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An unaccounted for N_2O sink in the surface water of the eastern subtropical South Pacific: Physical versus biological mechanisms ☆

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ABSTRACT

Nitrous oxide (N_2O) is a trace gas affecting atmospheric radiative forcing through its greenhouse effect in the troposphere and destroying the ozone in the stratosphere. The oceans account for one-third of the global atmospheric N_2O emissions, in which they are primarily cycled by nitrification and denitrification, with high N_2O production in the subsurface waters. The surface waters are generally reported to be in equilibrium or slightly supersaturated with respect to the atmosphere. However, surface N_2O sub-saturations have been observed in several regions of the world's oceans, such as off south-central Chile, which is bathed by the Sub-Antarctic Water Mass (SAAW), where N_2O subsaturations as low as 35% have been registered during the austral spring and summer. An analysis of the mechanisms driving such surface N_2O subsaturations (physical or biological) showed that physical mechanisms were not responsible for the high surface N_2O deficit. In contrast, *in situ* potential experiments in surface waters with $^{15}N_2O$ addition showed an active biological N_2O fixation (between 0.43 and 87.34 nmol/L/d), with the highest N_2O fixation rates associated with the SAAW (25.25–25.75 kg/m^3).

Additionally, incubation experiments with $^{15}N_2O$ in surface water samples from one oceanic station showed high ^{15}N-POM enrichment (0.44‰) and an inhibition of ^{15}N-POM enrichment when an additional nitrogen source was added (NO_2^- and NH_4^+). These results suggest the existence of a mechanism able to use several nitrogen sources, including N_2O. Molecular analyses (16S rRNA gene) from these experiments showed the presence of three major groups of bacteria: Gammaproteobacteria, Flavobacteria and Cyanobacteria, with Synechococcus sp. being the dominant group in the culture. However, the analysis of the *nif*H gene showed a taxonomic affiliation to the order *Stigonematales* associated with *Mastigocladus* sp. and *Fischerella* sp. and the order *Oscillatoriales* associated with *Trichodesmium* sp.

Finally, the oceanic region exhibiting surface N_2O subsaturations acts as a sink for atmospheric N_2O, consuming ~11.4 Gg of N_2O in a six-month period. The N_2O levels in the sink are 75% higher than those of the reported N_2O source from the coastal band. The balance between the oceanic region and the coastal band results in a sink region of 4.94 Gg of N_2O during this period.

Introduction

The global ocean is one of the primary natural sources of atmospheric N_2O (4–16 Tg N per year, Bange et al., 2010), a powerful greenhouse and ozone-lytic gas (Prather et al., 2001). The oceanic contribution of N_2O to the atmosphere is far from being homogeneous and presents a high spatial variability, responding to regional oceanographic conditions (e.g., upwelling, cooling, hypoxic–anoxic events, productivity, etc.). N_2O cycling occurs primarily in subsurface waters, where oxygen concentrations decrease and two microbial pathways, denitrification and nitrification, can take place, either separately or coupled. Accordingly, N_2O can reach the surface by diffusion and/or vertical transport through upwelling. In the eastern boundaries of the oceans, the upwelling processes are linked to oxygen minimum zones, the main zones of N_2O accumulation and an exchange to the atmosphere (Naqvi et al., 2005; Cornejo et al., 2007). These regions represent a small fraction of the oceans, whereas, indeed, ~80% of the surface waters globally appear to be close to the N_2O equilibrium with the atmo-

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☆ *Key Points:* Surface N_2O subsaturations in the oceanic surface layer of the ESP off central Chile were characterized, evaluated using hydrographic, wind data. The oceanic region was identified to be an important sink of atmospheric N_2O, whose main driving process is biological fixation rather than physical factors.

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sphere (e.g., gyres and polar oceans) (Nevison et al., 1995). However, although some marine environments have been reported as being sub-saturated with N_2O at the surface waters (from 59% of saturation; Aguilera et al., 1992; Rees et al., 1997; Walter et al., 2006), there is no reported area in the ocean that acts as a sink of atmospheric N_2O. The assumption that the surface layer is well mixed and oxygenated, providing conditions where N_2O consumption by canonical denitrification does not take place (until recently, this was considered to be the only strictly anaerobic pathway able to consume N_2O) (Codispoti et al., 2001), has led to little consideration of sub-saturation events. Thus, these low levels of N_2O, when considered, have been attributed to physical mechanisms such as fast cooling of the water mass, decreased salinity from mixing with melt water in polar regions (Rees et al., 1997), or the results of analytical errors (Cline et al., 1987).

An area that presents seasonal events of surface sub-saturation is the central Chilean coastal zone. There, every winter, during the non-upwelling period, a short sub-saturation event (2–3 months) is generally observed, behaving as a N_2O sink (from −9.8 μmol/m^2/d) (Cornejo et al., 2007). A characteristic feature of the surface sub-saturation events is an association with low density waters (sigma-*t* below 25.8 kg/m^3) known as the Sub-Antarctic Waters (SAAW) (Silva et al., 2009), which arrive to the coastal zone during winter, occupying most of the water column. However, the SAAW dominates the oceanic surface layer off central Chile (40°–28°S) throughout the year. Considering the N_2O behavior in the SAAW observed in the coastal zone, some questions arise: *Is the oceanic area off central Chile within the SAAW influence zone a sink for atmospheric N_2O?* If the oceanic region acts as a sink of N_2O, *is this region counterbalancing the coastal source?*

