Presence of nitrous oxide hotspots in the coastal upwelling area off central Chile: an analysis of temporal variability based on ten years of a biogeochemical time series

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Keywords: nitrous oxide hotspot, seasonal and inter-annual N2O variability, coastal upwelling, central Chile

Abstract
Seasonal and inter-annual variabilities of biogeochemical variables, including nitrous oxide (N2O), an important climate active gas, were analyzed during monthly observations between 2002 and 2012 at an ocean Time-Series station in the coastal upwelling area off central Chile (36° 30.8′; 73° 15′). Oxygen, N2O, nutrients and chlorophyll-a (Chl-a) showed clear seasonal variability associated with upwelling favorable winds (spring–summer) and also inter-annual variability, which in the case of N2O was clearly observed during the occurrence of N2O hotspots with saturation levels of up to 4849%. These hotspots consistently took place during the upwelling-favorable periods in 2004, 2006, 2008, 2010 and 2011, below the mixed layer (15–50 m depth) in waters with hypoxia and some NO2 accumulation. The N2O hotspots displayed excesses of N2O (ΔN2O) three times higher than the average monthly anomalies (2002–2012). Estimated relationships of ΔN2O versus apparent oxygen utilization (AOU), and ΔN2O versus NO3, suggest that aerobic ammonium oxidation (AAO) and partial denitrification are the processes responsible for high N2O accumulation in subsurface water. Chl-a levels were reasonably correlated with the presence of the N2O hotspots, suggesting that microbial activities fuelled by high availability of organic matters lead to high N2O production. As a result, this causes a substantial N2O efflux into the atmosphere of up to 260 μmol m−2 d−1. The N2O hotspots are transient events or hot moments, which may occur more frequently than they are observed. If so, this upwelling area is producing and emitting greater than expected amounts of N2O and is therefore an important N2O source that should be considered in the global atmospheric N2O balance.

1. Introduction
Nitrous oxide is a major climate modifying gas (Crutzen 1970) whose production depends on the concentration of O2, organic matter (OM) and bioavailable N compounds such as NH4+ and NO3− (Codispoti et al 2001). Globally, oceans are thought to add around 4 million tons of N2O to the atmosphere each year, but like other greenhouse gases, much of the N2O exchanged with the atmosphere comes from coastal regions such as upwelling systems (Nevison et al 1995, 2004). There, highly supersaturated N2O conditions in the surface waters mainly arise from the upwelled subsurface waters, where N2O production is accelerated by microbial activities occurring in sinking particles hosted in hypoxic waters (Codispoti et al 2001, Bange 2008). The properties (variables) of the ocean are changing taking into account acidification, increasing temperatures and deoxygenation, among other factors (Sarmiento et al 2004, Doney et al 2009, Keeling et al 2010), which may influence oceanic N2O inventories (Bange et al 2010). Some studies have showed that O2 minimum zones (OMZ) in the oceans are
expected to expand as the world warms (Stramma et al. 2008, Keeling et al. 2010) affecting N2O production. N2O, on the other hand, is mainly formed as a byproduct during nitrification, including the aerobic oxidation of NH4+ to NO3− (AAO) and nitrifier denitrification (i.e. NH4+ to N2O) (Poth and Focht 1985), nitrate-ammonification, the fermentative conversion of NO3− to NH4+ and partial denitrification, the anaerobic and dissimilatory reduction of NO3−/NO2− to N2O (Codispoti and Christensen 1985, Bange 2008). As O2 decreases to less than 20 μmol L−1, N2O production by AAO, associated with both archaea and bacteria (Santoro et al. 2011, Löscher et al. 2012), is intensely increased (Goreau et al. 1980). However, if O2 becomes exhausted and falls below suboxia, total denitrification takes place, a process by which N2O can be dissimilatively reduced to N2 (Codispoti and Christensen 1985). However, it is very difficult to determine whether these pathways operate individually or interact as coupled mechanisms.

Wind stress is the main physical factor regulating surface gas distribution and its outgassing (Wanninkhof 1992). This is particularly relevant in the coastal upwelling region; thus intensification/reduction of upwelling-favorable winds, a phenomenon that may be associated with global warming (Bakun 1990), could potentially affect microbial activities involved in N2O cycling, and therefore, its temporal dynamics could associate with global warming (Bakun 1990), upwelling-favorable winds, a phenomenon that may intensify the coastal upwelling region; thus intensification/reduction of upwelling-favorable winds, a phenomenon that may be associated with global warming (Bakun 1990), could potentially affect microbial activities involved in N2O cycling, and therefore, its temporal dynamics could act as coupled mechanisms.

