Processes and patterns of oceanic nutrient limitation

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Microbial activity is a fundamental component of oceanic nutrient cycles. Photosynthetic microbes, collectively termed phytoplankton, are responsible for the vast majority of primary production in marine waters. The availability of nutrients in the upper ocean frequently limits the activity and abundance of these organisms. Experimental data have revealed two broad regimes of phytoplankton nutrient limitation in the modern upper ocean. Nitrogen availability tends to limit productivity throughout much of the surface low-latitude ocean, where the supply of nutrients from the subsurface is relatively slow. In contrast, iron often limits productivity where subsurface nutrient supply is enhanced, including within the major oceanic upwelling regions of the Southern Ocean and the eastern equatorial Pacific. Phosphorus, micronutrients other than iron, and vitamins may also (co-)limit marine phytoplankton. However, the spatial patterns and importance of co-limitation remain unclear. Variability in the stoichiometries of nutrient supply and biological demand are key determinants of oceanic nutrient limitation. Deciphering the mechanisms that underpin this variability, and the consequences for marine microbes, will be a significant challenge. However, such knowledge will be crucial for accurately predicting the consequences of ongoing anthropogenic perturbations to oceanic nutrient biogeochemistry.

The biomass of all living organisms is comprised of approximately 30 of the 92 naturally occurring elements (Fig. 1a, Supplementary Table S1)[1,2]. All organisms must obtain chemical forms of these essential elements, termed nutrients, from their external environment. The key role that nutrients play in controlling upper ocean productivity has long been recognised[3-5]. However, research over recent decades has yielded significant new insights into nutrient biogeochemistry, including the importance of numerous trace metals[6], co-limitation by two or more nutrients[7,8] and variability in nutrient requirements related to microbial function, environment and evolution[2,7,9-11]. Here, we present an overview of these recent advances, with reference to key concepts of nutrient limitation, and new data syntheses. Anthropogenic forcing will increasingly influence oceanic nutrient cycling[12,13]. We consider the potential impact of such environmental changes on nutrient limitation, ocean biogeochemistry and the carbon cycle[14].

Concepts of nutrient limitation

Nutrient elements comprise one of three principal resources required for life, alongside space to live, and energy in the form of chemical reducing equivalents and high-energy bonds. Organisms that use light to fix carbon dioxide, termed photoautotrophs, are responsible for the vast majority of primary production, both on land and in the ocean. Consequently sunlight is the ultimate source of energy for most of the biosphere[15]. Energy imposes an important constraint on primary productivity in the open ocean**.** The rapid attenuation of light with depth restricts the growth of the oceanic photoautotrophic microbes, collectively termed phytoplankton, to a thin euphotic layer (0-~200m) (Fig. 2a). Phytoplankton and other microbes take up nutrients from this near-surface layer and assimilate them into macromolecules, resulting in the formation of particulate organic matter. Downward transport of organic material[16], combined with microbially-mediated remineralisation, enhances nutrient concentrations below the euphotic zone (Fig. 2a), while the biological uptake of nutrients in the surface can result in depletion to levels that restrict microbial processes[5] (Fig. 2a). Consequently, biological activity influences the cycling of nutrients throughout the ocean.

The term nutrient limitation (see Box 1) encompasses a hierarchy of different scales of biological and ecological complexity, from the single cell to the biosphere (Fig.

2b). At the cellular scale, external nutrient concentrations can be so low that rates of nutrient transport to the cell surface, and thus the cellular interior, restrict the internal nutrient pool (quota) and consequently the growth rate of the cell (Box 1)[17,18]. The depletion of external nutrient concentrations typically results from integrated nutrient assimilation by the whole microbial community. The availability of nutrients may thus also set a limit on the overall community biomass yield (Box 1)[18,19]. Within the diverse microbial communities which characterize oceanic systems, the degree of growth rate limitation may vary between populations[20-22], for example due to differences in cell size[23,24] and cellular element requirements[8,25] (Box 2).

Although the growth rate of individual cells may depend on nutrient availability, the net growth rate of cell populations is also profoundly influenced by predation and other loss processes[5]. Ecological interactions can thus influence nutrient limitation by modifying species composition and abundance at multiple scales (Fig. 2b). For example, the enhanced supply of a limiting nutrient may provide little immediate benefit for the organisms that initially dominate the community, as these may be highly adapted to the lack of this resource in the environment and/or under tight grazer control[22-24]. However, addition of any limiting nutrient could still increase community biomass, for instance by enhancing the growth rates of initially rare larger phytoplankton that are more resistant to grazing[18,22]. Such mechanisms are supported by the observation that mean community cell size generally increases in parallel with overall community biomass[26].

Interactions between multiple nutrients also strongly influence microbial dynamics. A range of different nutrients can potentially co-limit microbial processes in the ocean (Box 1)[7,8]. Moreover, the nutrients that exert direct (or proximal) control over a microbial cell or community may differ from those that set the ultimate constraint on system productivity[18,27]. A conceptual two nutrient, two organism model represents a well-studied case[4,27]. The ability to convert di-nitrogen gas into the other forms of nitrogen that are more readily available, collectively termed fixed nitrogen, is restricted to groups of microbes termed diazotrophs. Loss of fixed nitrogen through the microbial processes of denitrification and/or anammox thus creates a niche for diazotrophs, through effectively generating an excess of other nutrients (e.g. phosphorus). As a consequence, at least conceptually, fixed nitrogen levels can proximally limit non-diazotrophic phytoplankton, while the phosphorus inventory sets an ultimate constraint on the nitrogen inventory[27]. In the ocean other factors may complicate this simple scenario, including restrictions on diazotrophy when iron availability is low [28-31].

Establishing the identity of a single ultimate limiting nutrient may thus be less relevant than understanding the controls on, and feedbacks pertaining to, any given process (Fig. 2b). For example, within the modern ocean there is no single nutrient for which the surface concentration, or overall inventory (Supplementary Information), could be considered limiting in isolation. Given the range of usage, discussions of nutrient limitation should specify the process being considered[18], alongside the scales relevant to that process, to prevent conceptual misunderstandings.

