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Contribution to the Theme Section 'Implications of large-scale iron fertilization of the oceans'

Nitrogen fixation-enhanced carbon sequestration in low nitrate, low chlorophyll seascapes

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ABSTRACT: The magnitude of fluxes in the carbon cycle of subtropical and tropical marine habitats is determined by the supply of inorganic nutrients. These habitats have low sea-surface concentrations of nitrate (NO_3^-) and chlorophyll (dubbed LNLC regions), sustain relatively low rates of organic matter production and export, and represent global ocean minima in carbon sequestration potential. The low NO_3^- resupply should select for nitrogen (N_2)-fixing bacteria, termed diazotrophs, provided all other growth-limiting nutrients are available. Several recent field efforts have been aimed at enhancing N_2 fixation in LNLC regions through mesoscale fertilization with iron and phosphorus (or both) and we hypothesize herein that controlled upwelling of nutrient-enriched deep water may also be effective. Based on a quantitative assessment of the vertical distribution of NO_3^-, phosphate (PO_4^{3-}) and dissolved inorganic carbon (DIC) at Station ALOHA (22° 45′ N, 158° W), we hypothesize that the process of controlled upwelling of low NO_3^-:PO_4^{3-} seawater may lead to enhanced N_2 fixation, organic matter production and net carbon sequestration. Furthermore, based on a long-term (20 yr) data set from Station ALOHA, we predict that the upwelling of water from a depth of 300 to 350 m during summer months will trigger a 2-stage phytoplankton bloom. The first stage will be characterized by a NO_3^--supported Redfield ratio (e.g. C_{106}:N_{16}:P by atoms) diatom bloom. Following quantitative NO_3^- removal, the residual PO_4^{3-} from the low N:P (<16:1) upwelled nutrient pulse will stimulate a N_2-fixing bacterial bloom, leading to net sequestration of carbon. However, any strategic benefit of controlled upwelling for enhancing the long-term carbon sequestration will depend on the spatial and temporal uncoupling of organic matter production and remineralization, which is ultimately controlled by the microbial response to these perturbations.

KEY WORDS: N_2 fixation · Diazotroph · Nutrients · Carbon sequestration · North Pacific gyre

N_2 FIXATION IN OCEANIC HABITATS

Nearly 80 % of the surface waters of the global ocean are considered nitrate (NO_3^-)-depleted (≤1 µM NO_3^-). These regions, located between 30° N and 30° S in every ocean basin, are characterized by low NO_3^- delivery, low standing stocks of particulate matter (including living organisms) and low rates of new and export production (Longhurst 1998). A strong vertical stratification insulates the upper layers of these vast seascapes from the large pool of NO_3^- in deeper waters; e.g. in the North Pacific subtropical gyre (NPSG) a maximum concentration of approximately 42 µM NO_3^- is observed at 1000 m depth, whereas NO_3^- concentrations in the upper 100 m are generally <5 nM (Fig. 1). Chronic NO_3^- limitation in the upper layer of the water column, where light is available to support photosynthesis, should select for microorganisms that are able to utilize the fairly large, but as yet poorly characterized, pool of dissolved organic nitrogen (DON, ~5 µM) or the unlimited supply of dissolved N_2 (>400 µM) as a source of cellular N; the latter process is termed N_2 fixation and the microorganisms responsible for it are termed diazotrophs.

N_2-based new production requires an ample supply of energy, iron (Fe) and phosphorus (P). In marine ecosystems, the primary source of energy appears to be light; i.e. most marine diazotrophs are also phototrophs

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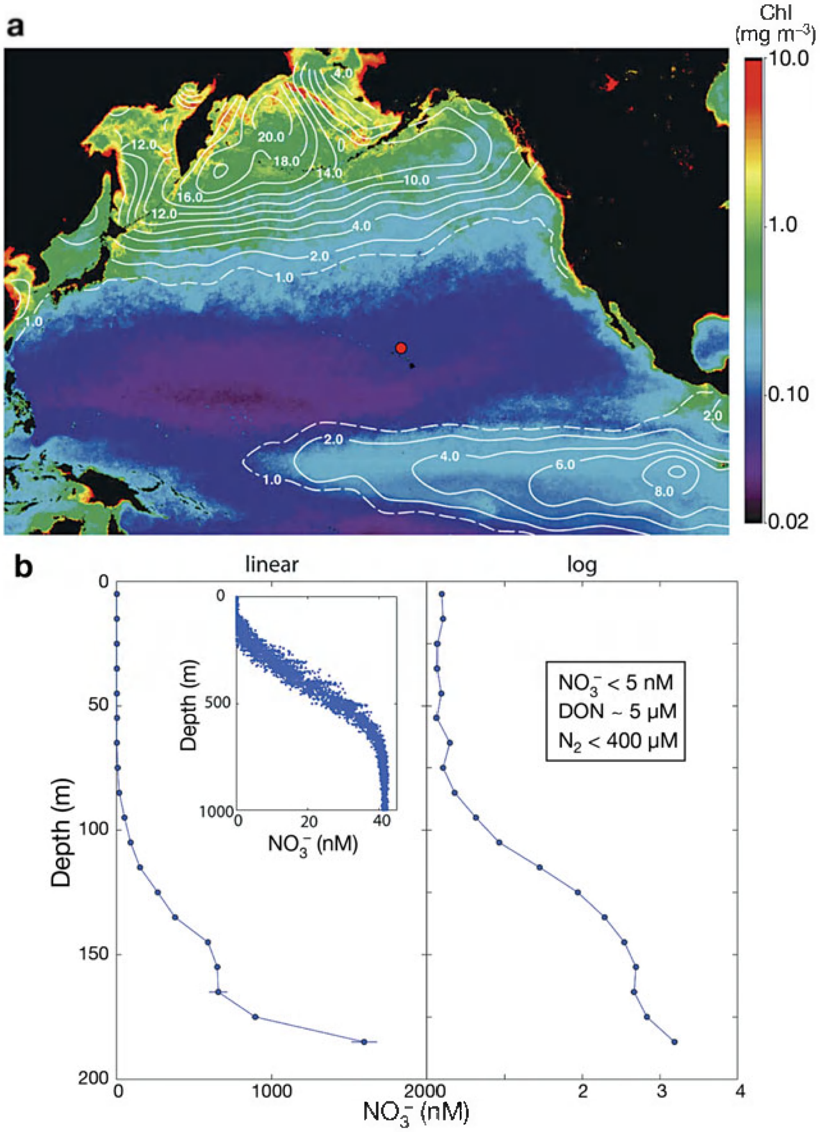

