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Methane production induced by dimethylsulfide in surface water of an upwelling ecosystem

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ABSTRACT

Coastal upwelling ecosystems are areas of high productivity and strong outgassing, where most gases, such as N_2O and CH_4, are produced in subsurface waters by anaerobic metabolisms. We describe seasonal CH_4 variation as well as potential mechanisms producing CH_4 in surface waters of the central Chile upwelling ecosystem (36°S). Surface waters were always supersaturated in CH_4 (from 125% up to 550%), showing a clear seasonal signal triggered by wind driven upwelling processes (austral spring–summer period), that matched with the periods of high chlorophyll-a and dimethylsulfoniopropionate (DMSP) levels. Methane cycling experiments, with/without the addition of dimethylsulfide (including ^{13}C-DMS) and acetylene (a nonspecific inhibitor of CH_4 oxidation) along with monthly measurements of CH_4, DMSP and other oceanographic variables revealed that DMS can be a CH_4 precursor. Net CH_4 cycling rates (control) fluctuated between −0.64 and 1.44 nmol L^{-1} d^{-1}. After the addition of acetylene, CH_4 cycling rates almost duplicated relative to the control, suggesting a strong methanotrophic activity. With a spike of DMS, the net CH_4 cycling rate significantly increased relative to the acetylene and control treatment. Additionally, the $\delta^{13}C$ values of CH_4 at the end of the incubations (after addition of ^{13}C enriched-DMS) were changed, reaching −32‰ PDB compared to natural values between −44‰ and −46‰ PDB. These findings indicate that, in spite of the strong CH_4 consumption by methanotrophs, this upwelling area is an important source of CH_4 to the atmosphere. The effluxes are derived partially from *in situ* surface production and seem to be related to DMSP/DMS metabolism.

1. Introduction

Methane is the most abundant hydrocarbon and a major greenhouse gas (20 times more effective in its radiative forcing than CO_2) which plays an important role in the Earth's radiative balance (Quay et al., 1999). It is mainly produced by biological (anaerobic) activity and accumulated through geological processes such as gas hydrate or other CH_4 deposition pathways (Wuebbles and Hayhoe, 2002). The ocean surface acts as a net source of atmospheric CH_4, although its release is minor compared with natural emissions on land (Cicerone and Oremland, 1988). Estimates of annual oceanic CH_4 emission vary greatly between 0.4 (Bates et al., 1996) and 11–18 Tg C y^{-1} (Bange et al., 1994) and part of this uncertainty could be associated with low spatial resolution. Regional CH_4 emissions fluctuate widely; for example, surface waters of subtropical oceanic gyres are almost in equilibrium (100%) or slightly supersaturated (>100%) with the atmosphere (Holmes et al., 2000) whereas coastal areas can attain up to 500% surface CH_4 saturation (Sansone et al., 2001; Rehder et al., 2002).

Coastal areas cover only a small portion of the world's oceans but release up to 75% of the overall marine CH_4 flux into the atmosphere. Among costal ecosystems, eastern boundary coastal upwelling areas could be major sources or hotspots of marine CH_4 (Monteiro et al., 2006; Kock et al., 2008; Naqvi et al., 2010). There, a combination of physical and biological processes must co-occur to produce high air–sea CH_4 efflux. Physical forcing related to favorable upwelling winds promotes intense vertical advection of subsurface waters, driving fertilization but also strong outgassing of N_2O and CH_4 (Hatton et al., 1999; Bange, 2006; Kock et al., 2008; Cornejo and Farias, 2012). On the other hand, high biological productivity linked to natural hypoxia occurs during coastal upwelling events, favoring the existence of anaerobic/microaerophilic processes which mediate CH_4 cycling; most of them in the sediments but also in free- or attached living pelagic or benthic microorganisms (Monteiro et al., 2006).

Considerable focus is placed on excess quantities of biogenic CH_4 in surface oceans, apparently generated by a local methanogenesis in oxygenated surface waters (Karl et al., 2008; Rudd and

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Taylor, 1980). Given that CH_4 microbial production does not occur in oxic environments, this phenomenon is called the "methane paradox" (Wolfe, 1971; Lamontagne et al., 1973). A number of studies suggest that CH_4 excess in surface waters results from several *in situ* sources other than vertical advection of subsurface waters (Scranton and Brewer, 1977). Earlier studies suggest that CH_4 production is carried out by microorganisms living within micro-environments which occur in dead cells, fecal pellets or large organisms (Scranton and Brewer, 1977). Other sites and mechanisms of CH_4 generation include zooplankton biomass (Traganza et al., 1979), the digestive tracts of fish (Lamontagne et al., 1973; Oremlad, 1979; Brooks et al., 1981) and the degradation of particulate organic matter (Karl and Tilbrook, 1994). Additional mechanisms for CH_4 formation in oxic environments are via methylphosphonate (MPn) cycling in subtropical gyres which are phosphate-stressed (Karl et al., 2008) and via DMSP in polar oligotrophic waters which are nitrate-stressed (Damm et al., 2010).

Methanogens are mainly Archaea that produce CH_4 by dismutation of acetate or pyruvate or by catabolism of a wide range of methylated compounds. However also bacteria may being methylotroph, using a series of methylated compounds, including methylated sulfur compounds such as dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS) (Neufeld et al., 2008). This metabolism is referred as methylotrophic methanogenesis (Sowers and Ferry, 1983).

Both DMSP and DMS are S- and C-containing organic compounds that are biosynthesized by phytoplankton (DMSP) and assimilated and degraded by bacterioplankton (Kiene et al., 2000). DMSP is transformed to DMS, a volatile compound influencing global temperature but with a cooling effect, through its effects on cloud cover (Andreae, 1990). Functional genomics applied to natural communities recently reveals two different DMSP degrading pathways mediated by different genes and, therefore, microorganisms (Moran et al., 2012); one of them is the demethylation of DMSP facilitating retention of carbon and sulfur in the marine microbial food web and another is by cleavage of DMSP to DMS with important consequences for the ocean–atmosphere sulfur flux.

In this study, we measured CH_4 cycling throughout processes that consume CH_4 as the only source of carbon (e.g. methanotrophy) and those that regenerate CH_4 in order to quantify net and gross rates of CH_4 in surface waters. More importantly, we present the first evidence that DMS cycling generates CH_4 in surface waters off central Chile (36°S), an area subjected to intense upwelling events which occur seasonally during austral spring–summer.

2. Methods

2.1. Study area

The study area is on the continental shelf off central Chile where the COPAS Time Series station is located (36°0.80′S–73°07.75′W, 92 m depth, Fig. 1). It is representative of the oceanographic conditions of the coastal upwelling system. Monthly cruises were conducted as part of the MILOCO time series program. Intensive MILOCO biogeochemical oriented cruises during upwelling periods were conducted between 2009 and 2011, in order to study the temporal variability in physical–chemical properties (field measurements) and elucidate the processes responsible for CH_4 cycling at surface waters (experimental assays).

