. The distribution of the nitrate assimilation genes also has a defined regional distribution, with a higher frequency in the Caribbean Sea and in the Indian Ocean. Martiny et al. (2009) connect this inhomogeneity in the metagenomic data to NO_3^- availability and, consequently, attribute the presence or absence on NR and related genes to differences in selective pressures in different regions of the oceans. According to this study, the *Prochlorococcus* NR genes form a distinct phylogenetic clade related to marine *Synechococcus* strains. The fact that in these strains the genomic location of the nitrate assimilation genes

is different from that in marine *Syneochococcus* suggests that these sequences were not recently transferred from *Synechococcus*.

Nitrite reductase (NiR) appears overall rather similar in plants and algae, both structurally and in terms of regulation; in both cases, it is plastidial, mostly constitutive and highly active. Since nitrite reductase is located in the plastid stroma of plants and eukaryotic algae, nitrite produced by cytosolic nitrate reductase or taken up from the medium must be transported into the plastid. High-affinity (half-saturation of about 0.1 mol m^{-3}) nitrite transport into isolated chloroplasts has been demonstrated (Anderson and Done, 1978; Brunswick and Cresswell, 1988). Sugiura et al. (2007) found two forms of the Nitr1 gene in *Cucumis sativa* (CsNitr1-L and CsNitr1-S); the proteins produced from both genes occur in the inner plastid envelope membrane. Heterologous expression of CsNitr1-S in *Saccharomyces cerevisae* plasmalemma catalyzed nitrite uptake with a half saturation concentration (Sugiura et al., 2007) similar to that previously shown for intact isolated chloroplasts by Anderson and Done (1978) and Brunswick and Cresswell (1988). Nitrite accumulates in the electrically negative compartment to which it is transported, showing that active transport is involved (Brunswick and Cresswell, 1988; Sugiura et al., 2007).

An interesting and direct link between sulfate and nitrate assimilation was observed in the extremophile red alga *Cyanidioschyzon merolae*, which inhabits acidic hot springs (Imamura et al., 2010). In this organism, no typical NiR was identified in the genome. However, two genes that closely resemble sulfite reductase (SiR) were found and one of these clusters close to nitrate-related (NAR) genes. The similarity between SiR and NiR is well known, but in all photosynthetic organism, the two genes are distinct; the specificity of SiR for NO_2^- can be dramatically increased by a single mutation (Nakayama et al., 2000) and NiR has been shown to function as a SiR in *Mycobacterium tuberculosis* (Schnell et al., 2005). The product of *C. merolae* SiR gene accumulates in the chloroplast when the alga is grown in the presence of nitrate and is repressed by ammonium; furthermore, *C. merolae* SiR functionally complements a NiR-deficient mutant of the cyanobacterium *Leptolyngbya boryana* (Imamura et al., 2010). No major differences in the glutamine synthetase/glutmine-oxoglutarate aminotransferase (GS/GOGAT) system of plants and algae have been identified; we shall therefore not deal with this portion of the N assimilation pathway, in this review.

5.2. Sulfur

Differing from nitrate, sulfate assimilation is induced by the lack of sulfate. In general, both algae and plants mostly use sulfate as the S source (Fig. 2). The standard reduction potential (E_0', 25 °C, 1 atm, pH 7, in aqueous solution) for the conversion of sulfate to sulfite is −454 mV, which is appreciably higher than that for the reduction of nitrate to nitrite (+421 mV; Rauen, 1964; Segel, 1975; Fig. 3). The reduction of sulfate to sulfite is therefore beyond the range of biological reductant potentials (Thauer et al., 1977). For this reason, in contrast to nitrate, sulfate must be "activated" before it can be reduced. The activation consists in the formation of adenosinephosphosulfate (APS), in which the mixed anhydride between phosphate and sulfate has a standard redox potential of about −60 mV, which is compatible with the use of thiols or pyrimidine as electron transporter ($NADH/NAD^+$ = −320 mV, $NADPH/NADP^+$ = −0.324; cysteine/cystine = −0.340; GSH/GSSG = −0.340 mV; Rauen, 1964; Segel, 1975). A similar situation occurs in the Calvin cycle, where the redox potential of the couples 3-phosphoglycerate/3-phosphoglyceraldehyde is in the order of −550 mV and PGA reacts with ATP to generate a lower potential intermediate that can be reduced by NADPH. The activation of sulfate is catalyzed by ATP

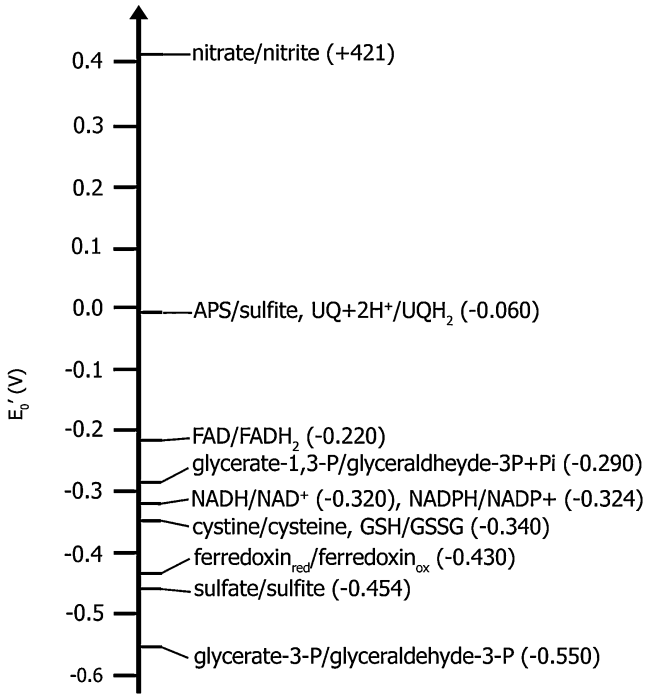

Fig. 3. Standard redox potential (E_0', 25 °C, 1 atm, pH 7, in aqueous solution) of cell compounds involved in redox reaction. The E_0' values were obtained from Rauen (1964) and Segel (1975).

sulfurylase (ATP-S; Takahashi et al., 2011). APS is the point of divergence between the sulfation pathways and S assimilation. In algae as in plant, sulfate reduction is primarily plastidial. Only one exception is known: in *Euglena gracilis*, S assimilation occurs in the mitochondrion.

Another major differences between algae and plants possibly resides in the control step of the S assimilation pathway. In plants, the key step in the pathway is believed to be the reaction catalyzed by APS reductase. So far, it has been assumed, although without much evidence that the same occurs in algae. Very recently, experiments conducted in M.G.'s lab, however, indicated that in most algae the first step in the pathway, the one catalyzed by ATP sulfurylase, may be under redox control, as it is shown by the strong stimulation of ATP-S activity in green algae, diatoms, and cyanobacteria of the *Synchococcus* genus by thiol reducing agents and the reversed effect of thiol oxidizing compounds. The same does not appear to be true for the dinoflagellate *Amphidinium klebsii* and for the freshwater *Synechocystis* PCC6803 (Prioretti and Giordano, unpublished). It is interesting that the redox sensitivity of ATP-S in algae is correlated with the number and location of cysteine residues in the protein sequence: a much larger number of cysteines (7–10; 5 of which in the same positions) are present in the ATP-Ses whose activity responds to thiol reduction/oxidation. The redox insensitive ATP-S of *Arabidopsis* and that of Rhodophyta only contains 2 cysteines; 4 cysteines are present in *Synechocystis* PCC6803 and in most freshwater and marine cyanobacteria of genera different form *Prochlorococcus* and *Synechococcus*; in *A. klebsii*, five of the 6 cysteines in the ATP-S protein sequence are in different positions from those of the other eukaryotic algae (Giordano and Prioretti, 2014). The phylogeny of ATP-S was thoroughly described by Patron et al. (2008): it is noteworthy that the genes encoding this enzymes in *Arabidopsis* have little sequence similarity with that of algae; the only exceptions are the sequences encoding the ATPS moieties of the fused ATP-S/APS kinase that are present in the genome of some haptophytes and heterokontophytes, which have

a high degree of similarity with *Arabidopsis* ATP-S genes (Bromke et al., 2013; Giordano and Prioretti, 2014).

Very little is known on the following steps of sulfur assimilation in algae; the few biochemical evidence and the genome data suggest that, as in plants, the sulfate in APS is reduced to sulfite by APS reductase; a highly active and nearly constitutive sulfite reductase leads to the production of sulfide, whose incorporation into cysteine is mediated by the *O*-acetyl serine thiol-lyase/serine acetyl transferase (OASTL/SAT) complex and by its regulation (Takahashi et al., 2011; Giordano and Prioretti, 2014; Fig. 2).