The central role of stoichiometry in microbe-nutrient interactions

Quantitative relationships between chemicals within both intracellular and extracellular nutrient pools (referred to as stoichiometry) are key determinants of oceanic biogeochemical cycles[1-4]. Six of the essential elements (C, H, N, O, P, S) constitute over 95% of organic matter by mass, principally in the form of the macromolecules: carbohydrates, proteins, nucleic acids and lipids[2,9]. In addition, all organisms require inorganic ions (for example of calcium, potassium and sodium) and a range of trace elements, including iron, manganese, cobalt, zinc and copper (Fig. 1a). The latter are found within a diverse array of metal-containing enzymes[6,8,32]. Mineral phases, such as silicon dioxide and calcium carbonate, are also essential for some organisms.

Redfield first drew attention to the co-variability of dissolved nitrate and phosphate in the ocean interior, and the similarity of this ratio to N:P ratios within particulate organic matter and cellular material[3]. Oceanographers have since employed the ‘Redfield Ratio’ of 106C:16N:1P as a key stoichiometric concept in ocean biogeochemistry[33,34]. Cellular ratios have also been extended to include other elements[35]. However, it has long been recognised that considerable variability is observed in the stoichiometric ratios for all the elements within cellular material[2,9,36-38] (Fig. 1a).

Variability in ocean geochemistry throughout evolutionary history has resulted in significant stoichiometric differences among taxa. In particular, changes in the relative availabilities of trace metals during redox transitions in ancient oceans have left imprints on the metal-binding proteomes, and hence trace-metal requirements, of modern organisms[1,10,32,39]. Ratios of C:N:P also vary between taxa, potentially reflecting ecological trade-offs in the allocation of carbon (and associated nutrients) amongst macromolecules associated with different functions[9-11,35]. The availability of nutrients in the environment also drives extensive phenotypic differences in cellular composition [2,9,36-38]. Other factors, including acclimation to light intensity, can also significantly influence cellular elemental composition[11,37]. As cells acclimate to environmental variability, plasticity in intracellular elemental stoichiometry broadly reflects the changing ratios of functional macromolecular pools (e.g. proteins and ribosomes), alongside the accumulation or depletion of energy (carbohydrates and neutral lipids) or nutrient reserves (such as polyphosphate, nitrate and ferritin)[2,9,11]. Substitutions between different metal-containing enzymes, or the metals bound by the enzymes, can also facilitate stoichiometric flexibility for these micronutrient elements[2,6,8,9,36].

The degree of variability in cellular elemental quotas appears to decline with increasing cellular requirements (Fig. 1a, Supplementary Fig. S1). Elements incorporated within larger biochemical pools will probably be involved in a wider range of metabolic processes. For example, nitrogen represents over 7% of cellular mass in marine microbes, being a major constituent of both proteins and nucleic acids[9]. Although there is scope for substitution and/or maintenance of metabolism at reduced cellular nitrogen concentrations[40], this appears relatively restricted. In contrast, phosphorus requirements, which typically account for around 1% of microbial mass, can be more significantly reduced under limiting conditions. For example, a substantial proportion of one of the major cellular pools, the phospholipids, can be substituted for non-phosphorus containing lipids[25]. The cellular C:N ratio thus appears more constrained than the C:P (and hence N:P) ratio[9,10]. Plasticity is even higher for many of the trace metals (Fig. 1a), as excess intracellular accumulation can occur when external availability is high, while substitutions can occur under limiting conditions[6,8,36].

Cellular stoichiometry and associated uptake ratios dictate how surface organic matter production both responds to and influences the differential availability of nutrients[2,4,7,41-44]. Assuming no other constraints operate, strict conservation of cellular stoichiometry would dictate that the nutrient in most deficient supply to the surface layer should limit the rate of new biomass production (see Box 1). Supply from the sub-surface will dominate for many nutrients (Fig. 2a). However additional inputs, for example from the atmosphere, may also be significant for some nutrients, influencing patterns of limitation[45,46]. Moreover, stoichiometric plasticity (Fig. 1a) and variable surface recycling rates for different elements[41] may further decouple the proximally limiting nutrient from what might be predicted on the basis of dissolved nutrient stoichiometry within local sub-surface pools (Supplementary Fig. S2)[41,47,48]. A significant proportion of the sub-surface pools of many nutrients results from remineralisation of organic matter produced in the surface layer (Fig, 2a, Supplementary Table 1). Consequently, the elemental composition of organic matter production (Fig. 1a), alongside any variability in remineralisation rates between elements[33,41], can also influence sub-surface nutrient stoichiometry[4,41-44].

Stoichiometry thus exerts a fundamental control on nutrient limitation and the coupling between the biogeochemical cycles of the different nutrients[2,4,44,49]. The majority of present day large-scale ocean biogeochemical models tend to assume fixed stoichiometries for many elements[45,50,51] (Supplementary Information). Although implementation of more realistic physiological parameterisations will be challenging[50], it may be necessary for improved representation of current nutrient limitation patterns in these models[45,46].

Patterns of phytoplankton nutrient limitation in the modern ocean

Over much of the surface ocean, non-nutrient constraints on net community growth, such as light levels, grazing and viral infection, appear insufficient to prevent the depletion of at least one nutrient to concentrations where experimental amendment can, at times, elicit a rapid biological response (Fig. 3). Large-scale spatial patterns of limiting nutrients have been inferred from multiple lines of evidence. Absolute surface nutrient concentrations or their stoichiometric ratios provide an indication of the potential for limitation or deficiency (cf. Box 1), respectively. Surface inorganic nitrogen and phosphorus concentrations are highly depleted throughout much of the low latitude oceans (Fig. 1b & c, Fig. 3). Phosphorus is typically in excess of nitrogen relative to cellular requirements[34] (Figs. 1 & 3). However this is not the case everywhere; both of the northern hemisphere oligotrophic gyres have lower surface phosphate concentrations than the southern gyres (Fig. 1b & c, Fig. 3b, Supplementary Fig. S2)[31,41,52]. Surface depletion of micronutrients, such as Fe, Co, Zn, Cu, Ni, Cd, is also observed in many regions[6]. Perhaps the classic example of how analytical advances can transform our understanding of oceanic nutrient cycles[5] concerns the so-called high-nitrate low-chlorophyll regions. The long-hypothesised deficiency of iron in these environments was only confirmed following improved sampling and measurement techniques[5,53,54] (Fig. 1d).