2.2. Field measurements

Water samples for gas (O_2, CH_4), DMSP, nutrients and pigments (in this successive order) were collected with a rosette (12-L Niskin bottles) equipped with a CTD (Seabird SBE 43). Discrete samples were obtained at nine depths between the surface and 90 m, with 3–4 sampling depths in the photic layer (2, 5, 10 or 15 and 20 m). For dissolved O_2 (triplicate), seawater samples were taken in an iodimetric flask and analyzed with an automatic version of the Winkler method, based on a photometric end point with a Dosimat 665. For dissolved CH_4 (triplicate), seawater samples were taken in 20 mL headspace vials and carefully sealed to avoid trapping air, with rubber stoppers and screw caps. The samples were poisoned with 50 μL saturated $HgCl_2$ and stored until their analysis in the laboratory. In the laboratory and just before analysis, 5 mL of water from the vial was replaced with helium in order to create a headspace. Then, the water-headspace sample was equilibrated at 40 °C (shaking continuously) and CH_4 from the headspace was manually analyzed with a gas chromatograph with a flame ionization detector (Schimadzu 17A) through a capillary column GS-Q (J & W, 0.53 mm × 30 m) at 30 °C oven temperature and a 4 ml min^{-1} column flow (Farías et al., 2009). A calibration curve was made with 4 points (He, synthetic air, 2 ppm and 10 ppm) and the detector linearly responded to this concentration range. The analytical error for the CH_4 analysis was less than 5%.

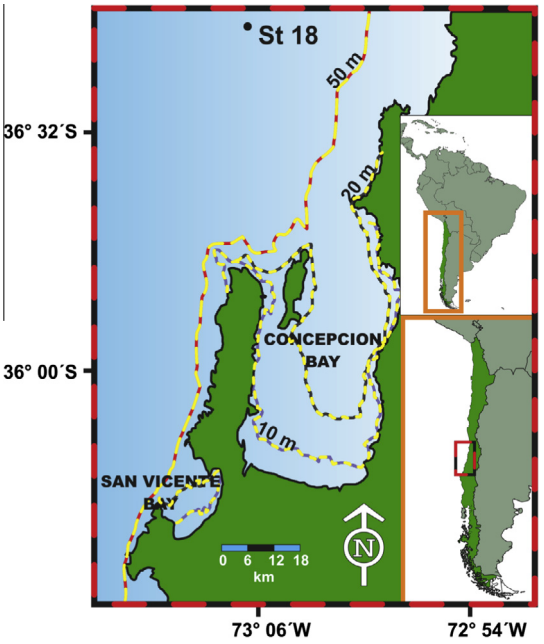

Fig. 1. Map indicating the location of Station 18 (36°30′S and 73°07′W) sampled monthly by the COPAS oceanographic center as part of a physical–chemical–biological integrated time-series study since 2002. Bathymetric lines (meters) are shown.

Dissolved dimethylsulphoniopropionate (DMSPd) was measured according to published procedures (Kiene and Slezak, 2006). DMSPt (DMSP total) samples were directly collected from Niskin bottles as unfiltered seawater samples and stored in the dark in 250 mL polycarbonate bottles and during the 2010–2011 upwelling season until their return to the laboratory (Dauphin Island Laboratories of the University of Alabama, USA) for further analysis. DMSPd and DMSPt samples were added to NaOH in a sealed vial and the resulting DMS quantified by GC (Kiene and Slezak, 2006; Rellinger et al., 2009). The difference between DMSPt and DMSPd results in DMSPp (particulate DMSP).

Nutrient measurements (NO_3^-, NO_2^- and PO_4^{3-}) were performed for each depth; samples were taken in 20 mL polyethylene flasks and analyzed after using an automatic SEAL Autoanalyser (analytical error better than 3%). Samples for Chlorophyll-a (Chl-a) was filtered onto a glass–fiber filter, and the filter in triplicate was

immediately frozen (−20 °C). Samples were kept until later analysis by fluorometry (Turner Design AU-10) previously acidification following Parsons et al. (1985).

The $\delta^{13}C$–CH_4 values were determined by a Delta XP plus Finnigan mass spectrometer. The extracted gas was purged and trapped with PreCon equipment (Finnigan) to pre-concentrate the sample. Depending on the CH_4 concentration, the reproducibility derived from duplicates was 0.5–1‰. All isotopic ratios were given a δ-notation relative to the Pee Dee Belemnite (PDB) standard (Craig, 1957; Damm et al., 2010).

2.3. Experimental assays

During biogeochemical oriented cruises undertaken in the upwelling season (see Table 1), on board CH_4 assessments were done in order to assess CH_4 cycling rates and determine the mechanism for CH_4 production and/or consumption. For this purpose, seawater taken at 10 m depth (a depth where a Chl-a peak is generally found) was dispensed into 2-L double-laminated aluminum–polyethylene bags. Each bag had a hose/valve with a septum through which all the different treatments were injected. A permeability test was performed prior to the experiment (for details see Farías et al., 2009). The net or gross CH_4 cycling rate was determined by incubating the bags for 12 h (total incubation time) at a temperature (14 °C) close to *in situ* conditions. Experiments without any additions (control) represent a net CH_4 cycling rate (production minus consumption); while experiments with additions and with ultrapure acetylene (C_2H_2 final concentration of 10% v/v), whose effect has been described as inhibiting the CH_4 monooxygenating (MMO) enzyme and the growth of methanotrophs (Prior and Dalton, 1985) represent gross CH_4 cycling rate. However, it is important to note that C_2H_2 is a very unspecific inhibitor, blocking the activity of methanogens (Sprott et al., 1982) and nitrifying microorganisms (Hyman and Arp, 1992) as well. Thus, if methanogens (mainly associated with micro-niches) are absent in the oxic water and if methanotrophs are active, and they are sensitive to C_2H_2, this treatment would lead to reduction of CH_4 consumption. Therefore, a CH_4 consumption rate by aerobic CH_4 oxidation could be estimated from the rate measured in C_2H_2 experiments minus the rate quantified in control experiments.

In parallel, ^{13}C-DMS (labeled in both C of methyls groups) experiments were conducted at one standard depth (10 m) in the time series station. For this purpose, an isotopic solution (5 μmol L^{-1}) was added with a spike of 1 mL until a final concentration ~6 nmol L^{-1} in order to determine whether CH_4 was produced by DMS through any reaction (e.g., demethylation); the CH_4 cycling rate was compared to net CH_4 cycling (control) and net apparent production (C_2H_2 experiment). Finally, poisoned treatments (with $HgCl_2$) were performed in order to assess whether biological processes or abiotic reactions were occurring. All additions were slowly injected into the bags through a septum with gastight syringes. Subsamples (triplicate) of each treatment were retrieved from the bags (one or two bags per experiment) into 20 mL GC vials for CH_4 analysis at different times (0, 6 and 12 h) by applying pressure on the bags; this water was poured into GC bottles (20 mL). For each incubation time, three GC bottles with incubated water were injected with 50 μL of saturated $HgCl_2$ in order to stop all biological reactions; the bottles were then sealed with rubber stoppers and metallic caps.