![Figure 1. Study area showing the continental shelf bathymetry off central Chile and the location of St. 18 (36°30.80'S-73°07.75'W).
](http://copas.udec.cl/)<br>

2. Material and methods

2.1. Sample locations

A fixed station, known as Station 18 (St. 18) at the COPAS center, was sampled regularly at approximately 30 day intervals from July 2002 to August 2012 (figure 1). St. 18 is located over the continental shelf off central Chile at 10 nm (~18 km) from the shoreline (36°30.80'S; 73°07.75'W) and 92 m depth (http://copas.udec.cl/). Continuous profiles of temperature, salinity, O2, fluorescence, and photosynthetically active radiation (PAR) were obtained using a CTD to measure conductivity, temperature and depth (CTD model SBE-25). Seawater was sampled using a 10 L Niskin bottle mounted on a 10 bottle rosette. Samples for gases (O2, N2O), nutrients and pigments (in this correlative temporal order) were obtained at nine depths distributed between the surface and 92 m depth (2/5, 10, 15, 20, 30, 40, 50, 65 and 80 m depth). Triplicate samples were taken for O2 (in iodimetric bottles immediately fixed) and for N2O (in 20 mL gas chromatography (GC) vials). Preservation was carried out through the addition of 50 μL of saturated mercuric chloride (Tilbrook and Karl 1995) and the GC vials were immediately sealed with a butyl-rubber septum and aluminum cap avoiding air bubbles and, then stored in darkness until analysis. From each sample depth syringes were connected directly to the spigot of the Niskin bottles to obtain triplicate nutrient samples (NO3−, NO2−, PO43− and Si(OH)4), filtered through a 0.45 μm Uptidisc adapted to the syringe, and then stored at −20 °C, whereas for Chl-a, 100 mL of seawater was filtered (triplicate) onto a glass-fiber filter (GF/F) and the filter was immediately frozen.

2.2. Chemical analysis

O2 was analyzed by the Winkler method using an automatic measurement system. For N2O analysis, within each GC vial, a headspace was created adding 5 mL of ultrapure helium (He) using a 5 mL gas-tight syringe and N2O dissolved in the seawater was measured through gas-liquid equilibration in the vial
at 40 °C for 15 min under agitation (using a headspace autosampler device, HP Agilent), followed by quantification via GC. N$_2$O was analyzed in a Varian 3380 GC with an electron capture detector (ECD) at 350 °C, and using a capillary column and injector operated at 60 °C and 250 °C, respectively, and connected to the mentioned autosampler (Farias et al. 2009). Five-point calibration curves were constructed using He and 0.1, 0.5, 1 and 100 ppm N$_2$O standards (Mathison gas mixture). Additionally, for the calibration curve, prepared compressed dry air was used with a 0.320 mole dry fraction of N$_2$O from NOAA (http://www.esrl.noaa.gov/gmd/hats/combined/N2O.html). The ECD detector linearly responded to this concentration range, which was equivalent to a range of dissolved N$_2$O in seawater of about 7–1500 nmol L$^{-1}$ (T ~ 18 °C and S ~ 35). The analytical error for the N$_2$O measurements was about 3%. The uncertainty of the measurements was calculated from the standard deviation of the triplicate measurements by depth. Samples with a variation coefficient higher than 10% were not taken into account for the N$_2$O database. Nutrients were analyzed by standard manual (from 2002 to 2009) or automated (from 2009 to 2012, autoanalyzer SEAL Analytical) colorimetric methods (Grasshoff et al. 1983). Chl-a was analyzed by fluorometry (Turner Design AU-10) according to standard procedures (Parsons et al. 1984).

2.3. Data analysis

In order to interpret the vertical and temporal variation of N$_2$O, the water column was divided as follows into two layers according to density and O$_2$ gradient (more details in Farias et al. 2009): 1) the mixed and illuminated layer delimited by the base of the mixed layer (ML); 2) the subsurface waters from the base of the ML to the water overlying the sediments (90 m depth). This layer included the oxycline (a layer where O$_2$ levels widely fluctuated but always remained higher than 4.4 μmol L$^{-1}$) and bottom water (a layer where levels of O$_2$ were as low as 4.4 μmol L$^{-1}$, generally from 65 to 90 m depth).

N$_2$O solubility was estimated according to Weiss and Price (1980) based on in situ temperature and salinity. Delta or excess of N$_2$O, a measure to diagnose the apparent production of N$_2$O in different water masses (Nevison et al. 1995, 2003) was calculated as ΔN$_2$O = [N$_2$O]$_{in situ}$ − [N$_2$O]$_{eq}$, where [N$_2$O]$_{in situ}$ is the measured concentration of N$_2$O, and [N$_2$O]$_{eq}$ is the concentration of N$_2$O in equilibrium with the atmosphere at the time of the last atmospheric contact. Atmospheric values, at the time in which samples were taken, came from the NOAA/ESRL program register of N$_2$O hemispheric and global monthly means (http://www.esrl.noaa.gov/gmd/hats/combined/N2O.html).

