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 (NO₃⁻), which is injected into the euphotic zone by mixing and vertical advection (upwelling), whereas ammonium (NH₄⁺) is derived from oceanic remineralization. The partitioning between these two fractions has been the subject of many studies, and the balance between them is determined by the rates of NO₃⁻ and NH₄⁺ assimilation and regeneration in the surface ocean (Dugdale & Goering 1967, Eppley & Peterson 1979).
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 archaia a competition advantage against heterotrophic bacteria and phytoplankton in open ocean systems (Martens-Habbema 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 aural 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$_2$O (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 the carbon fixation and affect the cycling of nutrients throughout the water column (Farias 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 sensorSatlantic 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-
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 NICCLEX 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; Farias 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$_4$Cl (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$_4$Cl (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,

![Fig. 1. Study area in the coastal upwelling system off central Chile](image)

<table>
<thead>
<tr>
<th>Targeted process</th>
<th>Treatment</th>
<th>Inhibitor</th>
<th>Expected result</th>
<th>Source</th>
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<tr>
<td>Nitrite oxidation</td>
<td>NO$_2^-$</td>
<td>Allylthiourea (ATU)</td>
<td>Inhibition of NH$_4$ox</td>
<td>Ginestet et al. (1998)</td>
</tr>
<tr>
<td>Ammonium oxidation</td>
<td>NH$_4^+$</td>
<td>Sodium azide (NaN$_3$)</td>
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<tr>
<td>Bacterial ammonium oxidation</td>
<td>NH$_4^+$</td>
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<td>Inhibition of archaeal biosynthesis</td>
<td>Jansson et al. (2000)</td>
</tr>
<tr>
<td>Net ammonium oxidation</td>
<td>NH$_4^+$</td>
<td>No inhibitor</td>
<td>Net nitrification control</td>
<td>Slawyk &amp; Raimbault (1995)</td>
</tr>
<tr>
<td>Control</td>
<td>NH$_4^+$</td>
<td>HgCl$_2$</td>
<td>Negative control</td>
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</table>

Table 1. Experimental setup for targeted experiments carried out during the NICCLEX I cruise at 30 and 80 m depth. NH$_4$ox: ammonium oxidation; NO$_2$ox: nitrite oxidation; denitrific.: denitrification
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 (pDIN, nmol l$^{-1}$ d$^{-1}$) was computed according to Eq. 1:

$$p\text{DIN} = \frac{R_{\text{PON}}}{R_{\text{DIN}} \times T} \times [\text{PON}]$$  (1)

where $R_{\text{PON}}$ and $R_{\text{DIN}}$ 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 $R_{\text{DIN}}$ in Eq. (1) equal to the mean value between initial and final enrichment value ($R_{\text{NH4}}$). Uptake rates are expressed as daily rates, taking the standard length of a solar day (12 h) into account (hourly rate $\times$ 12).

NH$_4^+$ regeneration ($r\text{NH4}$) and net nitrification ($r\text{NO3}$) 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$_4$)$_2$SO$_4$, 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 ($r\text{NO3}$, µmol l$^{-1}$ d$^{-1}$) were computed according to Eq. (2):

$$r\text{NO3} = \left(\frac{R_{\text{NO3}}}{R_{\text{NH4}} \times T}\right) \times [\text{NO3}]$$  (2)

where $R_{\text{NO3}}$ is the $^{15}$N atom percent excess enrichment in the (NO$_3^-$ + NO$_2^-$) pool, $R_{\text{NH4}}$ is the mean $^{15}$N atom percent excess enrichment of the NH$_4^+$ pool, and [NO3] 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):

$$r\text{NH4} = \left[\frac{[\text{NH4}]}{2T} \times \ln\left(\frac{R_{\text{NO3}}}{R_{\text{NH4}}}\right)\right]$$  (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 $R_{\text{NO3}}$ and $R_{\text{NH4}}$ 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$_3^-$ concentrations during the incubation were followed in the same experiment in order to estimate the net
consumption of \( \text{NH}_4^+ \) and production of \( \text{NO}_2^- \) with time.