Finally, the isotopic composition of CH_4 was measured during incubations in September 2010 and January 2011, before and after the addition of the ^{13}C-labeled compound with 99% of purity (Cambridge Isotope Laboratories, USA). Labeled DMS (^{13}C-DMS) was added to the bags at a final concentration of 6 nmol L^{-1} in order to directly assess the origin of CH_4 production from organic labeled compounds. The ^{13}C-DMS tracer additions was estimated to be close to 30–100% of ambient concentrations in surface waters which could fluctuated from <5 to 15 nmol L^{-1} in upwelling areas according to Andreae (1985) and Lana et al. (2011). In this study, DMS levels in seawater were not measured. Finally, sub-samples from the bags were taken similar to the experiments described previously, recovering subsample (triplicate) in 20 mL vials at zero and final time. These subsamples were immediately poisoned and the stable carbon isotopic composition of CH_4 accumulated inside the bags was determined.

2.4. Data analysis

In order to determine the CH_4 isotopic composition and its cycling rate (production and consumption rate experiments), CH_4 concentration during incubation (from different treatments and bags) were plotted against time and fitted to a linear $[A(t) = A_0 \pm m * t]$ model using the least squares method; where t is the incubation time, A_0 is the gas concentration at $t = 0$ (after DMS addition), and m is the slope ratio. Slope was calculated from the linear regression, and the rate uncertainties (±) were calculated from the linear regression errors. The rate uncertainty for control, C_2H_2 and DMS treatments was calculated directly from the standard error of the slope. Propagation of error was estimated for CH_4 oxidation rates (control minus acetylene treatments). Student's t-test and ANOVA were used to evaluate the significance of slopes and the differences between the treatments. Positive values represented CH_4 production or accumulation over time (as in the cases of inhibitors), whereas negative values indicated consumption. CH_4 concentration data were analyzed for differences between treatments using a two-way analysis of variance. Data were checked for normality using a Shapiro–Wilk test and for homogeneity with a constant variance test. Differences between treatments (Control, C_2H_2 and DMS-spike) were evaluated using one-way ANOVA.

To interpret vertical variation of CH_4 and how processes affect concentration, the water column was divided in two layers according with mixing layer (thermocline distribution): (1) well-mixed layer (and also photic) with high O_2 content; and (2) subsurface and aphotic layer with O_2 deficient waters (see criteria in Farías et al., 2009). CH_4 inventories were calculated by numerical integration of data from the surface and subsurface layers based on at

Table 1
Total and dissolved DMSP concentrations, DMSPp:Chl-a ratios and Pearson correlations values.

Month	DMSPd (nM)	DMSPt (nM)	DMSPp:Chl-a (nmol μg^{-1})	Correlations	
				DMSPd-Cl-a	DMSPt-Cl-a
September 2010	0.33–26.4 (5.11)	0.9–60 (17.36)	1.21	0.68	**0.84***
November 2010	0.2–21.5 (3.65)	1.65–43 (15.51)	11.8	**0.94****	**0.98****
December 2010	0.4–6.3 (1.65)	4.12–25 (12.54)	1.07	0.03	**0.69***
January 2011	0.35–20 (3.96)	5.10–109 (42.81)	28.1	**0.99****	**0.82***

Bold values are statistical significant.
* $p < 0.05$.
** $p < 0.01$.

least 4–6 sampled depths by layer. Saturation of CH_4 was estimated from the measured and expected CH_4 concentration in the water column, corresponding temperature and salinity, and based on equilibrium CH_4 concentration with atmospheric CH_4 calculations delineated by Weisenburg and Guinasso (1979).

3. Results

3.1. Temporal variability of physical–chemical variables (including CH_4)

Temporal variability of physical (temperature and salinity) and chemical (O_2 and CH_4) variables in the water column (from April 2009 to April 2011) is shown in Fig. 2. Temperature (Fig. 2A) is influenced by both the upwelling process (mainly at subsurface waters) and solar radiation (mainly surface waters), both being concurrent in spring–summer. These patterns give the system strong thermal stratification during spring–summer in comparison with fall–winter time. Salinity (Fig. 2B) is also affected by vertical advection of relatively high salinity waters during spring–summer time and by freshwater inputs (rainfall and river discharges) during late fall–winter time. This marked seasonality in terms of both variables is well documented in this region by Sobarzo et al. (2007).

Dissolved O_2 levels (Fig. 2C) are fully saturated (>200 μmol L^{-1}) at the surface throughout the entire study period, but displays a strong vertical gradient below the mixed layer (~20 m depth), reaching values as low as 1.0 μmol L^{-1} at bottom depths close to sediments during the upwelling periods (i.e., September–April). In contrast, during non-upwelling periods, i.e., fall–winter time (May–August), dissolved O_2 concentrations under 44 μmol L^{-1} are restricted to the bottom waters and high values throughout the water column were observed, usually coincident with periods of strong vertical mixing and winter storms. Dissolved CH_4 concentrations (Fig. 2D) show a clear seasonal signal consisting of accumulated CH_4 up to 65 nmol L^{-1} in bottom waters close to sediments during upwelling periods. This period matches low dissolved O_2 concentrations and the accumulation of biogenic organic particles from surface waters. In surface waters CH_4 levels fluctuate between 3.08 and 16 nmol L^{-1} (equivalent to 123–550% saturation), with maximum values found during the upwelling period.

3.2. Nutrient and chlorophyll-a

The temporal variability of nutrient and pigment variables in the water column (from April 2009 to April 2011) is shown in Fig. 3. NH_4^+ concentrations (Fig. 3A) in surface waters are highly fluctuating with submicromolar levels in summer time and as high as 1.8 μmol L^{-1} in winter time. NO_3^- and PO_4^{3-} classically considered as preformed nutrients also display high variability during the study period, ranging from 5.5 to 17 μmol L^{-1} for NO_3^- and from 0.9 to 1.5 μmol L^{-1} for PO_4^{3-}. Most of the time, surface waters never display depleted nutrient concentrations, in spite of high biological production and even strong NO_3^- and NH_4^+ uptake observed during the development of phytoplankton blooms (Fernandez and Farías, 2012). PO_4^{3-} is in excess relative to the Redfield ratio. Chlorophyll-a levels show peaks during each upwelling season with values fluctuating between 1 and 25.5 μg L^{-1} (Fig. 3D). It is important to note that in winter Chl-a levels are around 1 μg L^{-1}. These patterns appear to be associated with not only frequency of fertilizations by vertical advection driven by cumulative upwelling events, but also by phytoplankton structure and composition.

3.3. Vertical distribution of DMSPd, DMSPt and Chl-a

Vertical distribution of Chl-a and DMSPp are illustrated in Fig. 4. DMSPp (the difference between total and dissolved DMSP) levels, measured only in spring and summer time of the 2010–2011 years, varied from 7 to 80 nmol L^{-1}. DMSPp peaked at 10–20 m depth (Fig. 4A–C), except in January 2011 when the highest and variable DMSPp levels (up to 80 nmol L^{-1}) are found. In contrast, Chl-a profiles show exponential decreases with increasing depth (according to the photic layer).