6. Sensing and signalling

In plants as in algae, nitrate appears to act as a signal for the activation of a number of genes involved in its own acquisition and assimilation (Bouguyon et al., 2012; Fernandez et al., 2009, and references therein). It has been demonstrated that the initiation of the expression of nitrogen assimilation related genes depends on the intracellular nitrate; consequently some of the nitrate transporters (specifically system I, see below) are necessary for this signal transduction pathway, and may act as "transceptors", i.e. system with the dual function of transporters and receptors (Gojon et al., 2011). NIT2 is the only regulatory gene of N assimilation in photosynthetic eukaryotes known so far; this protein is a transcription factor whose transcription is enhanced in the absence of nitrate and is negatively regulated by ammonium (which is also thus involved in N-assimilation related signalling), although the negative regulation of nitrate assimilation by ammonium is probably still to be unveiled for the most part (Fernandez et al., 2009 and references therein). It is noteworthy that NIT2 is not present in the thermo-acidophylic red alga *Cyanidioschizon merolae* and in *Arabidopsis* (Imamura et al., 2009), which leads to the unavoidable conclusions that different transcription factors for nitrate assimilation exist in photosynthetic organisms. In *C. merolae*, for instance, at least in vitro, CmMYB1, a member of the myeloblastosis (MYB) transcription factor family, is able to bind to promoter regions of nitrogen assimilation genes and may play a role similar to NIT2 in *Chlamydomonas* (Imamura et al., 2009).

Nitrate also acts as a signal on a broader scale, possibly reflecting the complex interaction of N metabolism with other pathways (e.g. Huppe and Turpin, 1994; Stitt, 1999; Raven, 2012). In *Arabidopsis* up to 10% of the genome (i.e. more than 2000 genes) is under more or less direct NO_3^- control (e.g. Krouk et al., 2010a,b) and about 300 to 600 of these genes appear to be directly regulated by NO_3^- (Wang et al., 2004). The signal that NO_3^- carries does not necessarily lead to changes in gene expression. A number of NO_3^--mediated processes occur through regulation (sensu Giordano, 2013), often via phosphorylation/dephosphorylation (Engelsberger and Schulz, 2012; Gojon et al., 2011) and without changes in the expressed proteome (Krouk et al., 2010a,b). Similar responses, although on a smaller number of proteins, have been observed for NH_4^+ (Engelsberger and Schulz, 2012; Nacry et al., 2013). Also miRNAs constitute a target for NO_3^- as a signal.

A number of morphogenic processes driven by NO_3^- have been identified in terrestrial plants (see Nacry et al., 2013; Andrews et al., 2013 for an overview of such processes). An example of such process is offered by the mechanism of action of the previously mentioned root NO_3^- transporter NRT1.1 in *A. thaliana* (Gojon et al., 2011); this transporter can bind (and transport) both NO_3^- and the hormone auxin. At low NO_3^-, AtNRT1.1 transports auxin out of the lateral roots; when NO_3^- is abundant, auxin accumulates in the lateral roots facilitating their growth. AtNRT1.1 also interacts with the other root NO_3^- transporter AtNRT2.1, favouring its activation, at high NO_3^-. Also NRT2.1 appears to play a dual transport and signalling role, linking root growth with the availability of NO_3^- and sugar. Interestingly, as opposite to NRT1.1, NRT2.1 signalling function is independent of its function as a NO_3^- transporter (see Gojon et al., 2011 and references therein for details).

In algae, the signalling role of NO_3^- has mostly been studied in *C. reinhardtii*. In this green alga, NO_3^- regulates both metabolism and developmental processes (e.g. gametogenesis) (Fernandez et al., 2009). Nitrate and the product of the regulatory gene NIT2 have been shown to be involved in the regulation of C allocation to storage pools (i.e. carbohydrates and triacylglicerols); this is not surprising, given the very tight coupling of C and N in algal cells (Huppe and Turpin, 1994). In *C. reinhardtii*, NH_4^+ sensing, which is mostly involved in the negative regulation of nitrate assimilation genes, seems to be mediated by CDP1, a cysteine-rich protein with no homology with other known proteins, and CYG56, a NO-inducible guanylate cyclase (see below for NO signalling). The synthesis of cyclic GMP is believed to be a key step in the perception of NH_4^+ and in the NH_4^+ signal transduction chain (de Montaigu et al., 2011).

Nitric oxide (NO) is believed to be involved in a number of physiological and developmental responses in plants. Yet, in contrast to animals, no nitric oxide synthases (NOS) have been identified in plants so far (Fröhlich and Durner, 2011). According to Fröhlich and Durner (2011), a variety of alternative systems, operating either oxidatively (e.g. using arginine and hydroxylamine) or by nitrate reduction, may generate NO in plants, although the available information does not always allows to thoroughly describe their mode of action. In algae, a variety of pathways leading to NO production have been found. A NOS with about 45% similarity to human NOS has been identified in the green alga *O. tauri* (Foresi et al., 2010). In *Chlamydomonas*, it has been proposed that NO is produced from nitrite in the cytosol by the catalysis of NR (Sakihama et al., 2002). Similar systems have been postulated for vascular plants (Fröhlich and Durner, 2011 and references therein). Since NO inhibits the mitochondrial electron transfer, when intracellular nitrite concentration is high the provision of ATP from the mitochondrial transfer chain may be hampered. AOX1, one of the two AOX genes encoding for the mitochondrial alternative oxidases, is regulated by nitrate and may be critical to maintain ATP synthesis at high cell nitrite concentrations. In the green alga *Chlorella*, AOX has been suggested to be directly involved in NO production by catalyzing nitrite reduction to NO; in this organism, NR is not involved in NO production (Tischner et al., 2004).

Recently, Sanz-Luque et al. (2013) proposed that the well known inhibitory effect of NH_4^+ on the expression of several genes involved in N assimilation in *Chlamydomonas rehinhardtii* (Fernandez et al., 2009) is mediated by NO. Such mechanism reversibly controls nitrate reductase activity and high affinity uptake systems of both ammonium and nitrate/nitrite. Apparently an integer cell is required for the operation of this system, which may be the consequence of the involvement of different organelles in the signal transduction pathway.

Sulfate is known to affect a variety of processes, besides sulfate assimilation. Among these, secondary metabolic pathways (both directly employing S and of flavonoid metabolism), nitrogen metabolism, response to oxidative stress, auxin and jasmonate synthesis (Takahashi et al., 2011). In the case of *A. thaliana*, the SULTR1.2 transporter, is involved in the perception of sulfate and possibly initiate the signal transduction that leads to a large number of S-related responses (Maruyama-Nakashita et al., 2003; Zhang et al., 2014). In the green alga *C. reinhardtii*, sulfate sensing is based on a membrane bound SAC1 protein (Gonzalez-Ballester and Grossman, 2009), which, in addition to inducing the expression of protein specifically associated with S deprivation response, also down-regulates other processes, especially photosynthesis, to pace them to S assimilation. Also a protein kinase, SNRK2.1, plays a role in the signalling pathway that descends from S deprivation

(Gonzalez-Ballester and Grossman, 2009). The action of SNRK2.1 is repressed by the activity of another kinase, SNRK2.2, which controls SNRK2.1. In turn, SNRK2.2 is negatively controlled by SAC1. The mechanisms of perception and response to S availability in other algae are not known.

In plants, a role for sulfide as a gaseous signalling molecule, or gasotransmitters (Wang, 2003), is also slowly emerging, with involvement in a number of functions such as stomatal opening, chloroplast biogenesis and time of germination (Dooley et al., 2013; Lisjak et al., 2013 and references therein). In plants, H_2S appears to interact with NO signalling and to be involved in responses to oxidative stress, via modulation of glutathione levels and the regulation of a variety of enzymes. Fundamental plant processes, such as stomatal opening appear to be, to some extent, influenced by H_2S (Lisjak et al., 2013). However, the physiology of H_2S as a gasotransmitters in plants (and even more in algae) is still to be fully and convincingly elucidated; we therefore leave the mechanistic details of these aspect of S nutrition to future reviews. It is worthwhile mentioning, in the context of this paper, that the signalling role of H_2S has been put in connection with life in anoxic or sulfidic conditions, which, as it was mentioned before, have accompanied the evolution of photosynthetic organism for a substantial amount of time.

A signalling function has also been attributed to sulfated polysaccharides. This is for instance the case a molecule of this group obtained from Ulvacean algae that had phytohormone-like effects on the gene expression of *Medicago sativa* and *Arabidopsis* (Jaulneau et al., 2010). These sulfated polysaccharides are believed to act through the jasmonic acid pathway.

7. Conclusion

- Long term changes in nitrogen and sulfur availability may have contributed to the radiation of algae and plants. The heterogeneity in algal N and S metabolism possibly reflects both their phylogenetic diversity and the changes in ocean chemistry over the long term.
- Both nitrogen and sulfur metabolism appear to be more directly under redox control in algae than in plants. This is especially true for the initial steps of the pathway (i.e. the reaction catalyzed by nitrate reductase and ATP sulfurylase). Further and broader investigations are necessary to confirm this observation.
- Many of the conclusions come from work on relatively few species; this is especially the case for S; this makes generalizations difficult.
- Molecules such as NO, NO_3^-, S^{2-} are important component of cells signalling systems and their role in modulating metabolism and responses to environmental changes is probably great, although little studied.

