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Evidence for efficient regenerated production and dinitrogen fixation in nitrogen-deficient waters of the South Pacific Ocean: impact on new and export production estimates

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Abstract. One of the major objectives of the BIOSOPE cruise, carried out on the R/V Atalante from October-November 2004 in the South Pacific Ocean, was to establish productivity rates along a zonal section traversing the oligotrophic South Pacific Gyre (SPG). These results were then compared to measurements obtained from the nutrient – replete waters in the Chilean upwelling and around the Marquesas Islands. A dual $^{13}C/^{15}N$ isotope technique was used to estimate the carbon fixation rates, inorganic nitrogen uptake (including dinitrogen fixation), ammonium (NH_4) and nitrate (NO_3) regeneration and release of dissolved organic nitrogen (DON). The SPG exhibited the lowest primary production rates ($0.15\,g\,C\,m^{-2}\,d^{-1}$), while rates were 7 to 20 times higher around the Marquesas Islands and in the Chilean upwelling, respectively. In the very low productive area of the SPG, most of the primary production was sustained by active regeneration processes that fuelled up to 95% of the biological nitrogen demand. Nitrification was active in the surface layer and often balanced the biological demand for nitrate, especially in the SPG. The percentage of nitrogen released as DON represented a large proportion of the inorganic nitrogen uptake (13–15% in average), reaching 26–41% in the SPG, where DON production played a major role in nitrogen cycling. Dinitrogen fixation was detectable over the whole study area; even in the Chilean upwelling, where rates as high as $3\,nmoles\,l^{-1}\,d^{-1}$ were measured. In these nutrient-replete waters new production was very high ($0.69\pm0.49\,g\,C\,m^{-2}\,d^{-1}$) and essentially sustained by nitrate levels. In the SPG, dinitrogen fixation, although occurring at much lower daily rates ($\approx$1–2 $nmoles\,l^{-1}\,d^{-1}$), sustained up to 100% of the new production ($0.008\pm0.007\,g\,C\,m^{-2}\,d^{-1}$) which was two orders of magnitude lower than that measured in the upwelling. The annual N_2-fixation of the South Pacific is estimated to 21×10^{12}g, of which 1.34×10^{12}g is for the SPG only. Even if our "snapshot" estimates of N_2-fixation rates were lower than that expected from a recent ocean circulation model, these data confirm that the N-deficiency South Pacific Ocean would provide an ideal ecological niche for the proliferation of N_2-fixers which are not yet identified.

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1 Introduction

The nitrogen cycle in the oceanic gyres has been studied ever since the pioneering work of Menzel and Ryther in the Sargasso Sea (Menzel and Ryther, 1960; Ryther and Menzel, 1961). Several years later, Dugdale and Goering (1967) defined new and regenerated nitrogen and hence new production, fuelled by allochthonous N-sources (mainly NO_3^-) supplied by diffusion from the nitracline. Regenerated production, on the other hand, is fuelled by autochthonous N-sources (mainly NH_4^+), derived from biological processes (Harrison et al., 1987). The fraction of primary production derived from "new nutrients" is termed the f-ratio (Eppley and Peterson, 1979) and when in a steady state, accounts for the proportion of production available for export. Historically, measurements of the nitrogen cycle were primarily based on ^{15}N tracer techniques. New production estimates, computed in terms of carbon using the f-ratio and primary production, have shown considerable variations (Aufdenkampe et al., 2002), due to inaccuracies in estimating f and NO_3^- assimilation (Priscu and Downes, 1985; Ward et al., 1989; Gentilhomme and Raimbault, 1994; Raimbault et al., 1999; Diaz and Raimbault, 2000; Aufdenkampe et al., 2001).

The main sources of error in classical ^{15}N uptake experiments are still being debated, in particular nitrogen regeneration and the release of dissolved organic nitrogen (DON), which have evoked several revisions on the concepts of new and regenerated production. Ammonium regeneration is the

main source of regenerated nitrogen in the euphotic zone. By studying ammonium isotopic dilution (recycling of unlabeled substrate) during ^{15}N incubation experiments, Glibert et al. (1982), and Harrison et al. (1987) showed that ammonium regeneration can result in significant underestimations of regenerated production, which would also bias the assessment of the f-ratio. Nitrification (the oxidation of NH_4 to NO_3 by bacteria) is also an important variable, responsible for maintaining the deep sea nitrate reservoir and also believed to provide a source of "in situ" regenerated nitrate at the base of the euphotic zone (where phytoplankton is light limited and competes for NH_4^+; see review in Ward 2000). Until recently, nitrification was considered to be restricted to specific environments but over the last decade it has been found to be more widely distributed (Zehr and Ward, 2002; Lomas and Lipschultz, 2006). The failure in estimating dissolved inorganic nitrogen (DIN) taken up by phytoplankton and released as DON presents another and not entirely resolved source of error in DIN uptake rates (Bronk et al., 1994; Slawyk and Raimbault, 1995). However, the impact of such processes is rarely reported as a possible factor for overestimation in new production calculations. Finally, oceanographers have traditionally viewed the upward eddy-diffusive flux of nitrate as the exclusive source of new nitrogen supporting the export flux of biogenic particles in the open oceans. In fact, the ubiquitous pool of dinitrogen gas (N_2) dissolved in the sea can represent a significant source of new nitrogen. Recently, estimates of biological nitrogen fixation have been revised and are much higher than originally thought (Galloway et al., 1995; Grüber and Sarmiento, 1997; Capone and Carpenter, 1982). In the north subtropical and tropical Atlantic and Pacific Oceans, it has been estimated that N_2 fixation contributes to 50–180% of the nitrate flux into the photic zone (Capone et al., 2005), demonstrating that a large proportion of new primary production is fuelled by N_2 fixation rather than from deep nitrate diffusing from the deeper layers into the photic zone. While the large size classes (*Trichodesmium* and diatoms containing endosynbiotic *Richelia*) are thought to be responsible for the vast majority of N_2 fixation, recent work by Zehr et al. (2001) has found a nifH gene in the nanoplanktonic fraction. These small diazotrophic organisms, while present at low cellular concentration, could sustain a large proportion of the new production under nitrate-deplete conditions (Montoya et al., 2004; Falcon et al., 2004; Garcia et al., 2006, 2007). Representing 60% of the global ocean's area, the subtropical open-ocean ecosystems are the largest coherent biomes of our planet and the biogeochemical processes they support have global consequences (Karl, 2002). These environments provide an ideal ecological niche for the development of nitrogen-fixing organisms. To date, studies dedicated to nutrient control on nitrogen fixation have concentrated on the Northern Hemisphere and there is very little data available for the Southern Hemisphere, which contains the largest ocean area globally. Deutsch et al. (2007), using an ocean circulation model associated with the climatological distribution of nitrate and phosphate, demonstrated that nitrogen rates were highest in the Pacific Ocean and were closely related to the generation of nitrogen-deficient waters. In this context, the BIOSOPE (BIOgeochemistry and Optics South Pacific Experiment) cruise was scheduled to provide a complete data set of biogeochemical parameters in the South Pacific Ocean, characterized by a marked nitrogen-deficiency (Deutsch et al., 2007; Raimbault et al., 2007). In addition, the South Pacific Central Gyre has been described as the most oligotrophic zone in the world's oceans (Claustre and Maritorena, 2003), exhibiting extreme nutrient limitation. It is also one of the least studied areas of the Ocean (Daneri and Quinones, 2001). The 8000-km transect, stretching from the Marquesas Islands to the Chilean coast and crossing the centre of the South Pacific Gyre, was chosen because it is an ideal area for studying primary production and new production along extreme trophic gradients. This work focuses on the geographical distribution of photosynthetic carbon fixation, nitrogen assimilation (including dinitrogen fixation and release of dissolved organic nitrogen) and nitrogen regeneration in the photic zone of the South Pacific Ocean and across a section spanning a wide range of trophic status (from nutrient enriched to severely nutrient impoverished), in order to estimate primary production and the f-ratio correctly, i.e. determining the part that was sustained by "new" nitrogen. Additionally, it provides an objective methodological approach for estimating new production in oligotrophic systems.

2 Methods

This work was carried out on board the R/V Atalante during October–November 2004. Data was collected during the BIOSOPE (BIOgeochemistry and Optics South Pacific experiment) cruise carried out in the southeast Pacific Ocean along a transect stretching from the Marquesas archipelago to the Chilean coast (between 146.36 W and 72.49° W, Fig. 1).