DMSPp: Chl-a ratios range from 1.07 (December 2010) to 28 nmol μg^{-1} (January 2011). This strong variation during upwelling period appeared to be affected by changes in the phytoplankton composition as described by Anabalón et al. (2007). Concentrations of DMSPt and DMSPd, along with DMSPp:Chl-a ratios and correlations between Chl-a and DMSP are shown in Table 1. This reveals large variations of DMSP with minimum values <2 nmol L^{-1} in both fractions and maximum values of 42.81 and 5.11 nmol L^{-1} for DMSPt and DMSPd, respectively. Correlations among Chl-a content and DMSPp are always positively and significantly correlated (Table 1).

3.4. Methane cycling in surface water

Methane cycling rates in the control, acetylene (C_2H_2) and DMS spike treatments, as well as estimations of aerobic CH_4 oxidation rates for different sampling periods are presented in Table 2. Typical time course experiments based on described experimental set-up are illustrated in Fig. 5. Rates and standard errors are expressed in nmol L^{-1} d^{-1}. Net CH_4 cycling rates measured in control experiments (−2.64 to 1.44 nmol L^{-1} d^{-1}) are usually lower than those rates in the C_2H_2 treatments (0.96 up to 5.53 nmol L^{-1} d^{-1}), indicating that most of the time (5 of 7 experiments) there are a consumption of CH_4 at variable rates. Since the experiments with C_2H_2 addition are significantly different ($p < 0.05$) to the control (in 4 of 5 experiments), this trend suggests that there is a sensitive C_2H_2 microbial assemblage and that it always lead to a CH_4 accumulation higher than control. Thus, this treatment represents a gross CH_4 production and it is possible to estimate an aerobic CH_4 oxidation rate ranged from 2.91 to 6.50 nmol L^{-1} d^{-1} (through the difference between C_2H_2 minus control), precluding any effect of the methanogens if they were active.

The DMS-spiked experiments also increase significantly with respect to C_2H_2 and control experiments (in 5 of 7 experiments), with the CH_4 cycling rate fluctuating between 1.68 and 4.56 nmol L^{-1} d^{-1} and indicating extra CH_4 production using DMS as a substrate. The isotopic composition in CH_4 cycling experiments after the addition of similar doses of DMS (6 nmol L^{-1} final concentration) but with methyl groups labeled (^{13}C-DMS), shows a significantly heavier CH_4 isotopic composition ($\delta^{13}C$–CH_4 ~ −35.43‰ to −43.85‰ PDB) than the isotopic composition immediately previous to the tracer additions (time zero; $\delta^{13}C$–CH_4 ~ −44.71‰ to −45.72‰ PDB). Fig. 6 illustrates the temporal evolution of conversion/incorporation of the methyl groups to CH_4, shown by different extents of ^{13}C-enrichments in the end time incubation. Experiments with ^{13}C-labeled DMS show recovered dissolved CH_4 enriched in ^{13}C after the incubation time, which is significantly less negative than the early days of incubation (Fig. 6). Isotopic fractionation in experiments amended with $HgCl_2$ does not change after incubation (data not shown).

4. Discussion

4.1. Methane content in the water column

The continental shelf off central Chile is an ecosystem under the influence of wind-driven coastal upwelling, which drives a well-studied seasonal signal in most physical–chemical variables (e.g., Sobarzo et al., 2007). A typical consequence of the local wind forcing is the fertilization of surface water column that increases

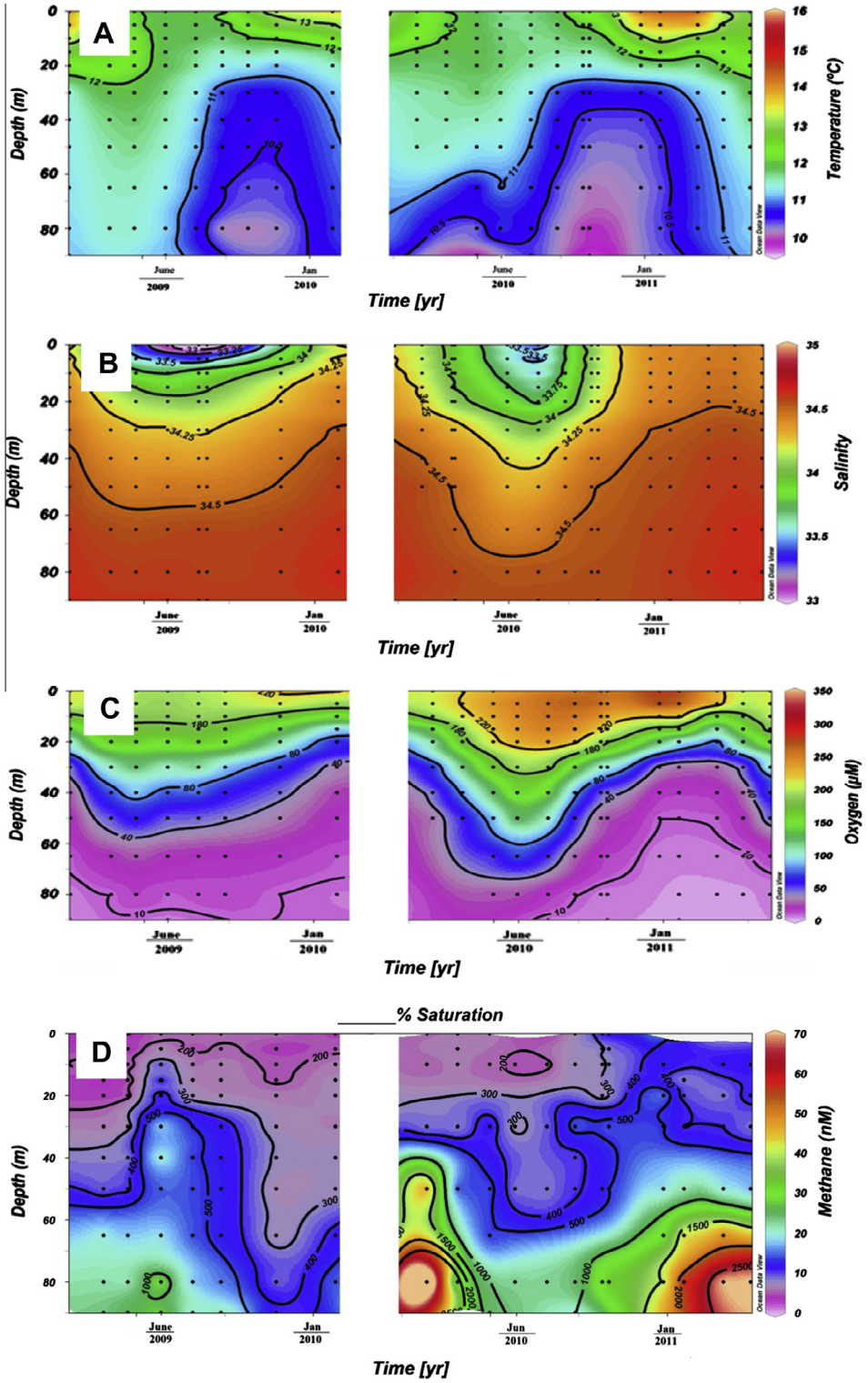

Fig. 2. Time series and vertical distribution of the oceanographic variables during July 2009–July 2011 at St. 18 off central Chile: (A) temperature °C, dashed line represents the 11 °C isotherm; (B) salinity; (C) dissolved oxygen concentrations µmol L^{-1}, the solid line shows 40 µmol L^{-1} O_2 concentrations; (D) methane concentrations (nmol L^{-1}), the lines indicate % saturations. Discontinuity in the data was caused by the February 27th 2010 Chilean earthquake and tsunami.

