8. Richter, P. H. & Ross, J. Concentration oscillations and efficiency: Glycolysis. *Science* **211,** 715–717 (1981).
9. Harris, D. C. *Quantitative Chemical Analysis* 5th edn 312–313 (Freeman, New York, 1999).
10. Edblom, E. C., Luo, Y., Orbán, M., Kustin, K. & Epstein, I. R. Kinetics and mechanism of the oscillatory bromate–sulfite–ferrocyanide reaction. *J. Phys. Chem.* **93,** 2722–2727 (1989).
11. Rábai, G., Orbán, M. & Epstein, I. R. Design of pH-regulated oscillators. *Acc. Chem. Res.* **23,** 258–263 (1990).
12. Berridge, M. J. Calcium oscillators. *J. Biol. Chem.* **265,** 9583–9586 (1990).
13. Szucs, A. *et al.* Interacting biological and electronic neurons generate realistic oscillatory rhythms. *Neuroreport* **11,** 563–569 (2000).

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Efficient export of carbon to the deep ocean through dissolved organic matter

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Oceanic dissolved organic carbon (DOC) constitutes one of the largest pools of reduced carbon in the biosphere. Estimated DOC export from the surface ocean represents 20% of total organic carbon flux to the deep ocean[1–3], which constitutes a primary control on atmospheric carbon dioxide levels[4]. DOC is the carbon component of dissolved organic matter (DOM) and an accurate quantification of DOM pools, fluxes and their controls is therefore critical to understanding oceanic carbon cycling. DOC export is directly coupled with dissolved organic nitrogen and phosphorus export. However, the C:N:P stoichiometry (by atoms) of DOM dynamics is poorly understood. Here we study the stoichiometry of the DOM pool and of DOM decomposition in continental shelf, continental slope and central ocean gyre environments. We find that DOM is remineralized and produced with a C:N:P stoichiometry of 199:20:1 that is substantially lower than for bulk pools (typically >775:54:1), but greater than for particulate organic matter (106:16:1—the Redfield ratio). Thus for a given mass of new N and P introduced into surface water, more DOC can be exported than would occur at the Redfield ratio. This may contribute to the excess respiration estimated to occur in the interior ocean[5]. Our results place an explicit constraint on global carbon export and elemental balance via advective pathways.

Accurate quantification of DOM pools, fluxes and their controls is critical to understanding oceanic carbon cycling and how the oceans will respond to increasing concentrations of atmospheric CO_2 and climate change[4,6,7]. The nitrogen and phosphorus cycles in the ocean are closely coupled with the carbon cycle and the Redfield ratio has been a unifying paradigm describing the stoichiometry of this coupling. It is an implicit assumption that the amount of carbon that can be exported to depth is directly related to the quantity of 'new' nutrients (NO_3^- and PO_4^{3-}) that are brought back to the surface through physical processes and added via nitrogen fixation and atmospheric deposition[8]. The production, export and

remineralization of particulate organic matter, which accounts for 80% of total organic carbon flux to the deep ocean, operate with Redfield stoichiometry. An extreme paucity of data on simultaneous measures of DOC, dissolved organic nitrogen and phosphorus (DON and DOP) and lack of information on production, decomposition and export processes have held back progress in understanding the coupled dynamics of these pools. Recent studies of DOM dynamics show large departures from Redfield trajectories. DOM pools in surface and deep-ocean waters deviate widely from the Redfield ratio with reports of C:N:P ratios in excess of 4,000:300:1 (refs 9–12). Production of new DOM is often N-poor relative to C, with elevated C:N ratios measured during and immediately after diatom blooms[13]. A number of studies have shown that N and P preferentially remineralize relative to C (refs 9–11 and 14–16). But how can overall organic matter export conform to the Redfield ratio while DOM stoichiometry apparently deviates so greatly? Is our understanding of the magnitude of C export relative to N and P correct? If estimates of DOC export are correct and the stoichiometry of DOM export deviates substantially from the Redfield ratio, we urgently need to understand the mechanisms that control the stoichiometry of DOM production, export and remineralization so that predictions of the response to climate and CO_2 changes can be made.