Variable cellular stoichiometry (Fig. 1a), the rapid turnover of nutrients within the foodweb[5,20], and the differing capacity of microbial groups to access different nutrient species (termed bioavailability[6], see Supplementary Information) complicate inferences of limitation or deficiency based on observed concentrations (Fig. 1b-d)**.** Consequently, experimental approaches to directly assess nutrient limitation of marine microbes have been employed for at least 50 years[55]. Nutrient manipulation experiments have included additions of specific nutrients to natural microbial communities enclosed in bottles[53] and *in-situ* enrichments in the open ocean[20,54]. A compilation of such experimental data for phytoplankton reveals coherent large-scale patterns, and a clear relationship between proximal nutrient limitation and the concentrations of nutrients (Fig. 1b-d, Fig. 3). These patterns appear robust to differences in employed methods, including variable time and space scales (Fig. 2b), and the wide range of ecophysiological and molecular techniques used to monitor responses.

In the low-biomass (oligotrophic) waters which dominate the low-latitude oceans, phytoplankton biomass and productivity typically only increase following experimental addition of nitrogen[47,52,56]. In contrast, in high-nitrate low-chlorophyll waters (Fig. 3), increases in phytoplankton biomass and productivity often result from the addition of iron alone, in both bottle and *in situ* experiments[53,54]. In certain oligotrophic regions, including the Eastern Mediterranean[20,48] and the sub-tropical North Atlantic[25,41,57,58], bioavailable forms of phosphorus can become severely depleted alongside nitrogen (Fig. 1c, Fig. 3) and microbial populations frequently display evidence of phosphorus stress (cf. Box 1)[20,25,57,58]. However, even in these systems, addition of phosphorus alone does not typically result in increased autotrophic activity or biomass[20,47,48,59]. Rather, once stratified oligotrophic conditions are well established, N- or NP- co-limitation seems to occur[47,48,59]. The higher bioavailability of organic phosphorus compounds[41,48] (see Supplementary Information), acclimation mechanisms for coping with phosphorus stress[25] and/or the selection for higher N:P stoichiometry under low nutrient conditions[11] could explain the tendency towards nitrogen limitation.

Other nutrients can be almost as deficient as nitrogen, iron and phosphorus (Fig. 1 b-d, Supplementary Figure S2). However, our understanding of the importance and geographic extent of multi-nutrient interactions (beyond those of nitrogen and phosphorus)[47,48,59] is less complete, due to a scarcity of experiments and the potential limitations of current methodologies[8]. Experiments that employ a factorial matrix of differing nutrients provide evidence of secondary- and/or co-limitation in both high-nitrate low-chlorophyll and oligotrophic environments (Fig. 3; Supplementary Table S2). However, such approaches are logistically challenging, and may not be capable of detecting a range of known metal-metal interactions, including the biochemical substitution of one metal for another[8] (Box 1).

Molecular diagnostics, including the expression of specific genes or the presence of biomarker proteins, hold promise for the future assessment of multi-nutrient interactions for phytoplankton[60] and other microbial groups. A variety of techniques could potentially be used to determine simultaneous multiple nutrient stress on individual phytoplankton taxa, circumventing logistical problems and caveats associated with bottle incubations. Individual diagnostics of both iron and phosphorus stress have already been employed[57,61], and emerging technologies will facilitate more in-depth sampling of nutrient-related gene expression[62] and protein abundance profiles.

Overall, our synthesis of experimental data (Fig. 3) supports prior model predictions[45,46], and can be used to infer that there are two broad nutrient limitation regimes in the modern ocean. Approximately 30% of the ocean's surface area consists of high-macronutrient, iron-limited systems, with most of the remaining low latitude oligotrophic systems being nitrogen (or in places NP co-) limited (Fig. 3). Certain phytoplankton groups may have additional specific requirements. For example, silicon availability may limit diatoms[21,45]. Emerging evidence also points to the potential for other micronutrients and vitamins, such as Co, Zn, Ni and vitamin B_{12}, to have secondary or subtle interactive influences beyond the primary N(P) or Fe limitations in some regions[7,8]. Variability in the stoichiometry of phytoplankton cellular elemental quotas (Fig. 1)[11,25] and upper ocean nutrient cycling[41,48], as well as physical mixing between water masses with contrasting nutrient stoichiometry (Fig. 3), may provide mechanisms for generating regions of co-limitation. The seasonal cycle can also influence patterns of (co-)limitation[21,48], as physical nutrient inputs and other drivers, including light levels, combine with biological cycling to alter nutrient availability.

Potential for change

Significant changes in nutrient biogeochemistry have occurred over glacial-interglacial cycles (Supplementary information), indicating the potential for altered patterns of upper ocean nutrient limitation[63-65]. A range of processes could influence nutrient availability in the future, including altered nutrient demands, increasing external nutrient inputs, and changes in surface ocean chemistry driven by anthropogenic increases in atmospheric carbon dioxide concentrations. Changes in ocean circulation could also play a major role, due to the dominance of physical supply from depth (Fig. 2a) on the fluxes of many nutrients to the euphotic zone[16]. At the global scale, physical resupply is dominated by the Southern Ocean, where strong upwelling brings macronutrient-rich deep waters to the surface[43]. These waters are deficient in those trace metals which have short oceanic residence times due to scavenging losses (Fig. 1a & d)[6,66], contributing to the tendency for iron limitation in the Southern Ocean (Fig. 3). A proportion of the surface macronutrients which remain unutilised in the Southern Ocean (Fig. 1d) are subsequently transported northwards within the thermocline, where smaller scale upwelling and mixing eventually supports production in the largely nitrogen-limited (Fig. 3) low-latitudes[43].

Anthropogenic CO_2 emissions and increasing temperatures

Continued anthropogenic carbon dioxide emissions and resulting ocean warming may influence oceanic nutrient cycles. Oceanic uptake of anthropogenic carbon dioxide directly alters ocean chemistry by changing inorganic carbon speciation, lowering pH and potentially affecting the speciation of some nutrients. For example, the bioavailability of iron[67] or the biological oxidation rate of ammonia[68], a fixed N species, might decrease. However, any co-occurring changes in nutrient supply and demand need to be taken into account when attempting to predict overall consequences for upper ocean nutrient limitation[68,69].