Twenty-four short-stay stations were sampled along an 8000-km transect crossing different oceanic regimes. These were the mesotrophic area associated with the plume off the Marquesas Island (141° W–134° W), the adjacent high nitrate – low chlorophyll waters (132°–123° W), the ultra-oligotrophic waters associated with the central part of the south pacific gyre (123° W–101° W), the oligotrophic eastern side of the gyre (101° W–81° W) and the Chilean upwelling (80° W–72° W). In addition, six experimental sites were specifically investigated with long-stay, fixed stations (over 2–5 days), representing sites of different trophic regimes: MAR = Marquesas archipelago (141.3° W; 8.4° S); HLNC = High Nutrient Low Chlorophyll area east of the Marquesas islands (136.8° W; 9° S); GYR = centre of the South Pacific Gyre 114° W, 26° S); EGY = eastern border of the gyre (91.4° W, 31.8° S), UPW and UPX situated in the area of a Chilean upwelling (73° W–34° S and 72.4° W–34.5° S). The

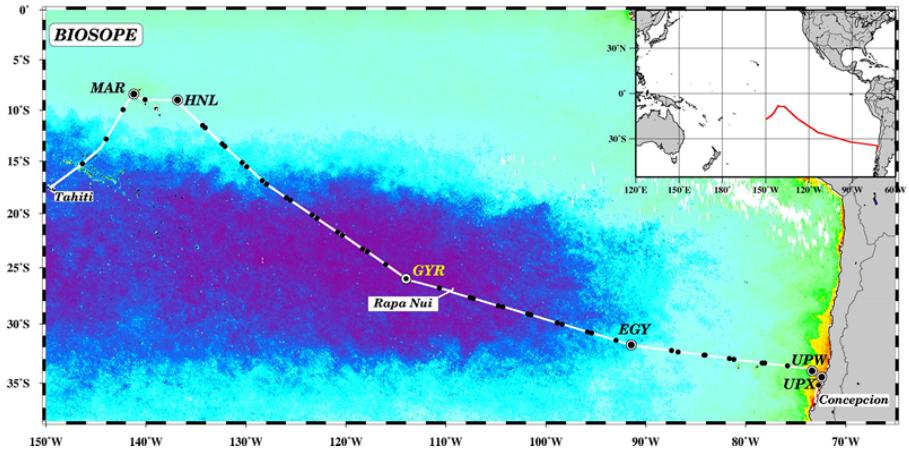

Fig. 1. Map showing the location of the BIOSOPE cruise from Marquises Island to Chile superimposed on a SeaWiFS surface Chl-*a* composite (November-December 2004). Locations of CTD casts are indicated by dark points and long time experimental stations by large circles. (MAR=141.3° W; 8.4° S; HLN=136.8° W; 9° S; GYR=114° W; 26° S); EGYR = eastern border of the gyre (91.4° W, 31.8° S; UPW=73° W–34° S and UPX=72.4° W–34.5° S).

station situated in the gyre was selected using ocean colour images, and has the lowest surface chlorophyll concentration in the world's ocean.

Nutrient measurements were performed at every station of the grid. For nitrate and nitrite determination, samples were taken into 250-ml polyethylene flasks and analyzed on board immediately using a semiautomatic Technicon Autonanalyser® II, according to Raimbault et al. (1990), for both low-nitrate waters (<500 nmoles l^{-1}; detection limit = 3 nmoles l^{-1}) and for high-nitrate waters (>500 nmoles l^{-1}; detection limit = 0.05 μmoles l^{-1}) according to Tréguer and le Corre (1975). Ammonium concentrations (40 ml collected in 50 ml Schott glass bottles) were measured using the fluorometric method (Holmes et al., 1999; detection limit = 0.005 μmoles l^{-1}).

At each productivity station, rates of carbon fixation (primary production), nitrate and ammonium uptake and dinitrogen fixation (diazotrophy) were measured using a dual $^{13}C/^{15}N$ isotopic technique. For this purpose, three 580-ml samples were collected before sunrise at 6 depths between the surface and 1% light irradiance/incidence and poured into acid-cleaned polycarbonate flasks. Bottles were rinsed after use with 10% HCl, then with distilled water from a Milli Q ion exchange unit. Labelled ^{13}C sodium bicarbonate ($NaH^{13}CO_3$ – 6 g 250 ml^{-1} deionized water – 99 at % ^{13}C, EURISOTOP) was added to each bottle in order to obtain an ≈10% final enrichment (0.5 ml 580 ml^{-1} sea water). $^{15}N_2$ gas (99 at % ^{15}N, EURISOTOP) was then bubbled into gas tight bottles (2 ml of gas 580 ml^{-1} sea water). We added a fixed quantity of $^{15}N_2$ gas and calculated the enrichment of each bottle on the basis of its volume and the solubility of N_2. We used the equations provided by Weiss (1970) to calculate the initial N_2 concentration, assuming equilibrium with the atmosphere. The $^{15}N_2$ enrichments ranged between 22% and 25% in seawater, where temperatures varied from 15°C (in the upwelling) to 27°C (in the subequatorial zone). The samples were then carefully shaken to ensure rapid equilibration between $^{15}N_2$ and natural N_2.

Nitrogen ^{15}N-tracer additions, $K^{15}NO_3$ or $^{15}NH_4Cl$ (99% at ^{15}N), were 10% or 20% of the ambient concentration based on real-time measurements. In nutrient impoverished waters, when concentrations were lower than the detection limit, additions of ^{15}N were fixed at ∼17 nmoles l^{-1} for ^{15}N-NO_3 and 43 nmoles l^{-1} for ^{15}N-NH_4.

Incubations were carried out immediately following tracer addition, just before dawn in an on-deck incubator. This consisted of 6–7 opaque boxes, each with a light screen, allowing 50%, 25%, 15%, 8%, 4%, 1% and 0.3% light penetration. The incubator was maintained at sea-surface temperature using pumped sea water. At each of the 5 experimental sites, incubations were performed in situ on a drifting rig situated at the same depth from which the samples were collected. After 24 h (dawn to dawn), final concentrations of NO_3^- and NH_4^+ were measured and samples were filtered through precombusted (450°C) Whatman GF/F filters (25 mm in diameter, nominal porosity ≈0.7 μm), using a low vacuum pressure (<100 mm Hg). The ^{15}N-NH_4 filtrates were collected in Duran Schott glass flasks and poisoned with 1 ml $HgCl_2$ (6 g l^{-1}) in order to prevent bacterial activity during storage; 200-ml aliquots of these filtrates were also filtered through 0.2 μm Teflon membranes. ^{15}N-nitrate filtrates were collected during in situ experiments, only. In this case 300 ml of GF/F filtrate was filtered through 0.2-μm Teflon membranes and stored as above. Following filtration, filters were placed into 2-ml glass tubes, dried for 24 h in a 60°C oven and stored dry until laboratory analysis. These filters were

used to determine the final $^{15}N/^{13}C$ enrichment ratio in the particulate organic matter and the concentrations of particulate carbon and particulate nitrogen.

The dual isotopic enrichment analysis was performed on an Integra-CN mass spectrometer, calibrated using glycine references for every batch of 10–15 samples. The accuracy of our analytical system was also regularly verified using reference materials from the International Atomic Energy Agency (AIEA, Analytical Quality Control Services). The mean ^{15}N atom % did not vary between 0.2 and 10 μmoles N. Thus, the low background of the system gave an accurate analyse for samples containing low nitrogen concentrations (0.1–0.2 μmole), values often observed in surface oligotrophic waters. The ^{15}N isotope enrichment of a sample is reported in terms of the ratio of ^{15}N atom % excess overtime, over the ^{15}N atom % in non-enriched samples taken from the same phytoplankton population at time zero. The value of time zero enrichment is vital and was determined using samples (same volume as the incubated sample) which were filtered immediately after isotope addition. For N_2 experiments, the time zero value, established using 8 samples, was 0.3676±0.007%. For ^{15}N-NO_3 and ^{15}N-NH_4 experiments, time 0 enrichment was 0.372±0.007%. We considered the results to be significant when ^{15}N excess enrichments were greater than 0.014% (two times the standard deviation obtained with time zero samples).

The transport rate of ^{15}N-labelled dissolved inorganic nitrogen (DIN), from the DIN pool to the PON pool, i.e. the net DIN uptake ($\rho^{\mathrm{net}}_{\mathrm{DIN}}$ in nmoles l^{-1} d^{-1}) was computed, according to Dugdale and Wilkerson (1986), from Eq. (1):

$$\rho^{\mathrm{net}}_{\mathrm{DIN}} = \frac{R_{\mathrm{PON}}}{R_{\mathrm{DIN}} \times T} \times [\mathrm{PON}], \quad (1)$$

where R_{PON} and R_{DIN} represent the ^{15}N atom % excess enrichment in the PON and DIN pools, respectively, [PON] represents the final PON concentration and T represents the incubation time (in days). To correct ammonium uptake rates for isotopic dilution, we made R_{DIN} in Eq. (1) equal to the mean value obtained between initial and final $R_{\mathrm{NH_4}}$. According to these experimental conditions, the detection limit for nitrogen uptake, calculated from significant enrichment (0.014% in excess) and lowest particulate nitrogen (0.2 μmole N) is estimated from Eq. (1) to be 0.12 nmol l^{-1} d^{-1} for nitrogen-fixation (mean R_{DIN}≈24%) and 0.03 nmol l^{-1} d^{-1} for nitrate and ammonium uptake in nutrient-depleted waters (R_{DIN}≈100%).

Carbon fixation rates were calculated according to Slawyk and Collos (1984), with a time 0 enrichment of 1.113±0.005% (n=8). This time 0 value is little higher than the natural abundance for phytoplankton (1.089), due to residual traces of ^{13}C tracer. It should be noted that the ^{13}C enrichment of samples was less problematic than the ^{15}N enrichment, since inorganic carbon is assimilated by the whole phytoplankton population and excess values ranged from 0.3 to 3.6%. Fixation rates for ^{13}C, i.e. primary production, were calculated from the mean of three replicates and are expressed in μg C l^{-1} d^{-1}. Applying a similar calculation as that for nitrogen, the detection limit is estimated to be 0.35 μg C l^{-1} d^{-1}.