We studied DOM pool and decomposition stoichiometry in continental-shelf, continental-slope and central-ocean-gyre environments. The observed C:N:P ratios for bulk DOM deviated substantially from the Redfield ratio. The average C:N:P ratio for all samples across all depths was 778:54:1. In general, C:N:P ratios were lower when DOM concentrations were higher and were higher with low DOM concentrations (Table 1). The average C:N, C:P and N:P ratios for surface waters with increased DOM concentrations were 14:1, 374:1 and 27:1, respectively, and these ratios differed only moderately between locations and time (Table 1). The average C:N, C:P and N:P ratios for deep waters, which have low DOM concentrations, were substantially higher, averaging 22:1, 3511:1 and 202:1, respectively.

The stoichiometry of production and decomposition is determined from the slope of DOM element–element plots for samples collected throughout the ocean, for example, DON versus DOC (Fig. 1). It is implicit in this type of analysis that the stoichiometry of production and decomposition is the same. The stoichiometry of the decomposable DOM pool averaged 199:20:1. Thus C:N and C:P ratios were significantly C-rich: 62% and 87% higher than the Redfield ratio. The N:P ratio was higher than the Redfield, but not significantly (20 versus 16:1). These results show that the C:N:P stoichiometry of DOM decomposition is much lower than the C:N:P ratios of surface or deep-water pools. Although the bulk DOM is extremely rich in C relative to N and N relative to P, the space-for-time patterns of DOM decomposition indicate that the stoichiometry of the degradable DOM pool is also C-rich but at a level intermediate to that of Redfield and bulk DOM.

The decomposition stoichiometry was also determined independently by directly observing changes in DOM composition during laboratory incubations of shelf and slope waters. The incubations revealed (Fig. 2) that the decomposable fraction of the bulk DOM varied for DOC, DON and DOP, but loss rates were similar[11]. The stoichiometry of the fraction that decomposed was substantially lower than that of the initial bulk pool and averaged 221:20:1. Laboratory decomposition studies agreed favourably with the field space-for-time approach, suggesting that the patterns found from field observations are robust.

Although there are numerous reports of greatly increased C:N ratios of DOM production, especially after phytoplankton blooms (see, for example, ref. 13), our results indicate that, on average, the stoichiometry is only moderately increased (10.7:1). The C:N:P of upwelled, deep-water DOM (3511:202:1) is brought closer to the Redfield ratio (C:N:P drops) in surface waters as new DOM is

biologically produced (at a C:N:P of 199:20:1) and added to the deep-water pool, which has an average age of ~4,000–6,000 yr (refs 17, 18). The resulting surface-water DOM is then decomposed at a similar stoichiometry, so pool stoichiometry again approaches that of deep water.

Our measures of N:P stoichiometry are similar to those of Jackson and Williams[19]. Williams et al.[9] suggested that elemental ratios of DOM increased with depth because of preferential remineralization of N or P in sediment pore waters, and diffusion of the resulting C-rich DOM into the overlying water. In contrast, our results suggest that it is the production and decomposition of a labile pool that differs from bulk stoichiometry that causes C:N:P ratios to change over depth and over time.

The global nature of DOM can be illustrated with a conventional two-pool model with refractory and labile DOM pools, which differ in C:N:P composition and in their role in nutrient regeneration and carbon export (Fig. 3). In reality DOM should be considered as a continuum of pools with decreasing lability on timescales ranging from seconds to thousands of years. As a simplification, we lump together all DOC of concentration greater than that in the deep ocean as 'labile' and the rest as 'refractory'.

Refractory DOM consists of old DOM (1–10 kyr, 4-kyr average age) that is carbon-rich and nutrient-poor. Most of the refractory DOM completes the mixing loop between surface and deep water several times[20], regenerating little inorganic nutrient. Labile DOM consists of young DOM (0–1 kyr) that has intermediate stoichiometry, C-rich relative to the Redfield ratio. Labile DOM does not complete a mixing cycle and regenerates essentially all the nutrients required to sustain its export. Labile DOM is replenished by autotrophic and heterotrophic activities in surface waters. Replenishment of refractory DOM is less clear and probably includes inputs from continental runoff as well as some abiotic and biotic conversion of labile DOM[21]. Global changes that might promote labile DOM export (such as increased temperature and ocean stratification) have the potential to increase the ability of the ocean to sequester CO_2 from the atmosphere. Changes that might promote the decomposition of refractory DOM (such as increased ultraviolet radiation and temperature) are likely to decrease CO_2 sequestration because of the extreme imbalance between the stoichiometry of refractory DOM decomposition and labile DOM production (3511:202:1 versus 199:20:1).