The climatic impacts of increased atmospheric carbon dioxide concentrations are expected to include a strengthening in the density stratification of the upper ocean in response to warming and intensification of the hydrological cycle[70]. Resulting changes in ocean circulation could influence nutrient cycling in several ways. Increased stratification may restrict the physical resupply of nutrients to surface waters[70,71], with some models predicting declines in global particulate organic matter export of around 10% by the end of the century as a consequence[51]. Stratification increases would likely involve expansion of the nitrogen-limited sub-tropical gyres[71] (Fig. 3). Although some observational data already supports such a trend[72], regional *in situ* records can differ[73] and longer time-series are needed[74].

Increased stratification and warming may also decrease deep-water oxygen concentrations[75]. Subsequent expansion of oxygen minimum zones could decrease the ocean inventory of fixed nitrogen species by increasing microbial denitrification and/or anammox. Expansion of oxygen minimum zones could also increase trace metal[66] and phosphorus inventories by increasing the release of these nutrients from sediments, as may have occurred over glacial-interglacial cycles (Supplementary Information). Such changes at depth could influence surface waters on timescales of decades or longer (Fig. 2a), being most significant in regions extending out from existing oxygen minimum zones in the eastern tropical Atlantic[66] and Pacific and northern Indian Oceans[75].

Changes in external nutrient supply

Anthropogenic activities have significantly influenced the biogeochemical cycles of many elements[14]. Terrestrial nutrients primarily reach the ocean through atmospheric and fluvial fluxes. These fluxes are of comparable magnitude (Table 1), but differ in their geographical distribution. Significant anthropogenic perturbations to both supply routes have increased the external supply of nutrient elements to the ocean (Table 1)[12,76-79]. Anthropogenic fixed nitrogen sources are comparable to that derived from biospheric nitrogen fixation[12,49], leading to enhanced fluvial fluxes of nitrogen to the ocean[49]. Riverine phosphorus fluxes have also increased by 50-300% over preindustrial levels and are expected to track future global population increases, unless declining mineral phosphorus reserves offset such changes[80]. Fluvial dissolved iron inputs are presently small relative to atmospheric inputs[81]. However, any change in the estuarine trapping efficiency of the much larger fluvial particulate iron fluxes could have a significant, but uncertain, impact on the supply of terrestrial iron to the open ocean[82].

Atmospheric bioavailable fixed nitrogen deposition to the open ocean has tripled since 1860 (Table 1), and a further 10-20% increase is expected by 2050 (ref. 12). At present, most of this anthropogenic nitrogen is deposited in low latitude nitrogen-limited regions (Fig. 3), with further increases predicted particularly to the Indian Ocean, tropical Pacific and the waters off southern Africa[12,13]. The total atmospheric flux of iron into the ocean is dominated by soil dust from desert regions, resulting in strong regional gradients in deposition[82], with high fluxes in the North Atlantic and western North Pacific from the Saharan and Asian deserts, and very low fluxes to the iron-limited Southern Ocean (Fig. 3). Although there is evidence that atmospheric dust has increased over the last century[78], the magnitude and even the sign of further changes, resulting from continued shifts in climate and altered land use in important source regions, is difficult to predict[78]. Depending on the regional distribution of changes to dust and other, anthropogenic, inputs of iron (Supplementary material), biological responses might principally be expected in iron-limited regions (Fig. 3)[13,46] and/or in low latitude waters where iron potentially limits diazotrophy[29,31,83]. Atmospheric deposition of phosphorus to the ocean predominantly comes from natural sources, and changes will likely parallel those of dust[77]. Experimental evidence has indicated the potential for a range of microbial responses to future changes in dust inputs (Supplementary Fig. S3). Overall, atmospheric fluxes are predicted to become increasingly enriched in nitrogen relative to both iron and phosphorus (Table 1).

Although the magnitude of likely changes in external nutrient inputs suggests only modest changes to whole ocean inventories on decadal timescales (Table 1)[12,13,46,78], the increased anthropogenic fluxes are still significant, particularly at local scales or when compared to natural inputs. For example, the enhanced fluvial nutrient inputs entering the coastal zone are responsible for significant eutrophication, contributing to the growth of low-oxygen waters in many regions[84]. Overall, anthropogenic inputs are predicted to continue increasing beyond the magnitude of natural external sources (Table 1)[12,76-79], representing a significant biogeochemical perturbation of the whole oceanic system. For example, in addition to the well-discussed fixed nitrogen inputs[12,46], the short residence time for iron (Fig. 1a) means that any future changes[78] could have significant impacts on upper ocean cycling over decadal-century timescales[13,46] (Table 1).

Altered nutrient demand

A range of other factors alongside nutrient availability - including temperature, light, inorganic carbon availability and grazing - interact to control the physiology, growth and abundance of different marine microbial groups[5,21,85-87]. Changes in these non-nutrient drivers may alter microbial activity and distribution, and hence influence overall biological uptake, a crucial determinant of nutrient limitation. For example, model studies indicate that direct physiological responses to increasing temperature could potentially influence primary (although not export) production by a magnitude comparable to the influence of increased stratification[71,88]. Future warming and higher carbon dioxide concentrations may also influence diazotrophic growth rates[85,87,89], potentially altering nitrogen inputs and/or phosphorus and iron uptake and hence the stoichiometry of N:P:Fe cycling in low-latitude nitrogen-limited regions (Fig. 1 b & c, Fig. 3). In the high latitudes, increases in stratification might increase seasonal light availability for phytoplankton and hence overall productivity[51]. However, the extent to which macronutrient drawdown might subsequently increase in different regions (Fig. 3) will depend on the current relative importance of light or iron availability as limiting factors, potentially alongside interactions with altered iron supply and other potential drivers[21,69].

Establishing the dominant environmental controls on the ecophysiology of different microbial groups in diverse regimes thus remains a crucial challenge. Biogeochemical responses are unlikely to be simply related to single environmental drivers. Mechanistic understanding of individual physiological responses and how they interact[21,50] will need to guide the next generation of numerical models if these are to provide accurate predictions of how future changes in patterns of nutrient supply and biotic demand will combine to alter regional nutrient limitation (Fig. 3)[13,46]. Geographical shifts in the boundaries between the two broad regimes of nutrient limitation apparent at basin scales (Fig. 3) would likely be one primary consequence of changes in either external inputs[46], internal transports related to oceanic circulation[71], or alterations in biological processes linked to other environmental drivers[21].