primary production during the austral spring–summer (with ranged from 1.2 to 10 g C m^{-2} d^{-1}, Farías et al., 2009) and also leads to strong outgassing of N_2O and CH_4 (Cornejo et al., 2007; Naqvi et al., 2010).

The sediments of the continental shelf harbor intense anaerobic organic matter remineralization processes as a consequence of strong organic matter sedimentation, which matched periods of suboxia/anoxia (upwelling periods). Thus, methanogenesis in sediments (Ferdelman et al., 1997), as well as the presence of cold seeps (Jessen et al., 2011; Sellanes et al., 2011) could mean that the sediments are an important CH_4 source toward the water column.

A marked increase of CH_4 with depth is observed in 75% of the sampling period. This CH_4 vertical trend is also associated with a

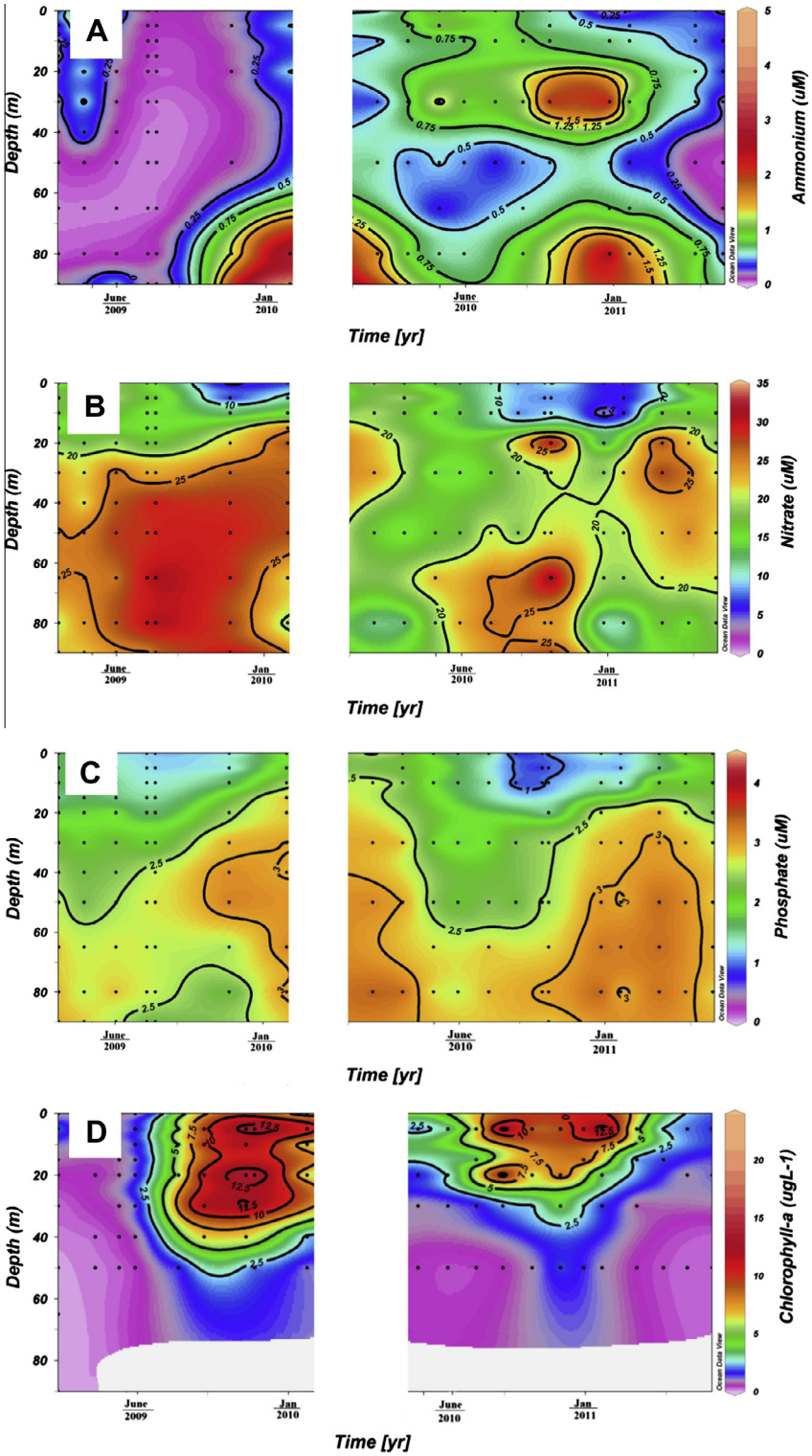

Fig. 3. Time series and vertical distribution of the oceanographic variables during July 2009–April 2011 at St. 18 off central Chile: (A) ammonium (μM), (B) nitrate (μM), (C) phosphate (μM), and (D) July 2009–December 2010, Chl-a concentration (μg L^{-1}).

market dissolved O_2 decreases towards the bottom waters, with dissolved O_2 concentration reaching values as low as 5 μmol L^{-1}. This vertical pattern suggests that the anoxic sediments and even the bottom waters are the main CH_4 source for the whole water column, which is subjected to an upward advection by upwelling. Table 3 shows CH_4 inventories in the mixed and subsurface layer, the latter spanning from the base of the thermocline (or the base of the mixed layer) down to the sediment interface. Estimated CH_4 inventories revealed that the subsurface CH_4 pool size is 4- to 6-fold greater than the CH_4 accumulated within the mixed layer, and that the CH_4 increased levels between early spring to late summer responded to successive organic matter cumulative events during the 2010–2011 upwelling season (see Table 3). The biogeochemical O_2 demand associated with organic matter decay, along

