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FEATURE ARTICLE

Assimilation and regeneration of inorganic nitrogen in a coastal upwelling system: ammonium and nitrate utilization

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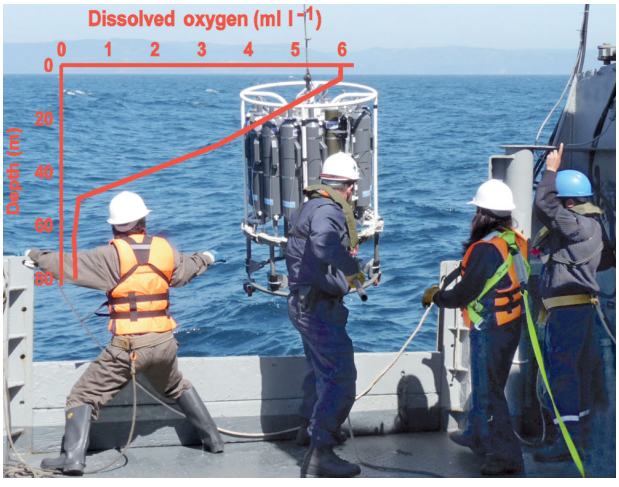
ABSTRACT: The main processes involved in nitrogen cycling (as NO_3^- and NH_4^+ assimilation and their regeneration) were studied using N-tracer experiments in the coastal upwelling system off central Chile (36° S). The study area shows seasonal surface fertilization and the development of subsurface hypoxia in austral spring and summer. The rates of NO_3^- uptake during active upwelling were 5 times higher than in non-upwelling seasons. Uptake of NH_4^+ was almost half that of NO_3^- uptake rates during upwelling periods, and similar to NO_3^- uptake rates in the absence of upwelling. Nitrification experiments showed higher rates during active, compared to non-active, upwelling seasons. NH_4^+ oxidation was coupled with NO_2^- oxidation in near bottom (suboxic) waters, while in the oxycline (hypoxic water), total fluxes of NO_3^- regeneration via nitrification resulted from higher activity of NO_2^- oxidation compared to NH_4^+ oxidation. On the other hand, archaeal NH_4^+ oxidation had the potential for processing a large fraction of NH_4^+ and could therefore co-occur with bacterial NO_2^- oxidation. NH_4^+ utilization in this coastal upwelling is thus in the same range as NO_3^- assimilation. NH_4^+ oxidation is affected by oxygen concentration in the water column, leading to occasional decoupling of NH_4^+ and NO_2^- oxidation, particularly at the oxycline. This study gives preliminary evidence of the importance of archaeal–bacterial interactions in the nitrification process and highlights the role of ammonium in fueling annual primary production in coastal upwelling systems.

KEY WORDS: Coastal upwelling · Nitrogen uptake · Ammonium oxidation · Archaea



Oxygen concentrations decrease rapidly in the water column off central Chile, and this modulates the activity of microbial communities involved in the nitrogen cycle.

Photo: C. Fernandez

INTRODUCTION

Research on nitrogen (N) cycling in marine ecosystems has been largely influenced by the conceptual partitioning between new and regenerated production, in which primary production supported by new N should be quantitatively equal to the organic material exported below the surface ocean (Dugdale & Goering 1967, Eppley & Peterson 1979).

New N is mainly represented by nitrate (NO_3^-), which is injected into the euphotic zone by mixing and vertical advection (upwelling), whereas ammo-

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nium (NH_4^+), the most common form of 'regenerated' N, is mainly released during bacterial remineralization of dissolved organic matter (DOM) in the water column (Bronk 2002, Mopper & Kieber 2002) as well as by zooplankton excretion (Alcaraz et al. 1994).

NO_3^- regeneration was not included in this balance and considered for long time as negligible for N fluxes in surface waters (Dugdale & Goering 1967). However, it is now widely recognized that nitrification plays an important role in surface N budgets through the stepwise oxidation of NH_4^+ into nitrite (NO_2^-) and NO_3^- (Yool et al. 2007).

The production of NO_3^- via nitrification results from a coupling between NH_4^+ and NO_2^- oxidizers in the marine environment, but the variability of this biogeochemical coupling is not fully understood. Both groups use oxygen as an electron acceptor and inorganic carbon as a carbon source and obtain their reducing power from NH_4^+ and NO_2^-, respectively.

Aerobic NH_4^+ oxidation includes NH_4^+ oxidizing bacteria (AOB) as well as NH_4^+ oxidizing Crenarchaeota (AOA; Könneke et al. 2005), while the oxidation of NO_2^- is performed only by nitrite oxidizing bacteria (NOB). Niche partitioning among nitrifying communities is beginning to be studied in detail; it is likely to be determined by the physiological characteristics of the community as a function of substrate and oxygen concentration. AOA possess a lower half-saturation constant and substrate threshold compared to bacteria, which indicates an enhanced capacity to adapt to oligotrophic conditions. This would confer to archaea a competition advantage against heterotrophic bacteria and phytoplankton in open ocean systems (Martens-Habbena et al. 2009). Also, AOA are particularly active in oxygen-deficient nutrient-rich waters and can be more abundant than AOB in coastal upwelling systems (Molina et al. 2010).

Nitrification could therefore play an important biogeochemical role in the ocean, not only as a remineralization process but also by supplying inorganic nutrients for photosynthesis and electron acceptors for NO_3^- reduction-denitrification in suboxic and hypoxic conditions such as those found in upwelling systems and Oxygen Minimum Zones (OMZ).

The coastal upwelling system off central Chile (36°S) shows significant rates of primary and secondary production (Daneri et al. 2000, Montero et al. 2007). Active upwelling occurs in austral spring and summer, when intensification of the south and southwest winds drives the upwelling of Equatorial Subsurface Water (ESSW) with high NO_3^- and low O_2 concentrations (Sobarzo & Djurfeldt 2004). During late autumn and winter, non-active upwelling conditions are observed, when northerly winds intensely mix Sub-Antarctic Water (SAAW), rich in dissolved O_2, causing biological production to slow down. The water column in this area presents distinct chemical gradients, which are separated by the oxycline into at least 2 layers: an oxygenated and illuminated layer, and an oxygen-limited, non-illuminated subsurface water where important accumulations of NH_4^+, NO_2^-, and N_2O (nitrous oxide) may result from the coupling of chemo- and heterotrophic processes. The water column also shows gradients in the richness of AOB and AOA populations, indicating the presence of differentiation niches along the oxygen and nutrient gradients. Recent observations also suggest that chemosynthetic processes such as nitrification and methane oxidation contribute significantly to of the carbon fixation and affect the cycling of nutrients throughout the water column (Farías et al. 2009b).

The objective of this study was to estimate autotrophic NO_3^- and NH_4^+ uptake in the coastal upwelling area of central Chile, taking the entire microbial community into consideration. We also studied the biogeochemical interaction between N uptake and regeneration, with special emphasis on the coupling within the nitrifying community (including AOA, AOB, and NOB). We report seasonal survey data and process-targeted experiments carried out during the spring–summer upwelling season.

MATERIALS AND METHODS

Study area and sampling strategy

Sampling was carried out monthly at the COPAS time series Stn 18 (36° 30.8′ S, 73° 07.75′ W) on board the RV 'Kay-Kay II' of the Universidad de Concepción. This observation site (92 m bottom depth, Fig. 1) was visited between December 2006 and May 2008. Hydrographic data (temperature, salinity, O_2, fluorescence) were obtained using a CTD (Seabird 25) with an O_2 probe and fluorescence and photosynthetically active radiation (PAR) sensors (e.g. optical sensor Satlantic for PAR) attached to a rosette. The depth of the euphotic zone was considered as the depth down to which irradiation is equivalent to 1% of its surface value based on the attenuation coefficient of downwelling scalar irradiance in the PAR region (400–700 nm waveband).