Monthly anomalies of environmental variables, for example N$_2$O, were estimated as

\[
\text{Anomaly} = \frac{xN_2O - \bar{X}_{N2O}}{\sigma_{N2O}}
\]

where, $xN_2O$ is the discrete value at a certain depth and time, and $\bar{X}_{N2O}$ is the average value for the whole 2002–2012 period, and $\sigma_{N2O}$ is the respective standard deviation of the dataset.

N$_2$O hotspots were defined as exhibiting a ΔN$_2$O three times higher than the average monthly ΔN$_2$O anomaly at each depth range, i.e.

\[
\frac{\Delta N_2O}{\bar{X}_{N2O}} > 3
\]

where, ΔN$_2$O is the monthly estimated N$_2$O anomaly, and $\bar{X}_{N2O}$ is the average N$_2$O anomaly for the entire water column.

N$_2$O flux through the air–sea interface was determined using the following equation, modified by Wanninkhof (1992):

\[
F = kw \cdot (T^*, \text{salinity}) \cdot \left( C_w - C_{eq} \right)
\]

where, kw is the transfer velocity from the surface water to the atmosphere, as a function of wind speed, temperature and salinity from the ML. $C_w$ is the mean N$_2$O concentration in the ML and $C_{eq}$ is the gas concentration in the ML expected to be in equilibrium with the atmosphere, according to Weis and Price (1980). The ML depth was calculated using a potential density-based criterion of Kara et al. (2003).

Gas transfer velocity was calculated according to Wanninkhof (1992) or W92, estimated to be the best parametrization for the study area. Wind speed and direction based on a six hourly register were obtained from a permanent meteorological station located at Carriel Sur (http://www.meteochile.gob.cl) that is considered to be a coastal station and meets with international standards. These data were compared with those obtained from satellite observations of ocean winds based on QuikSCAT (2002–2009) and ASCAT (2010–2012).

Cumulative alongshore (south–north) wind stress was obtained using a running mean (10-day) wind stress calculated to eliminate the intra- and inter-daily variability (Barth et al. 2007). Wind stress was calculated according to Nelson (1977):

\[
\tau_y = C_d \cdot \rho_{air} \cdot |V| \cdot \nu
\]

where, $C_d$ is the drag coefficient of wind at 10 m above sea level and corresponds to the value of 0.0013 (Kraus 1972), $\rho_{air}$ is the air density and corresponds to the value 1.22 kg m$^{-3}$, $|V|$ is the wind speed in m s$^{-1}$ and $\nu$ is the meridional component of the wind speed in m s$^{-1}$.

N$_2$O, O$_3$, NO$_3^-$, NO$_2^-$ and Chl-a inventories were calculated through numerical integration of data at 1 m increments (linear interpolation) based on at least 4–6 sampled depths per layer. Spearman correlations (Rho) were determined for N$_2$O, Chl-a, and nutrient inventories estimated in the surface and subsurface layers. The threshold value for statistical significance...
was set at p < 0.05. Multiple comparison procedures for all pairwise comparisons among seasonal means were made with a t-student test, with a previous application of the Shapiro–Wilks test to check a normally distributed population. The threshold value for statistical significance was set at p < 0.05.

3. Results

3.1. N$_2$O content in the water column

Time series vertical distributions of O$_2$, Chl-a, NO$_3^-$ and N$_2$O are shown in figure 2. Chl-a in surface waters fluctuated between 0.1 and 53.15 μg L$^{-1}$ (mean ± SD = 3.63 ± 5.65) and revealed a marked seasonal cycle with maximum values in spring–summer (upwelling period) and minimum values (<1.00 μg L$^{-1}$) in winter-time (non-upwelling period) (figure 2(a)). Temporal variability of O$_2$ stands out as the predominant seasonal signal of all oceanographic variables (figure 2(b)). Each year, a winter oxygenation throughout the water column is followed by a gradual deoxygenation (undersaturated levels) in the oxycline and the bottom layers, commencing in September/October and lasting until April. Each spring–summer, the oxycline and the bottom waters showed a decrease in the O$_2$ content reaching values as low as 1 μmol L$^{-1}$, creating a suboxic/anoxic environment in the bottom waters (close to the sediments), and also a marked oxycline in depths as shallow as 20 m (figure 2(b)).