Acknowledgements

The University of Dundee is a registered Scottish charity, No. SC 015096. MG's research on N and S was funded by the Italian Ministry for Agriculture (MIPAF, Bioforme project), by the Italian Ministry of Foreign Affairs (MAE, Joint Italian-Israel Cooperation Program) and by the Assemble program of the European Union.

References

Agostoni, M., Erdner, D.L., 2011. Analysis of ammonium transporter and urease gene expression *in Aureoumbra lagunensis*. Harmful Algae 10, 549–556.

Aguilar-Barajas, E., Díaz-Pérez, C., Ramírez-Díaz, M.I., Riveras-Rosas, H., Cervantes, C., 2011. Bacterial transport of sulfate, molybdate and related oxyanions. Biometals 24, 687–707.

Allen, S., Smith, J.A.C., 1986. Ammonium nutrition in *Ricinus communis*: its effect on plant growth of the chemical composition in the whole plant, xylem and phloem saps. J. Exp. Bot. 37, 1599–1610.

Allen, A.E., Vardi, A., Bowler, C., 2006. An ecological and evolutionary context for integrated nitrogen metabolism and related signaling pathways in marine diatoms. Curr. Opin. Plant Biol. 9, 264–273.

Anbar, A.D., Knoll, A.H., 2002. Proterozoic ocean chemistry and evolution: a bioorganic bridge? Science 297, 1137–1142.

Anbar, A.D., Duan, Y., Lyons, T.W., Arnold, C.A., Kendall, B., Creaser, R.A., Kaufman, A.J., Gordon, G.W., Scott, C., Garvin, J., Buick, R., 2007. A whiff of oxygen before the Great Oxidation Event? Science 347, 1903–1906.

Anderson, J.W., Done, J., 1978. Light-dependent assimilation of nitrite by isolated pea chloroplasts. Plant Physiol. 61, 693–697.

Andrew, C.S., Kipps, E.H., Barford, H., 1952. Plant response to nitrogen and sulphur on a heavy clay soil from the Darling Downs, south-east Queensland. Crop Pasture Sci. 3, 111–124.

Andrews, M., Raven, J.A., Lea, P.J., 2013. Do plants need nitrate? The mechanisms by which nitrogen form affects plants. Ann. Appl. Biol. 163, 174–199, http://dx.doi.org/10.1111/aab.12045.

Andrews, M., Lea, P.J., Raven, J.A., Azevedo, R.A., 2009. Nitrogen use efficiency. 3. Photon and water costs of N_2 fixation. Ann. Appl. Biol. 155, 1–13, http://dx.doi.org/10.1111./j.1744-7348.2009.00338.x.

Andrews, M., Maule, H.G., Raven, J.A., Mistry, A., 2005. Extension growth of *Impatiens glandulifera* at low irradiances: importance of nitrate and potassium accumulation. Ann. Bot. 95, 641–658.

Aquino, R.S., Landeira-Fernandez, A.M., Valente, A.P., Andrade, L.E., Mourão, P.A.S., 2005. Occurrence of sulphated galactans in marine angiosperms: evolutionary implications. Glycobiology 15, 11–20.

Arad, S., Levy-Ontman, O., 2010. Red microalgal cell-wall polysaccharides: biotechnological aspects. Curr. Opin. Biotechnol. 21, 358–364.

Asael, D., Tissot, F.L.H., Reinhard, C.T., Rouxel, O., Dauphas, N., Lyons, T.W., Ponzevera, E., Liorzou, C., Cheron, S., 2013. Coupled molybdenum, iron and uranium stable isotopes as oceanic palaeoredox proxies during the Palaeoproterozoic Shunga Event. Chem. Geol. 362, 193–210.

Bekheet, I.A., Syrett, P.J., 1977. Urea-degrading enzymes in algae. Br. Phycol. J. 12, 137–143.

Bekker, A., Holland, H.D., 2012. Oxygen overshoot and recovery during the early Paloproterozoic. Earth Planet. Sci. Lett. 118, 295–304.

Bekker, A., Karhu, J.A., Erikson, K.A., Kaufman, A.J., 2003. Chemostratigraphy of Paleoproterozoic carbonate successions of the Wyoming Craton: tectonic forcing of biogemochemical change? Precambrian Res. 129, 279–325.

Bekker, A., Holland, H.D., Wang, P.-L., Rumble, H.I., Stein, H.J., Hannah, J.L., Coetzee, L.L., Beikes, N.J., 2004. Dating the rise of atmospheric oxygen. Nature 427, 117–120.

Berger, J., Schager, l.M., 2004. Allelopathic activity of Characeae. Biologia, Bratislava 59, 9–15.

Berges, J., 1997. Algal nitrate reductase. Eur. J. Phycol. 32, 3–8.

Bochenek, M., Etherington, G.J., Koprivova, A., Mugford, S.T., Bell, T.G., Malin, G., Kopriva, S., 2013. Transcriptome analysis of the sulphate deficiency response in the marine microalga *Emiliania huxleyi*. New Phytol. 199, 650–662, http://dx.doi.org/10.1111/nph.12303.

Bouguyon, E., Gojon, A., Nacry, P., 2012. Nitrate sensing and signaling in plants. Semin. Cell. Dev. Biol. 23, 648–654, http://dx.doi.org/10.1016/j.semcdb.2012.01.004.

Bowler, C., Allen, A.E., Badge, J.H., et al., 2008. The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 456, 239–244.

Bowler, C., Vardi, A., Allen, A.E., 2010. Oceanographic and biogeochemical insights from diatom genomes. Annu. Rev Mar. Sci. 2, 333–365.

Bragg, J.G., Quigg, A., Raven, J.A., Wagner, A., 2012. Ptotein elemental sparing and codon usage bias are correlated among bacteria. Molecular Ecology 21, 2480–2487.

Broadley, M.R., Bowen, M.C., Cotterrill, H.L., Hammond, J.P., Meacher, M.C., Mead, A., White, P.J., 2004. Phylogenetic variation in the shot mineral concentration of angiosperms. J. Exp. Bot. 55, 321–336.

Britto, D.T., Kronzucker, H.K., 2006. Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci. 11, 529–534.

Bromke, M.A., Hoefgen, R., Hesse, H., 2013. Phylogenetic aspects of the sulfate assimilation genes from *Thalassiosira pseudonana*. Amino Acids 44, 1253–1265, http://dx.doi.org/10.1007/s00726-013-1462-8.

Brunswick, P., Cresswell, C.S., 1988. Nitrite uptake into intact pea chloroplasts. I. Kinetics and relationship with nitrite assimilation. Plant Physiol. 86, 378–383.

Busigny, V., Lebeau, O., Ader, M., Krapez, B., Bekker, A., 2013. Nitrogen cycle in the late Archean ferruginous ocean. Chem. Geol. 362, 115–130.

Buchner, P., Takahashi, H., Hawkesford, M.J., 2004. Plant sulphate transporters: coordination of uptake, intracellular and long-distance transport. J. Exp. Bot. 55, 1765–1773.

Callon, O., Bulte, L., Kuras, R., Olive, J., Wollman, F.A., 1993. Extensive accumulation of an extracellular L-amino acid oxidase during gametogenesis of *Chlamydomonas reinhardtii*. Eur. J. Biochem. 215, 351–360.

Cambridge, M.L., Fraser, M.W., Holmer, M., Kuo, J., Kendrick, G.A., 2012. Hydrogen sulfide intrusion from Shark Bay, Western Australia. Mar. Freshwater Res. 63, 1027–1038.