Dissolved oxygen concentrations were determined through the Winkler method using a semi-automatic system (AULOX) developed at the University of Concepción (LabPROFC). Samples for nutrient determi-

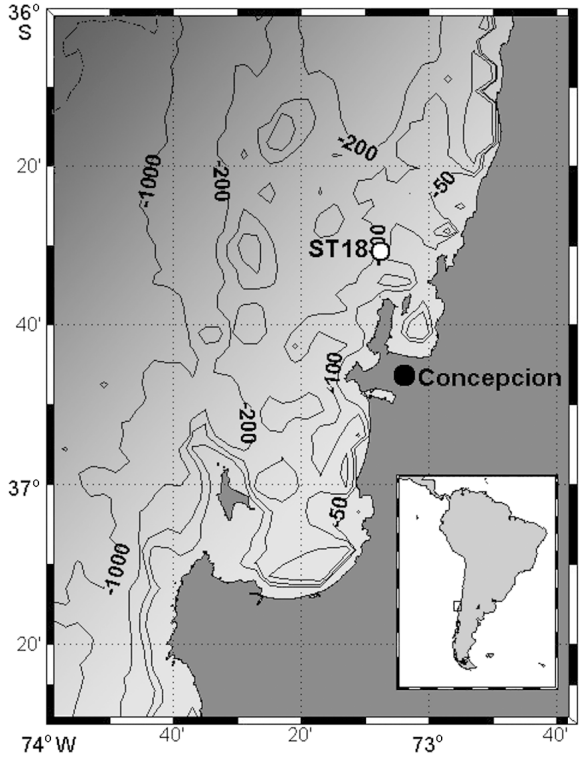

Fig. 1. Study area in the coastal upwelling system off central Chile

nation were taken and frozen in duplicate for laboratory analysis. Seawater samples were filtered (0.7 µm, GF/F) on board and stored frozen until analysis. Concentrations of dissolved NO_2^-, NO_3^-, and PO_4^{3-} (phosphate) were determined using standard manual colorimetric techniques following Grasshoff et al. (1983). For NH_4^+ concentrations, samples (in triplicate) were taken directly from the Niskin bottle in 50 ml Pyrex (Duran Schott) flasks. Each sample (40 ml) received 10 ml of working solution. Samples were then stored in the dark for 2 h and analyzed by the fluorometric method (Holmes et al. 1999) using a Turner design® fluorometer. The precision of NO_3^-, NO_2^-, and NH_4^+ in terms of the CV was better than ±10, ±3, and ±5%, respectively.

In addition to the time series program sampling, a process-oriented cruise was performed in January 2008 during the NICCHEX I cruise (RV 'Kay Kay II') during active upwelling conditions. During this cruise (immediately after the monthly sampling of Stn 18 by the COPAS program), water samples were taken from 2 depth levels: 30 m, which corresponds to the oxycline, and 80 m, which corresponds to near-bottom waters (free from the effect of particulate organic matter re-suspension as well as NH_4^+ efflux from the sediments; Farías et al. 2004). The samples were used for biogeochemical incubations using ^{15}N tracers and specific inhibitors as outlined in Table 1.

^{15}N uptake and regeneration experiments

Incubation experiments were performed monthly using an *in situ* mooring line at Stn 18. Samples were taken in acid-cleaned polycarbonate bottles (600 ml) at 5 depth levels (5, 15, 30, 50, and 80 m). Samples were amended with ^{15}N-labeled substrate and incubated *in situ* for 8 to 12 h (dawn to dusk). Incubations were terminated by gentle vacuum filtration (<100 mm Hg) through pre-combusted GF/F filters (450°C for 12 h). Filters were then dried at 60°C for 24 h and stored at constant temperature until laboratory analysis by continuous-flow isotope ratio mass spectrometry (IRMS; Finnigan Delta Plus).

^{15}N tracer additions were performed as $^{15}NH_4Cl$ (99% at 0.5 µmol ml^{-1}) or $K^{15}NO_3$ (99% at 0.5 µmol ml^{-1}). Samples taken for nutrient ambient concentrations were analyzed after the *in situ* experiments. The 10 yr old time series Stn 18 (COPAS) data set available allowed us to estimate the necessary tracer addition. Final tracer concentrations were variable but often close to 10% of ambient concentration. At low initial NH_4^+ concentrations (<50 nmol l^{-1}), minimum tracer additions as $^{15}NH_4Cl$ (99% at 0.5 µmol ml^{-1}) resulted in initial enrichments (T_0) exceeding 50%. In such cases, rates should be considered as potential uptake. It is important to note,

Table 1. Experimental setup for targeted experiments carried out during the NICCHEX I cruise at 30 and 80 m depth. NH_4ox: ammonium oxidation; NO_2ox: nitrite oxidation; denitrific.: denitrification

Targeted process	Treatment	Inhibitor	Expected result	Source
Nitrite oxidation	$^{15}NO_2$	Allylthiourea (ATU)	Inhibition of NH_4ox	Ginestet et al. (1998)
Ammonium oxidation	$^{15}NH_4$	Sodium azide (NaN_3)	Inhibition of NO_2ox and denitrific.	Ginestet et al. (1998)
Bacterial ammonium oxidation	$^{15}NH_4$	GC7	Inhibition of archaeal biosynthesis	Jansson et al. (2000)
Net ammonium oxidation	$^{15}NH_4$	No inhibitor	Net nitrification control	Slawyk & Raimbault (1995)
Control	$^{15}NH_4$	$HgCl_2$	Negative control	

however, that ambient NH_4^+ concentrations were generally above the detection limit and could often exceed 0.5 µmol l^{-1}. In contrast, high NO_3^- concentrations allowed keeping ^{15}N tracer additions as $K^{15}NO_3$ (99% at 0.5 µmol ml^{-1}) close to 10% of ambient values. Subsamples were taken directly from the incubation bottle before filtration and analyzed for determination of final NO_3^- and NH_4^+ concentrations.

The transport rate of ^{15}N-labeled dissolved inorganic N (DIN) to the particulate organic N (PON) pool, i.e. the net DIN uptake (ρDIN, nmol l^{-1} d^{-1}) was computed according to Eq. 1:

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

where R_{PON} and R_{DIN} represent the ^{15}N atom percent excess enrichment in the PON and DIN pools, and [PON] represents the final PON concentration; T represents the duration (h) of the incubation. The measurement of isotopic enrichment in the DIN pool provides a check on the isotope dilution of the ^{15}N tracer due to DIN regeneration and thus corrects for underestimation of DIN uptake rates. To correct NH_4^+ uptake rates for isotopic dilution (caused by *in situ* regeneration of organic matter during incubation), we made R_{DIN} in Eq. (1) equal to the mean value between initial and final enrichment value (R_{NH_4}). Uptake rates are expressed as daily rates, taking the standard length of a solar day (12 h) into account (hourly rate × 12).

NH_4^+ regeneration (rNH_4) and net nitrification (rNO_3) were measured for all $^{15}NH_4$ incubations by a triple diffusion isotopic method (Slawyk & Raimbault 1995, Raimbault et al. 1999). After ending the $^{15}NH_4$ incubations, 300 ml filtrates were recovered in Duran Schott flasks and amended with 1 ml $HgCl_2$ (6 g l^{-1}). This procedure does not affect the extraction efficiency and prevents losses of NH_4^+ by freezing the sample. Filtrates of $^{15}NH_4$ incubations were used to measure the final ^{15}N enrichment in the DIN pool, as outlined by Slawyk & Raimbault (1995). By removing all forms of DIN from the sample as $(NH_4)_2SO_4$, this procedure allows estimating sequentially the final ^{15}N enrichment of the DIN pool ($DIN^{15}N$) and the isotope dilution of the tracer due to NH_4^+ regeneration, then to estimate net nitrification via NH_4^+ oxidation to NO_2^- + NO_3^- and finally the loss of tracer as DON during the incubation process. Filters recovered after each step were dried at 60°C and analyzed by IRMS. The same procedure was applied to samples amended with allylthiourea (ATU) at a final concentration of 86 µmol l^{-1} (Ginestet et al. 1998). ATU inhibits NH_4^+ oxidation in AOB. However, it has not been well studied in AOA. Therefore, if AOB (and perhaps also AOA) are active in a water sample, samples amended with ATU are expected to accumulate $^{15}NH_4^+$ during incubation in the absence of NH_4^+ oxidation.