Implications for the carbon cycle

Ultimately, changes in oceanic nutrient cycles have the potential to influence atmospheric carbon dioxide concentrations, resulting in climate feedbacks[13,14,28,63]. Partitioning of carbon dioxide between the atmosphere and oceans is driven by interacting physical, chemical and biological processes. The downward transport of nutrients resulting from the sinking and remineralisation of particulate organic material formed in the surface ocean[16] (Fig. 2a) is associated with a flux of carbon, frequently termed the biological pump[90-92]. Physical processes also transport biologically unutilised (so-called 'preformed') nutrients into the ocean interior, leading to a decreased efficiency of the biological pump. Assuming constant stoichiometry and effective air-sea equilibration of gases in the surface ocean, the biological storage of carbon in the ocean is proportional to the total inventory of nutrients in the interior that arrived through the biological 'remineralised' pathway[90,92] (Supplementary information). Consequently, circulation patterns strongly dictate how changes in nutrient limitation can influence atmospheric carbon dioxide concentrations[91]. For example, the high-nitrate low-chlorophyll Southern Ocean currently represents the largest source of unutilised (preformed) macronutrients to the deep ocean[63,91]. As such, glacial-interglacial variations in atmospheric carbon dioxide levels have been linked to altered nutrient biogeochemistry in this region[63,64]. Past[64] or future increases in iron inputs to high-nitrate low-chlorophyll Antarctic waters could reduce excess surface macronutrients, decreasing the preformed nutrient contribution to the deep ocean and hence lowering atmospheric carbon dioxide concentrations[91,93]. In contrast, altered iron inputs to the high-nitrate low-chlorophyll Sub-Antarctic waters could influence (macro-)nutrient input into the thermocline[43], impacting phytoplankton productivity in low latitude nitrogen-limited regions (Fig. 3), while having less influence on the overall preformed nutrient pool and hence atmospheric carbon dioxide[43,91].

Altered external nutrient inputs (Table 1) influence oceanic carbon storage through impacts on nutrient inventories[46]. Any potential impact on atmospheric carbon dioxide concentrations thus needs to be considered from this perspective. For example, the nitrogen inventory is thought to be stabilised by feedbacks which keep biological di-nitrogen fixation in balance with the biological processes of fixed nitrogen loss[4,27,34,42,44]. Increasing anthropogenic atmospheric nitrogen inputs could directly increase the oceanic nitrogen inventory, driving a net drawdown of atmospheric carbon dioxide[12,13]. However, the associated increases in the ratio of external N:P inputs (Table 1) might also act to reduce the current excess of phosphorus in oligotrophic waters (Fig. 3). Consequently, depending on the dominant environmental control(s) on di-nitrogen fixation[21,87] - particularly the extent to which iron or phosphorus are currently limiting[29,31,57,61,83] - the realised niche for diazotrophy might decrease, partially counteracting any increase in the fixed nitrogen inventory[13]. Although stoichiometric variability (Fig. 1) in phytoplankton N:P uptake and/or remineralisation ratios[2,9,41,94] may also influence the niche for diazotrophs and the coupling of the nitrogen and phosphorus cycles both regionally[94] and globally[42].

The stoichiometries of biological matter production (Fig. 1) and cycling[2] couple the marine carbon cycle to that of nutrient pools and fluxes[49]. The relative constancy of the C:N ratio (Fig. 1) suggests that the total remineralised nitrogen pool could be considered the best measure of oceanic biological carbon storage (Supplementary Information). Variability in the overall C:N ratio could hence have a significant influence on carbon storage[49]. More broadly, a better understanding of the environmental controls on variable elemental stoichiometries of different microbial groups[2,25,42,94] would help constrain the potential magnitude of any future carbon cycle responses.

Future challenges

The last two to three decades have seen the emergence of a first order description of large-scale patterns of phytoplankton nutrient limitation in the upper ocean (Fig. 3), and the biogeochemical consequences of this limitation. However, clear gaps persist. Many regions remain under-sampled (Fig. 3), particularly with respect to the trace elements and the potential for interactive effects between multiple nutrients, including co-limitation[7,8]. Furthermore, our understanding of the extent to which important microbial groups such as diazotrophs and heterotrophs are nutrient-limited lags far behind that of (non-diazotrophic) phytoplankton. Recent research continues to highlight the magnitude, mechanisms and importance of variable nutrient stoichiometry at levels ranging from those of the microbial cell[2,7,9,11,25] (Fig. 1a) through to the availability and supply of multiple nutrients at ocean basin scales[42-44,46,66] (Fig. 1 b-d). However, application of such knowledge to our understanding of existing patterns of nutrient limitation (Fig. 3), as well as past and potentially significant future change (Table 1), remains rudimentary. The details of how biological processes both influence and respond to the availability of nutrients remain debated even in the most well studied case of nitrogen and phosphorus[3,4,31,42,44,94], let alone for a wider suite of elements (Fig. 1).

Addressing these challenges will require an interdisciplinary approach. New analytical techniques, together with observational platforms such as gliders and floats fitted with nutrient sensors, and co-ordinated international sampling surveys such as GEOTRACES[95] and the CLIVAR Repeat Hydrography Program, are all facilitating better descriptions of the oceanic distributions of many more nutrients (Fig. 1). Such data provide a basis for monitoring future change. From the biological perspective, even baseline information on the biogeographical distributions of key microbial groups, and associated metabolic rate processes, is lacking, while systematic evaluation of environmental controls, including nutrient limitation, lags even further behind. Applications of molecular tools should continue to yield insights, although linking such information to nutrient biogeochemistry in a quantitative way remains a challenge. Fuller exploitation of observations will also require better representation of trace metal cycling, flexible stoichiometry and multi-nutrient ecosystem interactions in numerical models. Only through synthesis of such diverse information will a more complete description of the physical-chemical-biological interactions driving oceanic nutrient biogeochemistry emerge.

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Figure Captions

Figure 1. Comparisons between intracellular and dissolved seawater elemental stoichiometry. a. Representative (circle) and observed (bar) range of elemental ratios in oceanic phytoplankton normalized to carbon (nutrient:C quotas) plotted against mean dissolved seawater concentrations. Colours indicate oceanic residence times (see Supplementary Table S1 for data and full list of references). Dark and light grey regions indicate <10 fold and <100 fold excesses and deficiencies relative to nitrogen, which is limiting over much of the ocean (Fig. 3). Elements to the top left of the shaded area are thus in great excess in seawater and biological processing has little influence on their distribution, while some of those in the shaded regions have the potential to become limiting. **b-d**. Intercellular quotas versus surface dissolved seawater concentrations (normalized to mean ocean nitrate) for three oceanic regions. For clarity, intercellular stoichiometric variability is neglected and only the macronutrients N, P, Si and the scavenged micronutrients, Co, Mn, Fe are indicated (for additional detail and references see Supplementary Fig. S2). Experimental addition of the nutrient indicated in red typically promotes the most immediate (proximal) biological response in each region (Fig. 3); with solid red, dashed and dotted diagonal lines ***[can only see solid lines – amend?]*** delineating elements which are equally deficient and 10 and 100 fold more replete than this nutrient respectively.