Canfield, D.E., 1998. A new model for Proterozoic ocean chemistry. Nature 396, 450–453.
Canfield, D.E., 2004. The evolution of the Earth surface sulfur reservoir. Am. J. Sci. 304, 839–861.
Chow, F., Cabral de Oliveira, M., 2008. Rapid and slow modulation of nitrate reductase activity in the red macroalga *Gracilaria chilensis* (Graciliariales, Rhodophyta): influence of different nitrogen sources. J. Appl. Phycol. 20, 775–782.
Chow, F., de Oliveira, M.C., Pedersén, M., 2004. In vitro assay and light regulation of nitrate reductase in red alga *Gracilaria chilensis*. J. Plant Physiol. 161, 769–776.
Cooper, R.M., Williams, J.S., 2004. Elemental sulfur as an induced antifungal substance in plant defence. J. Exp. Bot. 55, 1947–1953.
Coskum, D., Britto, D.T., Mingyuan, L., Becker, A., Kronzucker, H.J., 2013. Rapid ammonia gas transport accounts for futile transmembrane cycling under NH_3/NH_4^+ toxicity in plant roots. Plant Physiol. 163, 1859–1867.
Coyne, K.J., 2010. Nitrate reductase (NR1) sequence and expression in the harmful alga *Heterosigma akashiwo* (Raphidophyceae). J. Phycol. 46, 135–142.
Crawford, N.M., Glass, A.D.M., 1998. Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci. 3, 389–395.
Cuevasanta, E., Denicola, A., Alvarez, B., Möller, M.N., 2012. Solubility and permeation of hydrogen sulfide in lipid membranes. PLoS One 7 (4), e34562.
Czyzewski, B.K., Wang, D.N., 2012. Identification and characterization of a bacterial hydrosulphide ion channel. Nature 483, 494–497.
Dagestad, D., Lien, T., Knutseb, G., 1981. Degradation and compartmentalization of urea in *Chlamydomonas reinhardtii*. Arch. Microbiol. 129, 261–264.
de Hostos, E.L., Schilling, J., Grossman, A.R., 1989. Structure and expression of the gene encoding the periplasmic arylsulfatase of *Chlamydomonas reinhardtii*. Mol. Gen. Genet. 218, 229–239.
de Montaigu, A., SanzLuque, E., Macias, M.I., Galvan, A., Fernandez, E., 2011. Transcriptional regulation of CDP1 and CYG56 is required for proper NH_4^+ sensing in *Chlamydomonas*. J. Exp. Bot. 62, 1425–1437.
Derelle, E., Ferraz, C., Rombautz, S., et al., 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. Proc. Nat. Acad. Sci. U.S.A. 103, 11647–11652.
Dooley, F.D., Nair, S.P., Ward, P.D., 2013. Increased growth and germination success in plants following hydrogen sulfide administration. PLoS One 8, e62048.
Duarte, C.M., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquat. Bot. 65, 159–174.
Dyhrman, S.T., Anderson, B.M., 2003. Urease activity in cultures and field populations of the toxic dinoflagellates. Limnol. Oceanogr. 48, 647–655.
Elser, J.J., Hassett, R.P., 1994. A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems. Nature 370, 211–213.
Elser, J.J., Bracken, M.E.S., Cleland, E.E., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seablom, W.E., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142.
Engelsberger, W.R., Schulz, W.X., 2012. Nitrate and ammonium lead to distinct global dynamic phosphorylation patterns when resupplied to nitrogen starved Arabidopsis seedlings. Plant J. 69, 978–995.
Ermilova, E.V., Nikitin, M.M., Lapina, T.V., Zautskaya, Z.M., 2004. Chemotactic behaviour of *Chlamydomoans reinhardtii* is altered during gametogenesis. Curr. Microbiol. 46, 261–264.
Farias, E.H.C., Pomin, V.H., Valente, A.P., Nader, H.B., Rocha, H.A.O., Nourao, P.A.S., 2008. A preponderantly 4-sulphated, 3-linked galactan from the green alga *Codium isthmocladum*. Glycobiology 18, 250–259.
Falcão, V.R., Oliveira, M.C., Colepicolo, P., 2010. Molecular characterization of nitrate reductase gene and its expression in the marine red alga *Gracilaria tenuistipitata* (Rhodophyta). J. Appl. Phycol. 22, 613–622.
Falkowski, P.G., 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. Nature 387, 272–275.
Falkowski, P.G., Raven, J.A., 2007. Aquatic Photosynthesis, 2nd edition. Princeton University Press, Princeton, pp. xiii + 484.
Fernandez, E., Llamas, A., Galvàn, A., 2009. Nitrogen assimilation and its regulation. In: Stern, D.B., Harris, E.H. (Eds.), The Chlamydomonas source book, vol. 2. , second ed. Elsevier, Amsterdam, pp. 69–113.
Field, C.B., Beherenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science 281, 237–240.
Flynn, K.J., Blackford, J.C., Baird, M.E., Raven, J.A., Clark, D.R., Beardall, J., Brownlee, C., Fabian, H., Wheeler, G.L., 2012. Changes in pH at the exterior surface of plankton with ocean acidification. Nat. Clim. Change 2, 510–513.
Forde, B.G., 2000. Nitrate transporters in plants: structure, function and regulation. Biochim. Biophys. Acta 1465, 649–655.
Foresi, N., Correa-Aragunde, N., Parisi, G., Calo, G., Salerno, G., Lamattina, L., 2010. Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 22, 3816–3830.
Fröhlich, A., Durner, J., 2011. The hunt for plant nitric oxide synthase (NOS): is one really needed? Plant Sci. 181, 401–404.
Fry, B., Scalan, R.S., Winters, J.K., Parker, P.L., 1982. Sulphur uptake by salt grasses, mangroves, and seagrasses in anaerobic sediments. Geochim. Cosmochim. Acta 46, 1121–1124.
Galván, A., Quesada, A., Fernández, E., 1996. Nitrate and nitrite are transported by different specific transport systems and by a bispecific transporter in *Chlamydomonas reinhardtii*. J. Biol. Chem. 271, 2088–2092.
Garcia-Garcia, J.D., Olin-Sandoval, V., Saavedra, E., Girard, L., Hernández, G., Moreno-Sánchez, R., 2012. Sulfate uptake in photosynthetic *Euglena gracilis*. Mechanisms of regulation and contribution to cysteine homeostasis. Biochim. Biophys. Acta 1820, 1567–1575.
Gárcia-Sánchez, M.J., Jaime, M.P., Ramos, A., Sanders, D., Fernandez, J.A., 2000. Sodium-dependent nitrate transport at the plasma membrane of leaf cells of the marine higher plant *Zostera marina*. Plant Physiol. 122, 879–885.
Gattuso, J.-P., Frankignoule, M., Wollast, R., 1998. Carbon and carbonate metabolism in coastal aquatic ecosystems. Annu. Rev. Ecol. Syst. 29, 405–434.
Gill, B.C., Lyons, T.W., Saltzman, M.R., 2007. Parallel, high-resolution carbon and sulfur isotope records of the evolving Paleozoic marine sulfur reservoir. Palaeogeography, Palaeoclimatology, Palaeoecol. 256, 156–173.
Gill, B.C., Lyons, T.W., Young, S., Kump, L., Knoll, A.H., Saltzman, M.R., 2011. Geochemical evidence for widespread euxinia in the later Cambrian ocean. Nature 469, 80–83.
Gilleaudeau, G.J., Kah, L.C., 2013. Oceanic molybdenum drawdown by epeiric sea expansion in the Mesoproterozoic. Chem. Geol. 356, 21–37.
Giordano, M., 2013. Homeostasis: an underestimated focal point of ecology and evolution. Plant Sci. 211, 92–101, http://dx.doi.org/10.1016/j.plantsci.2013.07.008.
Giordano, M., Beardall, J., Raven, J.A., 2005a. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation and evolution. Ann. Rev. Plant Biol. 56, 99–131, http://dx.doi.org/10.1146/annurev.arplant.56.032604.144052.
Giordano, M., Chen, Y.N., Koblizek, M., Falkowski, P.G., 2005b. Regulation of nitrate reductase in *Chlamydomonas reinhardtii* by the redox state of the plastoquinone pool. Eur. J. Phycol. 40, 345–352, http://dx.doi.org/10.1080/09670260500334263.
Giordano, M., Norici, A., Hell, R., 2005c. Sulfur and phytoplankton: acquisition, metabolism and impact on the environment. New Phytol. 166 (2), 371–382, http://dx.doi.org/10.1111/j.1469-8137.2005.01335.x.
Giordano, M., Pezzoni, V., Hell, R., 2000. Strategies for the allocation of resources under sulfur limitation in the green alga *Dunaliella salina*. Plant Physiol. 124, 857–864.
Giordano, M., Prioretti, L., 2014. Sulphur and algae: metabolism, ecology and evolution. In: Borowitzka, M.A., Beardall, J., Raven, J.A. (Eds.), The Physiology of Microalgae. Springer, Dordrecht, The Netherlands (in press).
Gojon, A., Krouk, G., Perrine-Walker, F., Laugier, E., 2011. Nitrate transceptor(s) in plants. J. Exp. Bot. 62, 2299–2308.
Gonzalez-Ballester, D., Grossman, A.R., 2009. Sulphur: from acquisition to assimilation. In: Stern, D.B., Harris, E.H. (Eds.), The Chlamydomonas Source Book, vol. 2. , second ed. Elsevier, Amsterdam, pp. 69–113.
Goss, R., Wilhelm, C., 2010. Lipids in algae, lichens and mosses. In: Wada, H., Murata, M. (Eds.), Lipids in Photosynthesis. Advances in Photosynthesis and Respiration, vol. 30, p. 117.
Goss, R., Nerlich, J., Lepetit, B., Schaller, S., Vieler, A., Wilhelm, C., 2009. The lipid dependence of diadinoxanthin de-epoxidation presents new evidence foe a macrodomain organization of the diatom thylakoid membrane. J. Plant Physiol. 166, 1839–1854.
Habicht, K.S., Gade, M., Thamdrup, B., Berg, P., Canfield, D.E., 2002. Calibration of sulfate levels in the Archean ocean. Science 298, 2372–2374.
Hagelueken, G., Adams, T.M., Wiehlmann, L., Widow, U., Kolmar, H., Tuemmler, B., Heinz, D.W., Schubert, W.-D., 2006. The crystal structure of SdsA1, an alkylsulfatase from *Pseudomonas aeruginosa*, defines a third class of sulfatases. Proc. Acad. Sci. U.S.A. 103, 7631–7636.
Handley, L.L., Raven, J.A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ. 15, 965–985.
Hell, R., Hillebrand, H., 2001. Plant concepts for mineral acquisition and allocation. Curr. Opin. Biotechnol. 12, 161–168.
Hildebrand, M., 2005. Cloning and functional characterization of ammonium transporters from the marine diatom *Cylindrotheca fusiformis* (Bacillariophyceae). J. Phycol. 41, 105–113.
Hockin, N.L., Mock, T., Mulholland, F., Kopriva, S., Malin, G., 2012. The response of diatom central carbon metabolism to nitrogen starvation is different from that of green algae and higher plants. Plant Physiol. 158, 299–312.
Hoffmann, R., Bisson, M.A., 1990. *Chara buckellii*, a charophyte from an unusual saline environment. Plant Physiol. 93, 123–127.
Horita, J., Zimmermann, H., Holland, H.D., 2002. Chemical evolution of seawater during the Phanerozoic: implications from the record of marine evaporites. Geochim. Cosmochim. Acta 66, 3733–3756.
Holmer, M., Storkholm, P., 2001. Sulfate reduction and sulfur cycling in lake sediments: a review. Freshwater Biology 46, 431–451.
Huang, N.C., Liu, K.H., Lo, H.J., Tsay, Y.F., 1999. Cloning and functional characterization of an *Arabidopsis* nitrate transporter gene that encodes a constitutive component of low-affinity uptake. Plant Cell 11, 1381–1392.
Huppe, H.C., Turpin, D.H., 1994. Integration of carbon and nitrogen metabolism in palt and algal cells. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 577–607.
Imamura, S., Kanesaki, Y., Ohnuma, M., Inouye, T., Sekine, Y., Fujiwara, T., et al., 2009. R2R3-type MYB transcription factor, CmMYB1, is a central nitrogen assimilation regulator in *Cyanidioschyzon merolae*. Proc. Nat. Acad. Sci. U.S.A. 106, 12548–12553.
Imamura, S., Terashita, M., Ohnuma, M., Maruyama, S., Minoda, A., Weber, A.P.M., Inouye, T., Sekine, Y., Fujita, Y., Omata, T., Tanaka, K., 2010. Nitrate assimilatory genes and their transcriptional regulation in a unicellular red alga *Cyanidioschyzon merolae*: genetic evidence for nitrite reduction by a sulfite reductase-like enzyme. Plant Cell Physiol. 51 (5), 707–717, http://dx.doi.org/10.1093/pcp/pcq043.