Net nitrification rates (rNO_3, µmol l^{-1} d^{-1}) were computed according to Eq. (2):

$$\mathrm{rNO_3} = \left(\frac{R_{\mathrm{NO_3}}}{R_{\mathrm{NH_4}} \times T}\right) \times [\mathrm{NO_3}] \quad (2)$$

where R_{NO_3} is the ^{15}N atom percent excess enrichment in the (NO_3^- + NO_2^-) pool, R_{NH_4} is the mean ^{15}N atom percent excess enrichment of the NH_4^+ pool, and [NO_3] is the final NO_3^- concentration in the sample. Net rNH_4^+ was accounted for as specified in previous surveys in the area (Raimbault & Garcia 2008) and according to Eq. (3):

$$\mathrm{rNH_4} = \frac{[\mathrm{NH_4}]_0 + [\mathrm{NH_4}]_{\mathrm{F}}}{2T} \times \ln\left(\frac{R_{0(\mathrm{NH_4})}}{R_{\mathrm{F}(\mathrm{NH_4})}}\right) \quad (3)$$

where $[NH_4]_0$ and $[NH_4]_F$ represent the initial and final concentrations of NH_4^+ during the incubation experiment, respectively. The terms $R_{0(NH_4)}$ and $R_{F(NH_4)}$ represent the initial and final excess enrichments in $^{15}NH_4^+$ during the incubation period.

Aerobic NH_4^+ oxidation experiments

The vertical structure of the water column in the study area adds methodological complexities to our experimental setup, since some coupling between oxidative and reductive processes can occur under low oxygen conditions (i.e. NH_4^+ and NO_2^- oxidation with NO_3^- and NO_2^- reduction). To assess such difficulties, a series of experiments with amended substrates and specific inhibitors was carried out during the NICCHEX I cruise (Nitrogen and Carbon Chemosynthetic Experiment, January 2008, active upwelling season).

Incubations were planned with samples retrieved at 2 depth levels, 30 m (oxycline) and 80 m (near-bottom water). These depth levels are representative of hypoxic (O_2 < 2 ml l^{-1}) and suboxic (O_2 < 0.2 ml l^{-1}) conditions within the aphotic layer (according to PAR profiles). For that reason, all incubations were performed in duplicate for periods of 12 h (which included T_0, T_0+6 h, and T_0+12 h) and in dark and *in situ*-simulated temperature conditions (using a Velp® incubator). Changes in NH_4^+ and NO_2^- + NO_3^- concentrations during the incubation were followed in the same experiment in order to estimate the net

consumption of NH_4^+ and production of NO_2^- with time.

The targeted processes for these experiments are listed in Table 1. Net nitrification (or rNO_3 rate, performed by the total microbial community, which may include bacteria and archaea) was evaluated with $^{15}NH_4$ amendment as described in the previous section. On the other hand, gross and net NH_4^+ and NO_2^- oxidation were also evaluated separately (hereafter referred as NH_4ox and NO_2ox, respectively). To this end, samples were amended with $^{15}NH_4$ or $^{15}NO_2$ in order to recover $^{15}NO_2$ + $^{15}NO_3$ (via diffusion as described in the previous section). In addition, blockage of NH_4ox (as a control) was obtained in samples amended with $^{15}NH_4$ and ATU (see previous section). A further treatment was designed in order to evaluate bacterial nitrification in $^{15}NH_4$-amended samples, using an archaeal inhibitor (N1-guanyl-1,7-diaminoheptane, GC7) previously used in the study area (Levipan et al. 2007), which acts by arresting biosynthesis (Jansson et al. 2000). Samples amended with $^{15}NH_4$ were also treated with sodium azide (NaN_3) at a final concentration of 24 µmol l^{-1} (Ginestet et al. 1998) in order to block NO_2^- oxidation and disassimilative NO_3^- reduction, which should lead to an accumulation of NO_2^- while oxidation of NH_4^+ proceeds (also reflected by high $^{15}NO_2$ + $^{15}NO_3$ recovery). This approach was used to estimate NH_4ox. In all cases, separate rates of NH_4^+ and NO_2^- oxidation obtained by diffusion and rates obtained by nutrient evolution represent the difference between net rates of nitrification and rates obtained in inhibitor-amended samples. We acknowledge that the effect of each inhibition treatment cannot be warranted at 100% efficiency. Therefore, in some cases, rates of NH_4^+ or NO_2^- oxidation may proceed to some extent after inhibitor addition. As additional controls, amended and un-amended samples were systematically tested after the addition of ^{15}N-tracers, using $HgCl_2$.

Separated NH_4^+ and NO_2^- oxidation rates were estimated by the difference between the rates obtained from ATU-amended treatments and the net nitrification rate (Molina & Farias 2009):

$$NH_4ox = rNO_3 - Control - NH_4ox + ATU \quad (4)$$

NO_2ox rates were estimated by the difference between net nitrification rates and rates in inhibitor-amended samples:

$$NO_2ox = rNO_3 - Control - NH_4ox + azide \quad (5)$$

Potential NH_4ox rates by archaea were obtained by the difference between net nitrification and rates obtained from GC7-amended samples.

Because of the large number of incubated bottles and samples generated, this experiment was not replicated on a monthly basis, as was the case for our time series uptake and regeneration experiments (see previous section).

RESULTS

Seasonal oceanographic conditions

The variability of temperature, oxygen, and nutrients at the study area is shown in Figs. 2 & 3. During spring and summer (December 2006 to February 2007; September 2007 to February 2008), sea surface temperature (SST) oscillated between 13 and 14°C, and maintained those values down to 20 m depth, where the thermocline was located. Temperatures reached 11°C in near-bottom waters. The winter temperature distribution (May to July 2007) showed homogeneous values around 11 to 12°C throughout the water column.

Dissolved oxygen concentrations in the water column also varied seasonally (Fig. 2b). Maximum oxygen levels were observed in surface waters in winter time (>5 ml l^{-1}) and decreased to surface values <3 ml l^{-1} in spring and summer. During austral spring and summer, oxygen concentrations can be lower than 0.1 ml l^{-1} in near-bottom waters. The depth of the oxycline varied between 20 and 30 m depth in summer and winter, respectively.

NO_3^- concentrations were always above detection limits (Fig. 3a). Surface concentrations during spring and summer were <5 µmol l^{-1} but increased to >30 µmol l^{-1} through the oxycline and near-bottom waters. Concentrations during winter showed values between 20 and 25 µmol l^{-1} throughout the entire water column.

NH_4^+ concentrations were generally high within the euphotic zone (Fig. 3b); maximum values were observed in spring and summer. The highest values were observed in summer 2007 (maximum 2 µmol l^{-1} at 20 m) and 2008 (maximum 3.5 µmol l^{-1} in surface waters). Near the bottom, concentrations did not exceed 0.5 µmol l^{-1}.

The N:P ratio (the ratio between total inorganic N and PO_4^{3-}) varied between 15:1 and 12:1 (Fig. 3c) throughout the water column from December 2006 to May 2007. However, from August 2007 to February 2008, values in the water column were lower than the Redfield ratio of 16:1 (N:P ~10). Values close to 15 were only observed occasionally in the upper oxycline (Fig. 2b).