Fig. 1. (a) North Pacific Ocean with sea surface distributions of chlorophyll (mg m^{-3}) derived from the AQUA MODIS satellite-based sensor system (4 km resolution). Superimposed on ocean color, in white contour lines, is the mean annual surface nitrate concentration (mmol NO$_3^-$ mg m^{-3}) based on the World Ocean Atlas Ocean Climate Laboratory/NODC (http://www.nodc.noaa.gov/OC5/). Areas of high NO$_3^-$ (and presumably NO$_3^-$ flux) correspond to regions that are enriched in chlorophyll as a result of phytoplankton growth. Red circle indicates the approximate location of Station ALOHA 22° 45′ N, 158° W, a typical low-nitrate, low-chlorophyll (LNLC) habitat. (b) Mean concentrations of NO$_3^-$ versus water depth for samples collected at Station ALOHA during 1989–2006 plotted on both linear and logarithmic ($\log_{10}$) scales to show details of the profile. Inset (left-hand plot) shows the NO$_3^-$ concentration profile to 1000 m. Compared to the low (<5 nM) surface water concentrations of NO$_3$, dissolved organic nitrogen (DON) and dissolved N$_2$ are in ample supply but not readily available (see box in right-hand plot)

(Karl et al. 2002). Fe is an obligate co-factor for the enzyme nitrogenase and it has been estimated that diazotrophs may require an order of magnitude more Fe for growth than eukaryotic phytoplankton growing on ammonium as a source of nitrogen (Kustka et al. 2003). Although phosphate (PO_4^{3-}) is considered to be the preferred P substrate for the growth of marine microorganisms (including diazotrophs), selected P-containing organic compounds can also be used. In this study, we will use measured PO_4^{3-} concentrations as a lower limit on bioavailable P, acknowledging that the true concentrations may be at least twice as high (Björkman & Karl 2003). If solar energy, Fe and P all were present in excess, phototrophic diazotrophs would have a competitive growth advantage in low NO$_3^-$ habitats worldwide. As diazotrophs grow, their metabolism decouples the N and P cycles by providing new N in the absence of deep mixing and, over time, their net growth would remove all bioavailable P from the ecosystem as long as Fe and all other required nutrients are in sufficient supply. Any P remaining is termed residual P (r-P). Therefore, based on the constraints imposed by the C:P stoichiometry in the production of organic matter, the concentration of r-P in a given habitat can be used as a measure of the potential for CO_2 sequestration by N_2 fixation.

The pathways for Fe and P resupply to the surface ocean are governed by distinct environmental forcings (Karl 2002). Whereas both Fe and P are delivered from deep water to the surface by diffusion and turbulent mixing in the upper thermocline, Fe is also supplied from atmospheric deposition of dust. Furthermore, some dust sources (e.g. selected Saharan desert soils) contain both Fe and P (Ridame & Guieu 2002). Because the atmosphere-to-ocean Fe flux is approximately an order of magnitude larger in the northern hemisphere than in the southern hemisphere and is larger in the North Atlantic than in the North Pacific (Duce & Tindale 1991, Jickells et al. 2005), NO$_3^-$-stressed environments in the South Pacific gyre tend to have much higher r-P (>150 nM; Moutin et al. 2008) than either the NPSG (10–100 nM; Karl et al. 2001) or the North Atlantic subtropical gyre (<1 nM; Wu et al. 2000). Consequently, rates of N_2-based new and export production can vary geographically and may have regionally distinct environmental controls. Dissolved Fe concentrations in the NPSG exhibit 'nutrient-like' profiles with minimum concentrations (0.2 to 0.4 nM) in near-surface waters, increasing to values of 0.7 to 1.0 nM at approximately 1000 m (Johnson et al. 1997, Wu et al. 2001). In addition, and as a result of the atmospheric deposition of Fe-containing dust, near-surface waters have elevated — but much more time-variable — Fe concentrations relative to those found in the lower portion of the euphotic zone (Boyle et al. 2005). Nevertheless, quantifying bioavailability of Fe in marine ecosystems is difficult due, in part, to the presence of colloidal Fe-binding organic ligands (Wu et al. 2001) of unknown availability to microorganisms.

Superimposed on this complex network of Fe sources and sinks, r-P concentrations within the euphotic zone of the NPSG also display significant intra-seasonal as well as decadal-scale variability (Karl 2007a), emphasizing the temporal context of nutrient control of microbial dynamics. Ultimately, the concentration of r-P in low NO_3^- seascapes will depend on the extent of N_2-based new production, which, in turn, constrains carbon export and sequestration, and is controlled by both the availability of energy (e.g. solar radiation and upper water column stratification) and the resupply of Fe.

In theory, there are at least 2 experimental manipulations that could be employed to stimulate the growth of marine diazotrophs in well-stratified low-NO_3^-, low-chlorophyll (LNLC) subtropical pelagic habitats and promote the sequestration of CO_2: (1) fertilization of the surface ocean with Fe, PO_4^{3-} or both, depending upon the ambient concentrations and *in situ* fluxes of both elements relative to cell needs and (2) controlled upwelling of Fe-enriched deep waters that have a low NO_3^-:PO_4^{3-} molar ratio (<16:1) and, therefore, a relative excess of P. Successful application of either nutrient perturbation for the purpose of net CO_2 sequestration would be site- and depth-specific, and perhaps seasonally dependent. While the theory of N_2-based CO_2 sequestration appears sound, field application might not be as straightforward and the outcomes are difficult to predict given our current understanding of microbial biogeochemical processes and ecosystem function in LNLC habitats. For this reason, nutrient manipulations should be treated as hypotheses that are amenable to direct field experimentation and quantification.

OCEAN Fe/P FERTILIZATION

In well-illuminated and stratified NO_3^--depleted oceanic regions that contain r-P, the addition of Fe should enhance the growth of diazotrophs and promote N_2-based carbon export and sequestration. Target regions for these studies include Fe-depleted, denitrified water masses (e.g. eastern tropical North Pacific) or oceanic gyres far removed from continental dust sources (e.g. South Pacific subtropical gyre). Alternatively, the addition of PO_4^{3-} to Fe-containing, P-depleted waters also should stimulate N_2 fixation. Regions with these characteristics include oligotrophic waters downwind from continental dust sources (e.g. eastern Mediterranean Sea, North Atlantic subtropical gyre, South China Sea) or areas impacted by hydrothermal inputs of Fe from shallow underwater volcanoes. Furthermore, it is important to emphasize that PO_4^{3-} fertilization of Fe-sufficient regions may require much larger nutrient loads than Fe fertilization of P-sufficient habitats because of the relatively high P:Fe molar stoichiometry of living organisms (P:Fe $\geq 10^2$; Ho et al. 2003). The addition of PO_4^{3-} for the purpose of enhancing N_2-based carbon export and sequestration would, therefore, impose a significant logistical constraint and greater expense compared to Fe fertilization. However, even in seascapes that contain measurable r-P, the impact of Fe fertilization would be enhanced if additional PO_4^{3-} was added simultaneously due to the possibility of Fe and PO_4^{3-} co-limitation of N_2 fixation (Sañudo-Wilhelmy et al. 2001, Mills et al. 2004). Consequently, the autotrophic biomass response and concomitant CO_2 sequestration potential will ultimately scale on the total P, i.e. the sum of the r-P plus added PO_4^{3-} concentrations.

