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Redox control of N:P ratios in aquatic ecosystems

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ABSTRACT

The ratio of dissolved fixed inorganic nitrogen to soluble inorganic phosphate (N:P) in the ocean interior is relatively constant, averaging ~16 : 1 by atoms. In contrast, the ratio of these two elements spans more than six orders of magnitude in lakes and other aquatic environments. To understand the factors influencing N:P ratios in aquatic environments, we analyzed 111 observational datasets derived from 35 water bodies, ranging from small lakes to ocean basins. Our results reveal that N:P ratios are highly correlated with the concentration of dissolved O_2 below ~100 μmol L^{-1}. At higher concentrations of O_2, N:P ratios are highly variable and not correlated with O_2; however, the coefficient of variation in N:P ratios is strongly related to the size of the water body. Hence, classical Redfield ratios observed in the ocean are anomalous; this specific elemental stoichiometry emerges not only as a consequence of the elemental ratio of the sinking flux of organic matter, but also as a result of the size of the basins and their ventilation. We propose that the link between N:P ratios, basin size and oxygen levels, along with the previously determined relationship between sedimentary $\delta^{15}N$ and oxygen, can be used to infer historical N:P ratios for any water body.

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INTRODUCTION

With few exceptions, primary production in most aquatic ecosystems is limited by either P or N. While P serves many roles in biological processes, by far the major sink for the element in all cells is ribosomes: the nanomachines responsible for protein synthesis. Nitrogen is a major component of many important biological compounds, including nucleic acids and proteins, with the latter representing the largest sink for the element. Despite the ecological requirements for these two macronutrients, their biological and geological chemistries fundamentally differ. The primary sources and ultimate sinks of P and N in aquatic ecosystems have been the subject of discussion and debate for over a century (see Mills, 1989; Lea & Miflin, 2003). In this paper, we examine the underlying factors responsible for variations in N:P ratios in aquatic ecosystems, and the potential use of N isotopes to reconstruct nutrient and redox histories.

In 1934, an American animal physiologist, Alfred Redfield, who was visiting oceanographers and marine biologists at the Plymouth Marine Laboratories in England, noted that the ratio of fixed inorganic N to P found in sea waters below the upper mixed layer was surprisingly similar to the ratio of the elements in plankton[1] (Redfield, 1934). He made the rather bold suggestion that the elemental ratio of the inorganic nutrients in the ocean was due to the death, sinking and remineralization of organic matter produced in the upper ocean, i.e. that biological processes not only were responsible for creation of organic phases of the elements in organisms, but were also responsible for the observed N:P ratios in the inorganic phases in the ocean. These observations were recapitulated and strengthened in subsequent analyses (Redfield, 1958), and the notion of a canonical atomic ratio of ~16 N:1P, or the 'Redfield ratio', is firmly ingrained in the oceanographic literature (Redfield *et al.*, 1963; Broecker & Peng, 1982). It is still not well understood why the *average* N:P ratio of particulate organic matter in the ocean is ~16 N:1P by atoms (Falkowski, 2000), yet the coupling between the vertical flux of organic matter and the ratio of the two elements in the soluble inorganic phases as an 'emergent' property of the ocean is generally accepted (Lenton & Watson, 2000). N:P ratios in lakes appear to be more variant than oceanic ratios,

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[1] The term 'plankton' in those days referred to large phytoplankton and zooplankton, essentially organisms that could be caught in fine meshed nets.

though N and P uptake and remineralization are similar to Redfield proportions (Redfield *et al.*, 1963; Guildford & Hecky, 2000).

Regardless of the specific ratio of the two elements in organic matter, the geochemical sources and ultimate sinks (i.e. their elemental cycles) for N and P are totally different. Phosphorus is supplied to the water column via rock weathering. It is found in the aqueous phase as hydrated phosphate anions, which ultimately form complexes with Ca and/or Mg cations leading to the production of several primary minerals (especially the apatite series), and to a lesser extent secondary iron-bearing minerals such as strengite and vivianite. Under oxidizing conditions, the solubility of phosphate minerals is extremely low, but anaerobic or acidic conditions, which lead to hydrolysis or reduction of metal cations, promote dissolution and solubilization of phosphate. Hence, to first order, the P cycle is controlled primarily by abiological processes through acid/base chemistry, where low oxygen promotes the production of soluble phases of the P-bearing minerals (Froelich, 1988; van Cappellen & Ingall, 1994).

In contrast to P, the N cycle is driven by biological processes involving redox reactions. The vast majority of nitrogen on Earth is dinitrogen (N_2) gas: two nitrogen atoms with an oxidation state of zero, connected by a triple bond. Incorporation of N into biological molecules requires reduction of the gas to the equivalent of NH_3. Perhaps surprisingly, the reduction of N_2 to NH_3 has a negative Gibbs free energy:

$$N_2 + 3H_2 \rightarrow 2NH_3 \quad \Delta G^\circ_f = -16\ \text{kJ mol}^{-1} \qquad (1)$$

suggesting that the reaction should be easily catalyzed. However, the transition energy is thermodynamically formidable; the bond dissociation enthalpy for N_2 is 941 kJ mol^{-1}, making the molecule virtually inert under standard temperatures and pressures (as found on the Earth's surface). The biological reduction of N_2 comes at great metabolic cost. The process relies exclusively on the heterodimeric enzyme, nitrogenase, which contains 19 FeS clusters and either a Fe-Fe, V-Fe or Mo-Fe at the catalytic site. All three closely related forms of the enzyme are extremely sensitive to O_2 (Postgate, 1987). *In vivo*, the reduction of N_2 partially overcomes the enthalpy barrier by hydrolyzing approximately 16 ATP molecules per N_2 fixed; these hydrolytic reactions yield ~ 798 kJ. While the structure of the complex is known at 1.6 Å resolution, the exact mechanism for the reduction of N_2 remains obscure. This process is probably the oldest coupled electron-proton reaction involving nitrogen. Nitrogen fixation is limited to a subset of bacteria and archea, but is not found in any known eukaryotic genome; all nitrogen-fixing eukaryotes contain prokaryotic symbionts. In aquatic ecosystems, the predominant nitrogen fixers are cyanobacteria. The genes encoding for the two proteins that comprise nitrogenase are highly conserved in all nitrogen-fixing organisms, suggesting that nitrogen fixation evolved once and spread across several clades of bacteria and archea via lateral gene transfer (Zehr, 1995; Falkowski *et al.*, 2008).

In some aquatic ecosystems, nitrogen fixation may itself be limited by nutrients. For example, in lakes, N_2 fixation can be limited by P and/or Mo (Howarth & Cole, 1985; Howarth *et al.*, 1988). In the ocean, N_2 fixation appears to be limited by Fe (Paerl *et al.*, 1987; Reuter *et al.*, 1992; Falkowski, 1997; Wu *et al.*, 2000; Berman-Frank *et al.*, 2001; Berman-Frank *et al.*, 2007) and possibly P (Sañudo-Wilhelmy *et al.*, 2001; Mills *et al.*, 2004). In contemporary aquatic environments, nitrogen fixation is a significant process in warm, oligotrophic, N-limited environments (as signified by low N:P ratios) and sufficient micronutrient input, including N-deficient lakes (Howarth *et al.*, 1988) and tropical and subtropical surface ocean waters (Capone *et al.*, 1997; Karl *et al.*, 1997; Westberry & Siegel, 2006).