NO$_3^-$, which is a nutrient with a specific sensitivity to O$_2$ levels, showed highly variable concentrations, ranging from 0 to 11.7 μmol L$^{-1}$ (mean ± SD = 0.56 ± 0.99) (figure 2(c)). Extremely low values were detected in the surface layer, and some middle values up to 2.5 μmol L$^{-1}$ at the oxycline (figure 2(c)); whereas during the summer higher values (up to 11.7 μmol L$^{-1}$) were observed in the bottom layer (associated with suboxic waters). N$_2$O varied from 2.92 nmol L$^{-1}$ or 28.3% saturation (bottom water, 90 m depth) to 492 nmol L$^{-1}$ or 4849% saturation, having a mean ± SD of 39.4 ± 29.2 nmol L$^{-1}$ in the oxyclines (20–64 m depth) and a mean ± SD of 37.6 ± 23.3 nmol L$^{-1}$ in the bottom waters (65–90 m depth) (figure 2(d)). During mid-autumn and winter, N$_2$O levels were relatively low with occasional undersaturation in the surface layer. Conversely, in spring–summer a large accumulation of N$_2$O was observed as O$_2$ decreased at the oxyclines and the bottom layers (figure 2(d)); however in late summer and early autumn N$_2$O levels as low as 30% saturation were observed in the bottom waters close to the sediments, coinciding with an extremely low O$_2$ concentration (anoxia), and indicating that N$_2$O is being consumed in this layer.

Besides the seasonal cycles described for these variables, an inter-annual variability is also evident, particularly for N$_2$O. Monthly anomalies for Chl-a, O$_2$, NO$_2^-$, and N$_2$O are illustrated in figure 3. Inter-annual variability was clearly visible during the occurrence of N$_2$O hotspots (figure 2(d)), with very high saturation during December 2004 (up to 2442%), January 2006 (up to 1583%), January 2008 (up to 2097%), December 2008 (up to 2066%), January 2010 (up to 1574%) and November 2011 (up to 4849%). In addition, N$_2$O excess (ΔN$_2$O), the difference between observed N$_2$O and the expected N$_2$O concentration at equilibrium with the atmosphere, was analyzed to eliminate the majority of N$_2$O variability caused by changes in seawater temperature and salinity (figure 4(a)). The monthly anomalies of ΔN$_2$O highlighted the presence of N$_2$O hotspots which contained at least ΔN$_2$O three times higher than the average monthly ΔN$_2$O (figure 4(b)).

Oceanographic/biogeochemical profiles obtained during the presence of hotspots are shown in figure 5. In general, the N$_2$O hotspots developed at the oxycline layer, below the ML until 50 m depth and under variable O$_2$ conditions but always in a range of hypoxic conditions (higher than 4.4 μmol L$^{-1}$, except in November 2011) and with some NO$_3^-$ accumulation (from 0.53 to 2.53 μmol L$^{-1}$). Furthermore, the N$_2$O hotspots were always immered in a layer with high NO$_3^-$ and HPO$_3^{2-}$ content, ranging from 7.94 to 35.2 and from 1.03 to 2.32 μmol L$^{-1}$, respectively.

Table 1 presents the annual mean inventories of N$_2$O, O$_2$, Chl-a, and NO$_3^-$ estimated in the surface and subsurface layer from September to March during the sample period. Cumulated surface wind stress for the whole upwelling period is also included. Significant differences were found among mean inventories for all environmental variables estimated for the yearly spring–summer period (p < 0.05) and indicated differences between upwelling seasons. Additionally, table 1 shows that the majority of N$_2$O was accumulated in the oxyclines, which on average accounts for 72% of the total N$_2$O inventory in the water column over the study period, contrasting with 21% of the total inventory that accumulates in the bottom layer.

3.2. Air–sea N$_2$O exchange and relationships among environmental variables

The wind speed and alongshore wind speed ranged from 0.51 to 26.46 m s$^{-1}$ (figure 6(a)) and −15.47 to 9.36 m s$^{-1}$, respectively. The meridian component of the wind, which provoked favorable upwelling events, was observed more frequently in spring–summer and it occurred in total for approximately 61.26% of the whole year. The cumulated wind stress (10-day running mean) varied from −1.43 to 0.68 N m$^{-2}$ 10 days and clearly showed that the cumulative upwelling-favorable wind stress occurred during the spring–summer time (figure 6(b)). Sea surface temperature (SST in °C), measured at 10 m depth to avoid the effect of solar radiation, presented a similar pattern to the cumulated wind stress, indicating that cold subsurface water is being upwelled to the surface layer.
N2O fluxes varied between $-10.47$ and 260.2 $\mu$mol m$^{-2}$ d$^{-1}$ (mean ± SD = 27.88 ± 44.5) (figure 6(c)). Temporal trends in N2O fluxes also showed a marked seasonal pattern, being lower (influx) during the non-favorable upwelling periods and higher (efflux) during the favorable upwelling periods; this coincided with supersaturated N2O conditions in the ML, with levels ranging from 71.85 to 1428% saturation over the whole study period. The highest N2O fluxes coincided with the presence of the N2O hotspots (figure 6(d)).