There are strong parallels, but also contrasts, between oceanic and
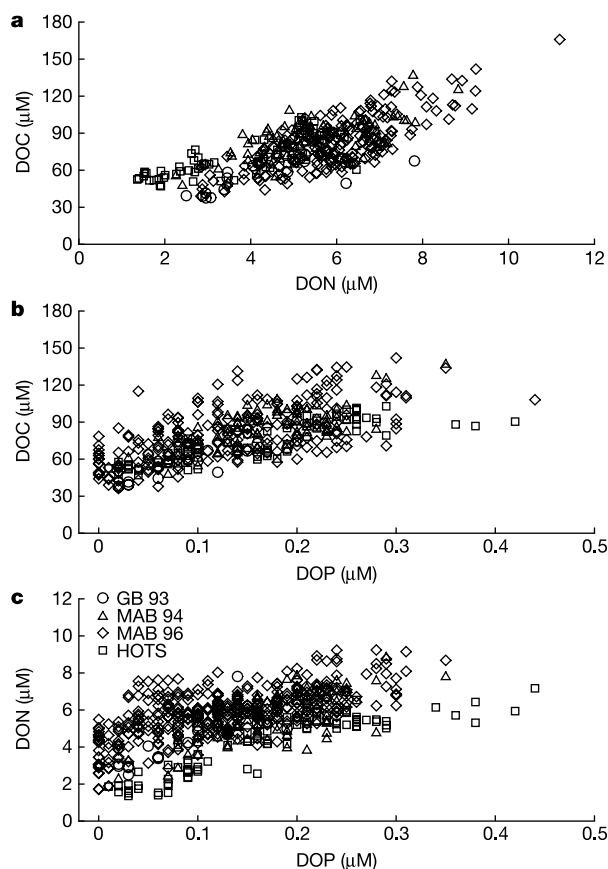
Figure 1 DOC, DON and DOP stoichiometry from field studies. Concentrations of DOC, DON and DOP from different oceanic regions and times are plotted relative to each other. C:N, C:P and N:P ratios for surface and deep-water pools and the decomposition stoichiometry were determined by regression analysis of these element–element plots. DOC concentration at time t, $C(t)$, can be described by $C(t) = C(t_0) + \gamma_{CN}(N(t) - N(t_0))$, where γ_{CN} is the C:N ratio of DOM production or consumption (DOM change). Consequently, plotting $C(t)$ versus $N(t)$ has a slope of γ_{CN} and y-intercept of $C(t_0) - \gamma_{CN}N(t_0)$. The slope describes the stoichiometry of both DOM remineralization and production. Data from: GB, Georges Bank; MAB94, Middle Atlantic Bight 4/1994; MAB96, Middle Atlantic Bight 8/1996; and HOTS, Hawaiian Ocean Time Series. All data are available in the Supplementary Information.
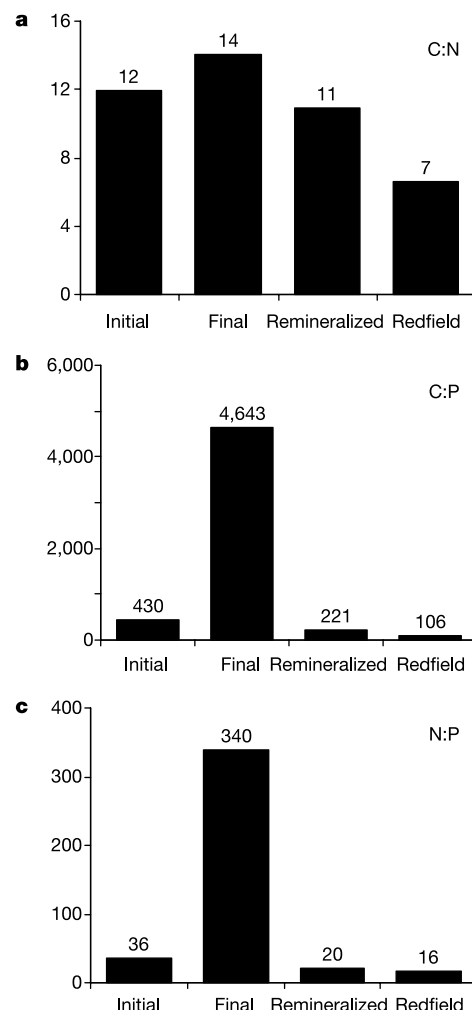
Figure 2 Decomposition stoichiometry determined from laboratory incubations. Average stoichiometry of DOM pools at the outset and conclusion of 0.5-yr-long incubations and of the DOM that was remineralized during this interval. The Redfield ratio is shown for comparison. The standard deviation of remineralization was C:N = 2.7, C:P = 52 and N:P = 3.8.