To date, there have been 2 open ocean field trials designed to assess the Fe/P fertilization effects on microbial assemblages and elemental fluxes: (1) PO_4^{3-} addition to an Fe-sufficient portion of the eastern Mediterranean Sea (CYCLOPS project; Thingstad et al. 2005) and (2) Fe and Fe/PO_4^{3-} additions to a region in the Northeast Atlantic Ocean (FeeP project; Rees et al. 2007).

Cycling of phosphorus in the Mediterranean (CYCLOPS) project

Following the addition of PO_4^{3-} to increase the r-P concentration by nearly 2 orders of magnitude (to 110 nM) in an approximately 16 km^2 patch within an ultraoligotrophic warm-core eddy, there was a 40% decrease (rather than a predicted 40-fold increase) of chlorophyll during the first 6 d, relative to an unfertilized control station (Thingstad et al. 2005). However, while parallel on-deck incubations amended with PO_4^{3-} did not display significant changes, those amended with PO_4^{3-} plus ammonium yielded a >9-fold increase in chlorophyll (Zohary et al. 2005), suggesting N and P co-limitation in selected non-diazotrophic phytoplankton taxa. Furthermore, *in situ* N_2 fixation was enhanced by only 48% following PO_4^{3-} addition and was unaffected by Fe addition (Rees et al. 2006), indicating that P, rather than Fe, was the primary limiting factor for N_2 fixation. These unexpected and still unexplained results from the CYCLOPS project require further elaboration and perhaps the development of a new trophic model (Thingstad et al. 2005). Additional considerations include the competition between phytoplankton and heterotrophic bacteria and the coupling to higher trophic levels, as well as the potential role of other essential trace metals and organic compounds (e.g. vitamins) in establishing the carrying capacity of the pelagic ecosystem.

FeeP project

During FeeP, 2 experimental patches, each approximately 25 km^2, were created and studied over a 3 wk period. The first patch contained only PO_4^{3-} while the second was seeded with Fe at first, followed by a pulse of PO_4^{3-} after 24 h. The experimental concentrations were ~200 nM PO_4^{3-} and 3 nM Fe:200 nM PO_4^{3-} in Patch 1 and Patch 2, respectively, compared to background concentrations of approximately 10 nM PO_4^{3-} and 0.3 nM Fe (Rees et al. 2007). Whereas total primary production showed only small deviations from the unamended control site, rates of PO_4^{3-} uptake and N_2 fixation in the 2 experimental treatments increased 6-fold and 4.5-fold, respectively (suggesting that only a small fraction of the photoautotrophic assemblage, probably composed of diazotrophs, responded initially to the nutrient enrichments); the dominant diazotroph in this habitat was a unicellular cyanobacterium (Rees et al. 2007). Particulate matter export to the deep sea was not measured during either the CYCLOPS or FeeP, so the potential impact on CO_2 sequestration from these fertilization experiments and the enhanced N_2 fixation they triggered is currently unknown.

CONTROLLED UPWELLING

Lovelock & Rapley (2007) recently suggested that the purposeful delivery of deep-water nutrients to the euphotic zone (i.e. artificial or controlled upwelling) might enhance primary and export production and thereby constitute an effective mechanism for CO_2 sequestration in the open sea. Upwelling of deep waters could be implemented using a passive, wave-powered pump (Isaacs et al. 1976) or via conventional mechanical pumping. Critics of this procedure for the purpose of CO_2 sequestration (e.g. Shepherd et al. 2007) were quick to point out that, along with the production-supporting nutrients (e.g. NO_3^-, PO_4^{3-}, Fe), deep waters also contain elevated concentrations of dissolved inorganic carbon (DIC, also referred to as total CO_2) derived from the long-term decomposition of sinking particulate matter (Fig. 2). Consequently, most natural upwelling regions are net sources of CO_2 to the atmosphere (Takahashi et al. 1997).

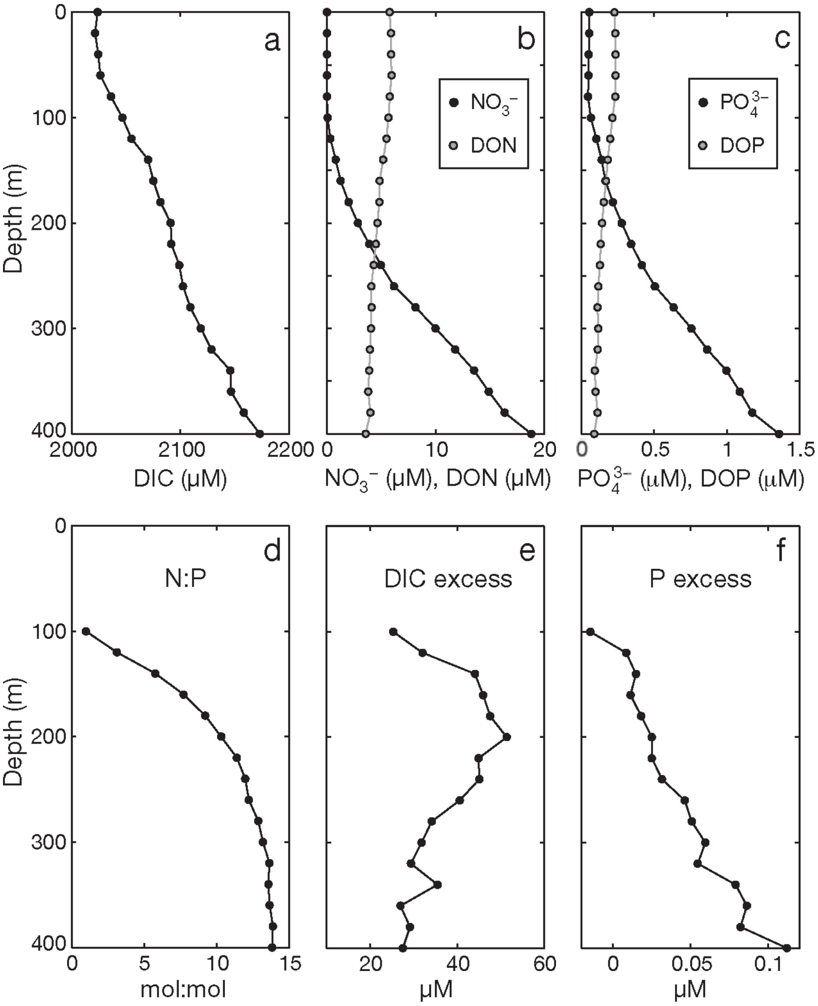

Fig. 2. Vertical profiles (a–c) of dissolved inorganic carbon (DIC) and dissolved nitrate and phosphate (NO_3^- and PO_4^{3-}, respectively) and dissolved organic nitrogen and phosphorus (DON and DOP, respectively) concentrations for the upper 400 m at Station ALOHA. (d–f) NO_3^-:PO_4^{3-} (N:P) molar ratios and excess DIC and excess P in the upper 400 m at Station ALOHA. Excess DIC and PO_4^{3-} were calculated as described in Table 1, and represent the DIC and P concentrations that would remain if the NO_3^- from each reference depth were assimilated into organic matter with a Redfield stoichiometry (C_{106}:N_{16}:P_1). All values shown are based on the long-term climatology (1989–2006) for this low-nitrate, low-chlorophyll (LNLC) station (data available at http://hahana.soest.hawaii.edu)

However, since the ratio of DIC:NO_3^-:PO_4^{3-} (C:N:P) in deep-sea waters is variable, the ecological consequences of a controlled upwelling experiment is predicted to be site- and depth-specific and, for some regions, seasonally dependent due to local variations in solar irradiance, surface mixed layer depth and temperature.