Table 2 shows Spearman correlations carried out among several environmental variables as cumulative wind stress, SST, Chl-a and inventories of N2O, NO$_2^-$
Cumulative wind stress correlated negatively with SST, whereas it correlated positively with Chl-a and N$_2$O inventories, but no correlations were found between wind stress and nutrient inventories (table 2). These correlations imply that more intensive upwelling favorable winds provoke a rise of cold and nutrient- and gas-rich subsurface water to the surface, fertilizing and allowing the accumulation of phytoplankton biomass in the surface water and accelerating the gas exchange across the air–sea interface (figure 6(d)). However, these nutrients were more rapidly consumed and assimilated by phytoplankton than the rates at which they were supplied at the surface by upwelling events. In addition, N$_2$O inventories in surface and subsurface layers correlated reasonably well with Chl-a inventories in the surface layer (table 2). Since the N$_2$O hotspots were only present during
upwelling-favorable winds, this pattern would suggest that these structures were controlled by the wind. However, no significant correlation was found between the N$_2$O inventory at surface layer and SST, suggesting that the dynamics of N$_2$O was not just controlled by physical processes (table 2). By contrast, the content of N$_2$O in surface and subsurface layer was related positively to Chl-a level and the NO$_2$- inventory at surface waters.

4. Discussion

The continental shelf off central Chile is subject to SW wind stress (figure 6(b)) that produces upwelling events during the austral spring–summer and affects most of the physical variables, such as temperature and salinity (Sobarzo et al 2007). The fertilization of the photic zone is the most notable consequence of this process, which can sustain high phytoplanktonic development, primary production, and the subsequent intense respiration of produced OM (figure 2) (Daneri et al 2012, Farias et al 2009). Thus, the phytoplankton standing stock increased throughout the upwelling season, as illustrated by the progressive accumulation of Chl-a in the surface water (figure 2(a)), with a subsequent buildup in the particulate OM occurring at the oxycline and the bottom layers (Farias et al 2009). This leads to an intense aerobic respiration of sinking organic particles, which along with the presence of the eastern Southern Pacific’s OMZ causes a decrease in O$_2$ to anoxic levels (Ulloa et al 2012). This stimulates dissimilative processes involved in N cycling, particularly those associated with N$_2$O production, such as nitrification and denitrification (Codispoti and Christensen 1985).

Some coastal upwelling areas show a rapid seasonal transition from oxic to anoxic conditions. In the study area, this phenomenon was observed throughout the vertical O$_2$ distribution (figure 2(b)), where conditions transitioned from relatively homogenous and oxygenated to a very shallow and sharp oxycline; as a result this influences the N$_2$O distribution (Cornejo et al 2007). This transition is accompanied by N$_2$O accumulation at the oxyclines (December–January) and consumption within the bottom layers (March–April) (figure 2(d)). Marked N$_2$O accumulation has previously been observed in only a few coastal time-series sites associated with the continental shelf and coastal upwelling, such as those off central Chile and West India, (Naqvi et al 2010). Such N$_2$O accumulations (up to several hundred nM) were related with low O$_2$ levels, where significant amounts of N$_2$O can temporarily accumulate during the short transition time, while the system is changing its oxygen regime (Bange et al 2010, Bakker et al 2014).

N$_2$O hotspots are defined as patches that show disproportionately high reaction rates relative to the surrounding water according to McClain et al (2003). In our study area (off central Chile), they have $\Delta$N$_2$O three times higher than the average monthly $\Delta$N$_2$O (figure 4). These hotspots were present between November and January at the oxyclines (15–50 m

Figure 4. Temporal variability in the water column of (a) N$_2$O excess (nmol L$^{-1}$); and (b) the anomaly of N$_2$O excess during the study period (August 2002–2012) at the St. 18.
range depth) with hypoxic rather than suboxic levels (higher than 4.4 μmol L\(^{-1}\)) and with some NO\(_2^-\) accumulation, which seems to form the primary NO\(_2^-\) maxima (figure 5). This contrasts with NO\(_2^-\) accumulation observed in the bottom water under suboxic/anoxic conditions (figure 2(b)) whose origin seems to be related to dissimilative NO\(_3^-\) reduction (Lomas and Lipschultz 2006). This situation was dissimilar to that reported by Naqvi et al. (2000) in the western Indian continental shelf; they found strong N\(_2\)O accumulation and the occurrence of sulphide in open coastal waters. However, in this case, the increased N\(_2\)O production seemed to be caused by the addition of anthropogenic NO\(_3^-\) associated with strong river runoff and a subsequent reduction to N\(_2\)O (i.e., partial denitrification).

According to the observed correlations, upwelling-favorable wind stress modulated the seasonal N\(_2\)O content in surface and subsurface waters (table 1). However the N\(_2\)O hotspots were not necessary associated with the strongest wind stress (figure 6(b)), nor with the colder waters (SST) upwelling/rising from the subsurface (figure 6(c)). The presence of the N\(_2\)O hotspots was well correlated with Chl-a (table 2), revealing the significance of the phytoplanktonic biomass in providing OM and the concomitant mineralization products (NH\(_4^+\) and N\(_2\)O).