Table 1 **DOM stoichiometry**

|	Sample location					
	Georges Bank 1993	Middle Atlantic Bight 4/1994	Middle Atlantic Bight 8/1996	Hawaiian Ocean Time Series	Mean	Standard deviation
C:N surface	12 (0.8)	14 (0.6)	14 (0.4)	15 (0.5)	14	1
C:N deep	15 (2.1)	21 (2.5)	16 (1.7)	34 (2.2)	22	7
C:P surface	444 (17)	374 (11)	398 (6)	281 (5)	374	59
C:P deep	2,035 (162)	2,370 (196)	4,869 (220)	4,768 (196)	3,511	1,314
N:P surface	35 (1.4)	26 (0.9)	27 (0.3)	19 (0.4)	27	6
N:P deep	139 (14)	110 (16)	394 (9)	163 (16)	202	113
Labile C:N	9.8 (1.4)	10.0 (0.9)	14.1 (0.6)	8.7 (0.6)	10.7	2.4
Labile C:P	245 (29)	193 (17)	203 (7)	154 (10)	199	37
Labile N:P	25 (2.5)	20.2 (1.1)	15.6 (0.4)	17.8 (0.5)	20	3

C:N:P stoichiometry of bulk DOM pools in surface (mixed layer) and deep-ocean waters and of the labile DOM pools for shelf, slope and open-ocean regions. The stoichiometry of the labile DOM is based on linear regressions of bulk pool data in Fig. 1 (see Methods). Numbers in parentheses are standard deviations.

terrestrial organic matter dynamics. Soil humics are the functional equivalent of refractory DOM, a large pool turning over on timescales greater than 1 kyr. But unlike refractory DOM, soil humics are relatively nutrient-rich and carbon-poor (C:N = 12–20). Remineralization of inorganic nitrogen from soil organic matter can promote a net CO_2 sequestration if incorporated into woody biomass (C:N = 300:1) of regrowing forests[22]. Decomposition of the oceanic refractory pool would have a much greater effect on atmospheric CO_2 concentrations than would soil humics, because the nutrients regenerated from the oceanic refractory pool would not be able to support significant new organic matter production as a result of insufficient N and P.

Our results show the elemental composition of oceanic refractory and labile DOM pools. The refractory pool is especially C-rich relative to N and P, and N-rich relative to P. More importantly, we find that the labile pool is also C-rich; the C:N and C:P ratios of the labile material are higher than the Redfield ratio. This pattern was observed in both field and decomposition analyses. Deviations from the Redfield ratio alter our perceptions of the coupling between the carbon, nitrogen and phosphorus cycles in the ocean. The difference between DOM and Redfield stoichiometry implies that DOM export is C-rich. Thus for a given mass of new N or P introduced into surface water, 62% or 88% more DOC can be exported than would occur at the Redfield ratio. Relative to particle export, which conforms to the Redfield ratio, we can consider DOM export to be more 'efficient'. Export of carbon-rich DOC may contribute to the excess respiration estimated to occur in the interior ocean[5].