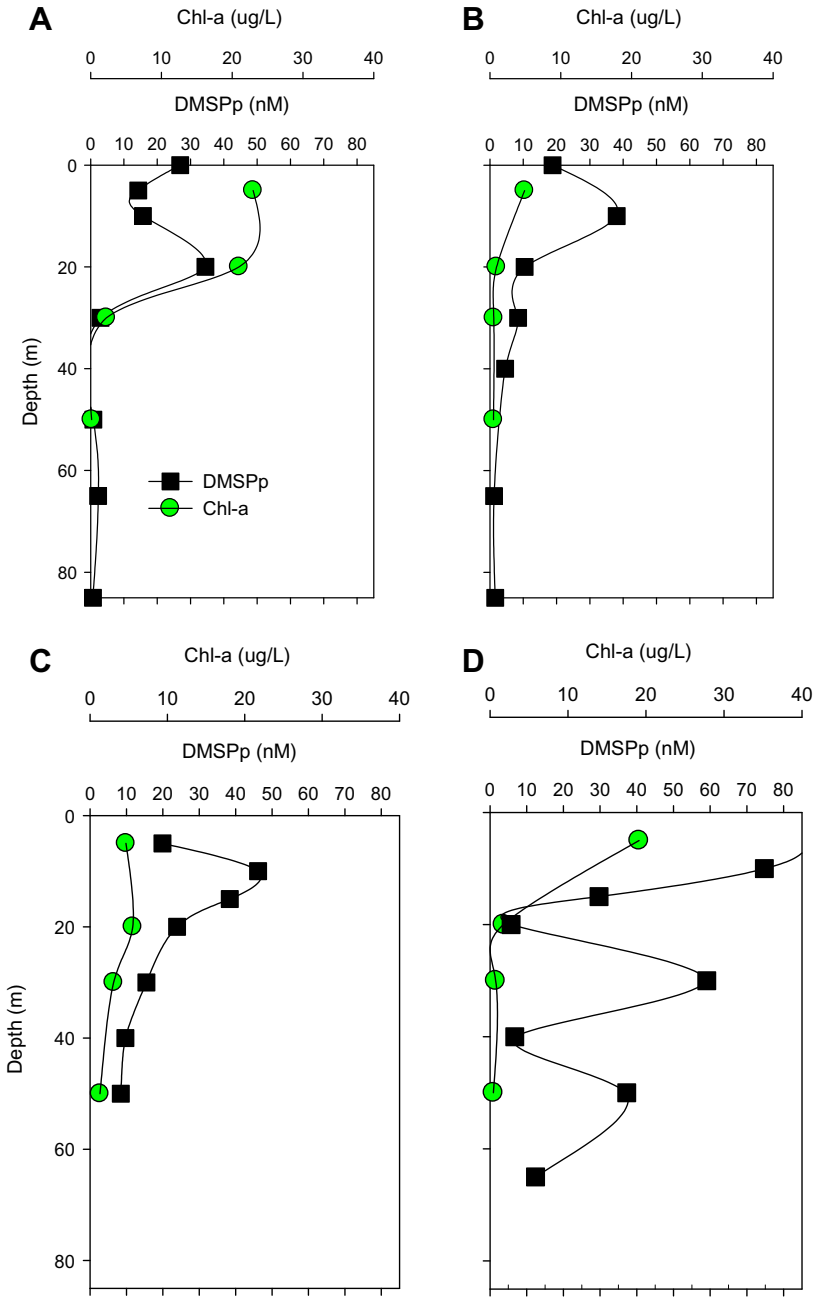

Fig. 4. Profiles of DMSP and Chl-a during the austral spring and summer (2009–2010) of: (A) September 2010; (B) November 2010; (C) December 2010; and (D) January 2011.

Table 2
Net CH_4 cycling rates and its net production, potential production and estimated CH4 oxidation rates obtained during favorable upwelling periods (austral spring–summer time) in two consecutive years (2009–2011).

Rates (nM d^{-1})	Period	2009		2010			2011	
	Treatments	August	September	September	October	December	January	March
Net CH_4 cycling	Control	−2.64 ± 1.44	1.44 ± 0.24	ND	1.2 ± 0.72	−0.64 ± 1.68	−0.29 ± 0.20	−0.41 ± 0.12
CH_4 production[a]	Acetylene	ND	0.96 ± 0.24 ($P < 0.001$)	5.53 ± 0.24 ND	7.08 ± 0.96 ($P = 0.00$)	4.0 ± 1.66 ($P < 0.05$)	2.61 ± 0.48 ($P < 0.001$)	ND
Potential CH_4 production	DMS Spike	−1.20 ± 1.20 ($P = 0.15$)	1.68 ± 0.07 ($P < 0.001$)	−0.96 ± 0.48 ($P < 0.05$)	2.64 ± 1.92 ($P = 0.002$)	4.56 ± 0.48 ($P < 0.005$)	−0.98 ± 0.30 ($P < 0.001$)	1.03 ± 0.64 P = ND
CH_4 consumption[b]	Estimation	NE	−0.48 ± 0.33	NE	5.82 ± 1.21	4.64 ± 2.36	2.90 ± 0.52	NE

Significant value (p-value) of comparison between acetylene and control treatments and, DMS spike and acetylene treatments are included in parenthesis. ND: No determined; NE: no estimated.

[a] Microbial assemblage sensitive to C_2H_2.

[b] Difference between acetylene minus control treatments.

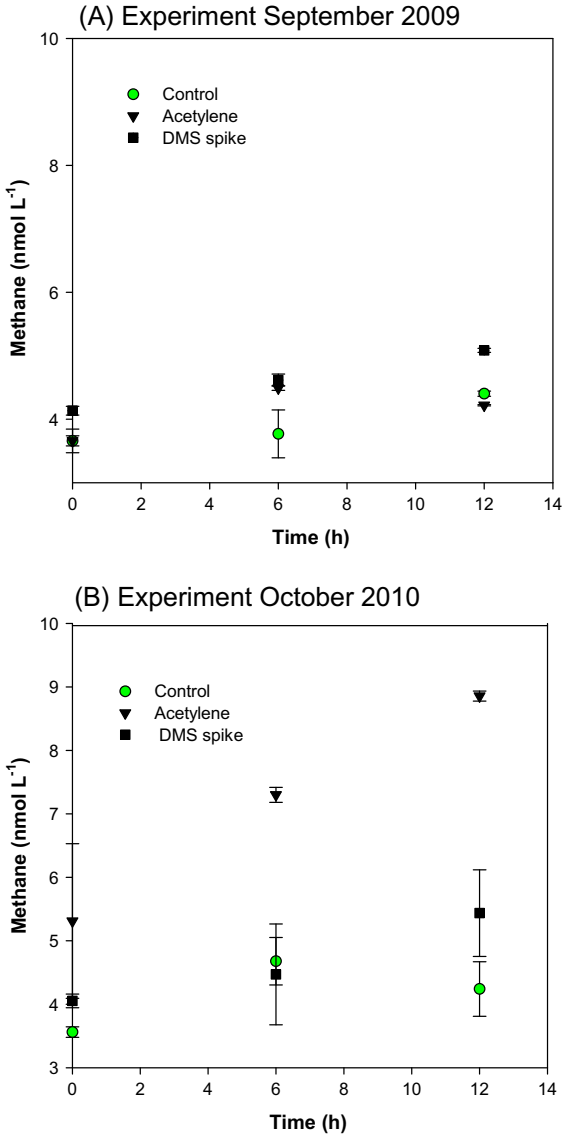

Fig. 5. Typical example of time course experiments of CH_4 cycling under different treatments (A) control (without any addition) represent net CH_4 cycling; (B) with C_2H_2 preventing potential consumption by methane oxidation; and (C) with a spike of DMS (final concentration of 6 nmol L^{-1}).