GF/F filtrates from the $^{15}NH_4$ incubations were used to measure the final ^{15}N enrichment 1) in the DIN pool and 2) in the <0.7 μm organic matter pool, as outlined by Raimbault et al. (1999). In this procedure, all forms of DIN are removed from the sample as $(NH_4)_2SO_4$, by successive diffusion and reduction processes. The first diffusion step enables us to quantify the final ^{15}N enrichment of the ammonium pool, and the estimation of the isotope dilution of the tracer due to NH_4 regeneration. During the second diffusion, the ^{15}N enrichment of the nitrate pool in the ammonium filtrates enables us to quantify the oxidation of ammonium to nitrate (nitrification). During the third diffusion, the ^{15}N enrichment was determined in the fraction passing through GF/F to estimate the rate of ^{15}N-NH_4 accumulation in the <0.7 μm organic matter (dissolved organic nitrogen +<0.7 μm particulate nitrogen). The <0.2 μm filtrates from ^{15}N-NH_4 and ^{15}N-NO_3 experiments were used to measure ^{15}N enrichment in the <0.2 μm fraction which only contains dissolved organic nitrogen (DON), in order to estimate tracer loss in terms of DON ($\rho^{\mathrm{loss}}_{\mathrm{DIN}}$).

Ammonium regeneration rates ($r\mathrm{NH_4}$ in nmoles l^{-1} d^{-1}) were estimated according to Laws (1984):

$$r\mathrm{NH_4} = \frac{[\mathrm{NH_4}]_I + [\mathrm{NH_4}]_F}{2 * T} * \ln\left(\frac{Ro_{\mathrm{NH_4}}}{Rf_{\mathrm{NH_4}}}\right), \quad (2)$$

where $[\mathrm{NH_4}]_I$ and $[\mathrm{NH_4}]_F$ represent initial and final concentrations of ammonium during the incubation experiment. $Ro_{\mathrm{NH_4}}$ and $Rf_{\mathrm{NH_4}}$ are the initial and final excess enrichments in ^{15}N-NH_4 for the incubation period.

Nitrification rates (ρ_{NIT} in nmoles l^{-1} d^{-1}) were computed according to Raimbault et al. (1999):

$$\rho_{\mathrm{NIT}} = \frac{R_{\mathrm{NO_3}}}{R_{\mathrm{NH_4}} \times T} \times [\mathrm{NO_3}], \quad (3)$$

where $R_{\mathrm{NO_3}}$ is the ^{15}N atom % excess enrichment in the (NO_3^-+NO_2^-) pool, $R_{\mathrm{NH_4}}$ is the mean ^{15}N atom % excess enrichment of the NH_4^+ pool, and [NO_3] is the final NO_3^- concentration in the filtrate.

The measurement of ^{15}N abundance in the organic matter collected in the <GF/F filtrate ($R_{<\mathrm{GF/F}}$) enabled us to calculate ammonium uptake in the <0.7 μm fraction ($\rho^{<\mathrm{GF/F}}_{\mathrm{NH_4}}$), calculated as following (Eq. 4):

$$\rho^{<\mathrm{GF/F}}_{\mathrm{NH_4}} = \frac{R_{<\mathrm{GF/F}}}{R_{\mathrm{NH_4}} \times T} \times \left[\mathrm{PON}_{<\mathrm{GF/F}}\right], \quad (4)$$

where $R_{<\mathrm{GF/F}}$ and $R_{\mathrm{NH_4}}$ are the ^{15}N atom % excess enrichment of the <GF/F fraction and final ammonium pool, respectively, and [$\mathrm{PON}_{<\mathrm{GF/F}}$] is the final particulate nitrogen in the GF/F filtrate. The measurement of ^{15}N abundance in

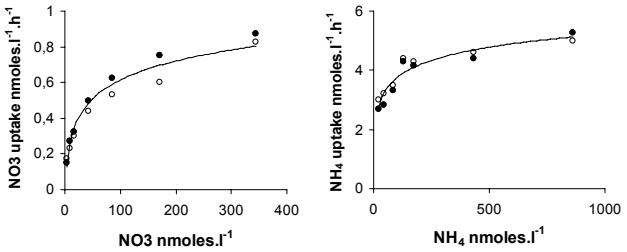

Fig. 2. Rates of nitrate and ammonium uptake (nmoles l^{-1} h^{-1}) as a function of nitrate and ammonium additions in nutrient-depleted waters. Experiments were performed during two successive days with surface waters of the South Pacific Gyre (114° W–26° S).

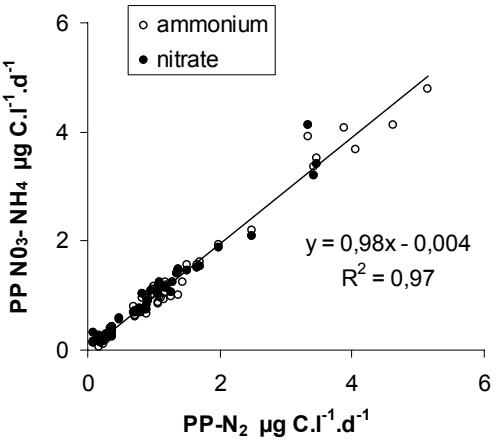

Fig. 3. Comparison between primary production (mg C m^{-3} d^{-1}) measured in nutrient-depleted samples, e.g. without nutrient additions (PP N_2), and those (PP NO_3-NH_4) spiked with nitrate (17 nmoles l^{-1}) or ammonium (43 nmoles l^{-1}). The solid line is the model II linear regression.

the extracellular DON pool (R_{DON}) enabled us to calculate the DIN (nitrate or ammonium) lost ($\rho_{\mathrm{DIN}}^{\mathrm{loss}}$) as DON (Eq. 5), calculated as outlined by Slawyk et al. (1998):

$$\rho_{\mathrm{DIN}}^{\mathrm{loss}} = \frac{R_{\mathrm{DON}}}{R_{\mathrm{DIN}} \times T} \times [\mathrm{DON}], \quad (5)$$

where R_{DON} and R_{DIN} are the ^{15}N atom % excess enrichment of the extracellular DON and DIN pool, respectively, and [DON] is the final extracellular DON concentration. The quantification of the $\rho_{\mathrm{DIN}}^{\mathrm{loss}}$ offers the possibility for calculating the gross uptake rate $\rho_{\mathrm{DIN}}^{\mathrm{loss}}$ as the sum of the net DIN uptake and the DIN loss:

$$\rho_{\mathrm{DIN}}^{\mathrm{gross}} = \rho_{\mathrm{DIN}}^{\mathrm{net}} + \rho_{\mathrm{DIN}}^{\mathrm{loss}}. \quad (6)$$

During this study, nitrate and ammonium concentrations were often lower than the detection limit (especially in the South Pacific Gyre) and it was experimentally impossible to reduce the addition of the tracer to the ideal level (<10% of ambient concentration). In these nutrient conditions, the tracer addition violates the general assumption that the tracer addition does not disturb the steady-state of the system and could well have evoked a major perturbation in the nitrate and ammonium uptake (Allen et al., 2002; Harrison et al., 1996). Despite this, the use of kinetic parameters, described by Harrison et al. (1996), enabled us to account for the uptake rate enhancement according to the following equation given by Rees et al. (1999),

$$\rho \mathrm{N}_H = \rho \mathrm{N}_0 / [\mathrm{N}_{sp} / (Ks + \mathrm{N}_{sp}) \times (Ks + \mathrm{N}_A) / \mathrm{N}_A], \quad (7)$$

where N is nitrate or ammonium, $\rho \mathrm{N}_0$ is the original uptake rate (nmoles l^{-1} d^{-1}), $\rho \mathrm{N}_H$ is the uptake rate adjusted for enhancement of tracer, N_{sp} is the ambient + tracer nutrient (nmoles), N_A is the ambient nutrient and Ks is the half-saturation constant. In this case, N_A was assumed to be 3 nmoles l^{-1} for nitrate and 5 nmoles l^{-1} for ammonium, corresponding to the detection limit of our analytical procedures. To quantify the affinity constant Ks, two kinetic studies were performed with 6 graduated additions of ^{15}N-labeled substrate (Fig. 2). A Monod equation $[V = VmS/(Ks+S)]$ was assumed, where V is the uptake rate for substrate concentration S, Vm is the saturated uptake rate and K_S, the affinity constant, e.g. the substrate concentration at half Vm. The kinetics constant K_S, needed for the use of Rees et al.'s model, was derived from the Wolf plot linear transformation of S/V vs. S. Ks obtained during 4 experiments performed in nitrogen-depleted waters (12.9 and 15 nmoles l^{-1} for nitrate and ammonium, respectively) were a little lower than those measured in other oceanic waters (around 25–30 nmoles l^{-1}) by Sahlsten (1987) and Harrison et al. (1996). As a first approximation, we assumed that DIN losses as DON ($\rho_{\mathrm{DIN}}^{\mathrm{loss}}$) and nitrification ($\rho_{\mathrm{NIT}}$) could be activated in the same way as net uptake rates and we applied the same procedure of correction to these processes.

In addition, spiked nutrient additions in nutrient-limited waters could also stimulate ^{13}C fixation. Thus samples enriched with ^{15}N-N_2, which did not significantly change the N_2 concentration of the samples, were used as a control for the stimulation of primary production by ^{15}N-tracer additions in oligotrophic waters (Fig. 3). Model II gives a regression coefficient of 0.98, revealing no significant stimulation of primary production by low nutrient addition, at least over the 24 h experiments. As the ^{13}C isotope is not routinely used for estimating marine productivity, especially in oligotrophic oceanic areas, our results (PP^{13}C) also offered the opportunity to carry out an extensive comparison with classical primary production measurements using a ^{14}C tracer (PP^{14}C) in the same conditions (T. Moutin, personal communication). On pooling all the data collected during in situ experiments and using the model II linear regression, we noted the significant relationships (PP^{13}C=1.02 PP^{14}C+0.14, r^2=0.98; n=50), which indicated the efficiency of the ^{13}C procedure in quantifying the photosynthetic carbon fixation in oligotrophic waters.