This new understanding of oceanic DOM stoichiometry has several important implications. The majority of oceanic DOM is largely unidentified, because of analytical limitations. The stoichiometry we describe for labile and refractory DOM pools might be useful in constraining the biochemical composition of the unidentified DOM in testable ways. We now know the
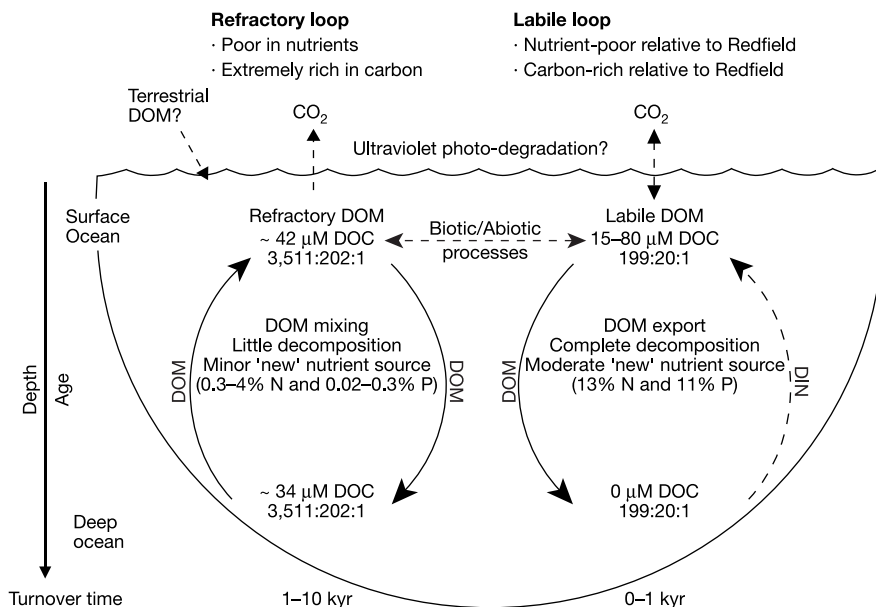
Figure 3 A simplified conceptualization of oceanic DOM pools using a conventional two-pool model. Labile and refractory oceanic DOM pools collectively comprise the bulk pool, upon which prior knowledge is based. Labile DOM produced in the surface ocean that is not decomposed in the surface ocean is exported to the deep ocean, where it resides for up to a thousand years while it is slowly remineralized to its inorganic components. Our study shows that labile DOM is produced and decomposed with a C:N:P stoichiometry of 199:20:1. This pool does not complete an ocean-mixing cycle and has an age of <1 kyr. The refractory pool has an average age of 4 kyr and has a C:N:P stoichiometry very different from labile DOM (3511:202:1). Only a small portion of the refractory DOM is remineralized on a single mixing cycle, thus generating little 'new' inorganic C, N or P. The relative contribution of DOM to the remineralization of inorganic N and P in deep water is based on the assumption that the DOC represents 20% of total C export.

stoichiometry of DOM and particulate organic matter export. This new knowledge of DOM now allows an explicit constraint and estimate to be made of global carbon export and elemental balance via advective pathways. Our new understanding also raises additional questions regarding the effect of global climate change on the relative importance of DOM and particulate organic matter export[12,23] and the stoichiometry of DOM production, as well as the vulnerability of the C-rich, refractory pool of DOM to degradation[21]. □

Methods

Study area

We studied DOM in a variety of environments, including continental shelf, continental slope and central ocean gyre. Shelf, slope-break and slope samples were collected over depth from along the northeastern USA, including Georges Bank and the Middle Atlantic Bight[10,11]. Additional data (October, November and December 1999) are from the Joint Global Ocean Flux Study (JGOFS) Pacific time-series station ALOHA, which is located in the subtropical gyre at 22° 45′ N, 150° W.

Approach

Patterns in the stoichiometry of DOM production and decomposition were determined from element–element plots of samples. Best-fit linear equations were determined for each data set (DOC versus DON, DOC versus DOP and DON versus DOP) from each cruise using a Type II regression[24], which accounts for errors in both coordinates. Analysis of residuals demonstrated the validity of a linear model. Slopes of the best-fit linear equations relating DOM concentrations (DOC versus DON, DOC versus DOP and DON versus DOP) describe the stoichiometry of DOM change that is due to production or decomposition. From these equations, we also calculated C:N:P ratios for surface waters where DOM concentrations are increased and for deep water where DOM concentrations are low. Radiocarbon dating of oceanic DOC indicates that the age of bulk DOC increases with depth[18,25], and so sampling over depth is a proxy for sampling over time (that is, space-for-time), which requires sampling globally representative regions for deep and surface water. With this approach it is necessary to sample enough surface and deep waters to get a statistically valid representation of the 'population' (all the DOC, DON and DOP molecules in the surface and deep ocean) of CNP elemental ratios of surface and deep waters. To characterize the 'population' we cast a wide sampling net that included a great number and diversity of deep and surface waters, so that statistically, water-mass variability effects could be minimized. With a large data set covering different water masses and depths, the slope of the combined data will approach that of the true slope. Therefore we sampled a wide variety of surface ocean environments, including coastal areas, continental shelf, western boundary currents and central ocean gyre. Deep samples included many water masses including North Atlantic Deep Water, Antarctic Intermediate and Bottom Water, Labrador Current and North Pacific Intermediate Water. The results are presented as a range of values for each region, and an average across regions proposed to be globally representative of labile DOM stoichiometry.