Jaulneau, V., Lafitte, C., Jacquet, C., Fournier, S., Salamagne, S., Briand, X., Esquerré-Tugayé, M.-Y., Dumas, B., 2010. Ulvan, a sulphated polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway. J. Biomed. Biotechnol., http://dx.doi.org/10.1155/2010/525291 (Article ID 525291), 11 pages.

Johnson, D.W., 1984. Sulfur cycling in forests. Biogeochemistry 1, 29–43.

Jones, M.R., Leith, I.D., Fowler, D., Raven, J.A., Sutton, M.A., Nemiz, E., Cape, J.N., Sheppard, L.J., Smith, R.I., Theobald, M.R., 2007a. Concentration-dependent NH_3 deposition processes for mixed moorland semi-natural vegetation. Atmos. Environ. 41, 2049–2060.

Jones, M.R., Leith, I.D., Fowler, D., Raven, J.A., Sutton, M.A., Nemiz, E., Cape, J.N., Sheppard, L.J., Smith, R.I., 2007b. Concentration-dependent NH_3 deposition processes for stomatal and non-stomatal moorland species. Atmos. Environ. 41, 8980–8994.

Kanamaru, K., Wang, R., Su, W., Crawford, N.M., 1999. Ser-534 in the hinge region I of *Arabidopsis* nitrate reductase is conditionally required for the binding of 14-3-3 proteins and the in vitro inhibition of NR. J. Biol. Chem. 274, 4160–4165.

Kah, L.C., Lyons, T.W., Frank, T.D., 2004. Low marine sulphate and protracted oxygenation of the Proterozoic biosphere. Nature 431, 834–838.

Kahnert, A., Kertesz, M.A., 2000. Characterization of a sulphur-regulated oxygenative alkylsulfatase from *Pseudomonas putida* S-313. J. Biol. Chem. 275, 31661–31667.

Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., Ohnishi, M., Mimura, T., Buchner, P., Hawkesford, M.J., Yamaya, T., Takahashi, H., 2004. Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in *Arabidopsis*. Plant Cell 16, 2693–2704.

Keller, M.D., Kiene, R.P., Matrai, P.A., Bellows, W.K., 1999. Production of glycine betaine and dimethylsulfonioprpionate in marine phytoplankton II. N-limited chemostat cultures. Marine Biology 135, 249–257.

Kertesz, M.A., 2000. Riding the sulfur cycle—metabolism of sulfonates and sulfate esters in Gram-negative bacteria. FEMS Microbiol. Rev. 24, 135–175.

Kirk, D.L., Kirk, M.M., 1978. Carrier-mediated uptake of arginine and urea by *Chlamydomonas reinhardtii*. Plant Physiol. 61, 556–560.

Kobayashi, M., Rodriguez, R., Lam, C., Omata, T., 1997. Involvement of the C-terminal domain of an ATP-binding subunit in the regulation of the ABC-type nitrate/nitrite transporter of the cyanobacterium *Synechococcus* sp. Strain PCC 7942. J. Biol. Chem. 272, 27197–27201.

Kojima, S., Bohner, A., von Wirén, N., 2007a. Molecular mechanism of urea transport in plants. J. Membr. Biol. 212, 83–91.

Kojima, S., Bohner, A., Gasser, B., Yuan, L., von Wirén, N., 2007b. AtDUR3 represents the major transporter for high-affinity urea transport across the plasmalemma of nitrogen-deficient *Arabidopsis* roots. Plant J. 52L, 30–40.

Kopriva, S., Mugford, S.G., Baranecka, P., Lee, B.R., Malthewman, C.A., Koprivova, A., 2012. Control of sulfur partitioning between primary and secondary metabolism in *Arabidopsis*. Front. Plant Sci. 3, 163, http://dx.doi.org/10.3389/fpls.2012.00163.

Koprivova, A., Giovannetti, M., Baraniecka, P., Lee, B.-R., Grondin, C., Loudet, O., Kopriva, S., 2013. Natural variation in the ATPS1 isoform of ATP sulfurylase contributes to the control of sulfate levels in *Arabidopsis*. Plant Physiol. 163, 1–9.

Kotur, Z., Siddiqi, Y.M., Glass, A.D.M., 2013. Characterization of nitrite uptake by *Arabidopsis thaliana*: evidence for a nitrite-specific transporter. New Phytol. 200, 201–210, http://dx.doi.org/10.1111/nph.12358.

Kronzucker, H.J., Britto, D.T., Davenport, R.J., Tester, M., 2001. Ammonium toxicity and the real cost of transport. Trends Plant Sci. 6, 336–337.

Krouk, G., Mirowski, P., LeCun, Y., Shasha, D.E., Coruzzi, G.M., 2010a. Predictive network modeling of the high resolution dynamic plant transcriptome in response to nitrate. Genome Biol. 11, R123.

Krouk, G., Crawford, N.M., Coruzzi, G.M., Tsay, Y.F., 2010b. Nitrate signaling: adaptation to fluctuating environments. Curr. Opin. Plant Biol. 13, 266–273.

Lamers, L.P.M., Govers, L.L., Janssen, I.C.J.M., Geurts, J.J.M., Van der Welle, M.E.W., Van Katwijk, M.M., Van der Heide, T., Roelofs, J.G.M., Smolders, A.J.P., 2013. Sulfide as a soil phytotoxin—a review. Front. Plant Sci. 4 (article 268).

Lambeck, I.C., Fischer-Schrader, K., Niks, D., Roeper, J., Chi, J.-C., Hille, R., Schwarz, G., 2012. Molecular mechanisms of 14-3-3 protein-mediated inhibition of plant nitrate reductase. J. Biol. Chem. 287, 4562–4571.

Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E., 2008. Plant nutrient-acquisition strategies change with soil age. Trends Ecol. Evol. 23, 95–103, http://dx.doi.org/10.1016/j.tree.2007.10.008.

Laudenbach, D.E., Grossman, A.R., 1991. Characterization and mutagenesis of sulphur-regulated genes in a cyanobacterium: evidence for function in sulphate transport. J. Bacteriol. 173, 2739–2750.

Leftley, J.W., Syrett, P.J., 1973. Urease and ATP: urea amidolyase in unicellular algae. J. Gen. Microbiol. 77, 109–115.