Seasonal nitrogen uptake and regeneration

NO_3^- uptake rates (expressed as ρNO_3) showed a seasonal trend with maximum values (average 1500 ± 2000 nmol l^{-1} d^{-1}) in spring and summer and minimum values in autumn and winter (average 100 ± 100 nmol l^{-1} d^{-1}; Fig. 4a). As a general trend, NO_3^- uptake was mainly concentrated in the first 20 to 30 m of the water column, being nearly an order of magnitude higher than rates obtained in subsurface layers.

NH_4^+ uptake rates (ρNH_4, Fig. 4b) throughout the water column showed lower values than ρNO_3 and were also concentrated in the first 30 m of the water column. The seasonal trend for ρNH_4 showed higher values during the spring–summer season (average 226 ± 400 nmol l^{-1} d^{-1}, maximum 1800 nmol l^{-1} d^{-1} in December 2007). However, contrary to what was observed for NO_3^-, NH_4^+ uptake showed high rates during winter, even higher than winter ρNO_3, averaging 133 ± 200 nmol l^{-1} d^{-1} (maximum 435 nmol l^{-1} d^{-1} in May 2007).

Net nitrification (rNO_3, Fig. 5a) was always detected during this study, but rates were higher during spring and summer compared to winter conditions. Values of rNO_3 averaged 74 ± 121 nmol l^{-1} d^{-1} in surface waters (maximum rate 316 nmol l^{-1} d^{-1} concentrated near the oxycline at 30 m in December 2006), while they were close to the detection limit in subsurface waters. Rates in winter averaged 21 ± 22 nmol l^{-1} d^{-1} in the entire water column, with higher rates in surface waters (20 ± 25 nmol l^{-1} d^{-1}) than in deeper waters (9 ± 10 nmol l^{-1} d^{-1}).

After inhibition of ATU-sensitive microorganisms (i.e. AOB and probably also AOA), partial nitrification showed the same pattern of distribution as net nitrification but mildly decreased to an average of 40 ± 79 nmol l^{-1} d^{-1} in the whole water column. Although no significant difference was observed in near-bottom waters, a 30% decrease was observed in NH_4^+ oxidation after ATU addition in surface waters (e.g. 80 versus 68 nmol l^{-1} d^{-1} in April 2008). Nevertheless, a local increase (~50 nmol l^{-1} d^{-1}) was detected in near-bottom waters during April and May 2007 (Fig. 5b).

Net N regeneration in the form of NH_4^+ (rNH_4) showed maximum rates between the surface and the oxycline and often exceeded 500 nmol l^{-1} d^{-1} (Fig. 5c). Average values for spring and summer reached 526 ± 584 nmol l^{-1} d^{-1}, while winter values reached 462 ± 360 nmol l^{-1} d^{-1}. Rates of rNH_4 peaked during spring 2007 and late summer 2008, with maximum rates exceeding 2000 nmol l^{-1} d^{-1} in the euphotic zone during March and April 2008 (Fig. 5c).

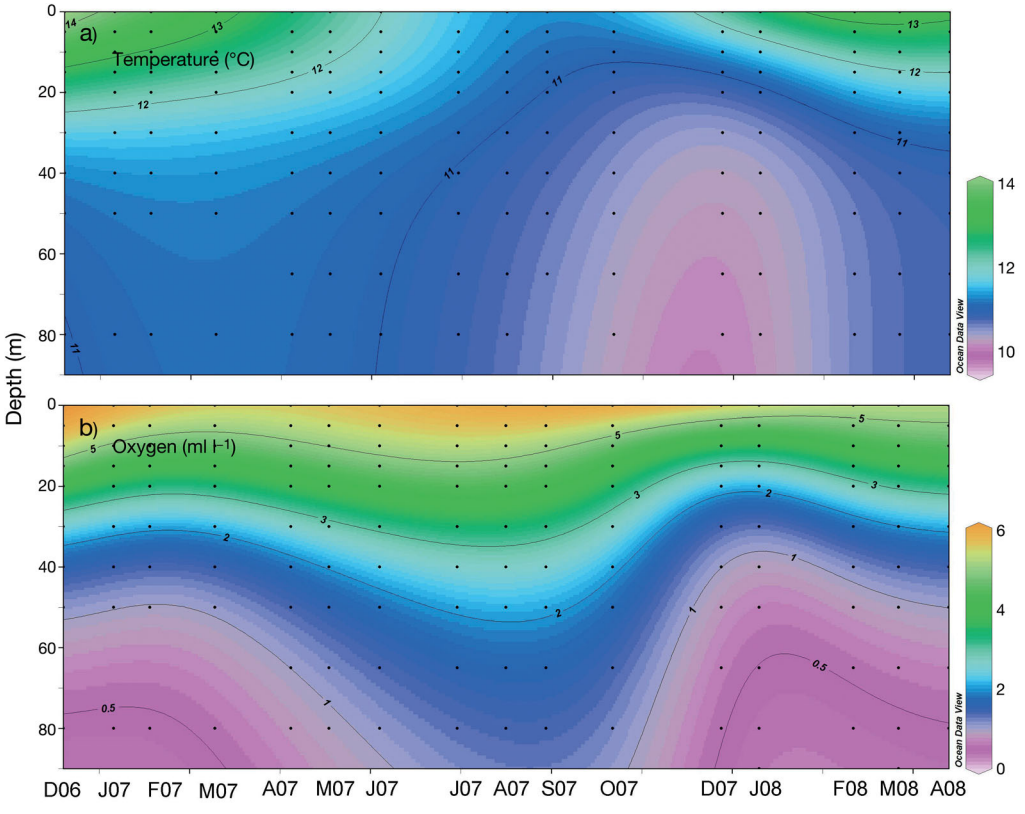

Fig. 2. Hydrographic parameters at time series Stn 18 (COPAS) between December 2006 and April 2008. (a) Temperature, (b) oxygen