4.1. Origin of N\(_2\)O accumulated in the hotspot

Based on the O\(_2\)/N\(_2\)O emission ratio observed in atmospheric records (Lueker et al. 2003), marine N\(_2\)O is predominately being sourced from nitrification. Considering correlations between ∆N\(_2\)O versus AOU and ∆N\(_2\)O versus NO\(_3^-\), it is possible to determine if N\(_2\)O production is related to O\(_2\) consumption and NO\(_3^-\) production, due to OM remineralization coupled with nitrification (Yoshinari 1976, Nevison et al. 2003). Figure 7 illustrates the relationship among these parameters and variables with all data (figures 7(a), (b)) a significant correlation of ∆N\(_2\)O.
Table 1. Monthly averages of nitrous oxide inventories. Chlorophyll-a, nitrate and nitrite estimated during the upwelling season (spring–summer) in both the mixed and subsurface layers, and the cumulated wind stress over the same period from the COPAS time-series station.

| Year   | Month | Nitrous Oxide nmol m⁻² | Chlorophyll-a mg m⁻²(c) | Nitrate μmol m⁻² | Nitrite μmol m⁻² | Wind Stress N m⁻² 210 days |
|--------|-------|------------------------|-------------------------|------------------|-----------------|--------------------------|
| 2002–2003 | ML    | 1036 ± 81              | 455 ± 55                | 205 ± 33         | 25 ± 4          | 1459 ± 15                |
|        | SSL   | 7293 ± 471             |                        | 5881 ± 101       | 233 ± 23        |                          |
| 2003–2004 | ML    | 1068 ± 117             | 280 ± 66                | 706 ± 112        | 13 ± 2          | 2253 ± 9                 |
|        | SSL   | 10440 ± 546            |                        | 8280 ± 152       | 212 ± 52        |                          |
| 2004–2005 | ML    | 2877 ± 417             | 454 ± 89                | 1060 ± 135       | 41 ± 5          | 2376 ± 11                |
|        | SSL   | 23 414 ± 2738          |                        | 10 492 ± 349     | 287 ± 30        |                          |
| 2005–2006 | ML    | 1203 ± 131             | 245 ± 38                | 160 ± 37         | 21 ± 5          | 3576 ± 9                 |
|        | SSL   | 13 171 ± 1089          |                        | 7726 ± 194       | 596 ± 121       |                          |
| 2006–2007 | ML    | 671 ± 44               | 152 ± 18                | 504 ± 62         | 15 ± 1          | 2446 ± 9                 |
|        | SSL   | 9642 ± 252             |                        | 10 824 ± 322     | 195 ± 24        |                          |
| 2007–2008 | ML    | 866 ± 67               | 236 ± 36                | 680 ± 62         | 15 ± 2          | 2457 ± 9                 |
|        | SSL   | 10 084 ± 528           |                        | 8176 ± 349       | 78 ± 6          |                          |
| 2008–2009 | ML    | 2317 ± 135             | 561 ± 81                | 1306 ± 147       | 30 ± 4          | 4096 ± 17                |
|        | SSL   | 20 302 ± 2599          |                        | 8778 ± 129       | 171 ± 41        |                          |
| 2009–2010 | ML    | 1076 ± 159             | 44 ± 3                  | 598 ± 308        | 14 ± 5          | 4013 ± 15                |
|        | SSL   | 10 928 ± 3026          |                        | 3834 ± 417       | 102 ± 42        |                          |
| 2010–2011 | ML    | 1950 ± 242             | 293 ± 66                | 849 ± 139        | 30 ± 6          | 3802 ± 11                |
|        | SSL   | 15 365 ± 1323          |                        | 7378 ± 282       | 253 ± 33        |                          |
| 2011–2012 | ML    | 2157 ± 229             | 384 ± 40                | 1182 ± 148       | 30 ± 4          | 1164 ± 3                 |
|        | SSL   | 29 795 ± 4912          |                        | 12 589 ± 306     | 328 ± 56        |                          |

* Denotes upwelling periods during N₂O hotspot presence;  
* Mixed layer (ML) and subsurface layer (SSL);  
* Inventories estimated in surface (the mixed layer) and subsurface waters (the latter included the oxycline and the bottom layer).