Based on our current understanding of marine microbial biogeochemistry, if deep waters are introduced to the surface ocean, a phytoplankton bloom (i.e. enhancement in the growth rate of one or more species leading to an increase in biomass; Richardson 1997) will result, following the stratification of the upper water column. It is well known that a sudden pulse of inorganic nutrients into the euphotic zone selects for rapidly growing, mostly eukaryotic phytoplankton (especially diatoms). Although the background microbial loop continues to be active, it is overprinted by larger phytoplankton species that can respond quickly to the introduction of allochthonous nutrients and have few predators, at least initially, allowing accumulation and subsequent sedimentation out of the euphotic zone (Karl 1999, Cullen et al. 2002). The nature of the upwelling nutrient supply, for example whether it is continuous or pulsed, can also influence the dynamical behavior and trophic structure of the resultant community (Wiegert & Penas-Lado 1995) and ultimately affect the C:N:P stoichiometry of the organic matter that is exported from the system. For any controlled upwelling experiment, the nutrient supply rate is a key design criterion.

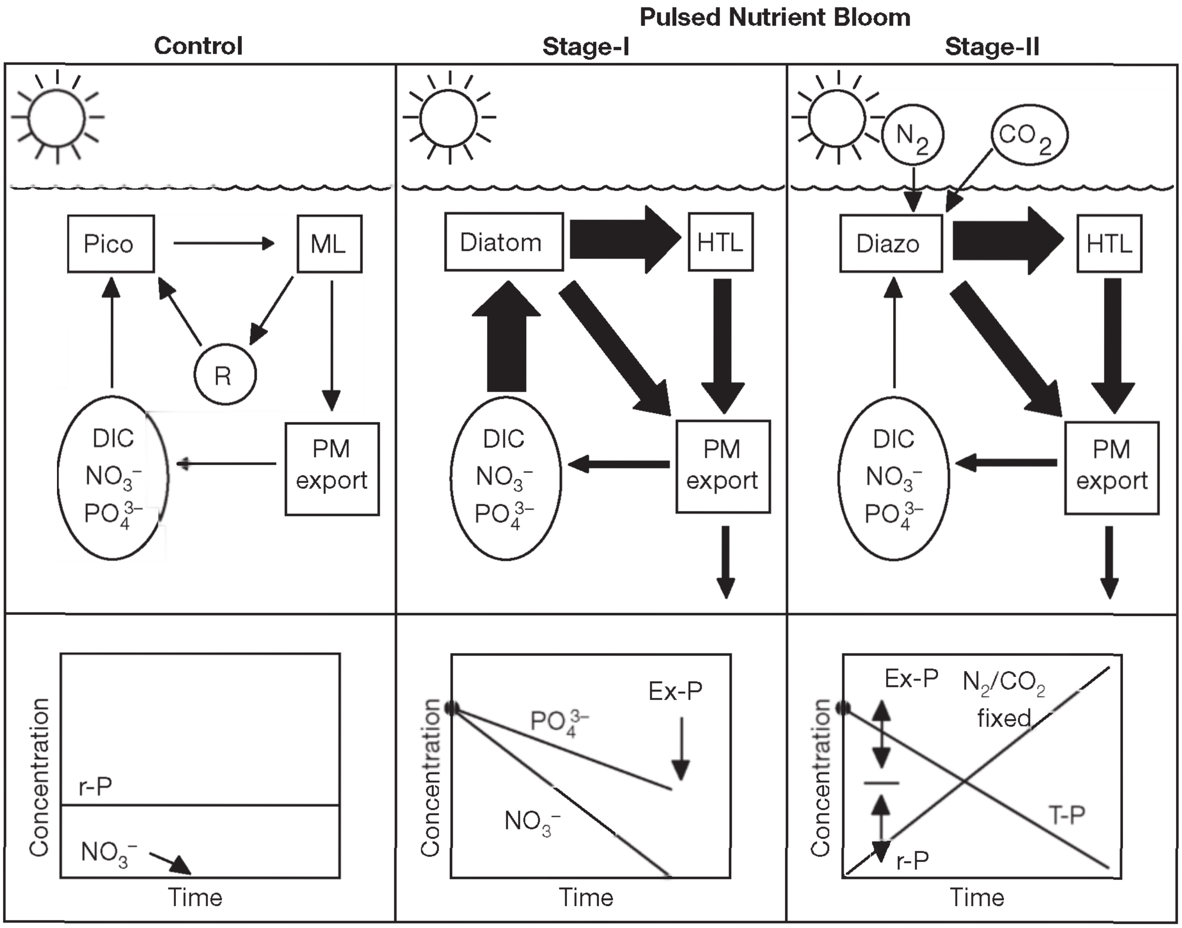

Fig. 3. Hypothesized ecosystem processes following a controlled nutrient upwelling event: (left) unamended control region, with low background nutrient fluxes from below the euphotic zone, a remineralization (R)-intensive picophytoplankton-based (Pico) microbial loop (ML) and low rates of particulate matter (PM) export mostly remineralized back to inorganic nutrients (dissolved inorganic carbon [DIC], NO_3^- and PO_4^{3-}) in the upper mesopelagic zone (plot at the bottom depicts the hypothesized time-dependent changes in NO_3^- and residual P [r-P]); (center) Stage I of the inorganic nutrient (DIC, NO_3^-, PO_4^{3-}) supported phytoplankton bloom of diatoms (~C_{106}:N_{16}:P_1) that exports additional PM partly by the combination of aggregation/sinking of intact cells and coupled higher trophic level (HTL) processes—based on the PM composition and sinking rates, a larger portion penetrates deeper into the water column facilitating long-term CO_2 sequestration (plot at the bottom depicts the excess P [Ex-P] that remains after all the NO_3^- in the upwelled water has been assimilated); (right) total P (T-P Ex-P + r-P) supports the Stage II N_2-based bloom of diazotrophs (~C_{331}:N_{50}:P_1) that assimilate N_2 and CO_2 and enhance the potential for CO_2 sequestration as described in section 'Controlled upwelling' and in Table 1