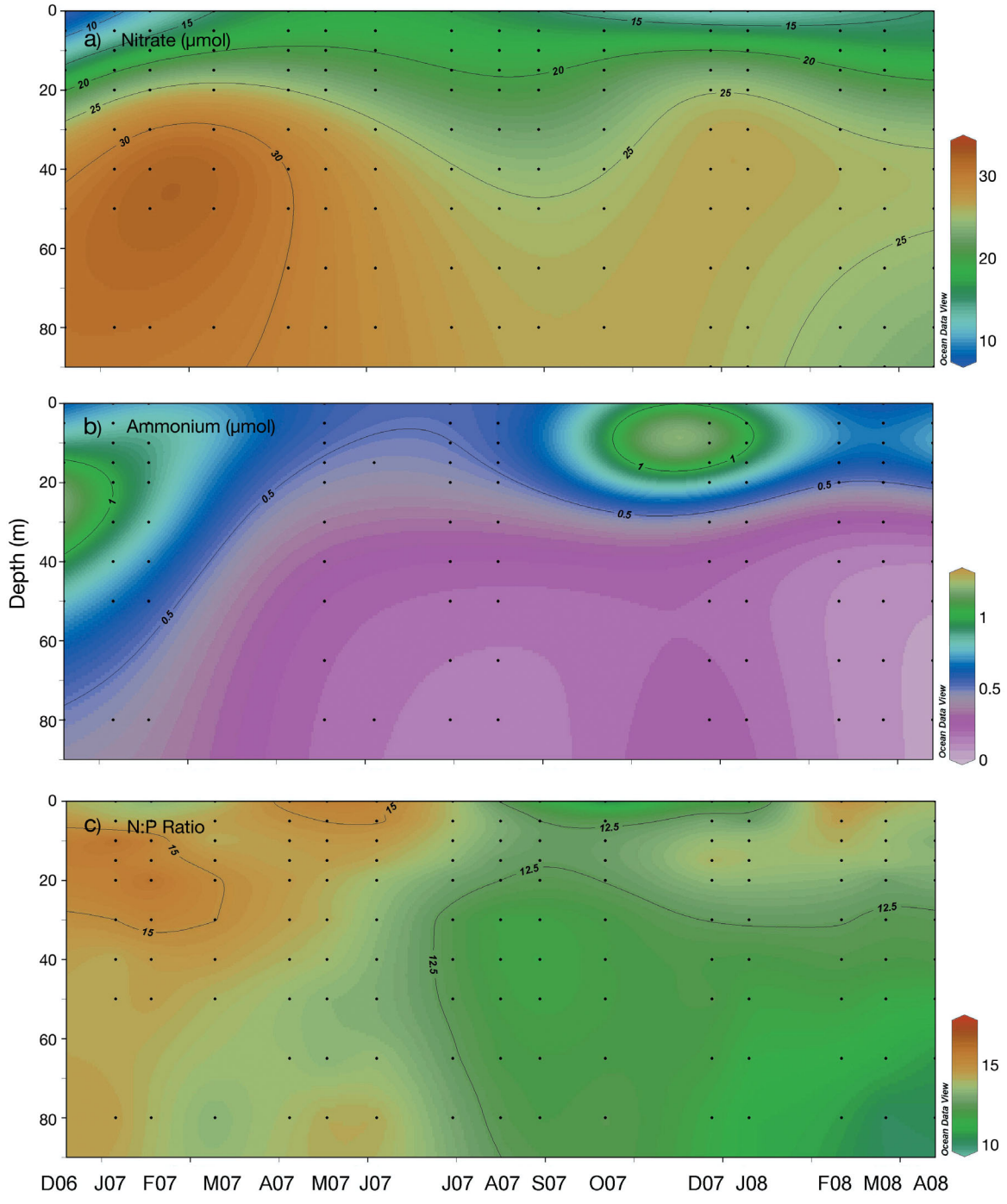

Fig. 3. Time series data of (a) NO_3^- (µmol l^{-1}), (b) NH_4^+ (µmol l^{-1}), and (c) N:P ratio at time series Stn 18 (COPAS) between December 2006 and April 2008

Nitrogen cycling during upwelling conditions

The NICCHEX I cruise was carried out in January 2008, during active coastal upwelling conditions. Surface temperature reached 13.9°C, while values decreased with depth to 10°C in near-bottom waters, indicating the presence of the ESSW (Fig. 6). The thermocline was located near 20 m depth while salinity remained constant around 34.5 in the entire water column. Dissolved oxygen

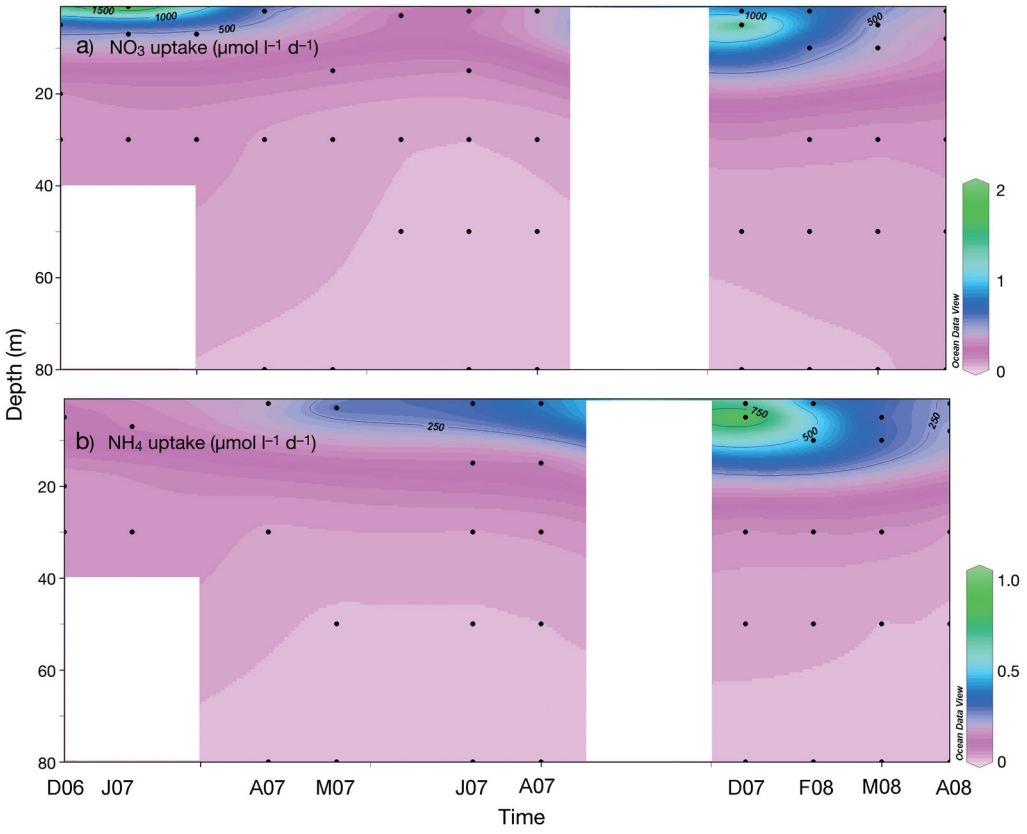

Fig. 4. Uptake rates of (a) NO_3^- and (b) NH_4^+ (in nmol l^{-1} d^{-1}) during the period December 2006 to April 2008 at Stn 18 of the time series program

exceeded 6 ml l^{-1} in surface waters but decreased dramatically with depth. The base of the oxycline was located near 15 m depth, with values close to 1 ml l^{-1}. Oxygen levels continued to decrease to <0.2 ml l^{-1} at 50 m and reached 0.3 ml l^{-1} at 80 m depth.

NO_3^- concentrations (Fig. 7) were close to 0.7 µmol l^{-1} in surface waters, indicating intense utilization by phytoplankton, compared to winter conditions. A sharp nitracline followed, reaching 30 µmol l^{-1} and coincided with the oxycline. NO_3^- concentrations decreased to 16 µmol l^{-1} at 50 m depth coinciding with minimum oxygen conditions and were followed by a 2-fold increase in near-bottom waters.

NO_2^- concentrations were close to 0.1 µmol l^{-1} in surface waters and increased to 0.5 µmol l^{-1} at 10 m depth, forming the primary NO_2^- maximum. A second peak (0.2 µmol l^{-1}) was observed at 70 m depth. NH_4^+ distribution followed the trend observed for NO_2^-, with maximum values (0.7 µmol l^{-1}) at 10 m depth. Concentrations then decreased with depth, although a local accumulation of 0.2 µmol l^{-1} was observed at 70 m depth.

During 12 h incubations using water from 30 m depth, net nitrification ($^{15}NH_4$ with no inhibitor) was detected at low rates (Fig. 8), although NO_2^- and NO_3^- accumulated and NH_4^+ levels remained constant (Table 2). In samples treated with $^{15}NH_4$ and sodium azide (NaN_3, for which NO_2^- oxidation was expected to be inhibited), NO_3^- + NO_2^- accumulated during the last 6 h of incubation. In this case we obtained a rate of net ammonium oxidation (NH_4ox) of 18 nmol l^{-1} h^{-1} (Fig. 8).

In contrast, the rate of change for NO_3^- + NO_2^- in samples where net NO_2ox was evaluated ($^{15}NO_2$ addition combined with ATU, Table 3) showed high net rates of NO_2ox (almost 60 nmol l^{-1} h^{-1}, Fig. 8) while NO_2^- concentrations in the sample decreased (Table 3). Samples amended with $^{15}NH_4$ and treated with GC7 (a treatment expected to inhibit archaeal NH_4^+ oxidation) revealed an accumulation of NO_3^- + NO_2^- that could be attributed to bacterial NH_4^+ oxidation (Table 2).