Figure 6. Time series of (a) wind speed (m s⁻¹); (b) along shore wind stress on surface (N m⁻² days); (c) sea surface temperature (SST) measured at 10 m depth (°C); and (d) estimated air–sea N₂O fluxes (μmol m⁻² d⁻¹), at St. 18. Vertical green lines indicate the dates at which the N₂O hotspots were present.
Table 2. Spearman correlation coefficients among cumulative wind stress, N$_2$O inventories and several biogeochemical variables in the COPAS time series station.

| Cumulative
| Wind Stress |
|------------|------------------|
| (Nm$^{-2}$ |
| 10 days)  | sur N$_2$O       |
|           | sub N$_2$O       |
| Rho       | p                 | Rho | p | Rho | p |
| SST$^a$   | −0.40 0.00        | −0.07 0.45 | −0.20 0.04 |       |
| Chl−a$^b$ | 0.51 0.00         | 0.47 0.00 | 0.24 0.01 |       |
| sur N$_2$O | 0.43 0.00         | −       | −       | −     |
| sub N$_2$O$^b$ | 0.24 0.01         | −       | −       | −     |
| sur NO$_2$$^b$ | 0.00 0.97         | 0.32 0.00 | 0.33 0.00 |       |
| sub NO$_2$$^b$ | −0.03 0.76        | −0.03 0.77 | 0.12 0.20 |       |
| sur NO$_3$$^b$ | 0.00 0.92         | 0.39 0.00 | −0.43 0.00 |       |
| sub NO$_3$$^b$ | 0.17 0.06         | −0.17 0.06 | 0.23 0.10 |       |

$^a$ SST in °C.
$^b$ Biogeochemical variables are estimated as inventories in surface (sur) and subsurface (sub) layer.

versus AOU and ΔN$_2$O versus NO$_3^−$ (respectively Rho = 0.40, p < 0.05 and Rho = 0.34 p < 0.05) is observed; similar results were obtained with data from the upper oxycline (i.e., 20 m depth, respectively, Rho = 0.55 p < 0.05 and Rho = 0.43 p < 0.05; figures 7(c), (d)), indicating that N$_2$O and NO$_3^−$ are being produced in parallel to O$_2$ consumption by nitrification. However, correlations between these parameters lose statistical significance as the estimates are carried out with data from the deepest layers (i.e., 50 and 80 m depth). In fact, a NO$_3^−$ consumption was observed at O$_2$ levels as low as ∼5 μmol L$^{-1}$, which implies that an advected/diffused denitrification signal from the bottom layer or sediment, and/or an in situ denitrification at the oxyclines is/are occurring simultaneously with the nitrification. Indeed, when these relationships (ΔN$_2$O versus AOU and ΔN$_2$O versus NO$_3^−$) were estimated from 50 m (respectively Rho = 0.20 p < 0.05 and 0.30 p < 0.05; figures 7(e), (f)) and 80 m depth (respectively, Rho = 0.10 p = 0.36 and Rho = 0.23 p < 0.05; figures 7(g), (h)), they revealed a strong denitrification observable by an intense NO$_3^−$ and N$_2$O consumption.

Regarding the processes responsible for the observed N$_2$O distribution in the study area, high AAO rates at the oxyclines were observed in association with high NH$_4^+$ regeneration rates (Fernandez and Farias 2012), however at the oxyclines and possibly also at the bottom waters, AAO appeared to be coupled to NO$_3^−$ and NO$_2^−$ reduction to N$_2$O (partial denitrification), which has been previously reported in the study area (Fernandez and Farias 2012, Galán et al 2014). With respect to the microbes involved in these processes, beside AAO bacteria, AAO archaea also play an important role as ammonia oxidizers at oxyclines in the central Chilean upwelling (Molina et al 2010). Some archaea have a high affinity for NH$_4^+$ (Martens-Habbena et al 2009), efficiently producing N$_2$O as a by-product (Santoro et al 2011); sulfur-oxidizing γ-proteobacteria are abundant and dominant at the oxyclines in our study area, and additionally, they have the ability to reduce dissimilative NO$_3^−$ and NO$_2^−$ and carry out a process known as chemolithothrophic denitrification (Murillo et al 2014), which could be producing N$_2$O throughout partial denitrification (Galán et al 2014).

4.2. N$_2$O exchange across air–sea interface

Another observed consequence of the upwelling processes was a strong outgassing. In fact, the contribution from coastal upwelling has been estimated as 0.2 ± 0.14 Tg N$_2$O-N yr$^{-1}$, which represents ∼5% of the total ocean source (Nevison et al 2004), despite representing less than 1% of the total surface of the ocean. Most of these coastal upwellings, which encompass eastern boundary systems, have been recognized as significant N$_2$O emission areas, such as those associated with the Arabian Sea (De Wilde and Helder 1997, Naqui et al 2005), the eastern South Pacific (Corneo et al 2007, Charpentier et al 2010) and west Africa, including the Benguela current and Mauritanian upwelling (Wittke et al 2010, Kock et al 2012).