Under anoxic conditions, fixed N is stable only in the –III oxidation state, either as organic compounds or as NH_3. In contrast, the products of aerobic nitrification, nitrate (NO_3^-) and nitrite (NO_2^-), are stable only in the presence of oxygen. Nitrification (i.e. the aerobic oxidation of ammonium) occurs in two steps: oxidation of ammonium to nitrite by one consortium of bacteria, then further oxidation to nitrate by a second bacterial group. Both reactions have small negative Gibbs free energy and low enthalpy, permitting the oxidation of nitrogen to be coupled to the reduction of inorganic carbon (i.e. nitrifying bacteria are chemoautotrophs). There is evidence that archea can also serve as nitrifiers in soils and aquatic environments (e.g. Hayatsu *et al.*, 2008; Horner-Devine & Martiny, 2008). The oxidized nitrogen compounds produced by aerobic nitrification are released into the environment where they ultimately are utilized by other organisms as a nutrient or energy source.

Under oxic conditions, nitrate is the most thermodynamically stable form of fixed nitrogen in the water column. Denitrification converts NO_3^- and NO_2^- back to N_2 gas (and N_2O, which can be reoxidized back to NO_3^-); the reaction is restricted to suboxic environments, as the presence of even micromolar amounts of oxygen can inhibit the denitrification enzymes (Codispoti *et al.*, 2001), but sufficient oxygen must be present to prevent reduction of the NO_3^-/NO_2^- species. Denitrification was probably the last of the biological N cycle processes to evolve, and there is a large amount of diversity in the enzymatic pathways and the type of organisms involved (Zumft, 1992). Low oxygen zones can occur due to permanent or seasonal density water column stratification or remineralization of large amounts of organic matter resulting in O_2 depletion. Denitrification can occur in sediments, where nitrate serves as an electron acceptor when sedimentary oxygen has been consumed. In sediments, denitrification generally goes to completion, consuming all of the nitrates that diffuse in from the overlying water column. Denitrification can also occur within the water column under low oxygen conditions, such as the oceanic oxygen minimum zones (OMZs), estuaries, organic-rich rivers and stratified lakes. Water column denitrification in both marine and freshwater systems (oceanic OMZs, lakes, rivers and estuaries) constitutes ~26% of the total global denitrification, removing approximately 155 Tg N/ year from these aqueous systems to the atmosphere (Seitzinger *et al.*, 2006). Of all the nitrogen pathways, denitrification may be the most impacted by the increase in fixed N concentrations and organic-rich waters resulting from human perturbation (Seitzinger *et al.*, 2006).

Less is known about anaerobic ammonium oxidation (anammox), which uses nitrite to oxidize ammonium to N_2 gas (Richards, 1965; Mulder *et al.*, 1995). There is evidence that the anammox reaction may be a significant factor in the removal of fixed N from aquatic ecosystems back to the atmosphere (e.g. Dalsgaard *et al.*, 2003; Engström *et al.*, 2005; Kuypers *et al.*, 2005), possibly contributing up to 30–50% of the N_2 produced in the ocean (Devol, 2003). The presence of anammox bacteria can be identified through ^{15}N tracer experiments (Thamdrup & Dalsgaard, 2002), 'ladderane' lipid biomarkers (Sinninghe Damasté *et al.*, 2002; Kuypers *et al.*, 2003), and DNA/RNA techniques (Freitag & Prosser, 2003; Kuypers *et al.*, 2003; Risgaard-Petersen *et al.*, 2004). Evidence for anammox has been found in several oxygen-limited environments including marine and freshwater sediments, anoxic fjords and basins, OMZ waters, and even arctic sea ice (Kuypers *et al.*, 2006 and references within). Potential controlling factors for the anammox reaction include Mn oxide concentration, presence of organic matter, relationships with denitrifiers, and oxygen levels, though not enough data exist to confirm these relationships (Kuypers *et al.*, 2006 and references within).

The circuit established between nitrogen fixation, nitrification, and denitrification/anammox reactions form the main nitrogen cycle in aquatic environments. The complete cycle involves a set of three coupled reduction/oxidation/reduction reactions, which are either alternatively sensitive to, or dependent on, oxygen. Fixed nitrogen enters the water column via nitrogen fixation, is transformed to a more stable inorganic form in oxic surface waters through nitrification, and then removed from the aquatic ecosystem by denitrification and/or anammox pathways. In a non-anthropogenically influenced system, the relative balance between nitrogen fixation and denitrification/anammox controls the net flow of nitrogen through the ecosystem. Changes in the relative strength of these two reactions may result in significant environmental changes, including the development of oceanic anoxic events (OAEs; Moore & Doney, 2007), sequestration of CO_2 (Falkowski, 1997), and perhaps even the evolution of oceanic oxygen in the Archean and early Proterozoic (Fennel *et al.*, 2005; Falkowski & Godfrey, 2008). Other nitrogen reactions include nitrate assimilation via photosynthesis to form organic N compounds and produce oxygen, and ammonium assimilation to organic matter. These reactions utilize nitrogen to drive metabolic growth but do not have a net effect on the global nitrogen cycle, since most of these organic compounds are remineralized to bioavailable inorganic forms.

Let us now consider the factors determining what controls N and P ratios in contemporary aquatic ecosystems.

VARIATIONS IN N:P RATIOS IN AQUATIC ECOSYSTEMS

We compiled data for dissolved inorganic nitrogen, phosphorus, and oxygen concentrations from a wide range of environments, including central ocean basins, restricted marine ecosystems and freshwater lakes (Table 1). Nutrient and oxygen concentrations were converted to common units of μmol L^{-1}. Dissolved inorganic nitrogen consists of measurements of nitrate, ammonium, and nitrite, where available; in some cases only nitrate, nitrate and nitrite, or nitrate and ammonium concentrations were measured. Dissolved inorganic phosphorus is generally in the form of phosphate, though a few studies measured soluble reactive phosphorus or total filtered phosphorus. All values were taken from deep waters to avoid recording the nutrient drawdown in the euphotic zone. Measurements range from averages over a survey track to basin-wide means over the course of several years. Table 1 should not be considered an exhaustive list of all major water bodies; we have chosen instead to collect data over a range of different environments. Although measured N:P ratios may be influenced by technical issues regarding the difficulty of measuring specific phases of one or both elements, we are searching for patterns across the spectrum of ecosystems.

The data in Table 1 reveal that N:P ratios vary by over six orders of magnitude, but are relatively normally distributed (Fig. 1). The highest value reported is from Lake Superior (N:P = 8700; Sterner *et al.*, 2007); the lowest is from the northern basin of Lake Lugano (N:P = 0.005; Barbieri & Simona, 2001). Generally, open ocean sites are very similar to the traditional Redfield N:P of 16, though there are slight variations both in site locations and depth. As nitrogen and phosphorus concentrations in the deep sea are influenced by both the surface water activity and the horizontal transport of particulate matter via water masses, this result is not unexpected (Fanning, 1992); however, while N and P concentrations are related to water masses, N:P ratios are not. N:P ratios for restricted basins have a greater degree of variation. The Mediterranean and Red Seas have generally higher than Redfield N:P ratios (N:P ~20), while suboxic/ anoxic water masses tend to have lower N:P than Redfield (N:P ~9 to 1). The range of N:P values for freshwater lakes is the largest of all three environments, as both the highest and the lowest recorded N:P values belong to lakes. The histogram of distributions of N:P ratios among the 35 water bodies examined suggests that the canonical Redfield ratio is anomalous; while ratios of ~16 N/1P dominate throughout the oceans, that specific ratio is rare in smaller basins and freshwater ecosystems (Fig. 1).

Table 1 Dissolved inorganic nitrogen, dissolved inorganic phosphorus, N:P ratios, and oxygen concentrations for a variety of open ocean, restricted basin/margin, and freshwater lake environments. All N measurements as total inorganic nitrogen and P measurements as phosphate unless otherwise noted (a = nitrate only, b = nitrate, nitrite, ammonium, c = nitrate, ammonium only, d = nitrate, nitrite only, e = total filtered phosphorus, f = soluble reactive phosphorus, g = total nitrogen). Data taken from references listed.