In experiments with water from 80 m depth, net nitrification rates for $^{15}NH_4$-amended samples were close to 20 nmol l^{-1} h^{-1} (Fig. 8), while nutrient evolution showed decreasing NO_3^- + NO_2^- concentrations

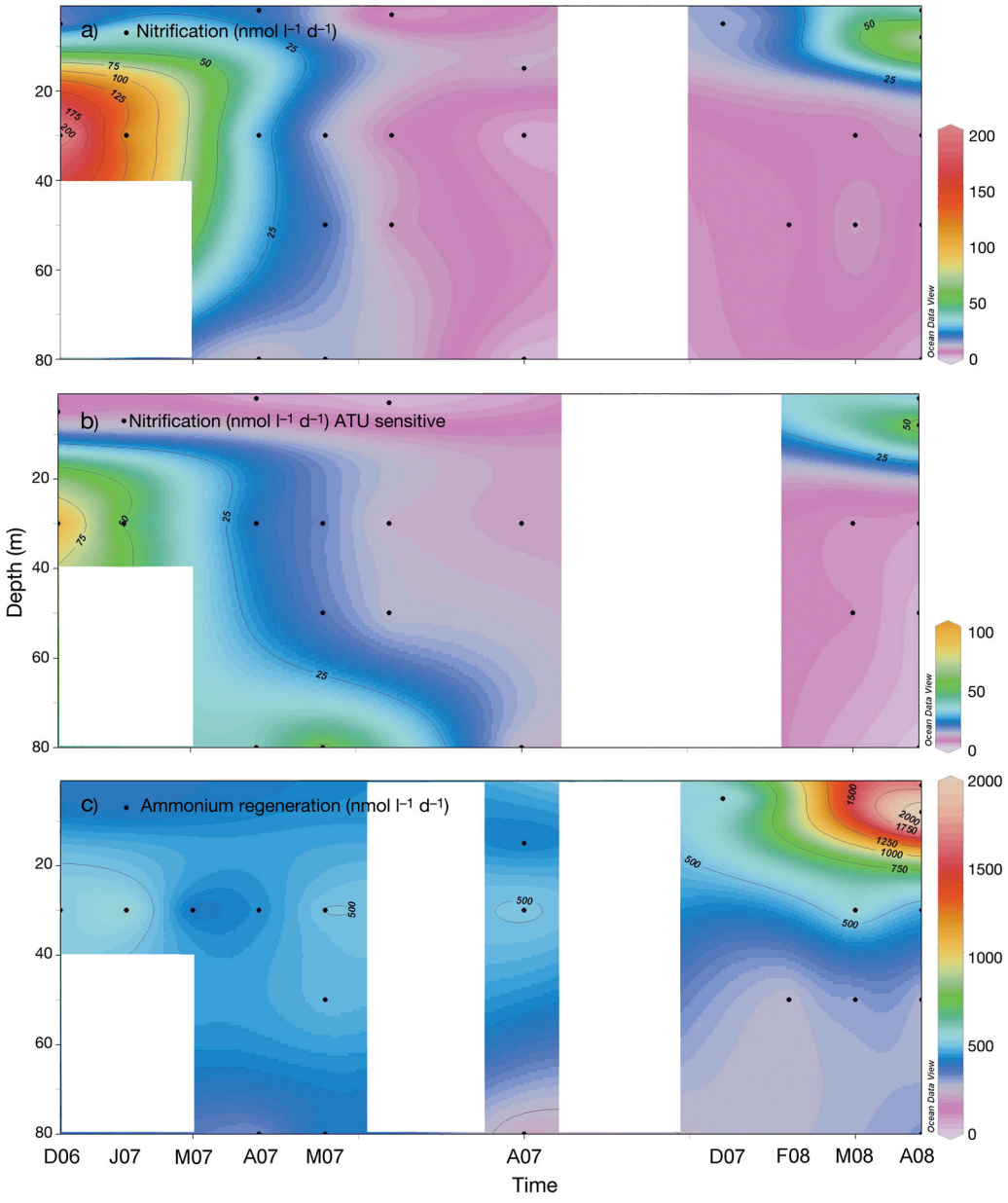

Fig. 5. Regeneration fluxes of NH_4^+ (rNH_4, nmol l^{-1} d^{-1}) and nitrification (rNO_3, in nmol l^{-1} d^{-1}) during the period December 2006 to April 2008. (a) Net nitrification, (b) nitrification after inhibition of allylthiourea (ATU)-sensitive microorganisms, (c) net N regeneration

and NH_4^+ levels showed constant values (Table 2). Net NH_4ox (obtained from samples amended with $^{15}NH_4$ and NaN_3) showed NH_4^+ oxidation rates of 12.5 nmol l^{-1} h^{-1} (Fig. 8). Samples amended with $^{15}NH_4$ and treated with GC7 (AOA inhibition) revealed an accumulation of NO_3^- + NO_2^-, while NH_4^+ decreased (Table 2). They also showed high rates of NH_4ox, exceeding 60 nmol l^{-1} h^{-1} (Fig. 8). This suggests that the bacterial nitrifying community has the potential for high rates of NH_4^+ oxidation, despite the low net rates obtained in the $^{15}NH_4$-only amended control. Concerning NO_2ox, samples amended with $^{15}NO_2$ and ATU showed a net estimated rate of NO_2ox of 15 nmol l^{-1} h^{-1} (Fig. 8), which is almost equivalent to net NH_4^+ oxidation rates for the same depth (14 nmol l^{-1} h^{-1}). In contrast, NO_2^- concentration did not vary significantly during the incubation period (Table 2), showing that consumption of NO_2^- was supported by its constant production (probably via archaeal NH_4^+ oxidation).

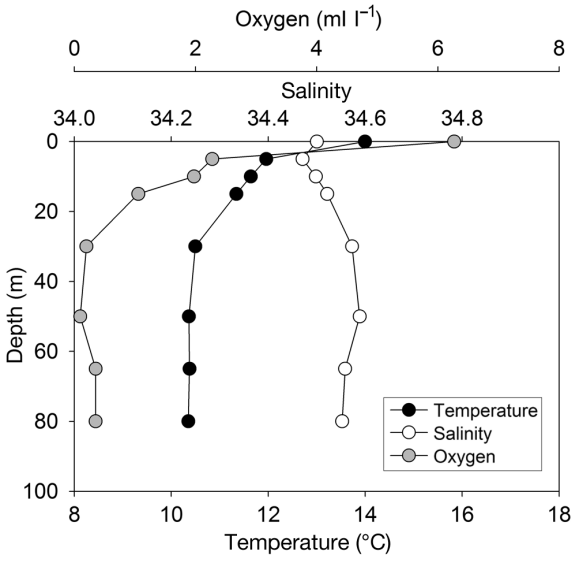

Fig. 6. Hydrographic conditions during the NICCHEX I experiment

DISCUSSION

During our study, the wind patterns and upwelling index off central Chile reflected the seasonality in the study area. Upwelling-favorable conditions were present during austral spring and summer, driven by southerly winds, and were followed by a northerly wind direction during winter (Sobarzo & Djurfeldt 2004). According to measurements which determined the timing of the spring transition and the upwelling period as well as upwelling-favorable winds, the prevailing conditions can be characterized as follows: upwelling from November 2006 to April 2007 and October 2007 to April 2008 (spring and summer), while the periods from May to September 2007 (fall and winter) did not have upwelling-favorable conditions (Galán et al. 2011).

Upwelling led to high levels of NH_4^+ (often exceeding 1 µmol l^{-1}). NO_3^- concentrations also showed very high values throughout the water column that resulted from the upwelling of NO_3^--rich ESSW. The availability of both substrates allowed intense assimilation by photoautotrophs, but also intense recycling via NH_4^+ regeneration and nitrification in the photic and aphotic layers of the water column (see Fig. 5).