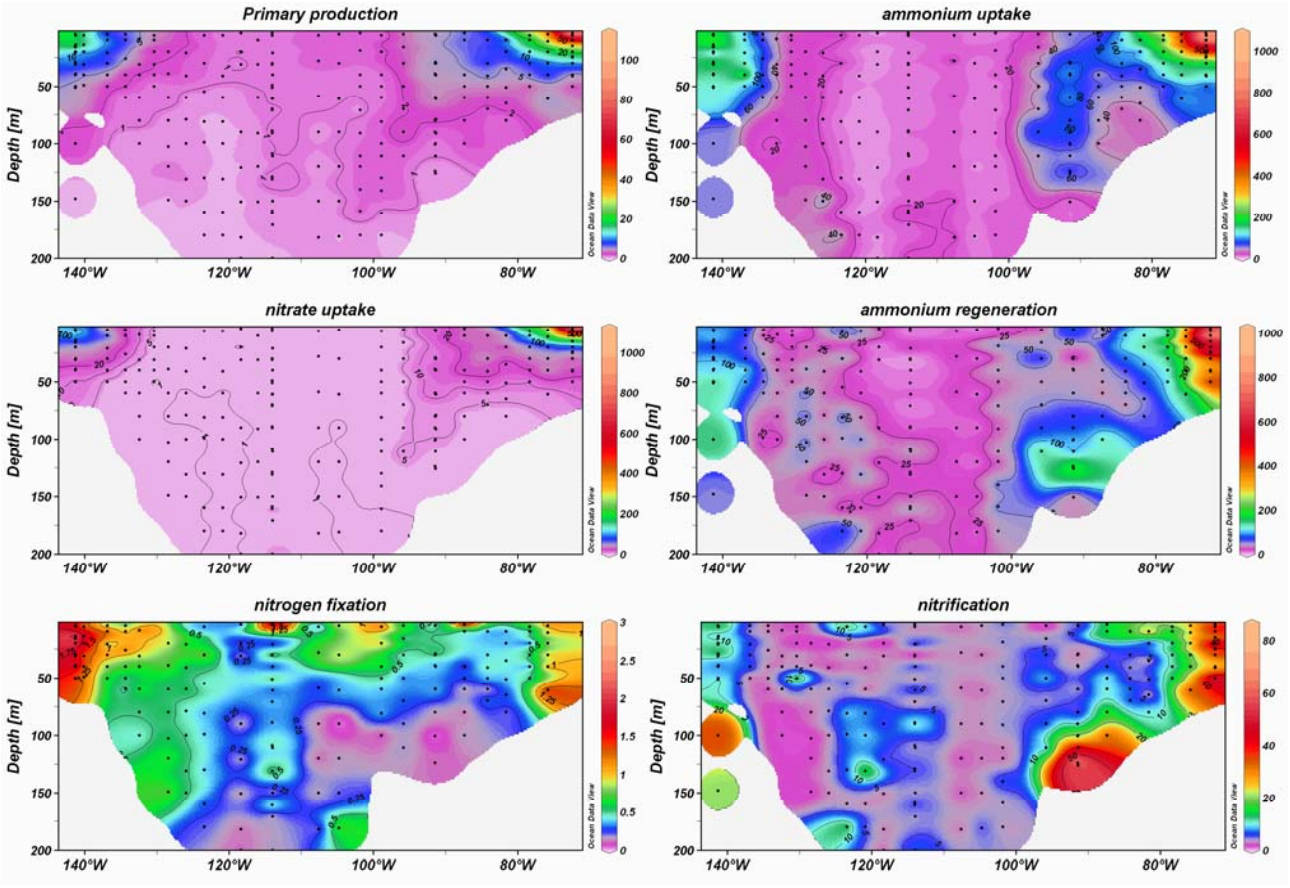

Fig. 4. Zonal sections of primary production (μg C l^{-1} d^{-1}), nitrate uptake (nmoles l^{-1} d^{-1}), ammonium uptake (nmoles N l^{-1} d^{-1}), nitrogen fixation (nmoles N l^{-1} d^{-1}), ammonium regeneration (nmoles N l^{-1} d^{-1}) and nitrification (nmoles N l^{-1} d^{-1}), along the BIOSOPE Transect. (Ocean Data View software (ODV), version 3.1; Reiner Schlitzer; http://odi.awi-bremerhaven.de/2005).

Surface light levels and the thickness of the euphotic layer were measured around noon using a spectroradiometer (LI.1800U.W, LI-COR instrument; Morel et al., 2007). The depth of the euphotic zone (Ze) was defined as the depth where the downward photosynthetical available radiation (PAR) irradiance was reduced to 1% of its surface value; For CTD casts performed early in the morning, Ze was computed using in situ TChl-*a* concentration profiles (see Ras et al., 2007), according to the model developed by Morel and Maritorena (2001).

3 Results

A detailed description of the geographical distribution of nutrients and biomass during this study can be found in Raimbault et al. (2007). Briefly, the zonal distribution of surface nitrate showed minimal values (lower than the detection limit of 3 nmoles l^{-1}) between 125° W and 95° W, i.e. in the South Pacific Gyre (SPG). Other regions (Marquesas Islands and Chilean upwelling) showed significant nitrate concentrations (>0.5 μmoles l^{-1}) in the surface. Chlorophyll biomass followed this general trend with very low values in the center of the SPG (0.023 μg l^{-1}), with levels reaching 0.3 μg l^{-1} near the Marquesas Islands and 1 μg l^{-1} in the Chilean upwelling. The photic layer located at 40–50 m around the Marquesas and upwelling regions deepened in the centre of the SPG, reaching 160 m between 120° W and 105° W, which could be expected in the clearest natural waters of the world (Morel et al., 2007; Tedetti et al., 2007). The incident solar radiation was more or less constant during the cruise (41±7 Em^{-2} s^{-1}) with the exception of three very cloudy days (9 to 11 November 2004, e.g. from 120° W to 117° W), where incident radiation decreased to 14–20 Em^{-2} s^{-1}.

Primary production and nitrogen uptake rates followed the same general distribution, responding to the nutrient variations observed in the photic layer along the transect (Fig. 4). A large central area was characterized by very low primary production rates and this was surrounded by two small regions: the subequatorial Marquesas region (in the west) where primary production reached 10 μg C l^{-1} d^{-1} in surface waters, and a part of the Chilean upwelling in the east, which was the most productive area, primary

production reaching more than $50\,\mu g\,C\,l^{-1}\,d^{-1}$. For the most part, the transect (e.g. the South Pacific Gyre = SPG) was characterized by very low carbon fixation rates, less than $2\,\mu g\,C\,l^{-1}\,d^{-1}$ between 130 and 95° W. Vertical variations in primary production were insignificant in the centre of the SPG, with the rates remaining more or less constant ($\approx$1–2 $\mu g\,C\,l^{-1}\,d^{-1}$) from the surface to the base of the photic layer. Nitrate and ammonium uptake rates followed the same general pattern as primary production. Nitrate uptake ranged from 100–500 nmoles $l^{-1}\,d^{-1}$ in the upwelling to less than 5 nmoles $l^{-1}\,d^{-1}$ in the SPG. Intermediate values were found near the Marquesas Islands ranging from 20 and 100 nmoles $l^{-1}\,d^{-1}$. Ammonium uptake rates (corrected from isotopic dilution) were always significantly higher than nitrate uptake rates. The upwelling presented the highest uptake rates (up to 500 nmoles $l^{-1}\,d^{-1}$), intermediate levels (up to 100 nmoles $l^{-1}\,d^{-1}$) were observed around the Marquesas Island and very low rates (<40 nmoles $l^{-1}\,d^{-1}$) in the SPG As for primary production, vertical distribution of ammonium uptake, as well as nitrate uptake were quite homogeneous in the SPG, with very low vertical variation and no clear surface or subsurface maximum. However, contrary to primary production, noticeable rates of ammonium and nitrate uptake (>60 and >5 nmoles $l^{-1}\,d^{-1}$, respectively) were detected, reaching depths of 100 m on the eastern edge of the SPG between 90 and 100° W.

Dinitrogen fixation rates showed a particular distribution, with rates always lower than ammonium and nitrate uptake. Firstly, the vertical extension of this biological process was more important to the west (until 150 m) than to the east of the investigated area. Secondly, nitrogen fixation was essentially located near the surface in the SPG, where a clear surface maximum (>1 nmoles $l^{-1}\,d^{-1}$) was detected. Rates decreased rapidly with depth and were <0.5 nmoles $l^{-1}\,d^{-1}$ below 50 m. The geographical gradient was weak since maximum surface rates measured in the upwelling (3.6 nmoles $l^{-1}\,d^{-1}$) were only two folds higher than the maximum rates measured in the SPG (1.8 nmoles $l^{-1}\,d^{-1}$). The Marquesas Island was marked by intermediate nitrogen fixation ($\approx$2 nmoles $l^{-1}\,d^{-1}$).

Ammonium regeneration rates showed the same regional variations. This process was very active in surface water near the upwelling (>200 nmoles $l^{-1}\,d^{-1}$), as well as around the Marquesas Islands with rates >100 nmoles $l^{-1}\,d^{-1}$until 135° W. Surface values decreased to 20 nmoles $l^{-1}\,d^{-1}$ in the SPG, but remained more or less constant between the surface and 200 m. As for ammonium uptake, a subsurface maximum (located around 125 m) was detected on the eastern edge (90° W) of the SPG. The significant ammonium regeneration measured at all stations, regardless of the trophic level, induced great variations of ammonium enrichment during the 24-h incubations. Figure 5 demonstrates that if isotopic dilution is not taken into account, then there is a massive underestimation of ammonium uptake of more than 50%. Moreover, there was no relationship between the magnitude of the underestimation and the uptake rates, clearly indicating that estimates of isotopic dilution are necessary in order to quantify ammonium uptake over 24-h incubations, even in oligotrophic waters.