Leran, S., Varala, K., Boyer, J.C., Chiurazzi, M., Crawford, N., Daniel-Vedele, F., David, L., Dickstein, R., Fernandez, E., Forde, B., Gassmann, W., Geiger, D., Gojon, A., Gong, J.-M., Halkier, B.A., Harris, J.M., Hedrich, R., Limami, A.M., Rentsch, D., Seo, M., Tsay, Y.F., Zhang, M., Coruzzi, G., Lacombe, B., 2014. A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. Trends Plant Sci. 19, 5–9.

Lillo, C., Meyer, C., Ruoff, P., 2001. The nitrate reductase circadian system. The central clock dogma contra multiple oscillatory feedback loops. Plant Physiol. 125, 1554–1557.

Lindberg, P., Melis, A., 2008. The chloroplast sulfate transport system in the green alga *Chlamydomonas reinhardtii*. Planta 228, 951–961.

Lisjak, M., Teklic, T., Wilson, I.D., Whiteman, M., Hancock, J.T., 2013. Hydrogen sulfide: environmental factor or signalling molecule? Plant Cell Environ. 36, 1607–1616.

Liu, K.H., Tsay, Y.F., 2003. Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. EMBO J. 22, 1005–1013.

Liu, L-H., Ludewig, U., Frommer, W.S., von Wirén, N., 2003. AtDUR3 encodes a new type of high-affinity urea/H^+ symporter in *Arabidopsis*. Plant Cell 15, 790–800.

Lopez-Lozano, A., Diez, J., El Alaoui, S., Moreno-Vivian, C., Garcia-Fernandez, J.M., 2002. Nitrate is reduced by heterotrophic bacteria but not transferred to *Prochlorococcus* in non-axenic cultures. FEMS Microbiol. Ecol. 41, 151–160.

Luo, G.M., Kump, L.R., Wang, Y.B., Tong, J.N., Arthur, M.A., Yang, H., Huang, J.H., Yin, H.F., Xie, S.C., 2010. Isotopic evidence for an anomalously low oceanic sulfate concentration following end-Permian mass extinction. Earth Planetary Sci. Lett. 300, 101–111.

Maberly, S.C., King, L., Dent, M.M., Jones, R.L., Gibson, C.E., 2002. Nutrient limitation of phytoplankton and periphyton growth in upland lakes. Freshwater Biol. 47, 2136–2152.

MacKintosh, C., 2004. Dynamic interactions between 14-3-3-proteins and phosphoproteins regulate diverse cellular processes. Biochem. J. 381, 329–342.

Mariscal, V., Moulin, P., Orsel, M., Miller, A.J., Fernandez, E., Galvan, A., 2006. Differential regulation of *Chlamydomonas* Nar1 gene family by carbon and nitrogen. Protist 157, 421–433.

Martin, A.P., Condon, D.J., Prave, A.R., Melezhik, V.A., Lepland, A., Fallick, A.E., 2013. Dating the termination of the Paleoproterozoic Lomagundi–Jatuli carbon isotope Event in the North Transfennoscandian Breenstone Belt. Precambrian Res. 224, 160–168.

Martinoia, E., Maeshima, M., Neuhaus, H.E., 2007. Vacuolar transporters and their essential role in plant metabolism. J. Exp. Bot. 58, 83–102.

Martiny, A.C., Kathuria, S., Berube, P.M., 2009. Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. Proc. Nat. Acad. Sci. U.S.A. 106, 10787–10792.

Maruyama-Nakashita, A., Inoue, E., Watanabe-Takahashi, A., Yamaya, T., Takahashi, H., 2003. Transcriptome profiling of sulfur-responsive genes in *Arabidopsis* reveals global effects of sulfur nutrition on multiple metabolic pathways. Plant Physiol. 132, 597–605.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105, 1141–1157, http://dx.doi.org/10.1093/aob/mcq028.

McClintock, M., Higginbotham, N., Uribe, E.G., Cleland, K.E., 1982. Active, irreversible accumulation of extreme levels of H_2SO_4 in the brown alga *Desmarestia*. Plant Physiol. 70, 771–774.

McDonald, S.M., Plant, J.N., Warden, A.Z., 2010. The mixed lineage nature of nitrogen transport and assimilation in marine eukaryotic phytoplankton: a case study of *Micromonas*. Mol. Biol. Evol. 27, 2268–2283.

Melis, A., Chen, H.-C., 2005. Chloroplast sulfate transport in green algae—genes, proteins and effects. Photosynth. Res. 86, 299–307.

Molis, M., Wesselss, H., Hagen, W., Karsten, U., Wiencke, C., 2009. Do sulphuric acid and the brown alga *Desmarestia viridis* support community structure of Arctic kelp patches by altering grazing impact, distribution pattern and feeding behavior of sea urchins. Polar Biol. 32, 72–81.

Moore, L.R., Post, A.F., Rocap, G., Chisholm, S.W., 2002. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol. Oceanogr. 47, 989–996.

Moss, B., 1969. Limitation of algal growth in some central African waters. Limnol. Oceanogr. 14, 591–601.

Muñoz-Blanco, J., Moyano, E., Cardenas, J., 1990. Extracellular deamination of amino acids by *Chlamydomonas reinhardtii* cells. Planta 182, 194–198.

Nacry, P., Bouguyon, E., Gojon, A., 2013. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370, 1–29.

Nakayama, M., Akashi, T., Hase, T., 2000. Plant sulfite reductase: molecular structure, catalytic function and interaction with ferredoxin. J. Inorg. Biochem. 82, 27–32.

Näsholm, T., Kielland, K., Haneteg, U., 2009. Uptake of organic nitrogen in plants. New Phytol. 182, 31–48.

Navarro, M.T., Guerra, E., Fernandez, E., Galvan, A., 2000. Nitrite reductase mutants as an approach to understanding nitrate assimilation in *Chlamydomonas reinhardtii*. Plant Physiol. 122, 283–290.

Navarro-González, R., McKay, C.P., Mvondo, D.N., 2001. A possible nitrogen crisis for Archaean life due to reduced nitrogen fixation by lightning. Nature 412, 61–64.

Newton, R.J., Reeves, E.P., Kafousia, N., Wignall, P.B., Bottrell, S., Sha, J.-G., 2011. Low marine sulfate concentrations and the isolation of the European epicontinental sea during the Early Jurassic. Geol 39, 7–10.

Norici, A., Hell, R., Giordano, M., 2005. Sulfur and primary production in aquatic environments: an ecological perspective. Photosynth. Res. 86, 409–417.

Orsel, M., Chopin, F., Leleu, O., Smith, S.J., Krapp, A., Deniel-Vedele, F., Miller, A.J., 2006. Characterization of a two-component high affinity nitrate uptake system in *Arabidopsis*. Physiology and protein–protein interaction. Plant Physiol. 142, 1304–1317.

Ottosen, L.D.M., Risgaard-Petersen, N., Nielsen, L.P., 1999. Direct and indirect measurements of nitrification and denitrification in the rhizosphere of aquatic macrophytes. Aquat Microb. Ecol. 19, 81–91.

Papineau, D., 2010. Global biogeochemical changes at both ends of the Proterozoic: insights from phosphorites. Astrobiology 10, 165–181.

Papineau, D., Purohit, R., Fogel, M.L., Shields-Zhou, G.A., 2013. High phosphate availability as possible cause for massive cyanobacterial production of oxygen in the Paleoproterozopic atmosphere. Earth Planet. Sci. Lett. 302, 225–236.

Partin, C.A., Bekker, A., Planavsky, N.J., Scott, C.T., Gill, B.C., Li, C., Podkovyroc, V., Maslov, A., Konhauser, K.O., Lalonde, S.V., Love, G.D., Poulton, S.W., Lyons, T.W., 2013. Large-scale fluctuations in Precambrian atmospheric and oceanic oxygen levels from the record of U in shales. Earth Planet. Sci. Lett. 369–370, 284–293.

Patron, N., Durnford, D., Kopriva, S., 2008. Sulfate assimilation in Eukaryotes: evolutionary origins and subcellular localisation. BMC Evol. Biol. 8, 39, http://dx.doi.org/10.1186/1471-2148-8-39.

Pelletreau, K.N., Muller-Parker, G., 2002. Sulfuric acid in the phaeophyte alga *Desmarestia munda* deters feeding by the sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 141, 1–9.

Petrychenko, O.Y., Peryt, T.M.T., Chechel, E.I., 2005. Early Cambrian seawater chemistry from fluid inclusions in halite from Siberian evaporites. Chem. Geol. 219, 149–161.

Pilgrim, M.L., Caspar, T., Quail, P.H., McClung, C.R., 1993. Circadian and light regulated expression of nitrate reductase in *Arabidopsis*. Plant Mol. Biol. 23, 349–364.

Planavsky, N.J., Rouxel, O.J., Bekker, A., Lalonde, S.V., Konhauser, K.O., Reinhard, C.T., Lyons, T.W., 2010. The evolution of the marine phosphate reservoir. Nature 467, 1088–1090.

Planavsky, N.J., Bekker, A., Hoffman, A., Owens, J.D., Lyons, T.W., 2012. Sulfur records of rising and falling oxygen and sulfate levels during the Lomagundi event. Proc. Nat. Acad. Sci. U.S.A. 109, 18300–18305.