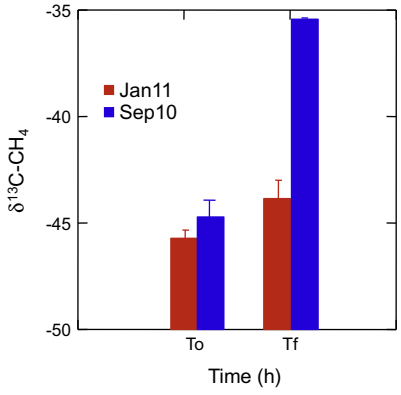

Fig. 6. Stable carbon isotopic composition of methane (expressed as delta) during sampling incubations, before and after the addition of ^{13}C-DMS at Station 18 on the continental shelf off central Chile. Experiments were carried out in small bottles (20 mL) during September 2010 (A), January 2011 (B).

with pre-existing low O_2 concentrations following the distribution of ESSW (Paulmier et al., 2006), triggered the high production and accumulation of CH_4 in the sediments and even within the water column.

In fact, an intense vertical advection by coastal upwelling accelerated the vertical transport of CH_4 towards the surface during the spring–summer season, also leading to the rise of thermocline (Fig. 2A), oxycline, (Fig. 2C) and nutricline (Fig. 3B and C). This process causes surface fertilization (reflected by Chl-a; Fig. 3D) and degasification (reflected by CH_4; Fig. 2D). Upon observing the CH_4 time series in surface waters, CH_4 concentrations were always oversaturated (123–550%), forming sometime structures with high CH_4 accumulation in the mixed layer, a pattern that may indicate of a local production.

Methane flux across the air–sea interface, estimated from the gas transference velocity (k cm h^{-1}), CH_4 concentration measured in the mixed layer and those CH_4 concentrations in relative equilibrium with the current atmospheric concentration, were calculated according Broecker and Peng equation modified by Wanninkhof (1992). These estimates indicated that gas exchanges were always positive (efflux) and ranged from 11 to as high as 115 μmol m^{-2} - d^{-1}, with high variability mainly controlled by favorable upwelling wind velocities and to a lesser degree by the CH_4 content in the mixed layer.

These effluxes were relatively high with respect to other coastal areas (see Bange, 1994) and comparable with CH_4 emissions from other coastal upwelling areas (Monteiro et al., 2006; Kock et al., 2008). CH_4 emission has been indirectly linked to high primary productivity, which favors CH_4 formation in sinking organic particles or in sediments (Owens et al., 1991; Sansone et al., 2001; Monteiro et al., 2006; Reeburgh, 2007). But as shown in Table 2, the experimental work in this study indicated that part of the CH_4 contents seems to come from the surface waters and that such produced gas could be emitted to the atmosphere. In fact, *in situ* CH_4 production at surface waters and vertical transport through the pycnocline should occur at higher rates in order to compensate CH_4 loss by effluxes and consumption (i.e., aerobic CH_4 oxidation). Thus, CH_4 effluxes represent 8–169% of depth integrated CH_4 production rates, which replace respective surface pools, between 0.8 and 5.2 days (see Table 3).

4.2. Rates of methane produced in surface waters

Methane formation is relatively certain in surface waters located outside anoxic areas. For instance, distinctive superficial peaks, some of which are located at the base of the photic layer, have frequently been described in marine environments (Lamontagne et al., 1973; Holmes et al., 2000), leading to the discussion on CH_4 sources in such layers. New insights reveal that CH_4 could be a by-product during the cycling of MPn or DMSP, but both mechanisms seem to be dependent on strong limitations or co-limitation of nutrients that trigger utilization of the compound (Karl et al., 2008; Damm et al., 2010). However, in the coastal upwelling off central Chile, high nutrient levels were observed throughout NO_3^- and PO_4^{3-} during the study period (Fig. 3), and never a nutrient depletion was found even during winter (Morales and Anabalón, 2012).

Our results indicate that net CH_4 production rates were positive (net accumulation) in most of the experiments done in surface waters during two consecutive upwelling periods, with rates fluctuating between 0.96 and 5.5 nmol d^{-1}. Moreover, DMS-spiked experiments produced higher CH_4 cycling rates, from 20% to 8-fold, compared with control experiments (see Table 2), suggesting that an increase of CH_4 production follows the addition of DMS.

Besides an increment of net production rates, the isotopic composition of CH_4 after the addition of DMS (99% enriched in ^{13}C)

Table 3
Surface daily integrated rates of net CH_4 production and consumption rates along with their respective turnover times based on surface pool size. It includes estimations of CH_4 air–sea exchange during coastal upwelling period (2010–2011).

Parameters	2010			2011	
	October	November	December	January	March
Integrated net production rate ($\mu mol\ m^{-2}\ d^{-1}$)	141 ± 19	ND	80 ± 33	52 ± 9.6	45 ± 4.0
Integrated net consumption rate ($\mu mol\ m^{-2}\ d^{-1}$)	116 ± 24	ND	93 ± 47	58 ± 16	53 ± 4.6
Surface inventory ($\mu mol\ m^{-2}$)	108	236	324	141	236
Subsurface inventory ($\mu mol\ m^{-2}$)	975	1716	1487	2470	2371
Air–sea CH_4 flux[a] ($\mu mol\ m^{-2}\ d^{-1}$)	11.4	124	41.1	20.8	76.3
Surface turnover[b] (days)	0.76 (0.92)	NE	4.0 (3.5)	2.7 (2.4)	5.2 (4.4)

[a] Air–sea fluxes based on mean CH_4 concentration in the mixed layer and piston velocities (wind velocities fluctuating 3.5 and 15.6 m s^{-1}) according Wanninkhof (1992).
[b] CH_4 turnover time based on surface CH_4 pool mixed layer of depth of 20 m according this study and previous data by Farías et al. (2009) divided by depth integrated production and consumption rates (parenthesis values). ND: no determined: NE: no estimated.

revealed a significant enrichment in its heavier Carbon isotope after 12 h of incubation (i.e. −35.43‰ to −43.85‰ PDB) in relation to the natural CH_4 isotopic signals and those obtained from experiments poisoned with $HgCl_2$ (a poison for most microbes) (Fig. 6). It is important to note that natural $\delta^{13}C$–CH_4 values (i.e. −44.71‰ to −45.72‰ PDB) are comparable to those values found in natural systems subject to considerable CH_4 oxidation (Sansone et al., 2001), as CH_4 oxidation leaves the residual CH_4 pool slightly enriched in ^{13}C in comparison to the atmospheric background of −47‰ PDB) because of the favored microbial use of ^{12}C relative to ^{13}C in CH_4 (Coleman et al., 1981; Sasakawa et al., 2008). This finding directly shows that part of the CH_4 came from DMS added to the experiment while the extent of the enrichments in relation to natural measured values suggests that CH_4 production varied according to the period of time.