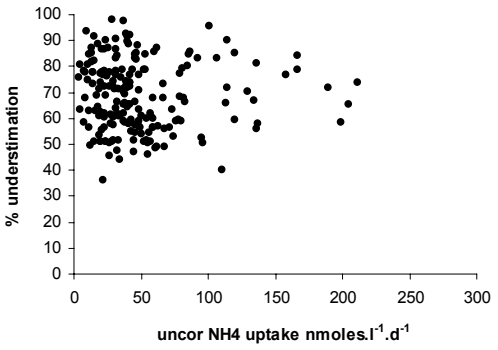

Fig. 5. Percentage of underestimation of ammonium uptake (% underestimation) when isotopic dilution is not included for calculating rates versus ammonium uptake rates not corrected from the isotopic dilution (uncor NH_4 uptake).

Nitrification showed a similar pattern to that of ammonium regeneration, indicating a tight coupling between the two processes, although rates, always significant, were globally one order of magnitude lower than those of ammonium regeneration. Highest nitrification rates (>30 nmoles $l^{-1}\,d^{-1}$) were observed in the upwelling with a westward extension to 90°W. The subsurface patch of ammonium regeneration at 90° W was also marked by active nitrification (up to 40 nmoles $l^{-1}\,d^{-1}$). The Marquesas region was characterized by relatively low nitrification rates (5–10 nmoles $l^{-1}\,d^{-1}$), while the lowest, but detectable values (<5 nmoles $l^{-1}\,d^{-1}$) were measured in the SPG. As observed for preceding biological processes, nitrification was again homogeneous throughout the water column with no significant maximum in the oligotrophic central region. Nitrate uptake in comparison to nitrification generally dominated the upper layer, except in the SPG, where both rates were similar. At the base of the photic layer, nitrification rates were 2 to 10 times higher than the corresponding nitrate uptake rates, especially in the upwelling area.

A significant proportion of the ammonium was assimilated by the particulate matter passing through the GF/F filters (Fig. 6). This <0.7 μm ammonium uptake was significant in the upwelling region, with rates higher than 25 nmoles $l^{-1}\,d^{-1}$ and up to 170 nmoles $l^{-1}\,d^{-1}$, but also significant in the SPG (10 to 20 nmoles $l^{-1}\,d^{-1}$). Some of the <0.7 μm uptake was ultimately found in the <0.2 μm fraction, i.e. in the dissolved organic nitrogen pool, rates here defined as ρ_{loss}. Ammonium losses in terms of DON were again highest in the upwelling area (50 to 100 nmoles $l^{-1}\,d^{-1}$), but highly significant in the oligotrophic SPG (5 to 20 nmoles $l^{-1}\,d^{-1}$). The lowest rates (<5 nmoles $l^{-1}\,d^{-1}$) were measured in the western part of

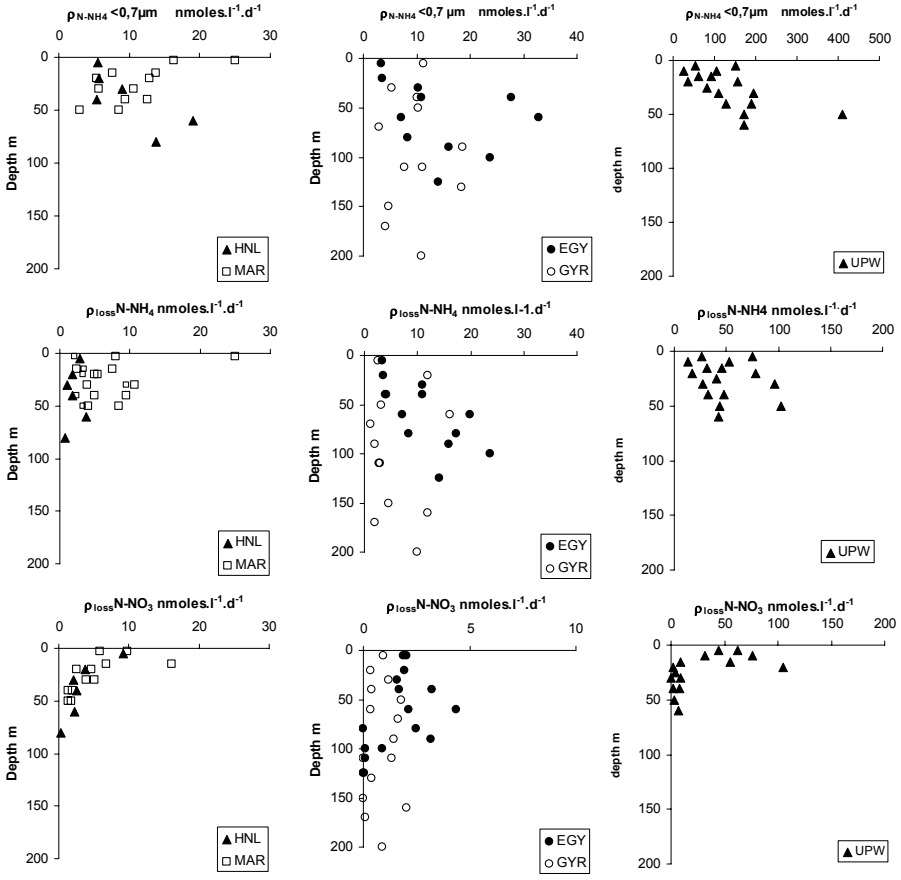

Fig. 6. Vertical profiles of ammonium uptake in the <GF/F fraction (ρ_{NH_4} <0.7 μm; upper panels), loss of ^{15}N-ammonium in terms of DON in the <0.2 μm fraction (ρ_{loss} NH_4; middle panels) and loss of ^{15}N-nitrate in terms of DON in the <0.2 μm fraction (ρ_{loss} NO_3; lower panels) at the five experimental sites.

the area. The nitrate losses were lowest in the SPG (1 to 5 nmoles l^{-1} d^{-1}) and highest in the surface waters of the upwelling. Some data in the SPG appeared close to zero and could be explained by the very low uptake rates of labeled nitrogen and then the very low excess enrichment in the <0.2 μm DON pool (close to 0.01%). Loss of nitrate generally tended to decrease rapidly with depth, as observed in the most productive region (Marquesas Islands and Chilean upwelling). The mean DIN percentage loss showed important regional variations (Table 1). The lowest productive system (SPG) revealed the most important loss of recent nitrogen uptake (>20%), while in the other regions percentage losses were less than 15%. It should be noted that the percentage of N-nitrate losses were equivalent to those of N-ammonium losses, except around the Marquesas Islands (Mar and HLN sites).

Several corrections have been applied to the f-ratio to take into account several processes that are rarely measured during nitrogen uptake experiments, such as nitrification, DIN loss and nitrogen fixation. Nitrification (as a source of nitrate) can induce overestimations of new production by adding NO_3^- to the nitrate pool, with some being derived from regenerated production (Dore and Karl, 1996; Ward et al., 1989; Priscu and Downes, 1985; Dugdale and Goering, 1967). Thus, the fraction of nitrate produced by nitrification (ρ_{NIT}) has to be subtracted from the total nitrate uptake (ρ_{NO_3}) in order to truly assess the uptake of "new" nitrate as classically defined by Dugdale and Goering (1967). As nitrogen fixation rates (ρ_{N_2}) were available, they were included in the estimate of new production (P_{new}), calculated as follows:

$$P_{new} = \rho_{N_2} + (\rho_{NO_3} - \rho_{NIT}). \quad (8)$$

When nitrification rates were higher than nitrate uptake ($\rho_{NO_3} - \rho_{NIT<0}$), P_{new} was estimated to be equal to ρ_{N_2}. According to this assumption, the f-ratio can be calculated as follows:

$$f = \frac{\rho_{N_2} + \rho_{(NO_3^-)} - \rho_{NIT}}{[\rho_{N_2} + \rho_{(NO_3^-)} + \rho_{(NH_4^+)}]}. \quad (9)$$

As ρ_{loss} was not available for all stations (especially from ^{15}N-NO_3 experiments), gross uptake rates were not used in the calculation of the f-ratio, which was only estimated with

Table 1. Percentages (mean values integrated over the photic layer) of ammonium uptake in the <GF/F filtrate ($\rho_{<0.7\,\mu m}$) and loss in terms of DON ($\rho_{DON<0.2\,\mu m}$) from ammonium and nitrate relative to gross uptake rates ($\rho_{gross}=\rho_{net}+\rho_{DON<0.2\,\mu m}$) for the five investigated oceanic regions. Values from nitrate values are available from only in situ profiles performed on the 5 experimental sites.