Seasonal trends in NH_4^+ and NO_3^- utilization

This study confirms that the coastal upwelling area off Concepción is a highly productive region (Daneri et al. 2000, Farías et al. 2009a,b). The NO_3^- uptake rates reported here are higher than those in the eastern South Pacific Ocean, which can reach 12.5 and 17.5 nmol l^{-1} h^{-1} off Peru and Costa Rica, respectively (Franck et al. 2005, Fernández et al. 2009). Although the spring season of 2007 is under-represented in our data (Fig. 4) and therefore the DIN uptake rates for that season might be underestimated, rates of NH_4^+ uptake presented here are also within the range of previous measurements in the Northeast Pacific (0.32 to 1.38 nmol l^{-1} h^{-1} in winter and 1.2 to 2.95 nmol l^{-1} h^{-1} in spring; Varela & Harrison 1999).

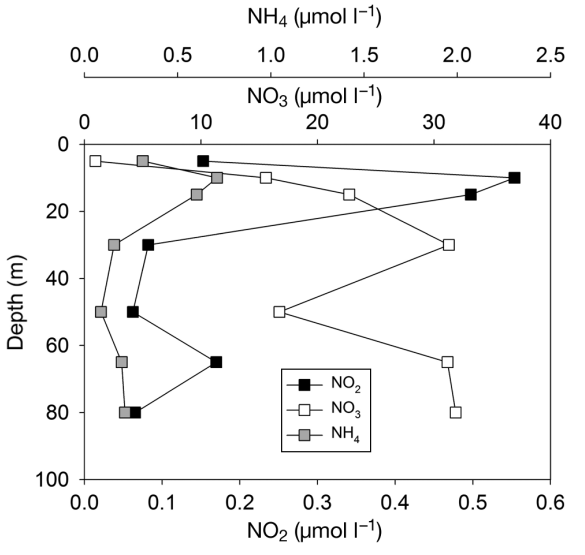

Fig. 7. Nutrient profiles of initial conditions for NICCHEX I experiments

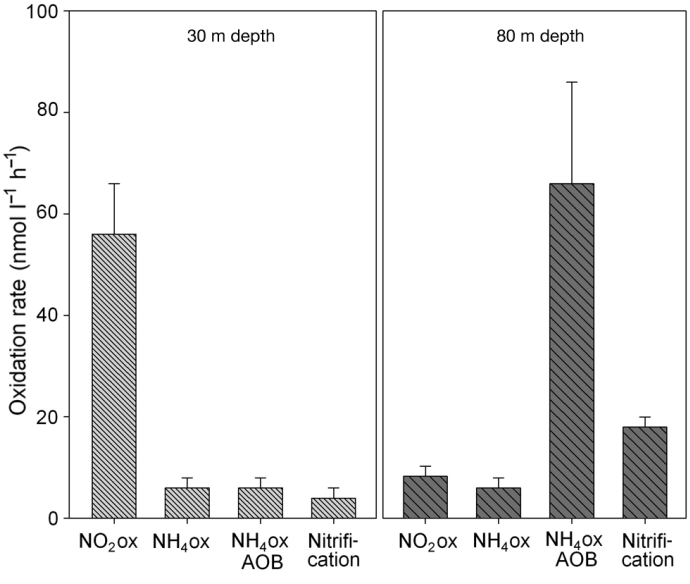

Fig. 8. Fluxes of net nitrification obtained through stable isotope assays. AOB: NH_4^+ oxidizing bacteria, NH_4ox: NH_4^+ oxidation; NO_2ox: NO_2^- oxidation

Table 2. Evolution of nutrient concentrations during tracer incubations carried out during the NICCHEX I experiments. Rates are reported for experiments carried out at 30 and 80 m depth. Values are reported as mean ± SD. NA: not applicable

Depth (m)	Treatment (tracer/inhibitor)	NO_3^- + NO_2^- concentration (µmol l^{-1})			NH_4^+ concentration (µmol l^{-1})		
		T_0	T_0 + 6 h	T_0 + 12 h	T_0	T_0 + 6 h	T_0 + 12 h
30	$^{15}NH_4$	20.14 ± 0.28	28.32 ± 0.23	28.97 ± 0.23	0.02	0.03 ± 0.02	0.02 ± 0.02
	$^{15}NH_4$ /azide	29.22 ± 0.25	18.74 ± 0.098	26.35 ± 0.011	0.058	0.003 ± 0	0.002 ± 0
	$^{15}NH_4$ /azide/GC7	24.9 ± 0.06	NA	32 ± 11.5	0.02	NA	3.68
80	$^{15}NH_4$	25.98 ± 0.08	26.21 ± 0.067	16.12 ± 0.23	2.56	4.05 ± 0.39	3.66 ± 0.085
	$^{15}NH_4$ /azide	20.23 ± 0.11	22.75 ± 0.61	26.69 ± 0.25	2.56	2.844 ± 0.45	2.95 ± 1.023
	$^{15}NH_4$ /azide/GC7	23.9	NA	22.61 ± 3.035	2.56	NA	2.09

Table 3. Evolution of NO_2^- concentrations during tracer incubations carried out during the NICCHEX I experiments. Values are reported as mean ± SD. ATU: allylthiourea

Depth (m)	Treatment (tracer/inhibitor)	NO_3^- + NO_2^- concentration (µmol l^{-1})			NO_2^- concentration (µmol l^{-1})		
		T_0	T_0 + 6 h	T_0 + 12 h	T_0	T_0 + 6 h	T_0 + 12 h
30	$^{15}NO_2$/ATU	27.11 ± 0.47	26.69 ± 0.47	19.42 ± 0.33	3.21	3.12 ± 0.02	0.19 ± 0.14
30	$^{15}NO_2$/ATU	26.75 ± 0.1	28.53 ± 0.38	22.45 ± 0.22	3.49±0.2	3.5 ± 0.2	3.6 ± 0.5

Maximum uptake of NO_3^- and NH_4^+ occurred during spring and summer, in accordance with the intensification of coastal upwelling and the subsequent increased availability of nutrients and chlorophyll *a* (chl *a*; Galán et al. 2011). Rates of surface ammonium assimilation (ρNH_4) were lower than NO_3^- uptake during summer, but very closely approached the values of ρNO_3 concentrations during the winter season (maximum 435 nmol l^{-1} d^{-1} for ρNH_4 versus 354 nmol l^{-1} d^{-1} ρNO_3, Fig. 4). The chl *a* levels reported for the study area for the same period (20 to 40 mg m^{-2} for winter versus 60 to 300 mg m^{-2} for summer; Galán et al. 2011) also support the idea that this system is sustained by regenerated production rather than by new production during the non-upwelling periods of the year. These observations suggest that total primary production levels encountered off central Chile are related not only to NO_3^- assimilation, but are also linked to concurrent and subsequent NH_4^+ uptake by phytoplankton, a feature that also characterizes other coastal systems (Dugdale et al. 2007). We cannot rule out that other groups intervene in the competition for NH_4^+ utilization in the euphotic zone in this system: picoeukaryots and fungi are potentially important components of the microbial community in the eastern South Pacific (Grob et al. 2007, Gutierrez et al. 2010), but more information is necessary to determine their role in the local N cycle.

DIN uptake during winter and spring is linked to N regenerating processes occurring simultaneously in the water column. Indeed, NH_4^+ regeneration activity was high and sustained during the entire year (Fig. 5), representing a constant source of substrate for photoautotrophic assimilation (Bode et al. 2004). Our data on NH_4^+ production by *in situ* microbial regeneration (exceeding 1 µmol l^{-1} d^{-1}) showed higher fluxes compared to the capacity of nitrification to remove NH_4^+ in the upper water column (Fig. 5), leading to strong sporadic NH_4^+ accumulation. Furthermore, NH_4^+ regeneration fluxes could support the demand of phytoplankton and picoplankton (archaea and bacteria) for N (maximum rates close to 1.8 µmol l^{-1} d^{-1} during spring 2007), which highlights the biogeochemical importance of this process.