Water body	Depth (m)/ pressure (dbar)	N µmol L^{-1}	P µmol L^{-1}	N:P	O_2 µmol L^{-1}	Area basin (km^2)	Reference
Open Ocean							
S. Atlantic Ocean	50–1000 m			17	233 ± 13	8.2×10^7	GEOSECS database: Bainbridge (Ed.) 1981;
	1000–2000 m			13	210 ± 14		Anderson & Sarmiento, 1994
Indian Ocean	50–1000 m			16	97 ± 42	7.3×10^7	GEOSECS database: Broecker *et al*., (Eds.) 1982;
	1000–2000 m			12.5	95 ± 25		Anderson & Sarmiento, 1994
	2000–3000 m			11	148 ± 12		
	3000–4000 m			13.5	174 ± 6		
Pacific Ocean	50–1000 m			14	197 ± 14	1.7×10^8	GEOSECS database: Weiss *et al*., (Eds.) 1983;
	1000–2000 m			12.5	156 ± 7		Anderson & Sarmiento, 1994
	2000–3000 m			13	151 ± 1		
	3000–4000 m			15	186 ± 22		
BATS time series 2002–2003	995.0–1102 m	22 ± 1[d]	1.49 ± 0.06	14.8	195 ± 10		BATS time series: www.bats/bios.edu/ bats_form_bottle.html
Southern Ocean (KERFIX 1992)	900–1300 dbar	34 ± 1[a]	2.4 ± 0.1	14.2	195 ± 8	2.0×10^7	KERFIX time series: Pondaven *et al*., 2000; Jeandel *et al*., 1998
Mid-Atlantic Bight, Cape Hatteras/ Chesapeake Bay (SEEP1 2/84-3/84)	1010–1518 dbar	13 ± 6	0.8 ± 0.1	16.3	274 ± 6		SEEP I database: SEEP I Investigators, 1984
Hawaii Time Station (2004–2006)	1000–2000 dbar	41.7[a]	3.1	13.5	61.1		HOT time series: www.hahana.soest.hawaii.edu/
	2000–3000 dbar	39.5[a]	2.8	14.1	109		hot/hot-dogs/bextraction.html
	3000–4000 dbar	37.4[a]	2.7	13.9	140.8		
	4000–4808 dbar	36.6[a]	2.6	14.1	155.2		
North Atlantic NABE spring 1989	1000–2000 m	18.4 ± 0.3	1.17 ± 0.02	15.7	268 ± 18		NABE database: Brewer *et al*., 2003
	2000–3000 m	19 ± 1	1.21 ± 0.07	15.7	272 ± 4		
	3000–3500 m	21.4 ± 0.7	1.42 ± 0.06	15.1	254 ± 4		
GEOSECS 115, N. Atlantic				15.2 ± 0.7			Bainbridge, 1981
Restricted Basins/Coastal Areas							
Mediterranean Sea						2.5×10^6	
Straits of Sicily	Deep water	5.5[a]	0.225	24.4			Denis-Karafistan *et al*., 1998; Karafistan *et al*., 2002
Cyprus eddy core station	550–2000 m	~5.5[d]	~0.24	27.5 ± 4			Krom *et al*., 1991
Cyprus eddy boundary station	300–1000 m	~6[d]	~0.22	27.3 ± 2.5			Krom *et al*., 1991
SE Levantine basin	200–2000 m			28.1 ± 3.0			Krom *et al*., 1991
GEOSECS 404 Sea of Crete	100–4000 m			24.3 ± 0.7			Spencer, 1983
Alboran Sea	100–2800 m			22.5 ± 1.6			Coste *et al*., 1984
S. Levantine Basin	Below 700 m						Kress & Herut, 2002
East		5.57 ± 0.30[a]	0.23 ± 0.03	24.7 ± 2.7	175.1 ± 2.9		
Central		5.56 ± 0.34[a]	0.22 ± 0.02	25.0 ± 2.4	177.3 ± 3.8		
West		5.55 ± 0.40[a]	0.22 ± 0.03	25.2 ± 2.8	179.9 ± 4.0		
West high latitude		4.80 ± 0.61[a]	0.19 ± 0.03	25.9 ± 2.7	191.4 ± 2.9		
Medatlante 1&2 (1988, 1989)	>300 m	8.5 ± 0.3	0.40 ± 0.2	21.3			Bethoux *et al*., 1992
Phycemed 1983 (west Med)	Deep water	7.8	0.39	20.0			Delmas & Treuger, 1984
Ionian Sea station (E med)	Deep water	4.4	0.2	22.0			Bethoux *et al*., 1992
SEMAPHORE 94	>800 m	8.35 ± 0.21	0.399 ± 0.02	20.9			Bethoux *et al*., 1998
Mediprod 1/1 1969	>400 m	6.32 ± 0.82	0.388 ± 0.048	16.3			Minas, 1971
Mediprod 1/2 1969	>400 m	6.58 ± 0.056	0.381 ± 0.056	17.3			Minas, 1971
Mediprod 3 1972	>400 m	7.68 ± 0.75	0.355 ± 0.055	21.6			
Mediprod 4 1981	>400 m	8.13 ± 0.4	0.387 ± 0.037	21.0			Coste *et al*., 1984

Table 1 *Continued.*

Water body	Depth (m)/ pressure (dbar)	N µmol L^{-1}	P µmol L^{-1}	N:P	O_2 µmol L^{-1}	Area basin (km^2)	Reference
Phycemed 2 1983	>400 m	7.77 ± 0.44	0.387 ± 0.024	20.0			Delmas & Treuger, 1984
Mediprod 5/1 1986	>400 m	8.59 ± 0.48	0.394 ± 0.022	21.8			Raimbault *et al*., 1990
Mediprod 5/2 1987	>400 m	8.16 ± 0.47	0.379 ± 0.012	21.5			Raimbault & Bonin, 1991
Medatlante 1 1989	>400 m	8.18 ± 0.31	0.392 ± 0.014	20.9			Bethoux *et al*., 1992
Medatlante 2 1989	>400 m	8.32 ± 0.23	0.378 ± 0.018	22.0			Bethoux *et al*., 1992
Mediprod 6 1990	>400 m	8.47 ± 0.27	0.389 ± 0.03	21.8			Raimbault & Bonin, 1991
Semaphore 1994	>400 m	8.68 ± 0.29	0.404 ± 0.024	21.5			Bethoux *et al*., 1998
Caspian Sea 2006						378400	
Middle (Divichi-Kenderli)	600 m	14[a]	1.6	8.8	44.6		Sapozhnikov *et al*., 2007
Middle (Divichi-Kenderly)	600 m	11[a]	1.5	7.3	66.9		Sapozhnikov *et al*., 2006
South (Kurinskii Kamen'-Ogurchinskii)	800 m	2[a]	1.8	1.1	0		Sapozhnikov *et al*., 2007
Middle Caspian							
1934	600 m	5[a]	1.6	3.125	44.6		Bruevich, 1937
1983	500 m (low sea level)	12[a]	1	12			Sapozhnikov *et al*., 2008
2000	600 m	10[a]	1.8	5.6			Sapozhnikov *et al*., 2008
2006	600 m	14[a]	1.6	8.75	26.8		Sapozhnikov *et al*., 2008
Black Sea		c				436400	
Suboxic sig = 15.8	See density	7.5[c]	1	7.5	15		Konovalov & Murray, 2001
Anoxic sig = 16.8	See density	25.25[c]	5	5.05	0		Konovalov & Murray, 2001
Black Sea 2001	Sigma-t						Knorr 2001 database: http://www.ocean.washington.edu/cruises/Knorr2001/
Oxic	~15	3 ± 2	0.6 ± 0.2	5.0	71 ± 17		
Suboxic	~16	1.4 ± 0.9	3 ± 1	0.47	6 ± 9		
Anoxic	~16.5	9 ± 5	4.8 ± 0.8	1.9	0		
Arabian Sea TTN						3862000	Arabian Sea database: Codispoti, 2000
Process cruise 1 all stations	1000–2000 m	38.9 ± 0.7	3.00 ± 0.08	13.	43 ± 28		
	2000–3000 m	38.1 ± 0.5	2.81 ± 0.08	13.6	106 ± 14		
	3000–4000 m	36.8 ± 0.4	2.63 ± 0.06	14.0	141 ± 9		
	4000–4300 m	35.61 ± 0.03	2.50 ± 0.01	14.2	162.0 ± 0.4		
Cariaco 10/05–9/06						103	Cariaco time series: http://www.imars.usf.edu/CAR/index.html
Oxic	100–200 m	10 ± 1	0.8 ± 0.3	12.5	105 ± 35		
Suboxic	200–300 m	4 ± 3	2.0 ± 0.6	2	8 ± 15		
Anoxic	300–1313 m	14 ± 8	3.2 ± 0.4	4.375	0		
VERTEX V, N.Pac Monterey							Broenkow & Reeves, 1985; Martin & Gordon, 1988
Station 1 upwelling	1090 m	45.8	3.31	13.8	36		
Station 2 Ca current	1080 m	46.6	3.36	13.9	24		
Station 4 NE Pac cent gyre	1085 m	44.9	3.5	12.8	22		
SEEP1 Feb–Mar 1984 margins							
Mid-Atlantic Bight, Cape Hatteras/Chesapeake Bay	1010–1518 dbar	13 ± 6	0.8 ± 0.1	16.25	274 ± 6		
Red Sea 2003	600 m	6.1 ± 0.5[a]	0.33 ± 0.03	19 ± 1	176 ± 11		Lazar & Erez, 2004
Freshwater Lakes							
Lake Baldegg, Switzerland	Average over depth	127	3.2	39.7	>100	5.2	Mengis *et al*., 1997
Lake Zug, Switzerland	Average over depth	30	5.1	5.9	50–0	38.3	Mengis *et al*., 1997
Lake Baikal (central basin)	1500 m	8[a]	0.67	11.9	319	30000	Killworth *et al*., 1996
Lake Lugano annual mean							Barbieri & Simona, 2001
Northern Basin	1–100 m	30[g]	1694[f]	0.018	168	27.5	
	101–288 m	42[g]	8372[f]	0.0050	0		