The targeted processes for these experiments are listed in Table 1. Net nitrification (or \( r\text{NO}_3^- \) rate, performed by the total microbial community, which may include bacteria and archaea) was evaluated with \( ^{15}\text{NH}_4 \) amendment as described in the previous section. On the other hand, gross and net \( \text{NH}_4^+ \) and \( \text{NO}_2^- \) oxidation were also evaluated separately (hereafter referred as \( \text{NH}_4\text{ox} \) and \( \text{NO}_2\text{ox} \), respectively). To this end, samples were amended with \( ^{15}\text{NH}_4 \) or \( ^{15}\text{NO}_2 \) in order to recover \( ^{14}\text{NO}_2 + ^{15}\text{NO}_3 \) (via diffusion as described in the previous section). In addition, blockage of \( \text{NH}_4\text{ox} \) (as a control) was obtained in samples amended with \( ^{15}\text{NH}_4 \) and ATU (see previous section). A further treatment was designed in order to evaluate bacterial nitrification in \( ^{15}\text{NH}_4 \)-amended samples, using an archaeal inhibitor (N1-guanyl-1,7-diaminoheptane, GC7) previously used in the study area (Leviperan et al. 2007), which acts by arresting biosynthesis (Jansson et al. 2000). Samples amended with \( ^{15}\text{NH}_4 \) were also treated with sodium azide (\( \text{NaN}_3 \)) at a final concentration of 24 µmol l\(^{-1} \) (Ginestet et al. 1998) in order to block \( \text{NO}_2^- \) oxidation and disassimilative \( \text{NO}_3^- \) reduction, which should lead to an accumulation of \( \text{NO}_2^- \) while oxidation of \( \text{NH}_4^+ \) proceeds (also reflected by high \( ^{15}\text{NO}_2 + ^{15}\text{NO}_3 \) recovery). This approach was used to estimate \( \text{NH}_4\text{ox} \). In all cases, separate rates of \( \text{NH}_4^+ \) and \( \text{NO}_2^- \) 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 \( \text{NH}_4^+ \) or \( \text{NO}_2^- \) oxidation may proceed to some extent after inhibitor addition. As additional controls, amended and un-amended samples were systematically tested after the addition of \( ^{15}\text{N} \)-tracers, using \( \text{HgCl}_2 \).

Separated \( \text{NH}_4^+ \) and \( \text{NO}_2^- \) oxidation rates were estimated by the difference between the rates obtained from ATU-amended treatments and the net nitrification rate (Molina & Farias 2009):

\[
\text{NH}_4\text{ox} = r\text{NO}_3^- - \text{Control} - \text{NH}_4\text{ox} + \text{ATU}
\]  

\( \text{NO}_2\text{ox} \) rates were estimated by the difference between net nitrification rates and rates in inhibitor-amended samples:

\[
\text{NO}_2\text{ox} = r\text{NO}_3^- - \text{Control} - \text{NH}_4\text{ox} + \text{azide}
\]  

Potential \( \text{NH}_4\text{ox} \) rates by archaea were obtained by the difference between net nitrification rates 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.

\( \text{NO}_3^- \) 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.

\( \text{NH}_4^+ \) 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 \( \text{N}:\text{P} \) ratio (the ratio between total inorganic N and \( \text{PO}_4^{3-} \)) 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 (\( \text{N}:\text{P} \approx 10 \)). Values close to 15 were only observed occasionally in the upper oxycline (Fig. 2b).
Seasonal nitrogen uptake and regeneration

$\text{NO}_3^-$ uptake rates (expressed as $\rho_{\text{NO}_3}$) showed a seasonal trend with maximum values (average $1500 \pm 2000 \text{ nmol l}^{-1} \text{ d}^{-1}$) in spring and summer and minimum values in autumn and winter (average $100 \pm 100 \text{ nmol l}^{-1} \text{ d}^{-1}$; Fig. 4a). As a general trend, $\text{NO}_3^-$ 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.

$\text{NH}_4^+$ uptake rates ($\rho_{\text{NH}_4}$, Fig. 4b) throughout the water column showed lower values than $\rho_{\text{NO}_3}$ and were also concentrated in the first 30 m of the water column. The seasonal trend for $\rho_{\text{NH}_4}$ showed higher values during the spring-summer season (average $226 \pm 400 \text{ nmol l}^{-1} \text{ d}^{-1}$, maximum $1800 \text{ nmol l}^{-1} \text{ d}^{-1}$ in December 2007). However, contrary to what was observed for $\text{NO}_3^-$, $\text{NH}_4^+$ uptake showed high rates during winter, even higher than winter $\rho_{\text{NO}_3}$, averaging $133 \pm 200 \text{ nmol l}^{-1} \text{ d}^{-1}$ (maximum $435 \text{ nmol l}^{-1} \text{ d}^{-1}$ in May 2007).

Net nitrification ($r_{\text{NO}_3}$, Fig. 5a) was always detected during this study, but rates were higher during spring and summer compared to winter conditions. Values of $r_{\text{NO}_3}$ averaged $74 \pm 121 \text{ nmol l}^{-1} \text{ d}^{-1}$ in surface waters (maximum rate $316 \text{ nmol l}^{-1} \text{ d}^{-1}$ 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 $21 \pm 22 \text{ nmol l}^{-1} \text{ d}^{-1}$ in the entire water column, with higher rates in surface waters ($20 \pm 25 \text{ nmol l}^{-1} \text{ d}^{-1}$) than in deeper waters ($9 \pm 10 \text{ nmol l}^{-1} \text{ d}^{-1}$).

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 $40 \pm 79 \text{ nmol l}^{-1} \text{ d}^{-1}$ in the whole water column. Although no significant difference was observed in near-bottom waters, a 30% decrease was observed in $\text{NH}_4^+$ oxidation after ATU addition in surface waters (e.g. 80 versus $68 \text{ nmol l}^{-1} \text{ d}^{-1}$ in April 2008). Nevertheless, a local increase (~$50 \text{ nmol l}^{-1} \text{ d}^{-1}$) was detected in near-bottom waters during April and May 2007 (Fig. 5b).