Although it is less intense compared to NH_4^+ regeneration, an important fraction of NH_4^+ utilization in this study can be attributed to nitrifying activity via NH_4^+ oxidizers. This process showed significant rates in the entire water column, particularly during spring and summer 2006 to 2007 and late summer 2008, but was mostly constrained to the oxycline and suboxic layers (Fig. 5), suggesting a non-negligible effect of dissolved oxygen concentrations on the intensity of this flux.

Our findings confirm studies in the eastern South Pacific, which showed that nitrification is an active process with maximum significance during spring and summer (Fernández et al. 2009). In particular, nitrifying activity might have the potential for removing up to 700 nmol l^{-1} d^{-1} of NH_4^+ off central Chile (Molina & Fariás 2009), supporting our view of the importance of NH_4^+ oxidizers (bacteria and archaea) for channeling NH_4^+ into chemosynthetic pathways. Regeneration of NH_4^+ is concurrent with intermittent

nitrification fluxes in surface and subsurface waters, with intense vertical variability that might be related to oxygen dynamics.

NH_4^+ and NO_2^- oxidation

In oxygen-deficient waters, nitrification can act as a critical process linking regeneration of N to its eventual loss as N_2 or N_2O via denitrification and anammox. Nitrification is carried out by 2 independent steps involving NH_4^+ and NO_2^- oxidation. It has long been considered as a tightly coupled process where the rate of NH_4^+ oxidation is equivalent to the rates of NO_2^- oxidation and NO_3^- production, therefore avoiding significant NO_2^- accumulation. However, field data provide increasing evidence of uncoupled functioning of nitrification in upwelling areas (Clark et al. 2011). Factors influencing the interaction of the 2 steps of nitrification (NH_4^+ and NO_2^- oxidation) and the capacity of the former to sustain the latter include variables such as light (Guerrero & Jones 1996a,b), pH, substrate availability (Grundle & Juniper 2011), and oxygen (Ward 2008). In the case of central Chile, hypoxic conditions prevail during the most productive season of the year, and oxygen levels in the water column can seasonally change from oxic in winter to suboxic (≤11 µmol l^{-1} O_2) or even anoxic levels in spring and summer. The response of nitrifying communities to such oxygen gradients might explain the variability in nitrification rates observed during this study.

Low oxygen levels can benefit nitrifying communities, as these often consist of microaerophiles. However, the specific effect of oxygen on nitrifying microorganisms has mostly been studied in NH_4^+ oxidizers. Carlucci & McNally (1969) and Goreau et al. (1980) found that aerobic AOB could remain active and even increase their growth rates and the ratio of N_2O/NO_2^- production at low O_2 concentrations. On the other hand, Ginestet et al. (1998) estimated kinetic parameters of both NH_4^+ and NO_2^- oxidation by oxygen depletion due to substrate consumption in pure cultures, and concluded that NH_4^+ oxidizers are more tolerant to low oxygen levels than NO_2^- oxidizers. The determination of the biomass index based on the substrate utilization rate revealed that low levels of O_2 doubled the growth yield of ammonium oxidizers, therefore compensating for the reduced specific substrate utilization. In contrast, NO_2ox was strongly inhibited by 0.5 mg l^{-1} of O_2, with no increase in growth yield.

In this study, NO_2ox decreased significantly as oxygen levels dropped, whereas NH_4ox did not show differences at different oxygen levels. At the oxycline, rates of NO_2^- oxidation seem to be uncoupled from NH_4^+ oxidation (Fig. 8), with NO_2^- oxidizers having a higher potential for producing NO_3^- than NH_4^+ oxidizers for producing NO_2^- (see Tables 2 & 3). The difference between rNO_3 and NO_2ox could also reflect that NO_2^--producing processes (such as denitrification or excretion by phytoplantkton) sustain NO_2^- oxidation, as has been suggested for other systems (Clark et al. 2011).

In near-bottom waters, on the other hand, NH_4^+ and NO_2^- oxidation fluxes seem to be coupled within the bacterial community despite low oxygen levels (Fig. 8 and Tables 2 & 3). Sporadic NO_2^- accumulations at this depth might result from the interaction of nitrification with denitrification and anammox occurring at high rates, as shown by data from sediment and bottom water in the study area (Farías et al. 2004, Galán et al. 2011).

Concerning nutrient availability as a controlling factor in nitrification coupling, nitrifying communities such as *Nitrosococcus oceani* may not depend on the direct release of NH_4^+ to survive and might be able to reduce urea into NH_4^+ in order to produce NO_2^- (Klotz et al. 2006). Concentrations of urea are high off central Chile, ranging between 0.2 and 1 µmol l^{-1} in winter and spring, respectively (Pérez-Aragón et al. 2011). Therefore, it is possible that NH_4^+-oxidizing communities use alternative substrates in this system.

Community composition also needs to be taken into account when analyzing these results, as the coupling between NOB and AOA has not yet been demonstrated experimentally. A first indication of efficient coupling between these communities comes from our experiments, which proved that a fraction of the ambient NH_4^+ was being oxidized by AOA (Fig. 5). Further evidence comes from a study carried out in the North Pacific, where a correlation was found between the distribution of close relatives of the NO_2^- oxidizer *Nitrospina* and AOA, which suggested possible interactions between these groups (Mincer et al. 2007), while a tight correlation was also observed between NO_2^- distribution and AOA activity (Beman et al. 2010). Therefore, it is possible that the coupling of both steps of nitrification (NH_4^+ and NO_2^- oxidation) can be carried out independently of the composition of the community of nitrifiers (i.e. the percentage of archaea within NH_4^+ oxidizing microorganisms in the sample).

Role of NH_4^+ oxidizing archaea and bacteria

The vertical variability of NH_4^+ and NO_2^- oxidation in the Chilean upwelling system seem to be related to the main groups intervening in DIN regeneration, and to the relative importance of Crenarchaeota aerobic NH_4^+ oxidizers (Könneke et al. 2005). As reported by recent studies in the area (Molina et al. 2010, Belmar et al. 2011), retrieved sequences of *amoA* (the gene involved in NH_4^+ oxidation) and cell counts indicate that Crenarchaeota could be highly abundant and diverse in Oxygen Minimum Zones of northern Chile. Furthermore, although the physiology of this community is not well understood, it is known that they are particularly active (based on qPCR assays) under oxygen-deficient, nutrient-rich conditions and can be equal or more abundant than AOB in this system (Molina et al. 2010). According to previous studies using GC7 as an AOA inhibitor (Farías et al. 2009b), their influence is also expected to be high in hypoxic waters off central Chile.

Some archaea have higher affinity for NH_4^+ than most AOB (Martens-Habbena et al. 2009), which would give this group a comparative advantage in NH_4^+ utilization. This is confirmed by our process-oriented experiments that allowed separately approaching the activity of AOA and AOB, as well as their possible interaction. Our results show that a variable fraction of the observed nitrification fluxes could have been performed by AOA. Also, the inhibition of archaeal ammonium oxidation resulted in high rates of bacterial nitrification in the suboxic near-bottom waters (80 m) as opposed to the oxycline (30 m), where no significant changes were observed (Fig. 8). Based on this, AOA groups might play a distinct role in both oxic and suboxic conditions, as found by Beman et al. (2008) and Santoro et al. (2010). However, bacterial NH_4^+ oxidation might benefit from low oxygen concentrations, as shown by high fluxes in near-bottom suboxic waters (Fig. 8).

In summary, this study suggests that the seasonal upwelling system off central Chile is a highly productive region, in which primary production is sustained by NO_3^- as well as NH_4^+ uptake in summer and winter. Also, DIN regeneration via nitrification is intense and is highly influenced by oxygen levels, which determine the capacity of the nitrifying community (NH_4^+ and NO_2^- oxidizers) to act at equivalent rates. Archaeal NH_4^+ oxidizers are potentially important players in oxic and oxycline conditions, while bacterial NH_4^+ oxidation is important in suboxic layers.

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