Table 1 *Continued.*

Water body	Depth (m)/ pressure (dbar)	N µmol L^{-1}	P µmol L^{-1}	N:P	O$_2$ µmol L^{-1}	Area basin (km^2)	Reference
Southern Basin						20.3	
Melide	0–85 m	82[g]	2126[f]	0.039	194		
Figino	0–95 m	85[g]	1860[f]	0.046	166		
Lake Lugano, S. basin	70 m	78.5[c]	1.7	46.2	62.5–234	48.9	Lehmann, *et al*., 2004
Lake Müggelsee, Germany						7.3	
Stratified	4.5–7.0 m (hypolimnion)	2–13[c]	0.13–4.6[f]	2.8–15	56–222		Driescher *et al*., 1993
Mixed		3.6–23.6[c]	1.1–8.9[f]	2.7–3.3			Wilhelm & Adrian, 2008
Lake Tahoe	400 m	1.5 ± 0.4[a]	0.09 ± 0.02	16.7	339 ± 7	500	Holm-Hansen *et al*., 1976
Lake Victoria, E. Africa	60 m	22.9	2.42[f]	9.5	41	68800	Lehman & Branstrator, 1994
	40 m	8.4	2.43[f]	3.5	56		
Plesné Lake							
Winter (ice)	12 m	35[c]	0.05	700	313	0.075	Kopacek *et al*., 2004
Summer (ice free)	12 m	40[c]	0.08	500	156		
Mirror Lake						0.15	Mirror Lake time series: Likens, 2005
1967–2005	10 m	9 ± 7	1 ± 3	75 ± 187	125 ± 125		
	8–9.5 m	6 ± 7	2 ± 10	53 ± 121	188 ± 94		
	6–7 m	5 ± 5	1 ± 6	55 ± 141	281 ± 63		
Lake Superior	>50 m	26[a]	0.003	8700	405 ± 9	82400	Sterner *et al*., 2007; RW Sterner, personal communication
North Temperate Lakes LTER							NTL LTER database: Stanley, 2008; Rusak, 2008
Allequash Lake	4 m	5.3 ± 3.7	0.3 ± 0.2[e]	37 ± 55	125 ± 63	1.7	
	6–7 m	15.1 ± 14.9	0.6 ± 0.7[e]	26 ± 22	125 ± 156		
Big Muskellunge Lake	16–17 m	9.8 ± 5.9	0.39 ± 0.06[e]	24 ± 15	94 ± 94	4.0	
	8–12 m	4.8 ± 3.4	0.3 ± 0.2[e]	29 ± 42	344 ± 63		
Crystal Lake	12–15 m	1.9 ± 1.6	0.19 ± 0.06[e]	9 ± 7	313 ± 94	0.37	
	8–10 m	2.7 ± 1.2	0.1 ± 0.1[e]	20 ± 18	375 ± 63		
Fish Lake	12–14 m	22.6 ± 10.7	0.7 ± 0.9[e]	68 ± 70	156 ± 156	0.87	
	8–10 m	13.3 ± 10.9	0.3 ± 0.2[e]	59 ± 72.6	219 ± 125		
Mendota Lake	12–16 m	51.7 ± 9.9	3.6 ± 2[e]	18 ± 7	63 ± 125	39.4	
	8–10 m	35.1 ± 19.9	1.9 ± 0.8[e]	33 ± 13	188 ± 156		
Monona Lake	14–16 m	77.3 ± 29.4	8.7 ± 4.2[f]	9 ± 2	22 ± 31	13.2	
	10–12 m	47.0 ± 12.9	4.4 ± 2.5[f]	13 ± 4	125 ± 156		
Sparkling Lake	15–17 m	11.1 ± 8.7	0.19 ± 0.06[e]	79 ± 88	94 ± 94	0.64	
	10–12 m	3.2 ± 2.6	0.16 ± 0.1[e]	35 ± 64	125 ± 156		
Trout Lake	27 m	9.4 ± 2.1	0.07 ± 0.02[e]	130 ± 48	125 ± 94	16.1	
	20 m	4.4 ± 1.1	0.2 ± 0.1[e]	26 ± 13	313 ± 94		
Lake Tanganyika						32600	Edmond *et al*., 1993
Kigoma Basin	110 m	8.91[b]	1.88	4.7	24.3		
	579 m	40.41[b]	5.94	6.8	0		
Kipili Basin	110 m	9.4[b]	1.83	5.1	54.3		
	594 m	34.9[b]	5.74	6.1	0		
Lake Malawi	100 m	4.5[c]	0.45[f]	10	160	28800	Bootsma & Hecky, 1993
	300 m	10[c]	1.1[f]	9.1	0		

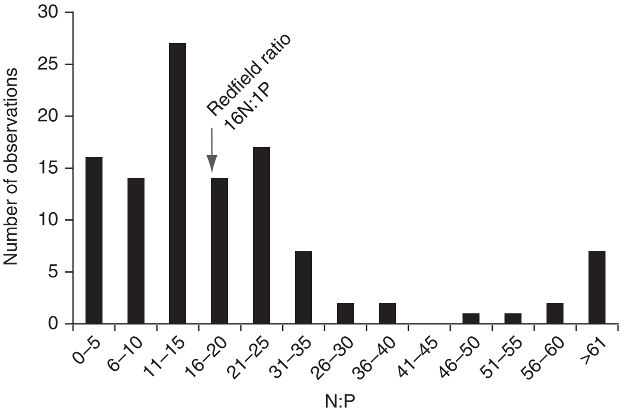

Fig. 1 The distribution of measured N:P ratios in Table 1 with respect to the number of observations. Most N:P measurements tend to fall in a roughly normal distribution between 0 and 30; however, the number of observed N:P ratios greater than 61 is significant, and could represent the effects of anthropogenic nitrogen loading. The Redfield ratio of 16 N:1P is marked on the histogram as a point of reference.