Net N regeneration in the form of $\text{NH}_4^+$ ($r_{\text{NH}_4}$) showed maximum rates between the surface and the oxycline and often exceeded $500 \text{ nmol l}^{-1} \text{ d}^{-1}$ (Fig. 5c). Average values for spring and summer reached $526 \pm 584 \text{ nmol l}^{-1} \text{ d}^{-1}$, while winter values reached $462 \pm 360 \text{ nmol l}^{-1} \text{ d}^{-1}$. Rates of $r_{\text{NH}_4}$ peaked during spring 2007 and late summer 2008, with maximum rates exceeding $2000 \text{ nmol l}^{-1} \text{ d}^{-1}$ in the euphotic zone during March and April 2008 (Fig. 5c).
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
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\(_4^{ox}\)) 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\(_2^{ox}\) was evaluated (\(^{15}\)NO\(_2\) addition combined with ATU, Table 3) showed high net rates of NO\(_2^{ox}\) (almost 60 nmol l\(^{-1}\) h\(^{-1}\), Fig. 8) while NO\(_3^−\) 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
and NH$_4^+$ levels showed constant values (Table 2). Net NH$_4$ox (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$_4$ox, 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$_2$ox, samples amended with $^{15}$NO$_2$ and ATU showed a net estimated rate of NO$_2$ox of 15 nmol l$^{-1}$ h$^{-1}$ (Fig. 8), which is almost equivalent to net NH$_4$ox 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). This suggests that consumption of NO$_2^-$ was supported by its constant production (probably via archaeal NH$_4^+$ oxidation).
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, Farias 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).
ity was high and sustained during the entire year linked to concurrent and subsequent NH4+ regeneration (exceeding 1 µmol l−1 d−1) showed higher fluxes compared to the capacity of nitrification to remove NH4+ in the upper water column (Fig. 5), leading to strong sporadic NH4+ accumulation. Furthermore, NH4+ 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 NH4+ regeneration, an important fraction of NH4+ utilization in this study can be attributed to nitrifying activity via NH4+ 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), supporting our view of the importance of NH4+ oxidizers (bacteria and archaea) for channeling NH4+ into chemosynthetic pathways. Regeneration of NH4+ is concurrent with intermittent
nitrification fluxes in surface and subsurface waters, with intense vertical variability that might be related to oxygen dynamics.

**NH₄⁺ and NO₂⁻ oxidation**

In oxygen-deficient waters, nitrification can act as a critical process linking regeneration of N to its eventual loss as N₂ or N₂O via denitrification and anammox. Nitrification is carried out by 2 independent steps involving NH₄⁺ and NO₂⁻ oxidation. It has long been considered as a tightly coupled process where the rate of NH₄⁺ oxidation is equivalent to the rates of NO₂⁻ oxidation and NO₃⁻ production, therefore avoiding significant NO₂⁻ 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 (NH₄⁺ and NO₂⁻ 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 O₂) 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 NH₄⁺ 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 N₂O/NO₂⁻ production at low O₂ concentrations. On the other hand, Ginestet et al. (1998) estimated kinetic parameters of both NH₄⁺ and NO₂⁻ oxidation by oxygen depletion due to substrate consumption in pure cultures, and concluded that NH₄⁺ oxidizers are more tolerant to low oxygen levels than NO₂⁻ oxidizers. The determination of the biomass index based on the substrate utilization rate revealed that low levels of O₂ doubled the growth yield of ammonium oxidizers, therefore compensating for the reduced specific substrate utilization. In contrast, NO₂⁻ox was strongly inhibited by 0.5 mg l⁻¹ of O₂, with no increase in growth yield.

In this study, NO₂⁻ox decreased significantly as oxygen levels dropped, whereas NH₄ox did not show differences at different oxygen levels. At the oxic-cline, rates of NO₂⁻ oxidation seem to be uncoupled from NH₄⁺ oxidation (Fig. 8), with NO₂⁻ oxidizers having a higher potential for producing NO₃⁻ than NH₄⁺ oxidizers for producing NO₂⁻ (see Tables 2 & 3). The difference between nNO₃ and NO₂⁻ox could also reflect that NO₂⁻-producing processes (such as denitrification or excretion by phytoplankton) sustain NO₂⁻ oxidation, as has been suggested for other systems (Clark et al. 2011).

In near-bottom waters, on the other hand, NH₄⁺ and NO₂⁻ oxidation fluxes seem to be coupled within the bacterial community despite low oxygen levels (Fig. 8 and Tables 2 & 3). Sporadic NO₂⁻ 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 (Farias 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 NH₄⁺ to survive and might be able to reduce urea into NH₄⁺ in order to produce NO₂⁻ (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 NH₄⁺-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 NH₄⁺ 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 NO₂⁻ oxidizer *Nitrospina* and AOA, which suggested possible interactions between these groups (Mincer et al. 2007), while a tight correlation was also observed between NO₂⁻ distribution and AOA activity (Beman et al. 2010). Therefore, it is possible that the coupling of both steps of nitrification (NH₄⁺ and NO₂⁻ oxidation) can be carried out independently of the composition of the community of nitrifiers (i.e. the percentage of archaea within NH₄⁺ 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 (Farias 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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