Based on field observations and experiments conducted at Station ALOHA (22° 45′ N, 158° W) over the past 2 decades (e.g. Karl 1999) and recent results of shipboard experiments that simulate upwelling (McAndrew et al. 2007), we hypothesize that controlled upwelling of Fe-replete deep water containing excess P (low NO_3^-:PO_4^{3-}) into the surface layers of the NPSG (a LNLC ecosystem) will lead to a 2-staged phytoplankton bloom (Fig. 3). Stage I is characterized by an immediate (hours to a few days) bloom of rapidly growing phytoplankton (typically diatoms; McAndrew et al. 2007) that assimilate DIC, NO_3^- and PO_4^{3-}, and trigger the export of particulate organic matter with a predictable stoichiometry of C_{106}:N_{16}:P_1 (so-called Redfield ratio), leaving excess P (and possibly excess DIC, depending on the DIC:PO_4^{3-} ratio and concentration of NO_3^- in the upwelled waters) in its wake. The excess PO_4^{3-} supplements the r-P, if any, already present in the surface waters, leading to a N_2-fixing cyanobacterial bloom, as described above. This Stage II bloom imports new N through the process of N_2 fixation, resulting in an enhanced sequestration of CO_2 over and above the DIC that was initially introduced to the euphotic zone by the upwelling event, depending upon the DIC:PO_4^{3-} ratio of the upwelled water and the C:P ratio of the exported organic matter (Fig. 3). The production of excess bioavailable N in the form of ammonium and dissolved organic nitrogen, a feature that is characteristic of diazotroph growth in general (Capone et al. 1994, Mulholland et al. 2004), will ensure the efficient scavenging of total P and lead to efficient carbon export, provided adequate light and Fe are both available. *In situ* observations of summer blooms dominated by diazotrophs in LCLN regions lend support to this hypothesis (Karl et al. 1992, Uz 2007, Dore et al. 2008).

The efficacy of C sequestration by N_2-based new production is enhanced by growth in P-limited environments. Diazotrophic growth is characterized by variable C:P stoichiometry in both the laboratory and the field, with C:P ratios ranging from 100 to 1000, with significantly reduced P cell quota under conditions of P stress (Karl et al. 1992, White et al. 2006). Even non-diazotrophic phytoplankton exhibit a P-sparing effect and synthesize new cell materials with elevated C:P when P becomes limiting (Bertilsson et al. 2003, Van Mooy et al. 2006). Consequently, this altered, C-enriched stoichiometry enhances the P-specific CO_2 sequestration potential of the Stage II N_2-based bloom. Characterizing and understanding the processes governing the plasticity of elemental coupling (stoichiometry) in the biologically mediated production, remineralization and export of organic matter in these oligotrophic environments is critical if we are to assess potential CO_2 sequestration by these ecosystems. From a biogeochemical perspective, there is nothing as fundamental as the elemental stoichiometry defined by biological systems through the coupling, storage and transport of nutrients.

N_2 FIXATION CASE STUDY: STATION ALOHA

The key to net CO_2 sequestration by controlled upwelling is the selection of an appropriate source water (geographic location and depth). Station ALOHA is the deep-water benchmark site of the Hawaii Ocean Time-series (HOT) program, where systematic observations of microbial biogeochemistry have been made since October 1988 (Karl & Lukas 1996). It is a typical LNLC, moderate r-P (30 to 50 nM) habitat with non-limiting total dissolved Fe concentrations (0.2 to 0.8 nM; Boyle et al. 2005). Assuming that a significant portion of the Fe is bioavailable, the well-illuminated surface waters of Station ALOHA should be a favorable habitat for the proliferation of N_2-fixing microorganisms. This prediction has been confirmed by direct measurements, including: (1) the abundances of the putative diazotrophs, (2) quantitative estimation of *nifH* gene abundances and expression, (3) $^{15}N_2$ tracer assimilation experiments, (4) N isotopic abundance of suspended and exported particulate matter, and (5) C:N:P stoichiometrics of dissolved and particulate matter pools (Karl et al. 1992, Letelier & Karl 1996, Karl et al. 1997, Church et al. 2005a,b). An intriguing observation from this ongoing 20 yr program is the reduction in r-P in the upper 60 m of Station ALOHA from a depth-integrated inventory of approximately 5 mmol P m^{-2} in 1989 to <2 mmol P m^{-2} in 2005 (Fig. 4). Within this long-term trend we also observe strong sub-seasonal variability in 0 to 60 m depth-integrated P with the lowest values observed following summer blooms of diazotrophs (White et al. 2007). Between 1989 and 2005 the total pool of particulate phosphorus (PP) has also decreased significantly, and the C:P and N:P ratios of the suspended particulate matter have systematically increased to values well in excess of the Redfield ratios that were observed 20 yr ago (Fig. 4). This long-term, decadal process can be viewed as a 'slow motion' bloom of N_2-fixing microorganisms controlled by some process other than P availability, possibly grazing, viral lysis, the bioavailability of Fe, or increased water column stratification (Karl 2007b). Top-down control by grazing or viral lysis is very likely in remineralization-intensive habitats like Station ALOHA. Bottom-up biomass control by Fe, or any other growth-limiting substrate, could occur independently or in conjunction with top-down control.

Based on our current understanding of this oligotrophic habitat, we predict that the addition of Fe to waters surrounding Station ALOHA may trigger a diazotroph bloom; if both Fe and P are added, the