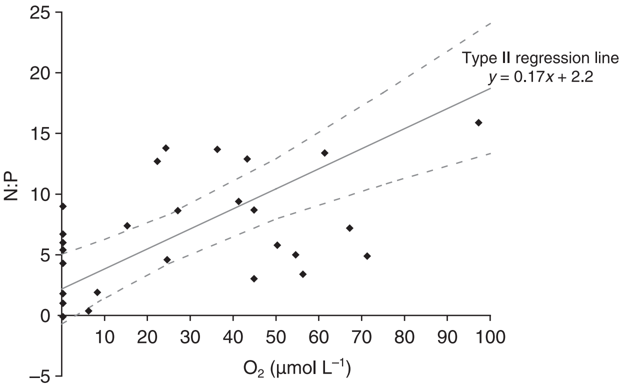

Fig. 2 The relationship between N:P and deep-water oxygen (μmol L^{-1}) concentrations for 12 suboxic/anoxic ecosystems (Caspian Sea, Black Sea, the Arabian Sea from 1000–2000 m, Cariaco Basin, northern Lake Lugano, the Indian Ocean from 50–1000 m, the HOT station from 1000–2000 dbar, the North Pacific off Monterey, Lake Tanganyika, Lake Malawi, Lake Victoria, and Lake Zug). N:P and O_2 are correlated by a type II regression line (solid gray) with slope 0.17. This is estimated by regressing N:P on O_2, then regressing O_2 on N:P. The type II slope is the geometric mean of the slopes of these two regression lines. The type II intercept is then calculated using the type II slope and the average N:P and O_2 values. Data references are listed in Table 1. The 95% confidence intervals (Student's t-test) are marked with dashed gray lines.

To examine the influence of oxygen on N:P ratios, we considered a subset of 27 data points from 12 basins and lakes, all of which have oxygen concentrations <100 μmol L^{-1} for at least some portion of the water column. These include the Caspian Sea, the Black Sea, the Arabian Sea between 1000–2000 m, the Indian Ocean from 50–1000 m, the HOT station between 1000–2000 dbar, the North Pacific off Monterey, Lake Tanganyika, Lake Malawi, the Cariaco Basin below 200 m, the northern section of Lake Lugano, Lake Victoria, and Lake Zug. Though Lake Monona also has low oxygen below 14 m, we did not include it in our analysis, since this depth is below the mean lake depth of 8.2 m, and the nutrient concentrations may be affected by sedimentary processes. The N:P values for these eight locations are low, generally under 13. This is particularly important when compared to the oxic lakes, where N:P ratios can be significantly higher. Other fully oxic environments have higher N:P ratios as well, though the difference is smaller; open ocean sites reflect Redfield N:P values, oxic enclosed basin values have measured N:P of approximately 20. If these 12 locations are representative of all low oxygen environments, it is clear that suboxic/anoxic conditions are characterized by lower deep-water N:P ratios.

There is a positive linear correlation between oxygen concentration and N:P for these 12 environments (Fig. 2). These results strongly support the concept that low oxygen concentrations promote loss of fixed inorganic nitrogen via denitrification and anammox reactions. For oxygen concentrations above ~100 μmol L^{-1}, there is no correlation between oxygen and N:P, though all samples with the exception of Lake Superior and Plesné Lake have N:P ratios <78.

For aquatic environments with O_2 concentrations >100 μmol L^{-1}, variations in N:P are related to the surface area of the basin. N:P ratios are generally more variable in lakes than in coastal or ocean sites (e.g. Hecky *et al.*, 1993; Hassett *et al.*, 1997). Smaller water bodies also tend to be more variable with regards to changes in water depth, nutrient input, productivity, redox state, and water turnover. The relationship between the above observations can be determined statistically by sorting the ecosystems by size, then dividing them into groups ranging from 0.1 km^2 to 10^9 km^2. The largest water bodies are the ocean basins (Atlantic, Pacific, Indian, Southern), progressing down in size through the Mediterranean and Black Seas and larger lakes (Superior and Victoria) down to the smallest lakes with surface areas of less than 1 km^2. For each size class, we calculated the coefficient of variation in N:P ratios as the standard deviation divided by the mean. In general, the coefficient of variation generally decreases as the size of the water body increases; i.e. N:P ratios in larger ecosystems are more consistent with each other (Fig. 3; black bars). This buffering of deep-water N:P ratios in water bodies with large surface area is due to the fact that these large basins remain relatively unaffected by small disturbances in factors such as nutrient input, productivity, and redox state. These water bodies are slow to change their redox conditions and have long periods of stable nutrient cycling. In contrast, the smallest water bodies have extremely variable N:P ratios; their small size makes them inherently unstable in their physical and chemical structures, and minor changes in nutrient input and/or physical ventilation can have an immediate effect in their redox state, thus affecting the distribution of N and P.

The one exception to the general trend of higher coefficient of variation for smaller water bodies is the 10^4 to 10^5 km^2 group (Fig. 3). Most of the variation in N:P ratios for this

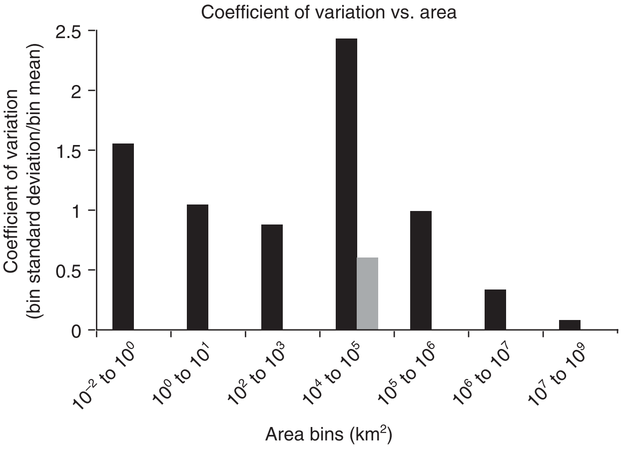

Fig. 3 The coefficients of variation for N:P ratios versus water body size. The samples in Table 1 were separated into bins based on the surface area of the basin. The mean N:P ratio and standard deviation were calculated for each of the bins. The coefficient of variation in N:P ratio for each bin was determined by dividing the standard deviation by the mean. The black bars include Lake Superior in the calculations; the gray bar for the 10^4 to 10^5 km^2 bin represents the coefficient of variation with the values for Lake Superior removed.

group can be traced to relatively anomalous N:P measurements for Lake Superior (N:P = 8700; Sterner *et al.*, 2007) compared to lakes with similar surface areas (N:P from 6.5 to 22.5). When the N:P for Lake Superior is removed from the group mean N:P and standard deviation calculations, the coefficient of variation for the group decreases to 0.6 (Fig. 3; gray bar), matching the trend of smaller coefficients of variation for larger lakes. Lake Superior has more nitrogen relative to phosphorus than other lakes in the size bin, resulting in a larger coefficient of variation for the group (Sterner *et al.*, 2007). The high nitrogen concentrations for Lake Superior are well documented as resulting from increased nitrification rates and anthropogenic nitrate input, particularly within the last several decades, combined with increasingly low phosphate concentrations that lie very close to the analytical detection limit (Sterner *et al.*, 2007). While it is likely that all measurements made in present-day environments are affected by human activities, the *in situ* nitrogen cycle within Lake Superior has changed significantly from the pre-anthropogenic conditions, making the comparison with other water bodies less applicable in the interpretation of natural ecosystems and their historical behavior.