As the exceeding rates of CH_4 production follows the addition of DMS (Table 2), we postulate that CH_4 formation came from during the demethylation of DMS due to an increase in the dissolved methyl group (R-CH_3), and its subsequent reduction (electron gain) during the oxidation of the methylated compound. This process may be mediated via methylotrophic microorganism activity.

Although coastal upwelling systems are not areas with high levels of DMS, in contrast to the Southern and Arctic Oceans (Gabric et al., 2005; Rellinger et al., 2009), they could have high DMSP and DMS transient accumulations and, therefore, their respective DMSP/DMS turnovers associated with microbiological communities (Vila-Costa et al., 2008) could be higher as well. In addition, the DMSPp:Chl-a ratio, used to estimate the proportion of algal DMSP-producers in natural phytoplankton assemblages, displayed great variation throughout this study (see Table 1), with ratios as high as 28 nmol μg^{-1} in January 2011, and less than 1 nmol μg^{-1} at the beginning of the upwelling season. This may correspond to a shift in phytoplanktonic communities from large pennate diatoms (during spring or early upwelling) to smaller dinoflagellates and picophytoeukariote assemblages during the maturation of upwelling (González et al., 2007; Morales et al., 2007; Collado-Fabbri et al., 2011).

These results raise questions regarding which microbes are benefited by the additions of S- and C-rich organic substrates, such as DMS, and how (functional pathways) they may be responsible for such CH_4 production. There are two possibilities: methanogens are present and active in the surface waters or other heterotrophic bacteria like methylotrophs are mediating CH_4 production. Most substrates for methanogens and methylotrophs, such as methylamine and methyl groups from glycine betaine (GBT), trimethylamine (TMA), trimethylamine N-oxide (TMAO), and DMSP/DMS (Cicerone and Oremland, 1988; Finster et al., 1992; Van der Maarel, 1997) are shared by both groups (except acetate used only by methanogens). It is important to remark that DMSP/DMS are among the most abundant compounds in the surface waters, being synthesized by phototrophs (Vila-Costa et al., 2008). DMS-degradation is ascribed to methanogenic archaea (Kiene et al., 1986; Kiene and Visscher, 1987) and methylotrophic bacteria (Vissher et al., 1994) that derive energy from the conversion of methyl into other products, and also use S as a source for the biosynthesis of methionine (Kiene et al., 1999).

Current studies based on natural and cultivated SAR11 Alphaproteobacteria (strain Ca. P. ubique HTCC1062; Sun et al., 2011) indicate that these microorganisms, some of the most abundant heterotrophic bacteria in surface waters, have genes that encode for oxidation pathways of a variety of one-carbon compounds, and they possess the capacity for demethylation and C1 oxidation, but do not incorporate C1 as biomass. These findings suggest the important role of this assemblage in obtaining energy from these compounds, and in mediating dissolved organic carbon cycling into CO_2 in the upper ocean (Sun et al., 2011). Neufeld et al. (2008) demonstrated that active marine methylotrophs belonging to the Alphaproteobacteria and Gammaproteobacteria are associated with phytoplankton blooms in coastal environments, which in turn, and depending on the phytoplankton species, provide substrates for methylotrophs and mediate CH_4 production. Additionally, in a microcosm experiment where CH_4 is formed in DMSP spiked seawater, the CARD-FISH analysis shows that *Archaea* remained negligible in the DMSP supplemented approaches while *Bacteria* became nearly 100% of the community and *alpha* and *gamma-proteobacteria* together accounted for more than 75% of the DAPI-stained cells (Damm et al., 2010). Recent observations indicated that Alphaproteobacteria (including SAR11) and Rodobacteria are dominant in upwelling system off central Chile (Pommier et al., 2007; Aldunate, personal communication). The cosmopolitan distribution of Alphaproteobacteria supports the hypothesis that these bacteria are able to produce CH_4 in surface waters. However, ongoing research is required to prove the proposed pathways.

4.3. Potential AMO contribution to methane consumption rates

Interestingly, although CH_4 microbial oxidation occurs throughout the water column and is recognized to be an important process that reduces CH_4 emissions (Reeburgh et al., 1993; Rehder et al., 1999), the microbial community mediating aerobic CH_4 oxidation has scarcely been investigated. If net cycling rates (production minus consumption) are corrected with the apparent CH_4 production (C_2H_2 experiments), an apparent aerobic CH_4 consumption rate could be obtained. Given that C_2H_2 is considered as an effective inhibitor of the MMO enzyme (Hanson and Hanson, 1996) and assuming that most methanotrophs are sensitive to C_2H_2, our cycling rates should represent methanotrophic activity.

Considering that our rates are representative of surface waters (Table 2), their integrated rates in surface or mixing layers can reduce CH_4 pool size between 0.9 and 4.2 days (Table 3), highlighting

the relevance of methanotrophic activity in partially reducing the emission of this gas towards the atmosphere. Although there are few measurements of aerobic CH_4 oxidation in marine environments, our estimated rates are considerably higher than CH_4 oxidation rates found in open systems under oligotrophic regimes (Tilbrook and Karl, 1995; Holmes et al., 2000), but are similar to those measured in the oxic waters of the Black Sea (Reeburgh et al., 1991; Gal'chenko et al., 2004). However, in these systems there is a marked increase in the magnitude of CH_4 oxidation rates towards the oxic/anoxic interface, suggesting that oxidation is stimulated in areas where strong redox gradients are present.

An early study reported mean aerobic CH_4 oxidation rates of 10 nmol d^{-1} when DIN levels were less than 5 μmol L^{-1}, however with DIN as high as 16 μmol L^{-1}, CH_4 oxidation rates were as high as 210 nmol d^{-1} (Sansone and Martens, 1978). These findings, along with physiological similarities between methanotrophic and ammonia oxidizing bacteria, suggest the possibility that ammonia oxidizing microorganisms could be utilizing CH_4 during their metabolic activities. Finally, our studied system, as seen in other systems with similar characteristics, is recognized as a hotspot of chemosynthetic activity, where high rates of dark carbon assimilation are measured, even in surface waters, given the high availability of electron donors other than organic matter in both surface and subsurface waters, such as NH_4^+ for ammonia oxidizing microorganisms (Farías et al., 2009) Here, large amounts of NH_4^+, CH_4 and even HS^- are able to channel large amounts of chemical energy for the generation of organic matter (dark carbon assimilation). This is also the case of the Cariaco Basin (Taylor et al., 2001), the Black Sea (Wakeham et al., 2004), the Arabian Sea (Owens et al., 1991).

5. Conclusion

The results found here are relevant to climate studies given that the amount of "unknown" CH_4 in surface layers may be substantial in areas where DMS has high turnovers. Thus, a positive climatic feedback mechanism could be produced, destroying DMS (with cooling effects) and producing CH_4 (with a greenhouse effect), thereby affecting the radiative balance of the atmosphere.

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