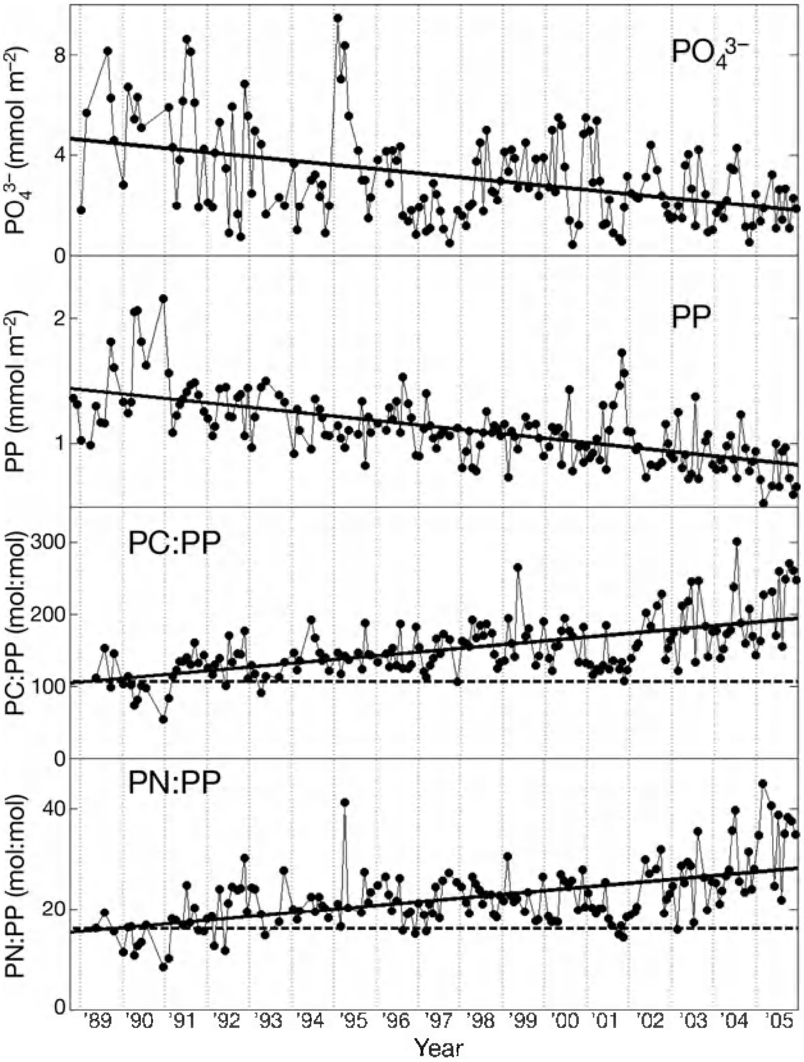

Fig. 4. Station ALOHA phosphorus pool dynamics. Shown is the 17 yr time-series of PO_4^{3-} integrated over the upper 60 m of the water column, particulate phosphorus (PP) integrated over the upper 75 m of the water column, and the average (0–75 m) C:P and N:P ratios of the particulate matter pools (PC:PP and PN:PP, respectively). Solid lines represent the best-fit linear regression analyses for each data set and the dashed line in the PC:PP and PN:PP plots is the global average stoichiometry (C_{106}:N_{16}:P_1) for marine particulate matter. The decreasing concentrations of SRP and PP, and increasing PC:PP and PN:PP ratios are all predicted from the hypothesized enhancement of N_2 fixation at Station ALOHA over the past 2 decades (Karl 2007a)

probability of a N_2-based bloom is even greater. However, it is obvious from the field observations at Station ALOHA that this seascape is not in biogeochemical steady state, but rather, supports a dynamic and time-variable ecosystem.

An important characteristic of the vertical nutrient structure at Station ALOHA is the low NO_3^-:PO_4^{3-} ratio in the upper 200 m of the water column (Fig. 2). Consequently, as deep waters are introduced into the near surface, whether by natural eddy-diffusion processes or by controlled upwelling, NO_3^- will be rapidly assimilated by phytoplankton along with DIC and PO_4^{3-} in relative proportions that approximate the Redfield ratio of C_{106}:N_{16}:P_1, the stoichiometry of average marine particulate matter (Stage I bloom; Figs. 3 & 5). Depending upon the depth of origin of the upwelled waters (controlled by experimental design) the complete assimilation of NO_3^- will leave behind excess PO_4^{3-} to supplement the r-P already present in the surface waters (Fig. 2, Table 1). Fe is also introduced as water is upwelled. However, both the biological requirements for Fe and the availability of the Fe upwelled are difficult to estimate, given the large range of Fe requirements reported in the literature (Sunda 1991) and the observation at Station ALOHA that a significant proportion of 'dissolved' Fe may be chelated with Fe-binding organic ligands (Wu et al. 2001). Nevertheless, the recurrent observation of diazotrophic summer blooms at Station ALOHA, followed by a significant reduction in r-P (White et al. 2007), suggests that Fe does not limit N_2-fixation at this site; most likely, there may be a temporal oscillation between Fe and P nutrient limitation, or co-limitation (Grabowski et al. in press).

The upwelled waters at Station ALOHA contain excess DIC (relative to NO_3^-, assuming Redfield stoichiometry) and variable excess DIC: excess P ratios depending on water depth (Fig. 2, Table 1). If the partial pressure of CO_2 in the surface waters is at or above equilibrium with the atmosphere, the addition of excess DIC may result in an ocean-to-atmosphere transfer of CO_2. However, at Station ALOHA, the surface waters are nearly always undersaturated with respect to atmospheric CO_2 (although the extent of undersaturation appears to have decreased between 1990 and 2000; Dore et al. 2003), so the excess DIC in upwelled waters (up to approximately 20 to 30 µM) will be retained unless the surface ocean warms. Furthermore, recent laboratory studies of N_2 fixation as a function of pCO_2 suggest that *Trichodesmium* spp. displays CO_2 limitation under present-day conditions (Hutchins et al. 2007). Consequently, an increase in surface pCO_2 by controlled upwelling may even stimulate nitrogen fixation in a well-stratified water column.

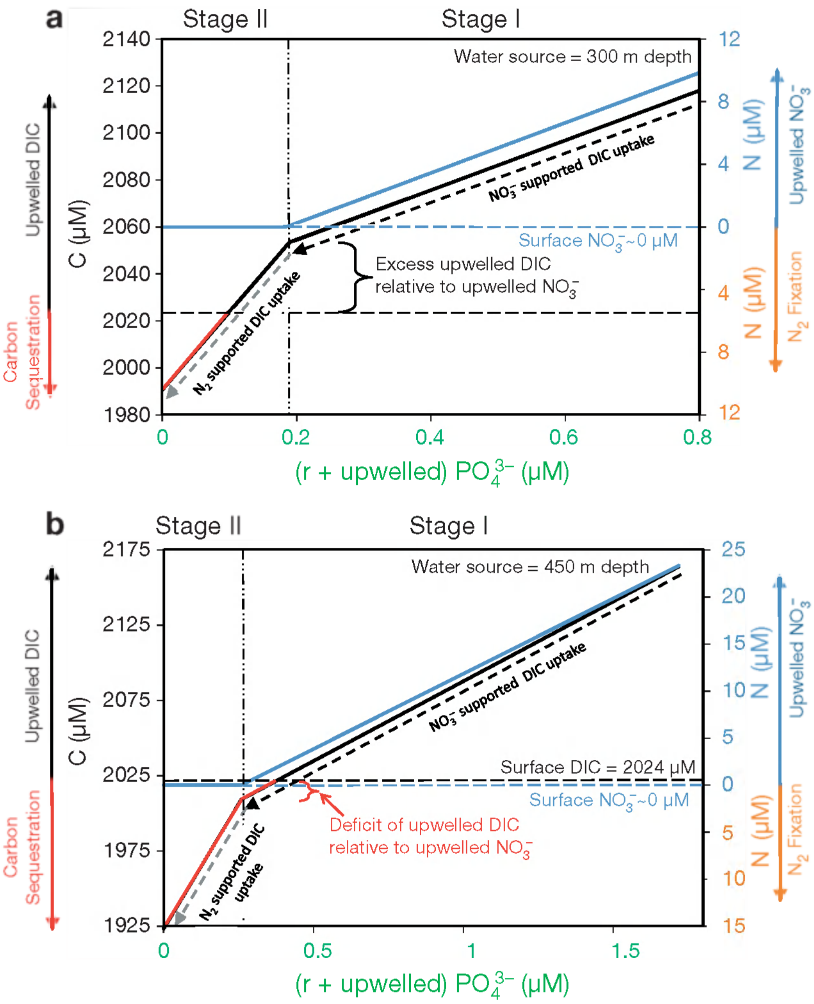

Fig. 5. Hypothetical evolution of DIC and NO_3^- concentrations as a function of total PO_4^{3-} concentration following the upwelling of (a) 300 m and (b) 450 m deep water into the upper euphotic zone at Station ALOHA. The increase of DIC (black arrow along left axis) and NO_3^- (blue arrow along white axis), relative to background surface ocean concentrations (horizontal dashed lines) will support a NO_3^- based bloom characterized by Redfield stoichiometry (Stage I bloom), reducing DIC and PO_4^{3-} until NO_3^- is depleted. Following NO_3^- depletion, the remaining PO_4^{3-} should support a N_2 based bloom (Stage II bloom and brown right axis) with an enhanced C:P and N:P stoichiometry, leading to the net sequestration of DIC in surface waters (red line within the graph). The vertical dash-dotted line marks the transition between Stage I and Stage II of the bloom. Note that while the 300 m upwelled water has excess DIC relative to NO_3^-, the water from 450 m has a deficit of DIC relative to NO_3^- leading to the removal of DIC even in the absence of N_2 fixation