We can use this information, along with the correlation between the coefficient of variation in N:P and basin surface area, to constrain the deep-water N:P ratio for any body of water for which the deep-water oxygen concentration and surface area are known. For oxygen concentrations of <100 μmol L^{-1}, the type II linear regression equation in Fig. 2 can be used to calculate the expected N:P ratio. If the basin is relatively large (>10 000 km^2), the calculated N:P ratio can be considered to be robust; if the basin is small, the calculated N:P ratio should be considered a general guideline only. The N:P ratios for basins with oxygen concentrations of >100 μmol L^{-1} are less predictable, though they are not expected to be greater than 78, unless the lake is highly affected by excess nitrogen input and nitrification, similar to Lake Superior, or has a significant amount of abiotic phosphorus removal like Plesné Lake (Kopacek *et al.*, 2004; Sterner *et al.*, 2007). Larger oxic basins, particularly those that have significant exchange with the ocean, are more likely to have N:P ratios similar to Redfield ratios than the smaller water bodies; however, the influence of anthropogenic activities may artificially alter the N:P ratios of even large basins.

We now examine if and how this information can guide the interpretation of δ^{15}N values in the sedimentary record on geological times scales.

NITROGEN ISOTOPES AND FRACTIONATION

The nitrogen cycle described earlier imprints an isotopic signature on the organic matter formed. The bond strength for ^{14}N is lower than for ^{15}N; thus, reactions in which a ^{14}N bond is broken will be energetically favored relative to ^{15}N. The isotopic fractionation for any particular reaction can be described as a fractionation factor (α) between reactants and products,

$$\alpha_{(\mathrm{N}_{(R)} \rightarrow \mathrm{N}_{(P)})} = \frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_R}{(^{15}\mathrm{N}/^{14}\mathrm{N})_P} \qquad (2)$$

where subscript R refers to the reactants and subscript P to products. A list of α values for each of the nitrogen processes is shown in Table 2. If no isotopic fractionation occurs during the reaction, then $\alpha = 1$. For most of the nitrogen reactions, the kinetic fractionation favors the ^{14}N atoms, resulting in products containing more ^{14}N than the initial reactants. As a result of this discrimination, the remaining reactants are enriched in ^{15}N. This results in a fractionation factor greater than 1. Only nitrogen fixation has an $\alpha < 1$, meaning that the pathway discriminates against the lighter isotope, resulting in products containing less ^{14}N than the initial reactants. Denitrification, in contrast, has the largest α values, indicating that the intensity of the isotopic discrimination is greater for this reaction than the others.

Since the amount of ^{15}N atoms is much smaller than the amount of ^{14}N, results of nitrogen isotopic measurements are reported as δ^{15}N in parts per thousand (per mil; ‰):

$$\delta^{15}\mathrm{N} = \left[\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{sample}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{standard}} - 1 \right] \times 1000 \qquad (3)$$

where the standard is atmospheric nitrogen, defined as $\delta^{15}\mathrm{N} = 0‰$. Samples with negative δ^{15}N values are relatively depleted in ^{15}N; positive values are enriched in the heavier isotope. Values for the organic products of nitrogen fixation have a δ^{15}N range of approximately $-2‰$ to $+2‰$. The

Table 2 Chemical equations and isotopic fractionation factors (α) for the major biologically mediated pathways for nitrogen cycling. Fractionation factors taken from [*]Talbot, 2001 and references within, [†]Wada 1980, and references within, and [‡]Delwiche & Steyn, 1970; anammox data taken from Kuypers *et al.*, 2006.

Pathway	N process	Fractionation factor (α)
Nitrate assimilation	NO_3^- reduced to cellular-N	1.000–1.019[†]
		1.011–1.023[*]
Ammonium assimilation	NH_4^+ converted to cellular-N	0.993–1.013[*]
		1.000–1.010[†]
		1.0011–1.0148[‡]
Nitrogen fixation	N_2 reduced to NH_4^+	0.994–1.008[†]
	N_2 reduced to cellular-N	0.996–1.0024[*]
Nitrate reduction	NO_3^- reduced to NO_2^-	1.000–1.029[†]
Nitrification	NH_4^+ oxidized to NO_2^-	1.02[*]
		1.026[‡]
	NH_4^+ oxidized to NO_3^-	1.000–1.021[†]
Denitrification	NO_3^- reduced to N_2	1.02[*]
		1.000–1.04[†]
		1.0173[‡]
Remineralization/decomposition	Cellular-N converted to dissolved organic N	0.995–1.002[†]
	Cellular-N oxidized to NO_3^-	0.998–1.006[†]
	Cellular-N converted to NH_4^+	1.001[*]
Anaerobic ammonium oxidation (anammox)	NH_4^+, NO_2^- converted to N_2	Unknown, suspected similar to denitrification

products of denitrification have a $\delta^{15}N$ of roughly – 0‰, and the residual nitrate left behind has a $\delta^{15}N$ of approximately + 25‰ (Brandes *et al.*, 1998). Raleigh fractionation dictates that if a reaction goes to completion (all the original reactant is utilized), the $\delta^{15}N$ of the products is the same as the initial substrate; the net change in $\delta^{15}N$ is zero.

Isotopic measurements can be performed on dissolved, particulate and sedimentary nitrogen compounds, and can provide information about the biological processes currently occurring in the water column, as well as a record of how the environment has changed. The $\delta^{15}N$ of nitrate in the surface water is dictated by the biological pathways present, as different reactions involving of nitrate fractionate the nitrate pool (Wada, 1980; Table 2). This nitrate pool is then assimilated by organisms into organic matter, a metabolic process that has minimal fractionation. As a result, the organic compounds synthesized in the euphotic zone bear the $\delta^{15}N$ signal of the nitrate pool, and thus reflect the nitrogen cycle in the surface waters. A small fraction of the organic nitrogen compounds produced are buried in the sediment. The organic nitrogen isotope ratio from well-preserved sedimentary organic matter is a reliable recorder of the water column nitrate isotope ratio, assuming complete nitrate assimilation (Altabet & Francois, 1994). It is assumed that sedimentary denitrification proceeds to completion, and thus does not alter the $\delta^{15}N$ of the preserved organic matter. Hence, in principle one can infer how nitrogen cycling at a particular location changed through time.

SEDIMENTARY NITROGEN ISOTOPE RECORDS

In the modern ocean, a well-preserved organic sedimentary isotope record at any location reflects the balance between two end-member processes: nitrogen fixation and denitrification. As mentioned above, nitrogen fixation is characterized by a small, mostly negative $\delta^{15}N$ signal. Remineralization of this isotopically light nitrogen, along with nitrification with minimal denitrification, results in a small, usually negative fractionation in the nitrate pool, which is then reflected in the sedimentary organic matter. In contrast, denitrification strongly prefers ^{14}N-nitrate, enriching the remaining nitrate in the heavier isotope. The isotopically heavy $\delta^{15}N$ signal is incorporated into organic matter and carried to the sediment. The sedimentary nitrogen profile at a particular location reflects the surface water nitrate pool through time, and thus the relative balance between nitrogen fixation and denitrification. In short, isotopically lighter $\delta^{15}N$ values indicate a surface ocean dominated by nitrogen fixation, while heavier values signify more denitrification.