Figure 2. Example time and space scales of nutrient related phenomena. a. Profile of the principal bioavailable form of nitrogen (nitrate) in the sub-tropical North Atlantic

Ocean (note logarithmic depth scale). Near complete depletion of nitrate occurs in the euphotic zone, while concentrations are enriched at depth due to both the remineralisation of particulate organic matter (N_{remin}) and physical transport of waters containing nitrate which was unutilized before these waters sank (N_{pre}). Representative time-scales for mixing back to the surface are indicated. **b.** Schematic illustrating the time and space scales of processes, levels of biological-biogeochemical organization and observational techniques related to oceanic nutrient cycling. Interactions and feedbacks occur over all scales. For example, large scale biogeochemistry is influenced by the integrated activity of the microbes operating at much smaller time and space scales. Abbreviations: nAxs, nutrient addition experiment; BCP, biological carbon pump; τ_S, turnover times of nutrients in the surface layer; $\tau_{r,Fe,N,P}$, whole ocean residence times of the key nutrients iron, nitrogen and phosphorous.

Figure 3. Patterns of nutrient limitation. Backgrounds indicate surface concentrations of nitrate (left) and phosphate (right) in umol/kg. To facilitate comparison nitrate is scaled by the mean N:P ratio of organic matter (i.e. divided by 16, Fig. 1)[34]. Symbols indicate the primary (central circles) and secondary (outer circles) limiting nutrients as inferred from chlorophyll and/or primary productivity increases following artificial amendment of: N (green), P (black), Fe (red), Si (orange), Co (yellow), Zn (cyan), vitamin B12 (purple). Divided circles indicate potentially co-limiting elements. White outer circles indicate that no secondary limiting nutrient was identified, which in many cases will be due to the lack of a test. See Supplementary Table S2 for references.

Table 1. External inputs of N, P and Fe to the oceans (in Gmol yr^{-1}) for ~1860 (pre-industrial), 2000, and 2050 (projected). Contributions to primary and export production and to the total inventory of each element are indicated.

					Totals as % of ‡		
		Fluvial	**Atmospheric**	**Glacial**	*Primary Production*	*Export production*	*Total inventory*
	~1860	1000 (79)#	1000 (12)	11 (96,97)	0.3%	1.5%	0.005%
Total N	2000	2100 (79)#	4800 (12)	11 (96)	1.2%	5.3%	0.017%
	2050	2300 (79)#	5500 (12)	11 (96)	1.3%	5.9%	0.019%
	~1860	23 (79)#	16 †	48 (98)	0.2%	1.1%	0.003%
Total P	2000	65 (79)#	21 †	48 (98)	0.4%	1.6%	0.005%
	2050	87 (79)#	21 †	48 (98)	0.4%	1.9%	0.006%
	~1860	2 (96)$	4.3 †	0.02 (81) $	2%	10%	0.8%
Dissolved Fe$	2000	2 (96)$	9.3 †	0.02 (81) $	4%	20%	1.5%
	2050	2 (96)$	8.8 †	0.02 (81) $	4%	20%	1.4%
	~1860	44:1	62:1	0.2:1			
N:P	2000	33:1	228:1	0.2:1			
	2050	26:1	258:1	0.2:1			

[How were these N:P ratios calculated, i.e. how do they differ from those directly above? And what does 'mean biological' stand for? Explain in legend?]

		Total inputs	**Mean biological**
	~1860	23:1	16:1
N:P	2000	52:1	
	2050	50:1	

Notes for Table 1.

Dissolved only. Particulate nitrogen and phosphorus (respectively estimated to be 960 and 210 Gmol yr^{-1} in 2000) are likely to be trapped in the near coastal zone. Total nitrogen and phosphorus analysis for 1860 is based on reanalysis of output from ref 79 (Seitzinger, pers. comm.).

$ Due to uncertainty concerning the bioavailability of particulate iron[81,82,96], we primarily consider dissolved inputs. For all glacial flows we assume no change in water flows, which in reality are likely to increase with future warming. Total (reactive particulate and dissolved) inputs are much higher, however the majority of the fluvial particulate iron input of around 627 Gmol y^{-1} is probably trapped on the shelf[82] , although much of the glacial reactive particulate Fe supply of 140 Gmol y^{-1} may reach the ocean[96]. We do not attempt to estimate the magnitude of potential changes in other dissolved iron inputs to the water column[81], including increases in the significant sedimentary source as a result of decreasing oxygen levels[66,75]. Iceberg associated fluxes are also an important contributor, particularly in the Southern Ocean[81].

† See supplementary material.

‡ Primary and export production are around 4.2 and 0.9 Pmol C y^{-1} respectively[12]. Percentage of upper ocean productivity supported by external nutrient inputs are calculated by scaling to representative biological ratios (Fig. 1), with estimates for iron in

particular only considered to be order of magnitude at best, due to known stoichiometric plasticity (Fig. 1). Cumulative anthropogenic inputs over century timescales are thus potentially equivalent in magnitude to 100%, 1-2 % and <0.5% of the oceanic iron, nitrogen and phosphorus inventories respectively.

Box(1): Concepts of nutrient limitation.

Nutrient scarcity may restrict a number of biological and ecological processes[18]. Thus, it is necessary to identify the specific process being "limited" and worth considering the different conceptual frameworks and terms which have been used in discussing nutrient limitation[5,7,8,17-19].

Blackman and Liebig. Low nutrient concentrations can limit the growth rate of individual cells, while the total available amount of a nutrient can also set an upper bound on the amount of new biomass that can be formed (the yield)[5,18]. These concepts are often referred to as **Blackman** and **Liebig** limitation[5,18], respectively, after two pioneers working on different aspects of plant production. F. F. Blackman[17] studied photosynthesis in leaves while J. von Liebig[19] worked on agricultural crop yields. The yield perspective could be extended to consider the total biomass that can be formed at all trophic levels, including heterotrophic microbes, zooplankton, and viruses.