	Ammonium % ρ_{gross}			Ammonium % ρ_{gross}		Nitrate % ρ_{gross}
Area	$\rho<0.7\,\mu m$	$\rho_{DON<0.2\,\mu m}$	Experimental site	$\rho<0.7\,\mu m$	$\rho_{DON<0.2\,\mu m}$	$\rho_{DON<0.2\,\mu m}$
MAR (141°–134° W)	7±4	4±2	12° S–138° W	6±3	4±2	11±4
HNL (133°–123° W)	17±14	10±9	9° S–136° W	7±4	1±1	10±7
SPG (123°–101° W)	41±19	28±13	26° S–114° W	26±14	20±7	19±12
EGY (100°–81° W)	25±15	15±13	32° S–91° W	16±14	12±7	11±9
UPW (80°–72° W)	26±36	12±9	34° S–73° W	55±61	16±14	12±8

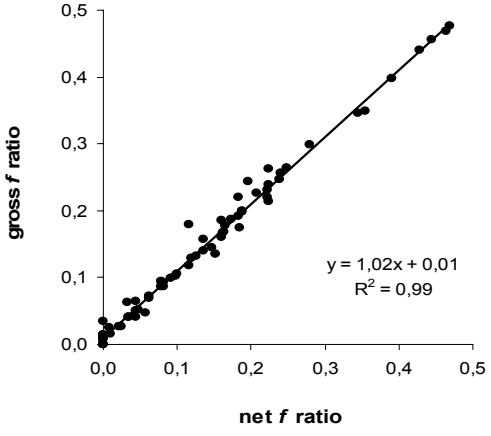

Fig. 7. Comparison of f-ratio calculating from gross nitrogen uptake (gross f-ratio) and from net nitrogen uptake rates (net f-ratio). The linear regression line was obtained from model II regression.

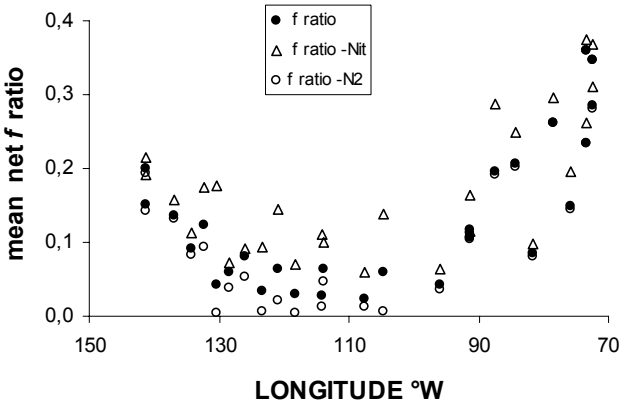

Fig. 8. Zonal evolution of net f-ratio calculating without nitrification (f-ratio – Nit), without dinitrogen fixation (f-ratio – N_2), or including both processes (f-ratio).

the net uptake rates. Using data from in situ experiments, where both losses of N-nitrate and N-ammonium were measured, the model II regression analysis gives a slope of 1.02 (Fig. 7). The slope was not significantly different from 1, indicating that by including DIN loss one does not change the estimates of the f-ratios. To assess the influence of nitrification and N_2-fixation on the magnitude of the f-ratio, we compared the f-ratio calculation, according to Eq. (5), to those calculated without nitrification (f_{-NIT}) or without N_2-fixation (f_{-N_2}). The possible overestimation due to the noninclusion of uptake of urea could not be evaluated, since fluxes of this organic nitrogen compound have not been investigated in this area.

The geographical variations of the f-ratios (averaged over the photic layer) reflect the zonal evolution of the trophic states (Fig. 8), with highest values (>0.30) observed in the upwelling. Waters surrounding the Marquesas Islands were characterized by f-ratios ranging between 0.1 and 0.2. In the SPG, the f-ratio was generally lower than 0.1, with very low values (0.02) found in the centre. The exclusion of nitrification rates (f_{-NIT}) significantly increased the f-values in the SPG (0.08–0.15). In the other regions nitrification, although acting at significant rates, did not modify the mean f-ratio. In the same way, the influence of nitrogen fixation was only sensitive in the SPG. In this oligotrophic area, f_{-N_2} down to very low values (often close to 0) clearly indicated that nitrogen fixation was the main process providing new nitrogen in this upper layer.

4 Discussion

This study confirms previous satellite observations suggesting very low productivity in the SPG, i.e. in the clearest waters of the world's ocean (Morel et al., 2007; Tedetti et al., 2007). Nixon (1995) assigned annual rates of carbon fixation of <100, 100 to 300 and 300 to 500 $g\,C\,m^{-2}\,y^{-1}$ in oligotrophic, mesotrophic and eutrophic areas, respectively. Following this criterion and assuming that our measured rates remained constant over the year, the upwelling

Table 2. Mean and standard deviation of integrated rates over the photic layer of nitrate (ΣNO_3), primary production (ΣPP), nitrate uptake ($\Sigma\rho_{NO_3}$), ammonium uptake ($\Sigma\rho_{NH_4}$), nitrogen fixation ($\Sigma\rho_{N_2}$), carbon/nitrogen ratio during uptake (C/N), ammonium regeneration (r_{NH_4}) and nitrification (ρ_{NIT}) pooled for each studied oceanic area.

Area	ΣNO_3 mmoles m^{-2}	ΣPP g m^{-2} d^{-1}	$\Sigma\rho_{NO_3}$	$\Sigma\rho_{NH_4}$ mmoles m^{-2} d^{-1}	$\Sigma\rho_{N_2}$	C/N	$\Sigma\rho_{NH_4}$ mmoles m^{-2} d^{-1}	Σr_{NH_4}	$\Sigma\rho_{NIT}$
MAR (141°–134° W)	128±24	0.66±0.1	2.2±1.1	8.7±3.6	0.11±0.03	6.3±2.6	6.3±1.6	5.8±1.9	0.82±0.4
HNL (133°–123° W)	49±73	0.25±0.12	0.5±0.7	4.6±4.3	0.07±0.07	5.2±2.2	2.6±0.8	4.8±3.1	0 .61±0.7
SPG (123°–101° W)	0.84±1.3	0.15±0.05	0.06±0.06	2.1±1.0	0.06±0.03	6.1±2.3	1.2±0.6	3.5±1.9	0 .76±0.4
EGY (100°–81° W)	141±67	0.33±0.6	0.82±0.4	6.0±2.3	0.03±0.005	4.4±1.4	2.9±0.4	6.3±2.0	0 .81±0.5
UPW (80°–72° W)	285±310	1.8±1.3	8.6±6.8	20±10	0.09±0.06	4.9±0.9	6.2±2.0	19 11	1.31±06

(660 g C m^{-2} y^{-1}) could be described as eutrophic, the Marquesas region as mesotrophic (270 g C m^{-2} y^{-1}), and the SPG (36 g C m^{-2} y^{-1}) as an "ultra" oligotrophic system.

The zonal variation of integrated rates over the euphotic layer, using the trapezoidal method, revealed some specific patterns (Table 2). For example, the increase in primary production and nitrogen uptake was not as pronounced as the increase in nutrient availability. Although integrated nitrate concentrations increased by a factor of 100 and 300 from the oligotrophic zone (0.9 mmoles m^{-2}) to the Marquesas or to upwelling regions (128 mmoles m^{-2} and up to 285 mmoles m^{-2}, respectively), integrated carbon fixation rates increased only by 10 to 20 fold. Nitrogen fixation appeared more or less constant around 0.03–0.11 mmoles m^{-2} d^{-1}, while integrated nitrate uptake increased 10 or 50 fold between the SPG (0.2 mmoles m^{-2} d^{-1}) and the Marquesas region (2.9 mmoles m^{-2} d^{-1}) or the upwelling (11 mmoles m^{-2} d^{-1}). The C/N uptake ratios were always lower than the conventional 6.6 Redfield ratio (Table 2). Such low C/N uptake ratios may suggest that some nitrogen assimilation (especially ammonium) was due to heterotrophic organisms. The importance of submicron heterotrophic organisms was emphasized by the large quantity of ^{15}N-NH_4 found in the <GF/F filtrates, i.e. the <0.7 μm size fraction, following 24-h incubations (Table 1). The low efficiency of the GF/F filters for PON retention compared to 0.2-μm membranes is now well-documented in a variety of marine environments (Altabet, 1990; Libby and Wheeler, 1997; Slawyk and Raimbault, 1995; Raimbault et al., 2000; Fernandez et al., 2007) and has been confirmed during this study (Raimbault et al., 2007). Our experiments showed that the use of GF/F filters can result in severe underestimations of ammonium uptake during high productivity, as well as under oligotrophic conditions. This emphasizes the importance of discussing the use of GF/F and 0.2-μm filters for tracer addition experiments. GF/F filters do, however, collect all particles containing chlorophyll, i.e photosynthetic organisms (Chavez et al., 1995; Raimbault et al., 2007) and this heterotrophic ammonium uptake should not be included in the estimates for regenerated primary production. Consequently, the underestimation of gross ammonium uptake rates by filtering through GF/F filters should not have any consequences for the f-ratio estimation. There are no current data available giving information on the possible nitrate uptake by the <0.7 μm size fraction; however, a study conducted in the equatorial Pacific has shown that submicron particles passing through the GF/F filters do not assimilate nitrate (Raimbault et al., 2000).

As our C/N uptakes deviate from the Redfield ratio, no relationship was found between new production estimates obtained using the ^{13}C fixation rates, multiplied by the independently estimated f-ratio, and those computed using direct measurements of new nitrogen multiplied by the 6.6 Redfield ratio (Table 3). The two estimates often disagreed by 25–50%, with the lowest (0.007–0.008 g C m^{-2} d^{-1}) and highest (0.53–0.69 g C m^{-2} d^{-1}) values found in the SPG and in the upwelling, respectively. The extremely low f-ratio obtained in the SPG (0.05±0.03) confirms that most of the primary production, which maintained relatively significant rates over the 0–180 m water column, was supported by regenerated nitrogen. This is indicated by the relatively high values of ammonium uptake (2.1 mmoles m^{-2} d^{-1}), as well as by significant ammonium and nitrate regeneration rates, 3.5 and 0.76 mmoles m^{-2} d^{-1}, respectively (Table 2). Rates of NH_4 regeneration are rare for oceanic waters but our results are in the range of the data available (e.g. Bode et al., 2002; Raimbault et al., 1999; Fernandez and Raimbault, 2007). Ammonium regeneration showed high rates (up to 500 nmoles l^{-1} d^{-1}) in the high productive Marquesas and upwelling regions (MAR and UPW), but significant activity was also measured in the SPG (10–20 nmoles l^{-1} d^{-1}); these rates were able to sustain the biological demand. This observation was substantiated by the picoplankton abundance which was strongly dominated by heterotrophic bacterioplankton along the whole transect (Grob et al., 2007).