Pomin, V.H., 2010. Structural and functional insights into sulfated galactans: a systematic review. Glycoconjugate J. 27, 1–12.

Popper, Z.A., Tuohy, M.G., 2010. Beyond the green: understanding the evolutionary puzzle of plant and algal cell walls. Plant Physiol. 152, 373–383, http://dx.doi.org/10.1104/pp.110.158055.

Pootakham, W., Gonzalez-Ballester, D., Grossman, A.R., 2010. Identification and regulation of plasma membrane sulphate transporters in *Chlamydomonas*. Plant Physiol. 153, 1653–1668.

Quesada, A., Gomez-Garcia, I., Fernandez, E., 2000. Involvement of chloroplast and mitochondria redox valves in nitrate assimilation. Trends Plant Sci. 5, 463–464.

Quigg, A., Irwin, A.J., Finkel, Z.V., 2011. Evolutionary inheritance of elemental stoichiometry in phytoplankton. Proc. R. Soc. London, Ser. B 278, 526–534.

Rai, A.N., Bergman, B., Rasmussen, U., 2003. Cyanobacteria in Symbiosis. Springer, Dordrecht, Netherlands.

Ratti, S., Giordano, M., 2008. Allocation of sulfur to sulfonium compounds in microalgae. In: Khan, N.A., Singh, S., Umar, S. (Eds.), Sulfur Assimilation and Abiotic Stress in Plants. Springer-Verlag, Berlin, pp. 317–333.

Ratti, S., Knoll, A.H., Giordano, M., 2011. Did sulfate availability facilitate the evolutionary expansion of chlorophyll *a* + *c* phytoplankton in the oceans? Geobiology 9, 301–312, http://dx.doi.org/10.1111/j.1472-4669.2011.00284.x.

Ratti, S., Knoll, A.H., Giordano, M., 2013. Grazers and phytoplankton growth in the oceans: an experimental and evolutionary perspective. PLoS One 8 (10), e77349, http://dx.doi.org/10.1371/journal.pone.0077349.

Rauen, H.M., 1964. Biochemisches Taschenbuch. Springer, Berlin.

Rausch, T., Wachter, A., 2005. Sulfur metabolism: a versatile platform for launching defence operations. Trends Plant Sci. 10, 503–509.

Raven, J.A., 1984. Energetics and Transport in Aquatic Plants. AR Liss, New York, NY.

Raven, J.A., 2003. Can plants rely on nitrate? Trends Plant Sci. 8, 314–315.

Raven, J.A., 2012. Protein turnover and plant RNA and phosphorus requirements in relation to nitrogen fixation. Plant Sci. 188–189, 25–35.

Raven, J.A., 2013a. The evolution of autotrophy in relation to phosphorus requirement. J. Exp. Bot. 64, 4023–4046.

Raven, J.A., Smith, F.A., 1976. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol. 76, 415–431.

Raven, J.A., 2013b. Half a century of pursuing the pervasive proton. In: Lüttge, U., et al. (Eds.), Progress in Botany, vol. 74. , pp. 3–34, Doi 10.1007/978-3-642-30967-0_1.

Raven, J.A., 1986. Biochemical disposal of excess H^+ in plants? New Phytol. 104, 175–206.

Raven, J.A., Wollenweber, B., Handley, L.L., 1992a. Ammonia and ammonium fluxes between photolithotrophs and the environment in relation to the global nitrogen cycle. New Phytol. 121, 5–18.

Raven, J.A., Wollenweber, B., Handley, L.L., 1992b. A comparison of ammonium and nitrate as nitrogen sources for photolithotrophic. New Phytol. 121, 19–32.

Raven, J.A., Scrimgeour, C.M., 1997. The influence of anoxia in plants from saline habitats with special reference to the sulphur cycle. Ann. Bot. 79 (Suppl A), 79–86.

Raven, J.A., Beardall, J., Larkum, A.W.D., Sanchez-Baracaldo, P., 2013. Interactions of photosynthesis with genome size and function. Philos. Trans. R. Soc. London, Ser. B 368, 2012.0264.

Ravina, C.G., Chang, C.-I., Tsakraklides, G.P., McDermott, J.P., Vega, J.M., Leustek, T., Gotor, C., Davies, J.P., 2002. The sac mutants of *Chlamydomonas reinhardtii* reveal transcriptional and posttranscriptional control of cysteine biosynthesis. Plant Physiol. 130, 2076–2084.

Rees, T.A.V., Cresswell, R.C., Syrett, P.J., 1980. Sodium-dependent uptake of nitrate and urea by a marine diatom. Biochim. Biophys. Acta 596, 141–144.

Reid, R.J., Mimura, T., Ohsumi, Y., Walker, N.A., Smith, F.A., 2000. phosphate uptake in *Chara*: membrane transport via Na/Pi cotramsport. Plant Cell Environ. 23, 223–228.

Reinhard, C.T., Planavsky, N.J., Robbins, C.J., Partin, C.A., Gill, B.C., Lalonde, S.V., Bekker, A., Konhauser, K.O., Lyons, T.W., 2013. Proterozoic ocean redox and biogeochemical stasis. Proc. Nat. Acad. Sci. U.S.A. 110, 5357–5362.

Rentsch, D., Schmidt, S., Tegeder, M., 2007. Transporters for uptake and allocation of organic nitrogen compounds on plants. FEBS Lett. 581, 2281–2289.

Rexach, J., Montero, B., Fernandez, E., Galvan, A., 1999. Differential regulation of the high affinity nitrate transport systems III and IV in *Chlamydomonas reinhardtii*. J. Biol. Chem. 274, 27801–27806.

Risgaard-Petersen, N., Jensen, K., 1997. Nitrification and denitrification in the rhizosphere of the aquatic macrophyte Lobelia dortmanna L. Limnol. Oceanog. 42, 529–537.

Ritchie, R.J., 1996. Sulphate transport in the cyanobacterium *Synechococcus* R2 (*Anacystis nidulens*, *S. leopolensis*) PCC 7942. Plant Cell Environ. 19, 1307–1315.

Robic, A., Rondeau-Mouro, C., Sassi, J.F., Lerat, Y., Lahaye, M., 2009. Structure and interactions of Ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). Carbohydr. Polym. 77, 206–216.

Rowell, P., James, W., Smith, W.L., Handley, L.L., Scrimgeour, C.M., 1998. ^{15}N -discrimination in molybdenum- and vanadium-grown N_2-fixing *Anabaena variabilis* and *Azotobacter vinelandii*. Soil Biol. Biochem. 30, 2171–2180.

Ruan, Z., 2013. Energy partitioning between the CO_2 concentrating mechanism and N assimilation in the cyanobacterium *Synechoccus* UTEX 2380 repercussions on cell composition and stoichiometry. In: Ph.D. Thesis. Università Politcnica delle Marche, Ancona, Italy.

Rubio, L., Linares-Rueda, A., García-Sánchez, M.J., Fernandez, J.A., 2005. Physiological evidence for a sodium-dependent high-affinity phosphate and nitrate transport at the plasma membrane of leaf and root cells of *Zostera marina*. J. Exp. Bot. 56, 613–622.

Sakihama, Y., Nakamura, S., Yamasaki, H., 2002. Nitric oxide production mediated by nitrate reductase in the green alga *Chlamydomonas reinhardtii*: an alternative NO production pathway in photosynthetic organisms. Plant Cell Physiol. 43, 290–297.

Sanz-Luque, E., Ocana-Calahorro, F., Llama, A., Galvan, A., Fernandez, E., 2013. Nitric oxide controls nitrate and ammonium assimilation in *Chlamydomonas reinhardtii*. J. Exp. Bot. 64, 3373–3383.

Sanchez-Baracaldo, P., Ridgwell, A., Raven, J.A., 2014. A Neoproterozoic transition in the marine nitrogen cycle. Curr. Biol. 6, 652–657.

Sasaki, H., Kataoka, H., Kamiya, M., Karai, H., 2002. Accumulation of sulfuric acid in Dictyotales (Pheophyceae): taxonomic distribution and ion chromatography of cell extracts. J. Phycol. 35, 732–739.

Schmalenberger, A., Noll, M., 2014. Bacterial communities in grassland turfs respond to sulphonate addition while fungal communities remain largely unchanged. Eur. J. Soil Biol. 61, 12–19.

Schmidt, S., Raven, J.A., Paungfoo-Lonhienne, C., 2013. The mixotrophic nature of photosynthetic plants. Funct. Plant Biol. 40, 425–438, Doi: 10.1071?FP13061.

Schnell, R., Sandalova, T., Hellman, U., Lindqvist, Y., Schneider, G., 2005. Siroheme- and [Fe4-S4]-dependent NirA from Mycobacterium tuberculosis Is a Sulfite Reductase with a Covalent Cys-Tyr Bond in the Active Site. J. Biol. Chem. 280, 27319–27328.