Stress and deficiency. The distinct concepts of stress and deficiency are also sometimes referred to as nutrient limitation. Although usage varies, we define **stress** as a physiological response to a nutrient shortage and **deficiency** as the stoichiometric lack of one element relative to another. For example, assuming a fixed biological N:P stoichiometry of 16:1 (cf. Redfield), if the concentration or flux of dissolved N falls below 16 times P, a system could be considered nitrogen deficient. Stress and deficiency are more closely related to, but clearly not synonymous with, the concepts of Blackman and Liebig respectively. There need not be a consistent relationship between the degree of physiological stress and growth rate[99], while the most deficient nutrient will still not become limiting if all the nutrients remain replete ***[could you expand on this? What do you mean by 'all'? All nutrients? And is this a continuation of the analogy with Blackman and Liebig?]]***.

Nutrient co-limitation. Conditions where two or more nutrients are **co-limiting** may be common in oceanic systems, however usage of the term varies greatly[7,8]. Nutrient co-limitation is typically ascribed to conditions where two (or more) nutrients have simultaneously been drawn down to levels where addition of both (or on some usage either) is required to stimulate growth. This may happen in a number of ways[7,8]. First, two or more nutrients can simply be drawn down to equally limiting levels so that both must be added to observe a growth response. Second, one limiting nutrient may be biologically substituted with another[36], either directly within the same macromolecule or indirectly by substituting one macromolecule for another. Third, the ability to take up low concentrations of one nutrient may require the availability of another nutrient[8]. Finally, one member of the microbial community may respond to the addition of one nutrient while another member responds to that of a different nutrient[7]. In the last three scenarios, addition of either nutrient elicits a growth response. Within nutrient addition experiments it may be difficult to distinguish the various types of co-limitation from each other, or from a secondary response due to addition of a nutrient depleted to levels where it is close to co-limiting[8,47].

Box(2): Competition in low nutrient environments.

Under stable low nutrient concentrations, the specific affinity (α, m^3 mol^{-1} s^{-1}), the slope of the relationship between growth rate and bulk concentration of a nutrient, represents a key parameter describing the competitive ability of microorganisms[100]. Assuming that diffusive transport towards a (for simplicity spherical) cell becomes rate limiting when the bulk concentration of the nutrient drops below some critical level, mechanisms for achieving a high α, and hence competitive advantage, can be understood by considering two readily derived expressions (Supplementary information):

$$\alpha = \frac{4\pi Dr}{Q}, \tag{1a}$$

or

$$\alpha = \frac{3D}{\sigma r^2}, \tag{1b}$$

where D is the diffusion constant for the nutrient (m^2 s^{-1}), r is the cell radius (m), Q is the cells nutrient quota (mol) and σ is the average whole cell concentration of the nutrient (mol m^{-3}), i.e. $3Q/4\pi r^3$ for a spherical cell.

At constant σ, small cells with high surface area to volume ratios will have a competitive advantage (Eqn. 1b). This size dependence of nutrient competition has likely been a key driver of phytoplankton evolution, explaining the dominance of pico-phytoplankton in very low nutrient oligotrophic systems[23,24]. In contrast, if constant Q can be maintained, increased size may actually represent an advantage (Eqn. 1a), a strategy that vacuolated organisms such as diatoms may adopt[100]. Finally, for a given size, minimisation of Q or σ (Eqns. 1 a or b) will maximise competitive ability, formalising the advantage gained from cellular substitutions of limiting nutrients[8,25] and other adaptive traits, including, for example, minimisation of protein nitrogen costs in oligotrophic waters[40].

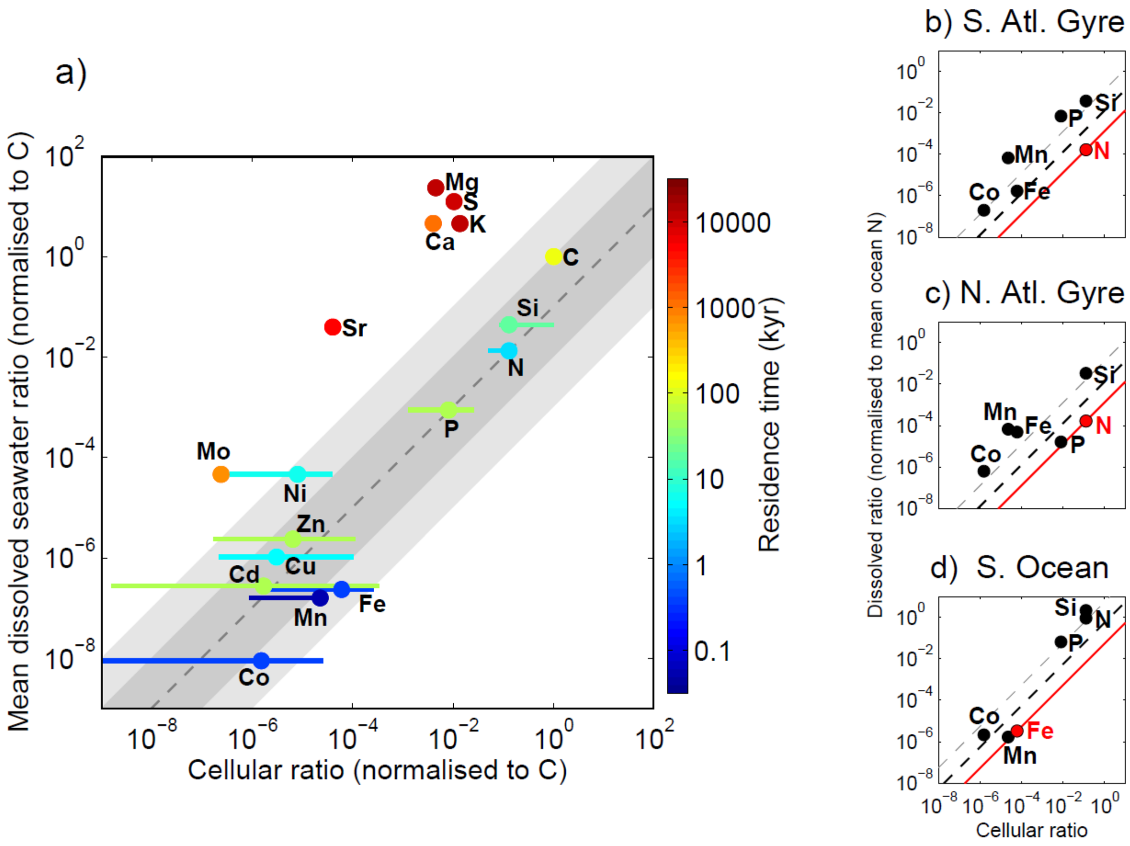
a)
Mean dissolved seawater ratio (normalised to C)
Cellular ratio (normalised to C)
Residence time (kyr)
10000
1000
100
10
1
0.1
Mg
S
K
Ca
C
Sr
Si
N
P
Mo
Ni
Zn
Cu
Cd
Fe
Mn
Co
b) S. Atl. Gyre
c) N. Atl. Gyre
d) S. Ocean
Dissolved ratio (normalised to mean ocean N)
Cellular ratio