Many researchers have taken advantage of marine sedimentary $\delta^{15}N$ records to determine how the nitrogen cycles changed on a glacial to interglacial time scale (Ganeshram *et al.*, 1995, 2000, 2002; Calvert *et al.*, 2001). As yet, however, only a few $\delta^{15}N$ profiles have been measured for time periods earlier than the Holocene, and most have focused on specific global events, such as mass extinctions (Quan *et al.*, 2008), and OAEs (Rau *et al.*, 1987; Jenkyns *et al.*, 2001; Ohkouchi *et al.*, 2006; Jenkyns *et al.*, 2007; Juniam & Arthur, 2007). Investigations of specific nitrogen biomarkers such as porphyrins and chlorophylls have measured the $\delta^{15}N$ of individual compounds or parts of molecules formed directly in the euphotic zone and preserved unchanged in the sediments (Chicarelli *et al.*, 1993; Sachs & Repeta, 1999; Ohkouchi *et al.*, 2006; Kashiyama *et al.*, 2008a, b). These biomarkers are thus immune from any diagenetic changes to the nitrogen through the water column and/or in the sediment, allowing a more direct measurement

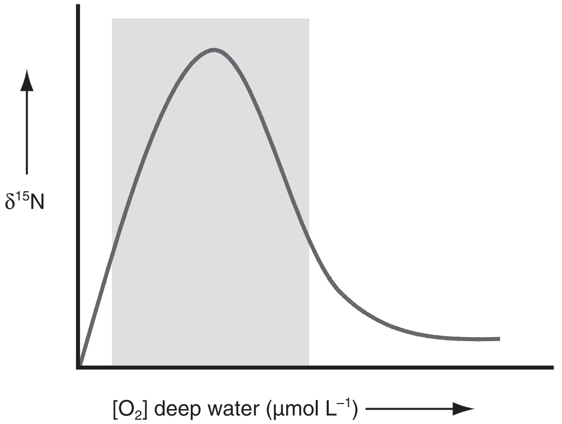

Fig. 4 Conceptual model of the correlation between $\delta^{15}N$ and deep-water oxygen concentration. Enriched $\delta^{15}N$ values are limited to a narrow range of oxygen concentrations that lie within the shaded hypoxic/suboxic range. Figure adapted from Quan *et al.*, 2008. The linear relationship between O_2 under ~100 μmol L^{-1} and deep-water N:P means that $\delta^{15}N$ can theoretically be linearly correlated to N:P ratio up to an N:P ratio of approximately 13.

of surface water nitrate. Recent measurements of the porphyrin fraction versus the bulk nitrogen $\delta^{15}N$ for a core through OAE2 indicate a constant difference between the two, indicating that nitrogen trends interpreted from bulk nitrogen records are real (Quan TM, Ostertag-Henning C, Hinrichs K-U, Ohkouchi N, Ogawa NO, Falkowski PG, unpublished).

NITROGEN AS A PALEOREDOX PROXY

Because the biological pathways that dictate the nitrogen isotopic content of sediments are also related to environmental oxygen concentrations, $\delta^{15}N$ can be used to predict the redox state of past environments. The theoretical relationship between sedimentary $\delta^{15}N$ and water column oxygen concentration is presented in Fig. 4 (adapted from Quan *et al.*, 2008). When there is no oxygen present in the environment, nitrate cannot be formed, and the $\delta^{15}N$ remains close to zero. As the oxygen level increases, the nitrate concentration also rises, resulting in optimal conditions for denitrification to predominate. The presence of denitrification is reflected in the increasingly enriched $\delta^{15}N$ values. Increases in oxygen and nitrate concentrations, along with enriched $\delta^{15}N$ values continue until oxygen levels reach a ‘tipping point’. This occurs when denitrification and the $\delta^{15}N$ of the system are in kinetic equilibrium. Further increases in the oxygen content of the surface waters beyond this critical threshold starts to inhibit the denitrification enzymes. Oxygen also inhibits the nitrogenase enzyme required for nitrogen fixation, but nitrogen fixers appear to better isolate this enzyme from oxygen within the cell (Berman-Frank *et al.*, 2001). As a result, the relative importance of denitrification decreases while nitrification and nitrogen fixation increase, leading to the gradual decrease in the $\delta^{15}N$ values. The relationship between oxygen and $\delta^{15}N$ shown in Fig. 4 implies that enriched $\delta^{15}N$ values are limited to a narrow range of oxygen concentrations. Oxygen levels must be high enough to establish a significant steady-state reservoir of nitrate, but low enough not to poison the denitrification reaction. A positive shift in the $\delta^{15}N$ signal in a sedimentary record implies increased denitrification, and thus low oxygen levels in at least part of the water column. In this way, nitrogen isotopes can be used as a paleoredox proxy.

This theoretical model of the correlation between oxygen concentration and $\delta^{15}N$, and the validity of nitrogen isotopes as a paleoredox proxy, has been confirmed for two types of events: global mass extinctions and glacial–interglacial cycling. The nitrogen isotope profile from a core from Mingolsheim, Germany, through the Triassic–Jurassic (T-J) mass extinction showed heavier $\delta^{15}N$ values in the Early Jurassic, indicating increased denitrification and lower oxygen levels (Quan *et al.*, 2008). This enriched $\delta^{15}N$/low O_2 period also contained increasing concentrations of other redox-sensitive elements (Cd, Mo, U, and V), along with a species shift seen in the palynology, confirming a reducing water column during this time period (Quan *et al.*, 2008; van de Schootbrugge *et al.*, 2008). The change in water column redox state from oxic through the T-J boundary to hypoxic/anoxic in the Early Jurassic is thought to be due to increased weathering brought about by events caused by Central Atlantic Magmatic Province degassing.

Shifts in $\delta^{15}N$ coincident with glacial–interglacial cycling in the Black Sea further corroborate the relationship between $\delta^{15}N$ and oxygen concentration (Quan TM, Wright JD, Falkowski PG, unpublished). The Black Sea transitions from an anoxic, marine basin during interglacials to a freshwater, oxic lake during glacial periods, with periods of transitional redox states in between. These shifts in redox states are mirrored in the $\delta^{15}N$ profile: enriched nitrogen isotopic values during the transition periods, and lower $\delta^{15}N$ during both the oxic glacial and the anoxic interglacials. This indicates that denitrification was more intense during the transition between the two redox end-members (when the oxygen concentration was low but > 0) than during the oxic glacials and anoxic interglacials (where oxygen concentrations were too high and too low, respectively). The timing of the glacial–interglacial events in the Black Sea is constrained by total organic carbon and Fe/Al ratios, along with the presence or absence of marine planktonic foraminifera, freshwater ostracods, and benthic foraminifera. The extreme changes in redox state in the Black Sea provide confirmation of the $\delta^{15}N$-O_2 correlation. Repeated cyclical patterns of $\delta^{15}N$ values are seen in profiles on glacial–interglacial time scales from Saanich Inlet and the Mexican, Californian, Peru, and Indian Ocean margins (Ganeshram *et al.*, 1995, 2000, 2002; Calvert *et al.*, 2001). While the absolute values differ, all studies find higher $\delta^{15}N$ during periods of lower water column oxygen concentration, and lower $\delta^{15}N$ during more oxic times. Measurements of individual porphyrin biomarkers also confirm this trend (Ohkouchi *et al.*, 2006; Kashiyama *et al.*, 2008a, b).

Based of this evidence, the theoretical model outlined in Fig. 4 appears to be robust, though further investigations clearly are needed.