Regardless of excess DIC concentration, excess P in the upwelled waters would most likely control the dynamics of N_2-based new production and carbon export (Figs. 2 & 3, Table 1). Therefore, the key factors for determining CO_2 sequestration via N_2-fixation in subtropical oligotrophic regions based on the long-term observations at Station ALOHA are (1) the excess DIC:excess P ratio in the upwelled waters, (2) the r-P concentration, (3) the saturation state of the surface ocean with respect to atmospheric CO_2, (4) the stratification of the upper water column, and (5) the C:P ratio of exported organic matter. The relative excess of P in upwelled waters (Fig. 2, Table 1) would ensure a significant net sequestration of CO_2 as long as N_2-based production and remineralization of the organic matter are uncoupled in time and with depth. Furthermore, the surface waters at Station ALOHA always contain r-P (although the amount appears to be decreasing over the past 2 decades), so the excess P delivered via upwelling would supplement the r-P, further enhancing N_2-based production and export, especially following the initial Redfield bloom, until either P or Fe becomes limiting for diazotrophic growth.

Based on well-constrained environmental observations at Station ALOHA (http://hahana.soest.hawaii.edu), we can provide estimates of the CO_2 sequestration potential following controlled upwelling from a variety of target depths using reasonable biogeochemical assumptions (Table 1). The derived values reported are CO_2 sequestered m^{-3} of upwelled water, provided sufficient Fe is available. There is an important tradeoff in the selection of the optimal source water between the NO_3^-:PO_4^{3-} and the excess DIC:excess P ratios (Table 1). If our assumptions and calculations are reasonable, then the delivery of deeper waters (>300 m) would appear to be more efficient at removing CO_2 than delivery of waters from immediately below the euphotic zone (e.g. 140 to 200 m), where dissolved Fe is at a minimum (Johnson et al. 1997) and the excess DIC:excess P ratio is at maximum. Zonal and meridional transects in the region surrounding Station ALOHA, as well as long-term observations at this benchmark confirm that low NO_3^-:PO_4^{3-} concentration ratios in subeuphotic-zone water depths are a characteristic feature of the NPSG (Fig. 6); hence, it is likely that the calculations from Station ALOHA might be appropriate for the regional gyre scale. However, over extended temporal scales (decades to centuries) of continuous upwelling, the export and remineralization of particulate organic matter with elevated C:P

Table 1. Nutrient balance and net CO_2 sequestration potential for precision upwelled waters at Station ALOHA. DIC: dissolved inorganic carbon; r-P: residual phosphorus

Source Water (m)	Total nutrient concentration (µM)			Excess nutrient concentration[a]			Total P (excess plus r-P, µM)	Total N_2 fixation[b] (µM)	Net C sequestered (mmol C m^{-3} upwelled)[c]
	DIC	NO_3^-	PO_4^{3-}	DIC (µM)	P (µM)	DIC:P (mol:mol)			
100	2046	0.06	0.062	22	0.058	371:1	0.111	5.6	15.2
120	2055	0.32	0.101	29	0.081	357:1	0.134	6.7	15.5
140	2070	0.79	0.137	41	0.088	465:1	0.141	7.0	5.8
160	2075	1.26	0.163	43	0.084	512:1	0.137	6.8	2.5
180	2082	1.98	0.215	45	0.091	492:1	0.144	7.2	2.9
200	2091	2.84	0.276	48	0.099	489:1	0.152	7.6	2.0
250	2100	5.58	0.461	39	0.112	348:1	0.165	8.3	15.7
300	2119	9.99	0.757	29	0.133	217:1	0.186	9.3	32.7
350	2146	14.22	1.044	28	0.155	179:1	0.208	10.4	41.2
400	2173	18.82	1.361	24	0.185	132:1	0.238	11.9	54.5
450	2164	23.35	1.667	–15	0.208	–71:1	0.261	13.0	101.1
500	2199	28.00	2.033	–11	0.283	–37:1	0.336	16.8	121.8
750	2313	40.90	2.985	18	0.429	42	0.482	24.1	141.5
1000	2337	41.58	3.006	38	0.407	92	0.460	23.0	114.9

[a] Excess nutrient is defined as concentration of DIC and PO_4^{3-} at the target depth that would remain in the surface if all the upwelled NO_3^- is removed during the hypothesized Stage I bloom with Redfield stoichiometry (C_{106}:N_{16}:P_1) after correcting for the average nutrient concentration that is observed in the upper 25 m at Station ALOHA between 1989 and 2005 (DIC = 2024 µM, r-P = 0.053 µM). For example, excess DIC at 300 m is: (2119 µM – 2024 µM) – (9.99 × [106 ÷ 16]) = 29 µM

[b] Total N_2 fixed = total P × (N:P ratio); assumes a molar N:P ratio of 50 (White et al. 2006)

[c] Net C sequestered = total P × (C:P ratio) – excess DIC for that depth; assumes a molar C:P ratio of 331 (White et al. 2006)

and N:P ratios may eventually alter the nutrient ratios of subeuphotic zone waters, thereby reducing the efficacy of controlled upwelling as a method of net CO_2 sequestration (Karl 2002).

The primary uncertainties in these first-order calculations of CO_2 sequestration potential are: (1) the role of dissolved organic N and P (DON and DOP, respectively) in supporting bloom dynamics or, for DOP, as a supplemental reservoir of r-P to further enhance N_2/CO_2 fixed, (2) the type of diazotroph community that is established during Stage II of the upwelling-induced bloom and (3) the C:N:P stoichiometry of the organic matter that is produced and exported. With respect to the role of DON/DOP, it is important to emphasize that these organic nutrient pools are the dominant reservoirs of N and P in surface waters at Station ALOHA (Fig. 2). However, their bioavailability, especially for DON, and the N:P ratio of the bioavailable fraction are not known or even well-constrained at present. Nevertheless, it has been demonstrated that approximately 50% of the total P assimilated in the surface waters at Station ALOHA is derived from DOP (Björkman & Karl 2003), so at least a fraction of the fairly large DOP pool in surface waters is also expected to support net CO_2 uptake and a concomitant carbon export to the deep sea.