Figure 1

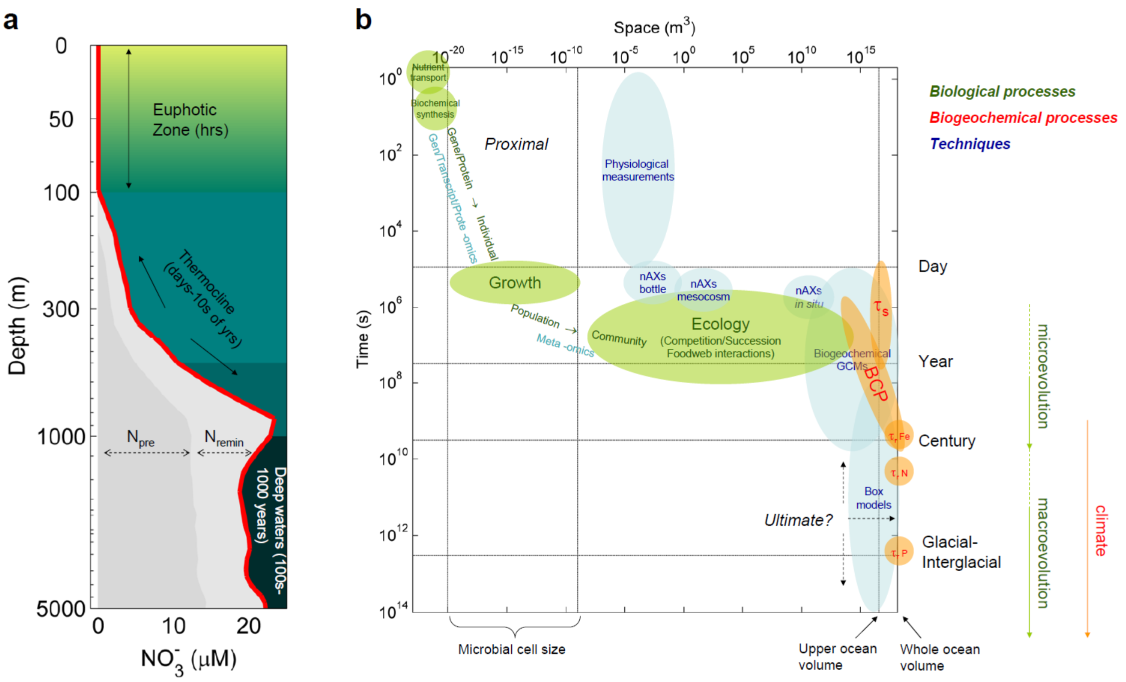
a
0
50
100
300
1000
5000
Depth (m)
Euphotic Zone (hrs)
Thermocline (days-10s of yrs)
N_{pre}
N_{remin}
Deep waters (100s-1000 years)
0
10
20
NO_3^- (µM)
b
Space (m^3)
10^{-20}
10^{-15}
10^{-10}
10^{-5}
10^{0}
10^{5}
10^{10}
10^{15}
Time (s)
10^{0}
10^{2}
10^{4}
10^{6}
10^{8}
10^{10}
10^{12}
10^{14}
Nutrient transport
Biochemical synthesis
Gene/Protein → Individual
Gen/Transcript/Prote -omics
Proximal
Physiological measurements
Growth
Population →
Meta -omics
Community
Ecology
(Competition/Succession Foodweb interactions)
nAXs bottle
nAXs mesocosm
nAXs in situ
Biogeochemical GCMs
τ_s
BCP
τ_r Fe
τ_r N
τ_r P
Box models
Ultimate?
Day
Year
Century
Glacial-Interglacial
Microbial cell size
Upper ocean volume
Whole ocean volume
Biological processes
Biogeochemical processes
Techniques
microevolution
macroevolution
climate

Figure 2

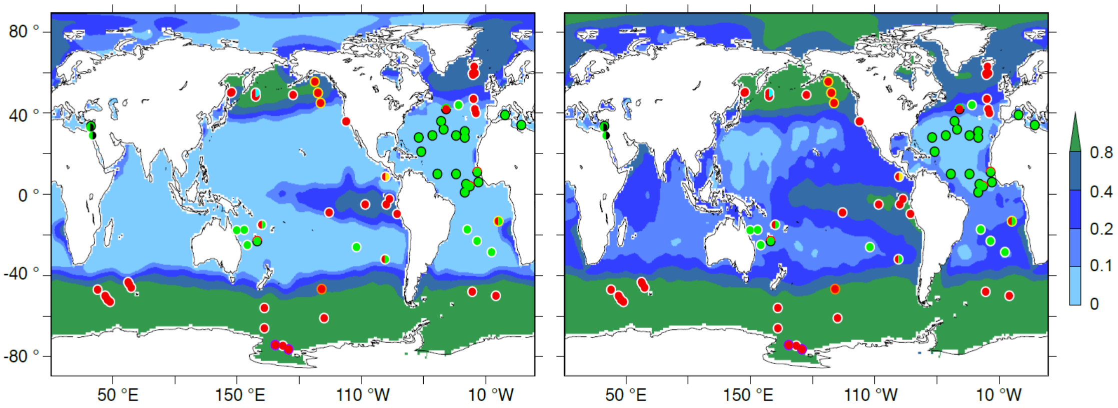

Figure 3