Freshwater nitrogen cycling and the related isotopic fractionation patterns are similar to that of marine ecosystems, though the influence of terrestrial and anthropogenic input is greater. As a result, well-preserved lake sediments contain a mixture of autochthonous organic matter (C:N ~6–20; Redfield *et al.*, 1963; Hecky *et al.*, 1993) with terrestrial compounds (mean C:N = 160; Schlesinger, 1997) and soil humics (mean C:N ~15; Schlesinger, 1997), and the amount of nitrogen preserved can vary greatly between lakes. Isotopically, soil organic matter has a $\delta^{15}N$ of approximately +5‰, while terrestrial plants have a $\delta^{15}N$ range of +2 to +10‰; sedimentary $\delta^{15}N$ values usually range from –5 to +20‰ (Owens, 1987; Talbot, 2001). The $\delta^{15}N$ of water column particulate organic matter collected using sediment traps can vary in seasonal cycles (Hodell & Schelske, 1998), which can be attributed to changes in inorganic N input sources, seasonal productivity cycles, and water column stratification. Sedimentary nitrogen isotope measurements for freshwater bodies have primarily focused on identifying and characterizing nitrogen cycle changes due to anthropogenic influence on a decadal time scale, such as eutrophication and decreases in bottom-water oxygen content. Sediment cores have also been used to describe the effects of longer term climate change and seasonal cycles on the ecology of the water body, including variations in water depth, alkalinity, and vegetation of the surrounding land mass (e.g. Talbot & Johannessen, 1992; Yoshii *et al.*, 1997; Wolfe *et al.*, 1999).

NITROGEN AS A PALEO-N:P PROXY

While sedimentary $\delta^{15}N$ profiles can be used to identify past water column oxygen concentrations, there is no way to evaluate past N:P ratios, which serve as indicators of nitrogen cycling, limiting nutrients, and the trophic state of a water body (Guildford & Hecky, 2000). While we can generally predict the biological response in the present-day system to perturbations in the nutrient input and N:P ratios in water bodies, past nitrogen and phosphorus cycles may have been significantly different and resulted in unexpected changes in the N:P ratios in the deep sea. For example, we know that the water column was primarily anoxic in the Archean and the Early Proterozoic, and that shorter perturbations in the water column ecosystem may have occurred during mass extinction events or OAEs. Predictions of the past N:P ratios would depend in part on the Redfield stoichiometry of the organisms that populate the past oceans, which were higher for N:P and C:P c. ~1000 My than present-day Redfield ratios (Quigg *et al.*, 2003). These enriched elemental ratios would then have altered the stoichiometric ratios of the deep water by increasing the N:P ratio (Lenton & Klausmeier, 2007). This increase in N:P ratio due to differences in phytoplankton species is then countered by the predicted decrease in N:P due to the lower intensity of phosphorus flux from weathering prior to the development of terrestrial biota (Lenton & Watson, 2000, 2004). The degree to which these two effects balanced each other out, and the conditions under which one may have predominated over the other, are not understood. Unfortunately, reliable proxies for NO_3^-, PO_4^-, and deep N:P ratios have not been available for older sediment samples, thus limiting the evaluation of the effect of weathering versus phytoplankton in controlling paleo-N:P deep-water ratios. N:P ratios during OAEs were modeled to determine if this ratio changed during the periods of extensive sustained anoxia; while the consensus is that the N:P ratio will have decreased due to the increased loss of N, the degree of this increase ranges from drastic to barely significant (Handoh & Lenton, 2003; Wallman, 2003; Kuypers *et al.*, 2004). Using a sedimentary nitrogen proxy may help to constrain the projected N:P ratios, and thus determine the paleonutrient cycling during this time.

One way to evaluate past N:P ratios would be to find a correlation between $\delta^{15}N$ and deep-water N:P. The reactions that controlled the N:P ratios of the oceans in the past also shaped the nitrogen isotopic composition of the organic matter deposited in the sediments via fractionation. The enzymatic and cellular mechanisms involved in nitrogen fixation evolved early in Earth's history, and have not changed significantly on a genetic level (Falkowski, 1997). It is likely that the isotopic fractionation inherent in the nitrogen fixation pathway would remain similar in intensity ($\delta^{15}N$ ~–2‰ to +2‰). Similarly, we would also expect the isotopic fractionation for denitrification to remain the same through time, as even with multiple pathways the $\delta^{15}N$ of the residual nitrate averages +25‰. In a pre-anthropogenic system, both sedimentary $\delta^{15}N$ and N:P ratios should reflect the same set of biological pathways, mainly controlled by nitrogen's ability to act as a redox proxy. Loss of N via denitrification/anammox under extensive suboxic conditions would appear as an increase in the $\delta^{15}N$ of particulate organic matter and a decrease in the deep sea N:P ratio. More oxic environments would be more likely to have higher N:P ratios, particularly for watersheds affected by anthropogenic nitrogen input.

The linear equation relating N:P ratio and oxygen concentrations can be a first step to correlate sedimentary $\delta^{15}N$ values to N:P ratios, similar to the relationship between $\delta^{15}N$ and oxygen shown in Fig. 4. Since the relationship between N:P and oxygen concentration is linear for oxygen concentrations <100 μmol L^{-1}, the oxygen axis in Fig. 4 can be replaced by an axis representing N:P values. The relationship between $\delta^{15}N$ and N:P should be linear up to a N:P of approximately 13 to 14, after which $\delta^{15}N$ and N:P would become decoupled. Thus we can expect that extremely low N:P ratios will have $\delta^{15}N$ values close to zero, and that as the isotopic values rise, so will N:P ratios. The maxima in $\delta^{15}N$ reflects a situation where denitrification predominates in the water column; the N:P ratio associated with this isotopic maxima is probably 13 to 14. Under oxygen-replete conditions, N:P ratios and $\delta^{15}N$ values become uncoupled, and N:P values can rise while the isotopic fractionation remains relatively constant. If this hypothetical model for the relationship between $\delta^{15}N$ and N:P is confirmed, profiles of sedimentary $\delta^{15}N$ could then be used to determine trends in past N:P ratios as well as paleoredox state. Knowing how N:P changes at one location through time could provide additional information to interpret past changes in nutrient cycles and productivity. The relationship between $\delta^{15}N$, N:P, and O_2 can also help refine the theoretical model of how $\delta^{15}N$ records changes in redox state (Fig. 4). Based on the predicted linear relationship between N:P and $\delta^{15}N$, the maximum $\delta^{15}N$ values should occur at oxygen concentrations of 100 μmol L^{-1}, though modeled scenarios suggest maximum $\delta^{15}N$ at lower O_2 values (Fennel *et al.*, 2005). This information would be particularly applicable for determining redox state and nutrient cycling during periods when the oceans underwent significant changes in redox state, such as mass extinctions, OAEs, and the great oxidation event of the Proterozoic.

While the correlation between oxygen concentration and N:P (and thus $\delta^{15}N$ and N:P) has yet to be absolutely proven, the theoretical basis for the relationship appears to be sound. The effects of anthropogenic nitrogen loading on the relationship between N:P ratios and $\delta^{15}N$ values in the present day, particularly for lakes, has yet to be fully determined. Hopefully, additional data will develop a more concrete correlation, and the use of sedimentary $\delta^{15}N$ can be utilized as a paleo-N:P proxy.

CONCLUSIONS

In this paper, we explain the concept of nitrogen isotopes as a paleoreodox proxy, based on the known isotopic fractionations and the dependence of biologically mediated nitrogen reactions upon the oxygen concentrations in the water column. We have further analyzed the relationship between nitrogen and oxygen both in the present-day and in past environments, and outlined the possibilities for future studies. In addition, we suggest that sedimentary nitrogen isotopic values could also serve as a proxy for N:P ratios in past environments, as the reactions that dictate the $\delta^{15}N$ values should also affect the relative balance of nitrogen added to or removed from an ecosystem. Our compilation of N:P values and oxygen concentrations from a variety of environments indicates a linear relationship between N:P and O_2 levels, particularly for suboxic/anoxic systems. While this relationship remains to be definitively proven, the known relationships between nitrogen cycling and oxygen concentration imply that additional data will only confirm this correlation. The connection between oxygen concentration and both $\delta^{15}N$ and N:P indicates a correlation between sedimentary $\delta^{15}N$ and paleo-N:P values, allowing further characterization of past ecosystems and the role of both nutrient nitrogen and phosphorus in the early ocean.

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