There are at least 3 fundamentally different groups of diazotrophs at Station ALOHA: (1) small, free-living unicellular cyanobacteria (*Crocosphaera*-like), (2) large filamentous and colonial morphologies of the cyanobacteria *Trichodesmium* spp. and (3) *Richelia*-like cyanobacteria living as ecto- and endosymbionts with several species of large, aggregate-forming diatoms (e.g., *Rhizosolenia* spp., *Hemiaulus hauckii*). The N_2 and CO_2 fixed by each of these groups will have a different impact on export, despite the fact that all belong to the same diazotroph guild. Finally, the C:P ratio of newly synthesized and exported organic matter is a critical consideration for CO_2 sequestration. As mentioned previously, diazotrophs, as well as many eukaryotic phytoplankton, exhibit a P-sparing effect (a reduction in P cell quota) under conditions of P limitation, thereby increasing the P-specific sequestration of CO_2.

The best proof of this concept is to 'study nature, not books,' as Louis Agassiz advised many years ago. N_2-based, high C:P export/efficient CO_2 sequestration Stage II phytoplankton blooms occur at Station ALOHA, usually in summer when the water column is well stratified (White et al. 2007, Dore et al. 2008, Fong et al. 2008). While the mechanisms that establish and sustain the periodic greening of the oligotrophic oceanic desert are not yet known, nutrient loading, especially of Fe and PO_4^{3-}, either from above (dust deposition) or below, is the most likely cause (Dore et al. 2008). However, the dynamical coupling between nutrient delivery and excess carbon export is not well constrained either for Station ALOHA or for any other

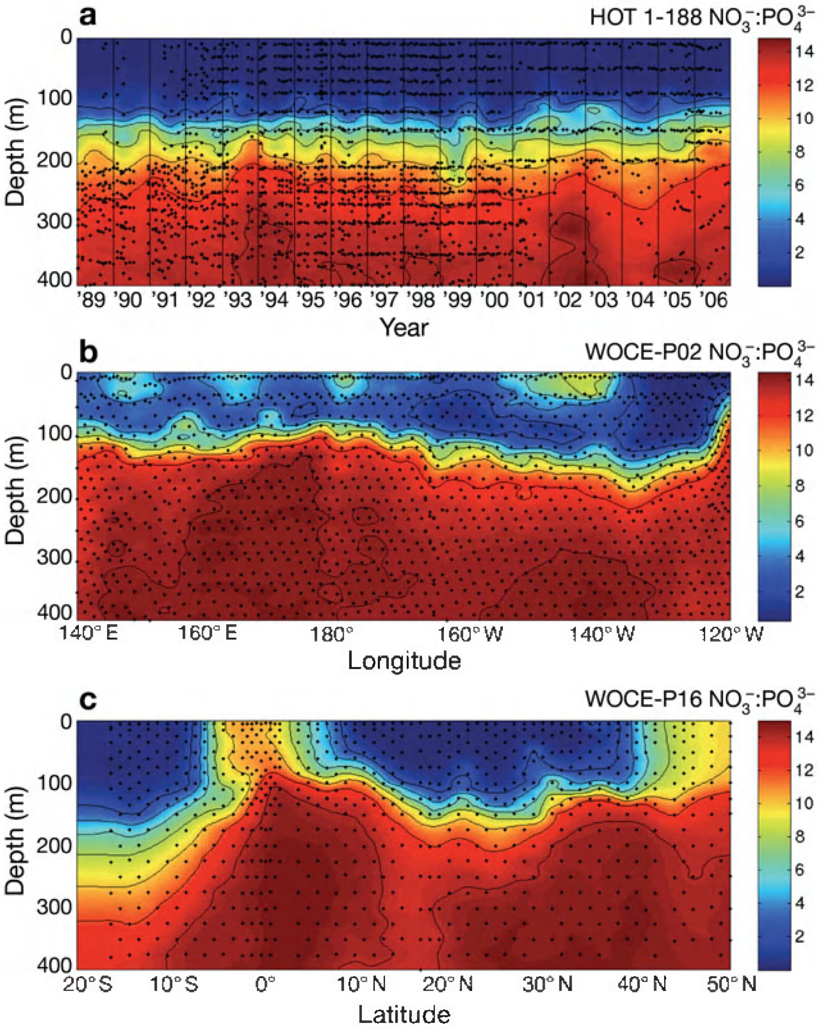

Fig. 6. Contour plots of $NO_3^-:PO_4^{3-}$ for the North Pacific Ocean: (a) time-series of $NO_3^-:PO_4^{3-}$ ratio at Station ALOHA for 1989–2006; (b) meridional section along 30° N, World Ocean Circulation Experiment (WOCE) line P02; (c) section along 150° W, WOCE line P16. Hawaii ocean time-series (HOT) data from http://hahana.soest.hawaii.edu and WOCE data from http://WOCE.nodc.noaa.gov/wdiu

marine ecosystem. Recently, Rodier & Le Borgne (2008) proposed a similar nutrient-trigger hypothesis to explain aperiodic blooms of *Trichodesmium* spp. in coastal waters of New Caledonia. In their study, *Trichodesmium* spp. blooms followed phosphate and chlorophyll enrichments with a 3 to 7 d lag, not unlike our hypothesized Stage I/Stage II progression.

FUTURE PROSPECTS

In promoting natural seascape engineering projects to enhance the efficiency for the ocean's biological carbon pump, one must always be vigilant of unintended consequences (Fuhrman & Capone 1991, Powell 2008). For the application of N_2 fixation-based methods, potential secondary impacts might include the production of nitrous oxide (N_2O) via coupled N_2 fixation-nitrification ($N_2 \rightarrow NH_4^+ \rightarrow N_2O$) and the aerobic production of methane (CH_4) from enhanced cycling of methylphosphonate, a likely component of the DOP pool (Karl et al. 2008). Either one of these unintended consequences, or others not yet considered, could negate the impact of CO_2 sequestration because both N_2O and CH_4 are potent greenhouse gases. Toxin production by both of the probable bloom-forming algal assemblages, diatoms and diazotrophs, is also possible and may have significant ecological consequences. Well-designed and comprehensive nutrient perturbation experiments that examine all aspects of microbial metabolism likely to be influenced by controlled upwelling, not just the intended consequences, need to be conducted before diazotroph manipulation can be promoted as a potential climate stabilization wedge (Socolow & Pacala 2006).

Human-induced climate change is already having measurable environmental consequences that need to be addressed by the scientific community. Sitting idle or criticizing those who do take a position on geo-engineering are not good options, in our opinion. The global scale and trajectory of climate change suggest that it may not be possible to address the problem without further affecting natural ecosystems. For this reason, understanding the ecosystem response to human-induced perturbations at a local scale must be a priority in order to develop the bases for risk assessment analyses and to minimize the impacts of geoengineering implementation on a basin-wide or global scale.

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