Another important finding was the magnitude of the nitrification process in the euphotic layer. Although the potential significance of "regenerated" nitrate in the euphotic zone has been acknowledged for some time (Dugdale and Goering, 1967; Ward et al., 1989; Dore and Karl, 1996) and nitrification studies have been performed in a variety

Table 3. Mean and standard deviation of integrated rates over the photic layer of chlorophyll-*a* (ΣTchlo), f-ratio calculated with net uptake rates of dissolved inorganic nitrogen (f_{net}), new production calculated by multiplied rates of dissolved inorganic nitrogen rates by the Redfield ratio 6.6 (New PP) or by multiplying the primary production by the f-ratio (PP$\times f$) for the five investigated oceanic regions. Mean "net f-ratio" (f_{net}) and "gross f-ratio" (f_{gross}) are compared with data obtained during in situ experiments performed at 5 experimental sites.

Area	Tchlo mg m^{-2}	f_{net} mean	New PP g C m^{-2} d^{-1}	PP$\times f$ g C m^{-2} d^{-1}	experimental sites	f_{net}	f_{gross}
MAR (141°–134° W)	27±5	0.18±0.04	0.18±0.10	0.13±0.02	12° S–138° W	0.18	0.19
HNL (133°–123° W)	18±4	0.08±0.04	0.04±0.06	0.02±0.02	9° S–136° W	0.09	0.10
SPG (123°–101° W)	11±2	0.05±0.03	0.008±0.007	0.007±0.007	26° S–114° W	0.05	0.06
EGY (100°–81° W)	16±4	0.12±0.07	0.07±0.03	0.04±0.03	32° S–91° W	0.11	0.11
UPW (80°–72° W)	44±28	0.27±0.08	0.69±0.49	0.53±0.47	34° S–73° W	0.27	0.28

of marine environments (Ward et al., 1984; Codispoti and Christensen, 1985; Ward and Zafiriou, 1988), observations in the open ocean are rare (Lomas and Lipschultz, 2006). Nitrification rates presented here (Table 2) are in the range of previous observations (e.g. Ward and Zafiriou, 1988; Bianchi et al., 1994; Raimbault et al., 1999; Fernandez and Raimbault, 2007). Regeneration processes were active throughout the water column, even in the oligotrophic waters of the SPG and were largely able to sustain the phytoplanktonic demand in the photic layer (Table 2). Rates measured in the top 100 m suggest that about 80 to 100% of nitrate uptake in the surface waters was supported by nitrification in the SPG. This result confirms the significant role of nitrification in the upper layers of oceanic waters, as previously reported in the equatorial Pacific, where 20 to 100% of the total nitrate demand was fueled by nitrification (Raimbault et al., 1999) and 142% at station ALOHA (Dore and Karl, 1996). The role of nitrification in providing regenerated nitrate in the euphotic zone has been confirmed by recent findings in the Atlantic, as well as in the Mediterranean Sea, where nitrification can support 25 to 100% of the new production (Diaz and Raimbault, 2000; Fernandez and Raimbault, 2007). A logical conclusion is that there could be overestimations in new production data when all the nitrogen regeneration processes are not taken into account. In terms of integrated values over the photic layer, overestimation was only significant in the SPG, where rates of new nitrate assimilation were lower than expected, even close to zero. Consequently, average f-ratio values were very low in the SPG but there were no zero values since significant new production was sustained by N_2-fixation, which appeared as the only source of new nitrogen in the upper layer of the SPG (Fig. 8).

Along the investigated area, primary production remained quantitatively related to new production (Fig. 9), revealing that a threefold increase in total production in oligotrophic waters would result in a tenfold increase of new production. Such a relationship between primary production and new production corresponds with the data of Dugdale et al. (1992) and Raimbault et al. (1999) for the equatorial Pacific (until 16° S), but were much lower (up to 10 fold) than those estimated by the model of Eppley and Peterson (1979). Moreover, in this very oligotrophic water, exhibiting strong nutrient gradients, there was no evident pattern between nitrate concentration and new production, as suggested by Platt and Harrison (1985). When mean f-ratio values are plotted against integrated primary productivity values there is a positive relationship (Fig. 10). The initial linearly slope (0.0003) for new production <500 mg m^{-2} d^{-1} ($\approx$200 mg m^{-2} y^{-1}) is close to that of Dugdale et al. (1992) for the equatorial Pacific (0.00063±0.00036), but much lower than the 0.0025 proposed by Eppley and Peterson (1979). The model proposed by Eppley and Peterson (1979) does not work in these oceanic waters, as nitrate uptake in coastal regions is very different from that in oligotrophic waters.

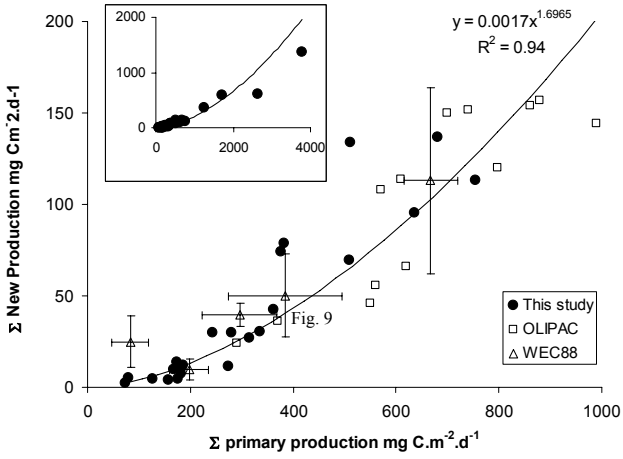

Fig. 9. Plot of integrated new production (ΣNew production) versus integrated primary production (Σprimary production). OLIPAC data are from Raimbault et al. (1999); WEC 88 data are from Dugdale et al. (1992). The power relationship is calculated from data of this study (dark points). Insert shows present data for primary production higher than 1000 mg C m^{-2} d^{-1}.

The f-ratio provides an indirect estimation of export rates of particulate organic matter toward the deep ocean only when suitable time scales are considered. This concept is

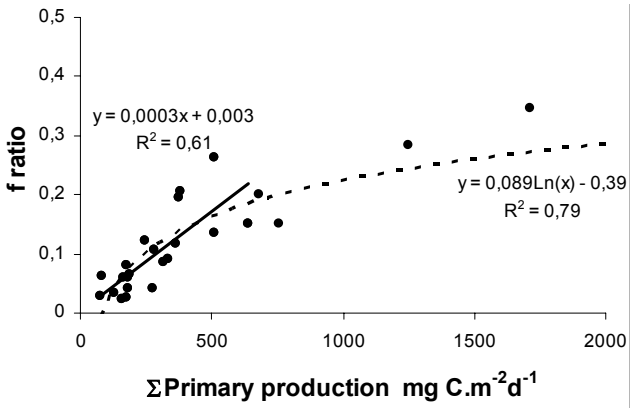

Fig. 10. Plot of mean f-ratio (f-ratio) versus integrated primary production (Σprimary production). Dashed curve is the best relationship calculated for all the data. Straight line is the linear relationship calculated for primary production lower than 500 mg C m^{-2} d^{-1}.

supported by the assumption that the input by advection and diffusion of nitrate towards the surface should be balanced by the losses of particulate and dissolved matter to the deep sea. Sinking fluxes of POC (0.14–1.15 mg C m^{-2} d^{-1}) and PON (0.04–0.2 mg N m^{-2} d^{-1}) measured in the SPG by sediment traps (J. C. Miquel, personal communication) appear to be much lower than the new production rates obtained using tracer uptake experiments (7–8 mg C m^{-2} d^{-1}; 1.7 mg N m^{-2} d^{-1}). There are several reasons to explain this discrepancy. The main one is the export of dissolved organic matter (by vertical mixing or horizontal advection) which can exceed fluxes of sinking particles and thus appear as the major fate of new production (Copin-Montégut-and Avril, 1993; Peltzer and Hayward, 1996). According to Toggweiler (1989), the most realistic balance is obtained when half of the new production sustained by upwelled nutrients goes into a pool of dissolved organic compounds. In fact, our data demonstrated that a significant part of the DIN taken up was lost as dissolved organic nitrogen (DON) during the 24-h incubation experiments. It is difficult to ascertain whether the ^{15}N-tracer detected in the DON pool was transferred solely by the direct and active release from living phytoplankton cells. The ^{15}N excess enrichment in the DON pool may have resulted from cell rupture, by sloppy-feeding, and from cell analysis due to viral infection (Bronk et al., 1994; Procter and Fuhrman, 1990). Pooling all available data, DI^{15}N ultimately found in the extracellular DON pool (ρ_{loss}) represented, on average, 13 to 15% of the nitrate and ammonium, respectively. Values were often higher in the SPG, up to 46% in the subsurface layer. It appears that the ρ_{net}:ρ_{gross} ratios were in the range of values found in literature, i.e. between the low loss rates (<15%) found in the equatorial Pacific (Raimbault et al., 1999, 2000) and the very high ones (74%) found in the Southern California Bight by Bronk and Glibert (1991). They are also in the same order of magnitude as those found in the Mediterranean Sea during spring (Diaz and Raimbault, 2000) and in the Atlantic Ocean (Fernandez and Raimbault, 2007).