Considering the classical view of natural N_2O consumption through denitrification only under suboxic and anoxic conditions, the mechanism responsible for N_2O subsaturations in temporal (winter time) and spatial scales in the oxygenated surface waters is an issue of considerable interest. However, in recent years a novel biological mechanism consuming N_2O has been described in soils and oceans known as N_2O fixation (Vieten, 2008; Farías et al., 2013). In the oceans, this apparently ubiquitous pathway occurs not only in subsurface waters but also in the oxygenated surface layer and from the eutrophic coastal zone of Northern Chile to the oligotrophic region of the eastern South Pacific (ESP). Culture experiments with cyanobacteria (*Trichodesmium* and *Crocosphaera*) showed that they were able to fix N_2O into particulate organic matter, suggesting its utilization as a substrate instead of N_2. Thus, another question arises: W*hat is producing the N_2O subsaturation in the surface layer dominated by SAAW (physical or biological mechanisms)?*

Here, we determine the role of the oceanic waters off central Chile (until 78°W) on the atmospheric N_2O sink and present insight that a biological process is responsible for subsurface N_2O levels rather than physical mechanisms. The analyses were made using field measurements and on-board experiments and using lab culture experiments with samples taken from oceanic waters.

Methods

Area of sampling

To determine the occurrence and extension of N_2O subsaturations in the ESP and to determine the processes responsible for N_2O subsaturations, several cruises between 33° and 40°S and from the coastline to 78°W were conducted during the spring–summer season over a six-year period (2005–2011) (Table 1; Fig. 1). Surface N_2O along with physical (temperature, salinity) and biogeochemical (oxygen and nutrients) variables were sampled. For each cruise, stations located 50 km off the coast were selected to avoid the influence of coastal upwelling according to the Rossby ratio estimated for the area (Pizarro, 1999). FIP cruises (Fondo de Investigaciones Pesqueras) (2005–2009) consisted of several transects perpendicular to the coastline between 35° and 40°S and from the coastline to 77.8°W, whereas the Tara Oceans cruise consisted of three stations between 40° and 30°S. In addition, winter samples from the COPAS Time Series (36.5°S/73°W), the seasonal period when SAAW reaches the coast, were used to characterize the SAAW conditions.

Sampling and laboratory analysis

Vertical profiles taken with a CTD with an oxygen sensor were used to characterize the surface structure of the oceanic region. In addition, triplicate surface samples for the N_2O and nutrients determination were obtained from 12 L Niskin bottles or by a rosette. N_2O samples were poisoned with 50 μL of saturated mercuric chloride and stored in the dark until measurement. N_2O was determined using the headspace technique in a gas chromatograph equipped with an ECD (Shimatzu 17A). A four-point calibration curve was determined with air and N_2O standards of 0.1, 0.5 and 1 ppm (Scotty gas mixture).

Nutrient samples (NO_3^-, NO_2^- and PO_4^{-3}) were taken in duplicate from seawater samples filtered with GF-F 0.7-μm filters (Whatman®) and stored at −20 °C until analysis. Nutrient analyses (NO_3^-, NO_2^- and PO_4^{-3}) were performed according to colorimetric techniques (Grasshoff et al., 1983).

To determine the natural signal of ^{15}N in the organic matter, ~800 mL of surface sea water was filtered through precombusted 0.7 μm glass fiber filters (Whatman® GF-F) and stored at −20 °C until analysis. Isotopic composition was performed with a continuous-flow isotope ratio mass spectrometry (IRMS, Finnigan Delta Plus). Reproducibility for ^{15}N was greater than 0.02‰ and isotope ratios were expressed as per mil deviations from the isotopic composition of air (Böhlke, 2005).

Field experiments

Field experiments were conducted on two oceanographic cruises (FIP 2009 and Tara Oceans) and three daily cruises to the time series station 18 from the COPAS program (May and July 2011). Duplicate experiments were performed with surface water samples incubated in 2-L transparent bags (Kynar bags), which were subjected to different treatments: (1) a control (without any addition); (2) the addition of $^{15}N_2O$ (1 mL of 99% $^{15}N_2O$); (3) the addition of $^{15}N_2O$ (1 mL of 99% $^{15}N_2O$) incubated in the dark; and (4) the addition of $^{15}N_2O$ along with a nitrogen source (NO_3^-, NH_4^+, NO_2^-). The addition of a nitrogen source was performed considering a final NO_3^- or NH_4^+ concentration of 2 and 0.2 μM, respectively. Bags were placed in an on-board incubator that was circulated with sea surface water, which maintained the field temperature and light condition (except for experiments under darkness). After 24 h of incubation the water inside the bag was filtered through a precombusted 0.7 μm glass fiber filter (Whatman GF-F) and stored at −20 °C until analysis. The ^{15}N percentage of the organic matter content in the filters was obtained in the same way as the natural sample filters. Differences between treatments were obtained from the enrichment, quantified as the percentage of ^{15}N atoms and the percentage of excess PON compared to the natural isotopic background of PON.

In addition, experiments of $^{15}N_2O$ fixation kinetics were conducted in two field cruises at station 18, during the COPAS Time Series on May and July 2011, to determine the response of N_2O fixation to the variation of the $^{15}N_2O$ availability. The experiments consisted of the incubation of 2-L bags of surface sea water (5 m depth) and the addition of different doses of $^{15}N_2O$ (0, 10.4, 13.0,