The 'global statistical' approach we used integrates over broad spatial and temporal scales. A limitation of this approach is that it cannot be applied to specific trajectories at small temporal or spatial scales (for example, seasonal cycles or specific sites). We acknowledge that there are deviations in DOM stoichiometry during events, such as phytoplankton bloom crashes, that cannot be isolated with the global statistical approach. Sampling along isopycnals (regions of constant density) would be a more appropriate approach for discerning the smaller-scale trajectories.

Direct measures of decomposition stoichiometry were made from 180-day laboratory incubations[11]. Incubations serve as a check on the space-for-time approach. 180 days approximates the residence time of shelf water and central-gyre surface water. Nitex screened (108-μm) water was incubated in 50-ml flame-sealed glass ampoules. Triplicate samples were frozen for later analysis after 0, 3, 12, 31, 90 and 180 days. Decomposition samples were collected from 11 stations along the width and length of the MAB on two occasions—March and August 1996. Changes in concentration of DOC, DON and DOP over time were used to estimate DOM decomposition rate and C:N:P stoichiometry[11].

For the continental shelf, slope break and slope DOM and nutrient analysis, we collected water at depths ranging from the surface to 1,500 m in depth on the RV *Oceanus* in April 1993 (GB93), RV *Columbus Iselin* in April 1994 (MAB94), RV *Endeavor* in March 1996 and the RV *Seward Johnson* in August 1996 (MAB96). Samples were vacuum-filtered using precombusted 47-mm Whatman GF/F glass fibre filters. For DOC analysis, samples were acidified, sealed and refrigerated at 4 °C (samples up to and including 1994) or frozen without acidification (1996) until analysis. In 1994, samples for DOC analysis were also analysed onboard ship within a short time of collection. Comparisons between filtered and unfiltered and between stored and unstored samples demonstrated that filtration and storage had no effect on concentration. For inorganic nutrient and total dissolved N and P analysis, filtered water was poured directly into acid-washed and deionized-water-rinsed 500-ml polycarbonate or polypropylene bottles, and stored frozen until analysis.

Similar protocols were used by the JGOFS programme at station Aloha.

Chemical analyses

DOM concentrations were determined following the procedure described in ref. 11. Basically, DOC was analysed by high-temperature oxidation with a Pt catalyst[26]. DON and DOP were determined as the difference between total dissolved N and P and inorganic N and P (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}). For DON and DOP, we followed the ultraviolet oxidation procedure of ref. 27, with colorimetric analysis of NH_4^+, and PO_4^{3-}. A chemiluminescence nitrogen oxide analyser was used to measure NO_3^- following the conversion to NO gas[28]. DOC, DON and DOP were analysed similarly for HOTS samples (high temperature[29] and ultraviolet oxidation[30]).