Scott, C., Wing, B.A., Bekker, A., Planavsky, N.J., Medvedev, P., Bates, S.M., Yun, M., Lyons, T.W., 2014. Pyrite multiple-sulfur isotope evidence for rapid expansion and contraction of the early Paleoproterozoic seawater sulfate reservoir. Earth Planet. Sci. 389, 95–104.

Segel, I.H., 1975. Biochemical Calculations. Wiley, New York, NY.

Shen, Y., Canfield, D.E., Knoll, A.H., 2002. Middle Proterozoic ocean chemistry: evidence from the McArthur Basin, Northern Australia. Am. J. Sci. 302, 81–109.

Sherameti, I., Sopory, S.K., Trebicka, A., Pfannschmidt, T., Oelmüller, R., 2002. Photosynthetic electron transport determines Nitrate Reductase gene expression and activity in higher plants. J. Biol. Chem. 29, 46594–46600.

Shibagaki, N., Grossman, A.R., 2010. Binding of cysteine synthase to the STAS domain of sulfate and its regulatory consequences. J. Biol. Chem. 285, 25094–25102.

Sirko, A., Hryniewicz, M., Hulanicka, D., Böck, A., 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the cysTWAM gene cluster. J. Bacteriol. 172, 3351–3357.

Smith, F.W., Ealing, P.M., Hawkesford, M.J., Clarkson, D.T., 1995. Plant members of a family of sulfate transporters reveal functional subtypes. Proc. Nat. Acad. Sci. U.S.A. 92, 9373–9377.

Sohm, J.A., Webb, E.A., Capone, D.G., 2011. Emerging patterns of marine nitrogen fixation. Nat. Rev. Microbiol. 9, 499–508.

Solomon, C.M., Collier, J.L., Berg, G.M., Glibert, P.M., 2010. Role of urea in microbial metabolism in aquatic systems a biochemical and molecular review. Aquat. Microb. Ecol. 59, 67–88.

Soupene, E., King, N., Field, E., Liu, P., Niyogi, K.K., Huang, C.H., Kusti, S., 2002. Rhesus expression in a green alga is regulated by CO_2. Proc. Nat. Acad. Sci. U.S.A. 99, 7769–7773.

Soupene, E., Inweed, W., Kustu, S., 2004. Lack of the Rhesus protein Rh1 impairs growth of the green alga *Chlamydomoans reinhardtii* at high CO_2. Proc. Nat. Acad. Sci. U.S.A. 101, 7787–7792.

Stefels, J., 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J. Sea Res. 43, 183–197.

Stewart, J.J., Coyne, K.J., 2011. Analysis of raphidophyte assimilatory nitrate reductase reveals uniquederein architecture incorporation a 2/2 hemoglobin. Plant Miol Biol. 77, 5565–5575.

Stitt, M., 1999. Nitrate regulation of metabolism and growth. Curr. Opin. Plant Biol. 2, 178–186.

Takahashi, H., 2010. Sulfate transport and assimilation in plants. Int. Rev. Cell Mol. Biol. 281, 129–159.

Sugiura, M., Georgescu, M.N., Takahashi, M., 2007. A nitrite transporter associated with nitrite uptake by higher plant chloroplasts. Plant Cell Physiol. 48, 1022–1035, http://dx.doi.org/10.1093/pcp/pcm073.

Takahashi, H., Buchner, P., Yoshimoto, N., Hawkesford, M.H., Shiu, S.-H., 2012. Evolutionary relationships and functional diversity of plant sulfate transporters. Front. Plant Sci. 2, 119, http://dx.doi.org/10.3389/fpls.2011.00119.

Takahashi, H., Kopriva, S., Giordano, M., Saito, K., Hell, R., 2011. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. Annu. Rev. Plant Biol. 62, 157–184, http://dx.doi.org/10.1146/annurev-arplant-042110-103921.

Thauer, R.K., Jungermann, K., Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41 (1), 100–180.

Thompson, A.W., Foster, R.A., Krupke, A., Carter, B.J., Musat, N., Vaulot, D., Kuypers, M.M.M., Zehr, J.P., 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337, 1546–1550.

Tischner, R., Planchet, E., Kaiser, W.M., 2004. Mitochondrial electron transport as a source for nitric oxide in the unicellular alga *Chlorella sorokiniana*. FEBS Lett. 576, 151–155.

Ullrich, W.R., Larsson, M., Larsson, C.M., Lesch, S., Novacky, A., 1984. Amnonium uptake in *Lemna gibba* G1, related membrane potential changes, and inhibition of anion uptake. Physiol. Plant. 61, 369–376.

Van Mooy, B.A.S., Rocap, G., Fredricks, H.F., Evans, C.T., Devol, A.H., 2006. Sulfolipids dramatically decrease phosphorus demand in oligotrophic marine environments. Proc. Nat. Acad. Sci. U.S.A. 103, 8607–8612.

Van Mooy, B.A.S., Fredricks, H.F., Pedler, B.E., Dyhrman, S.T., Karl, D.M., Koblizek, M., Lomas, M.W., Mincer, T.J., Moore, L.R., Moutin, T., Rappe, M.S., Webb, E.A., 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458, 69–72.

Vieler, A., Wilhelm, C., Goss, R., Süß, R., Schiller, J., 2007. The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella menghiniana* investigated by MALDI-TOF MS and TLC. Chem. Phys. Lipids 150, 143–155.

Walker, N.A., Reid, R.J., Smith, F.A., 1993. The uptake and metabolism of urea by *Chara australis*. IV. Symport with sodium–a slip model for the high and low affinity systems. J. Membr. Biol. 136, 263–271.

Wang, R., 2003. The gasotransmitter role of hydrogen sulfide. Antioxid. Redox Signaling 5, 493–501.

Wang, R., Tischner, R., Gutierrez, R.A., Hoffman, M., Xing, X., Chen, M., Coruzzi, G., Crawford, N.M., 2004. Genomic analysis of the nitrate response using a nitrate reductase-null mutant of *Arabidopsis*. Plant Physiol. 136, 2512–2522.

Wang, W-H., Köhler, B., Cao, F-Q., Liu, L-H., 2008. Molecular and physiological aspects of urea transport in higher plants. Plant Sci. 175, 467–477.

Wang, R., Liu, D., Crawford, N.M., 1998. The Arabidopsis CHL1 protein plays a major role in high-affinity nitrate uptake. Proc. Natl Acad. Sci. USA 95, 15134–15139.

Waraich, E.A., Ahmad, R., Saifullah Ashraf, M.Y., Ehsanullah, 2011. Role of mineral nutrition in alleviation of drought stress in plants. Aust. J. Crop Sci. 5, 764–777.

Weiss, M., Haimovich, G., Pick, U., 2001. Phosphate and sulfate uptake in the halotolerant alga *Dunaliella* are driven by Na^+-symport mechanism. J. Plant Phys. 158, 1219–1525.

Werner, A.K., Medina-Escobar, N., Zulawski, M., Sparkes, I.A., Cao, F.Q., Witte, C.-P., 2013. The ureide-degrading reactions of purine ring catabolism employ three amidohydrolases and one aminohydrolase in *Arabidopsis*, soybean, and rice. Plant Physiol. 163, 672–681.

White, P.J., Bowen, M.C., Marshall, B., Broadly, M.R., 2007. Extraordinarily high leaf selenium to sulfur ratios define 'Se-accumulator' plants. Ann. Bot. 100, 111–118.

Williams, S.K., Hodson, R.C., 1977. Transport of urea at low concentrations in *Chlamydomonas reinhardtii*. J. Bacteriol. 130, 266–273.

Witte, C.-P., 2011. Urea metabolism in plants. Plant Sci. 180, 431–438.

Wium-Andersen, S.S., Anthoni, U., Christophersen, C., Houen, G., 1982. Allelopathic effects on phytoplankton of substances isolated from aquatic macrophytes (Charales). Oikos 39, 187–190.

Yano, T., Kaiya, M., Arai, S., Kawai, H., 2004. Morphological homoplasy in Japanese *Plocamium* species (Plocamiales, Rhodophyta) inferred from the Rubisco spacer sequence and intracellular acidity. Phycologia 43, 383–393.

Young, E.B., Dring, M.J., Savidge, J.G., Birkett, D.A., Berges, J., 2007. Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant Cell Environ. 30, 764–774.

Zehr, J.P., 2011. Nitrogen fixation by marine cyanobacteria. Trend Microbiol. 19, 162–173.

Zhang, B., Pasini, R., Dab, H., Joshi, N., Zhao, Y., Lesustek, T., Zheng, Z.-L., 2014. Aberrant gene expression in the *Arabidopsis* SULTR1;2 mutants suggests a possible regulatory role for this sulfate transporter in response to sulfur nutrient status. Plant J. 77, 185–197.

Zhou, J.J., Theodoulou, F.L., Muldin, I., Ingemarsson, B., Mille, A.J., 1998. Cloning and functional characterization of a *Brassica napus* transporter that is able to transport nitrate and histidine. J. Biol. Chem. 273, 12017–12023.