Given the proximity of the N$_2$O hotspots to surface water (figure 5), they could be shifted closer to the surface by the piston effect of the SW wind. Indeed, air–sea N$_2$O fluxes vary in magnitude throughout the year, changing from negative to positive values (figure 6(d)); however, for the majority of the year there is a strong efflux of N$_2$O, indicating that the area behaves as a strong gas source towards the atmosphere. It is important to note that fluxes were estimated with wind data that came from a continental location (close to the coast); this may indicate the occurrence of a retarding or frictional force on the wind that could reduce the wind strength magnitude. In fact, comparison of these data with those obtained from satellites indicates that these data underestimate the N$_2$O air–sea exchange by about 20%. Taking into consideration that wind is highly variable with bursts of intensity (up to 26 m s$^{-1}$), the parametrization of W-92 should be the most appropriate for the study area.

During the presence of hotspot structures, N$_2$O effluxes were up to one order of magnitude higher than the average flux estimated during the entire decade (figure 6(d)), and almost two orders of magnitude higher than the average flux estimated during the entire decadal, seasonal, and inter-annual variation into decadal, seasonal, and inter-annual variation facilitates a more precise temporal scaling to assess the impact of the gas exchange on the atmosphere. If we consider that the observed dynamic is occurring on Chile’s continental shelf (41 105 km$^2$), this coastal area contributes, based on a weighted average (upwelling and non-upwelling representative of 60% and
40% of the year, respectively), 20.7 Gg N$_2$O-N per year, which is a significant N$_2$O contribution/source that should be considered in the global balance of atmospheric N$_2$O. Furthermore, if we take into consideration that the N$_2$O hotspots may occur more frequently than observed during this study’s sampling cycle (at intervals of 30 days or more), this contribution appears to be further underestimated. In fact, when the N$_2$O efflux data was removed for periods during the N$_2$O hotspots’ presence, the annually weighted average was reduced to 13.5 Gg N$_2$O-N; therefore, the importance of the area as a source of N$_2$O could be scaled up. As a result transient events rather than long-term steady-state conditions have been proposed to govern N$_2$O production in coastal areas subjected to eutrophication and hypoxia (Naqvi et al 2000).

There exists the possibility that N$_2$O dynamics act as hot moments, which are defined as short periods of time exhibiting disproportionately high reaction rates relative to longer intervening time periods (McClain et al 2003). In this sense, high frequency events (at the weekly and intra-seasonal scales) could be affecting the presence of hot moments. In general, upwelling-favorable winds work with quasi-weekly upwelling pulses, consisting of an alternation in the winds and/or change in wind intensity; with respect to the southern hemisphere, this may vary from southerly and intense (active upwelling) to northern and decreased winds (upwelling relaxation) (Send et al 1987, Rutllant and

Figure 7. Relationship found between $\Delta$N$_2$O (nmol L$^{-1}$) versus AOU (µmol L$^{-1}$), and $\Delta$N$_2$O versus NO$_3$ (µmol L$^{-1}$) in the water column with, respectively, (a), (b) the whole data set taken from 2002 to 2012; (c), (d) the data from 20 m depth; (e), (f) data from 50 m depth; and (g), (h) data from 80 m depth. Each graph includes a linear regression model between both parameters and the black line indicates a linear regression line.
Montecino 2002). Over a 5- to 10-day time scale this active-relaxing cycle affects both microbial communities and the accumulation of biomass, and primary production rates and microbial community composition (Rutllant and Montecino 2002, Wilkerson et al 2006, Du and Peterson 2009). For example, for effective Chl-a accumulation an optimal window of 3–7 days of relaxed winds was required following an upwelling pulse. Thus, N2O hot moments may occur during the relaxing phase of the upwelling process, and therefore would require a weekly and intra-seasonal study scale in order to resolve the frequency of these events.

Within the study region there are no clear decadal trends of an increase in phytoplanktonic biomass or an intensification of hypoxic conditions in subsurface waters regarding the analysis of the COPAS time series; however, phenomena such as the cooling of surface waters and intensification of coastal wind patterns have been reposted by Falvey and Garreaud (2009) in the study area. Therefore, we recommend further study on the physical forcing (i.e. ENSO and even global warming) mechanisms that may be affecting biogeochemical processes involved on N2O production and exchange across the air–sea interface.

Acknowledgments

Both the crew of R/V Kay Kay (II) and the Dichato Marine Station of the University of Concepción provided valuable help during fieldwork, as well as all participating colleagues in the COPAS time series (COPAS program), who provided the core measurements, particularly Carmen Morales and Valeria Anabalon who carefully checked the Chl-a data. We also appreciate the work done since the beginning of COPAS TS by Mauricio Gallegos and Juan Faundez. This research was funded by the Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) grant no. 1120719. Also we appreciate the support of Aldo Montencinos who gave us the wind data. This is a contribution to the Fondo de Financiamiento de Centros de Investigación en Areas Prioritarias (FONDAP) program no. 1511009 and ICM 120019 project (IMO).

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