1. Six, K. & Maier-Reimer, E. Effect of plankton dynamics on the seasonal carbon fluxes in a ocean general circulation model. *Glob. Biogeochem. Cycles* **10**, 559–583 (1996).
2. Carlson, C., Ducklow, H. & Michaels, T. Annual flux of dissolved organic carbon from the euphotic zone of the northwest Sargasso Sea. *Nature* **371**, 405–408 (1994).
3. Hansell, D. A. in *Biogeochemistry of Marine Dissolved Organic Matter* (eds Hansell, D. A. & Carlson, C.) 685–716 (Academic, New York, 2002).
4. Sarmiento, J. & Siegenthaler, U. in *Primary Productivity and Biogeochemical Cycles in the Sea* (eds Falkowski, P. & Woodhead, A.) 317–332 (Plenum, New York, 1992).
5. del Giorgio, P. & Duarte, C. Respiration in the open ocean. *Nature* **420**, 379–384 (2002).
6. le, B. & Williams, P. J. Evidence for the seasonal accumulation of carbon-rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. *Mar. Chem.* **51**, 17–29 (1995).
7. Emerson, S. *et al.* Experimental determination of the organic carbon flux from open-ocean surface waters. *Nature* **389**, 951–954 (1997).
8. Eppley, R. & Peterson, B. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* **282**, 677–680 (1979).
9. Williams, P. M., Carlucci, A. & Olson, R. A deep profile of some biologically important properties in the central North Pacific gyre. *Oceanol. Acta, 1980*, 471–476 (1980).
10. Hopkinson, C. S., Fry, B. & Nolin, A. Stoichiometry of dissolved organic matter dynamics on the continental shelf of the Northeastern U.S.A. *Contin. Shelf Res.* **17**, 473–489 (1997).
11. Hopkinson, C., Vallino, J. & Nolin, A. Decomposition of dissolved organic matter from the continental margin. *Deep-Sea Res. II* **49**, 4461–4478 (2002).
12. Church, M., Ducklow, H. & Karl, D. Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical gyre. *Limnol. Oceanogr.* **47**, 1–10 (2002).
13. Søndergaard, M. *et al.* Net accumulation and flux of dissolved organic carbon and dissolved organic nitrogen in marine plankton communities. *Limnol. Oceanogr.* **45**, 1097–1111 (2000).
14. Martin, J., Knauer, G., Karl, D. & Broenkow, W. VERTEX: carbon cycling in the northeast Pacific. *Deep-Sea Res.* **34**, 267–285 (1987).
15. Clark, L., Ingall, E. & Benner, R. Marine phosphorus is selectively remineralized. *Nature* **393**, 426 (1998).
16. Loh, A. N. & Bauer, J. Distribution, partitioning and fluxes of dissolved and particulate organic C, N, and P in the eastern North Pacific and Southern Oceans. *Deep-Sea Res.* **47**, 2287–2316 (2000).
17. Williams, P. M. & Druffel, E. R. M. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature* **330**, 246–248 (1987).
18. Bauer, J., Williams, P. & Druffel, E. ^{14}C activity of dissolved organic carbon fractions in the central North Pacific and Sargasso Sea. *Nature* **357**, 667–670 (1992).
19. Jackson, G. & Williams, P. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. *Deep-Sea Res.* **32**, 223–235 (1985).
20. Carlson, C. in *Biogeochemistry of Marine Dissolved Organic Matter* (eds Hansell, D. A. & Carlson, C.) 91–151 (Academic, New York, 2002).
21. Mopper, K. *et al.* Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. *Nature* **353**, 60–62 (1991).
22. Melillo, J. M. *et al.* Soil warming and carbon-cycle feedbacks to the climate system. *Science* **298**, 2173–2176 (2002).
23. Karl, D. M. A sea of change: biogeochemical variability in the North Pacific Subtropical Gyre. *Ecosystems* **2**, 181–214 (1999).
24. Press, W. H. & Teukolsky, S. Fitting straight line data with errors in both coordinates. *Comput. Phys.* **6**, 274–276 (1992).
25. Bauer, J., Druffel, E., Williams, P., Wolgast, D. & Griffin, S. Temporal variability in dissolved organic carbon and radiocarbon in the eastern North Pacific Ocean. *J. Geophys. Res.* **103**, 2867–2881 (1998).
26. Peltzer, E. T. & Hayward, N. A. Spatial and temporal variability of total organic carbon along 140 degree W in the Equatorial Pacific Ocean in 1992. *Deep-Sea Res.* **43**, 1155–1180 (1996).
27. Walsh, T. Total dissolved nitrogen in seawater: a new high-temperature combustion method and a comparison with photo-oxidation. *Mar. Chem.* **26**, 151–159 (1989).
28. Garside, C. A chemiluminescence technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater. *Mar. Chem.* **11**, 159–167 (1982).
29. Qian, J. & Mopper, K. Automated high-performance, high-temperature combustion total organic carbon analyzer. *Anal. Chem.* **68**, 3090–3097 (1996).
30. Tupas, L., *et al.* *Hawaii Ocean Time-series Program Data Report 8: 1996* (SOEST Tech. Report 97–7, School of Ocean and Earth Science and Technology, Univ. of Hawaii, Honolulu, 1997).