However, one should keep in mind that ρ_{loss} does not represent the total flux of nitrogen from the particulate organic matter (PON) in the DON pool (Slawyk et al., 2000). The true DON release (ρ_{DON}) is greater than our measured loss of DIN (ρ_{loss}). Quantification of ρ_{DON} depends on the ^{15}N enrichment in the intracellular DON ($R_{\text{DON}i}$). $R_{\text{DON}i}$ is not experimentally accessible, but it is related to the initial ^{15}N enrichment DIN pool (R_{DIN}), according the relationship defined by Raimbault et al. (2000),

$$\rho_{\text{DON}} = \rho_{\text{loss}} \times R_{\text{DIN}}/R_{\text{DON}i}. \quad (10)$$

Due to of the dilution of the ^{15}N tracer by intracellular nitrogen during uptake, $R_{\text{DIN}}/R_{\text{DON}i}$ is greater than 1. This relationship indicates that; 1) the total fluxes of organic nitrogen from particulate matter to extracellular DON would be higher than the loss of tracer in the form of DON, as measured here and 2) the lower the final enrichment of the intracellular DON pool, the greater the difference between DIN loss and DON release. Assuming phytoplanktonic growth rates calculated from the residence time of chlorophyll-containing particles (Raimbault et al., 2007), the $R_{\text{DIN}}/R_{\text{DON}i}$ ratio would be 3 to 6 folds higher in the SPG than in the productive regions (Marquesas Islands and Chilean upwelling). Consequently, we can hypothesize that production of DON was proportionally much higher in the oligotrophic waters than in productive systems, and consequently, this process could be a significant way for export production in the SPG.

The availability of high-precision isotope ratio mass spectrometers, combined with sensitive field tracer methods (Montoya et al., 1996) enable $^{15}N_2$-tracer incubations to be carried out on unconcentrated natural water samples, with minimal disturbance of the system. However, measurements of N_2-fixation in oceanic waters are rare and the data reported here are the first available for the South Pacific. The iron supply from atmospheric dust deposition at the sea surface has been hypothesized to favour N_2-fixation in the areas influenced by the continents, e.g. the Atlantic Ocean. N_2-fixation, due to high phosphorus requirements, could lead to a decrease in the soluble reactive phosphorus until a shift from N to P limitation occurs, as hypothesized by Béthoux and Copin-Montégut (1986) and Karl et al. (2002) for the Mediterranean Sea and the North Pacific Oceanic, respectively. Elevated N to P in the dissolved inorganic is often considered as indicative of N_2 fixation (Grüber and Sarmiento, 1997). From this point of view, the waters of the South Pacific, characterized by very low dust depositions (Mahowald et al., 1999) and by having sufficient phosphorus concentrations and very low nitrate/phosphate ratios (Raimbault et al., 2007) do not seem to be favourable regions for N_2-fixers. Nevertheless, significant rates were measured throughout the transect, confirming results from recent work using ocean

circulation modelling (Deutsch et al., 2007). N_2-fixation was the weakest, but the most stable biological process along the 8000-km investigated area, in spite of large deviations in nutrient biomass and primary productivity. In the SPG, N_2-fixation (0.06 mmoles m^{-2} d^{-1}) was essentially located in the upper layer (0–50 m), where irradiance was higher and atmospheric deposition, even though very weak in this region, was more readily available. The highest N_2-fixation rates (0.09–0.11 mmoles m^{-2} d^{-1}) were observed around the Marquesas Islands and in the Chilean upwelling (Table 2). The most surprising feature was the high N_2-fixation rates found in the cold and nutrient-replete waters along the Chilean coast, since nitrogen fixation is commonly associated with certain cyanobacteria, essentially *Trichodesmium* sp., that inhabit the warm and nutrient-depleted subtropical waters (Capone et al., 2005; Mahaffey et al., 2005). But this observation confirms the result of Deutsch et al.'s model (2007) that provides evidence that biological fixation could also be intimately linked with marine nitrogen removal in the South Pacific Ocean.

Unfortunately, the organisms responsible for this process were not identified in this study. The organisms responsible for N_2 fixation are taxonomically, physiologically, and ecologically diverse, including bacteria (phototrophs, heterotrophs, chemolithotophs) heterocystous and nonheterocystous cyanobacteria and Archeae (Karl et al., 2002). But much of the oceanic research about N_2 fixation has been focused on the filamentous cyanobacteria Trichodesmium (Capone et al., 2005). However, due to the absence of diazotrophic *Trichodesmium* populations during this cruise, we can postulate that N_2-fixation was executed by nanoplanktonic and picoplanktonic organisms, recently revealed by new molecular biological techniques (Zehr et al., 1998, 2000) and by direct measurements (Montoya et al., 2004; Garcia et al., 2007; Biegala and Raimbault, 2008). The discovery of potentially important marine diazotrophs other than *Trichodesmium* opens up a new area when studying the importance of nitrogen fixation in the ocean. Due to their ability to fix N, these nanoplanktonic cyanobacteria can contribute substantially to the input of new nitrogen into nutrient-depleted waters, even if the rates of fixation measured are typically quite low relative to the apparent N demand of the ecosystem. Our integrated values (30–910 μmol m^{-2} d^{-1}) are the same order of magnitude as found in the literature concerning dinitrogen fixation by nanoplankton, with the exception of the high value (3955 μmol m^{-2} d^{-1}) found along the Australian coasts by Montoya et al. (2004). Zehr et al. (2001) give an integrated value of 92 μmol m^{-2} 12 h^{-1} for the North Pacific. This value is a little higher than those measured by Montoya et al. (2004) in the same region (24–66 μmol m^{-2} d^{-1}). Similar results have been found in the Southwest Pacific around New Caledonia (Garcia et al., 2007), with nitrogen fixation occurring in the $<10\,\mu$m fraction, i.e. not associated with *Trichodeminum* populations, ranging from 40–300 μmol m^{-2} d^{-1}. Falcon et al. (2004) found a daily range of 62–167 μmol m^{-2} d^{-1} for the north Atlantic. Recent equivalent rates ($\approx$50 μmol m^{-2} d^{-1}) were obtained in the Mediterranean Sea during the oligotrophic summer period (Garcia et al., 2006), representing up to 40% of new production. These small nanoplanktonic cyanobacteria were not observed during the BIOSOPE cruise. But high density populations of phycoerythrin containing cyanobacteria were detected along the transect, with a maximum being observed in the Chilean upwelling, and most of these cyanobacteria (up to 50% in the SPG) forming colonies (Masquelier and Vaulot, 2007). Thus, as suggested by these authors, it is tempting to hypothesize that these colonial cyanobacteria could be the organisms responsible of the observed N_2-fixation. N_2-fixation sustained a large fraction of new production (up to 100%) in the oligotrophic SPG and this nitrogen pool represents a potentially important nitrogen source for other organisms in the pelagic food web, especially where any input of other forms of new nitrogen is excluded. The light $\delta^{15}N$ isotopic signal observed in the SPG (Raimbault et al., 2007) suggests that nitrogen fixation provides a local dominant supply of nitrogen to phytoplankton in this isolated region, where the vertical flux of nitrate from below the thermocline is extremely low due to the deep thermocline. Finally, our observations confirmed the global distribution diagnosed by Deutsch et al. (2007), providing evidence that elevated N_2-fixation rates are closely linked to areas of denitrification, as encountered along the Peruvian and Chilean coasts. According to the model of Deutsch et al. (2007), annual fixation rates in the SPG and in the Chilean upwelling range from 40–160 mmoles m^{-2} y^{-1} and 20–120 mmoles m^{-2} y^{-1}, respectively. Our direct measurements, assuming no seasonal variation, are at the low end of these ranges (22 ± 12 mmoles m^{-2} y^{-1} and 33 ± 22 mmoles m^{-2} y^{-1}, respectively). The basin scale N_2-fixation for the South Pacific Gyre, representing 1.1% of the global oceanic surface (4.10^6 km^2), is estimated to be about 1.34×10^{12} g N y^{-1} and corresponds to about 1% of the global N_2-fixation calculated by Deutsch et al. (2007). Finally, our direct estimates of N_2 fixation for the entire South Pacific basin (21.10^{12} g yr^{-1}) was two folds lower than expected by Deutsch et al. (2007). This apparent inconsistency could be due to uncertainties regarding ocean circulation and precise nutrient distribution (Deutsch et al., 2007) and also seasonal variations of N_2-fixation as observed in the southwest and North Pacific (Garcia et al., 2007; Dore et al., 2002). Nevertheless, our basin-scale budget are close to recent estimates for the North Atlantic Ocean (4.2 to 85×10^{12} g $yr^{-}1$, complied in Hansell et al., 2007), considered as an important region of N_2 fixation given the enhanced inputs of atmospheric dust from the Sahara. This indicates that the South Pacific Ocean would provide an ideal ecological niche for the proliferation of N_2-fixers (still unknown), even if our "snapshot" estimates of N_2-fixation rates were lower than could be expected from the ocean circulation model. Finally, oceanic N-fixation is a

process which is more important than initially thought and not restricted to the warm- and nutrient-depleted tropical areas. This source of new nitrogen will support net production and export of organic matter from the euphotic zone, with a direct effect on the carbon cycle. This fact leads to relevant consequences in relation to the N_2 fixation–climate feedback hypothesis proposed by Michaels et al. (2001). Clearly, the budget of N_2 fixation remains highly uncertain and so more widespread research needs to be carried out on marine N-fixers in order to quantify their role in the global oceanic biogeochemistry cycle.

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