**Biogeosciences
Discussions**

High production of nitrous oxide (N$_2$O), methane (CH$_4$) and dimethylsulphoniopropionate (DMSP) in a massive marine phytoplankton culture

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High production of N$_2$O, CH$_4$ and DMSP in phytoplankton culture

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Abstract

The production of large amounts of algal biomass for different purposes such as aquaculture or biofuels, may cause impacts on the marine environment. One such impact is the production of radiatively active trace gases and aerosols with climate cooling (dimethyl sulfide DMS and its precursor DMSP) and warming (N$_2$O and CH$_4$) effects. Total and dissolved DMSP, N$_2$O and CH$_4$, together with other environmental variables were monitored daily for 46 days within a massive microalgae monoculture of *Nannochloris* (Chlorophyceae) in an open pond system. The growth of this green microalgae was stimulated by the addition of N- and P-rich salts, resulting in exponential growth (growth phase) during the first 17 days observed by cell abundance (1×10^6 to 4.4×10^6 cell mL^{-1}) and Chl-*a* levels (from 1.4 to 96 mg Chl-*a* m^{-3}), followed by a decrease in both Chl-*a* and cell abundance (senescence phase). Total DMSP (from 6.3 to 142 µmol m^{-3}), dissolved DMSP i.e. 5.8 to 137 µmol m^{-3} and N$_2$O (from 8 to 600 µmol m^{-3}) abruptly peaked during the senescence phase, whereas CH$_4$ steadily increased between 2 and 10 µmol m^{-3} during the growth phase. Different ratios between tracers and Chl-*a* during both phases reveal different biochemical processes involved in the cycling of these gases and tracers. Our results show that despite the consumption of large quantities of CO$_2$ by the massive algal culture, a minor amount of DMS and huge amounts of greenhouse gases were produced, in particular N$_2$O, which has a greater radiative effect per molecule than CO$_2$. These findings have important implications for biogeochemical studies and for environmental management of aquaculture activities.

1 Introduction

Interest in the domestication of the oceans is growing. It is seen as a possible way to increase global food supply and avoid the "Malthusian paradigm" that could face humanity in coming years (Duarte et al., 2007). Recently, ocean domestication in the

form of industrial aquaculture has been one of the fastest growing economic sectors, making up around 10% of the global food industry (FAO, 2001). The environmental impacts of aquaculture are diverse and include important modification and acceleration of biogeochemical fluxes with respect to natural aquatic systems. The largest chemi-
5 cal fluxes are released as carbon (C), nitrogen (N), phosphorus (P) and sulfur (S), all metabolic byproducts of living organisms. If the fluxes of these compounds into the environment exceed the assimilative capacity of the ecosystem, they can severely affect the chemistry of the water column and the sediments, causing eutrophication, oxygen depletion and the consequential disruption of local biodiversity, among other effects.
10 Unicellular marine algae are widely used as food in the hatchery production of filtering organisms (e.g., mollusks). Nowadays, most microalgae are massively grown in open ponds, which are cost efficiento build and operate. Through microalgae culture, light energy is converted into biomass, removing large amounts of carbon dioxide (CO_2) from the atmosphere. However, it is not yet known whether these systems
15 lead to the emission of other trace gases and aerosols such as nitrous oxide (N_2O), methane (CH_4) and dimethylsulfonium propionate (DMSP), the latter is a precursor of dimethyl sulfide (DMS) that affect the global radiation balance. Furthermore, the massive culture of algae for different purposes (aquaculture or even biofuels) has not been included (nor have other aquaculture activities) in emissions inventories of global trace
20 gases product of anthropogenic activity (e.g., Seitzinger et al., 2000). Interest in measuring CH_4 and N_2O has increased due to the importance of both gases in atmospheric chemistry (having to ability to destroy direct or indirect the stratospheric ozone layer) and in global warming (both are greenhouse gases with, respectively 20 and 200 times greater radiative effect than CO_2 (Shindell et al., 2009). Also, little is known about mi-
25 crobial CH_4 production and consumption in the surface ocean (Karl et al., 2008). This situation also applies to N_2O, several studies have shown that much of the N_2O entering the atmosphere comes from the ocean product of microbial processes, but there is no certainty regarding the processes (e.g., nitrification, denitrification) that recycle this gas (Codispoti et al., 2001; Bange et al., 2005). Finally, DMS through its precursor

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Interactive Discussion

This research considers trace gas emissions with climatic effects from a massive cultivation system of *Nannochloris* microalgae, which is used to feed filter-feeding organisms such as *Mytilus sp*. Many of the algae used to feed species of *Mytilus chilensis, Mytilus galloprovincialis* and *Choromytilus chorus* are marine in origin, of which *Nannochloris* (Trebouxiophyceae, Chlorophyta) is one of the smallest and simplest forms of phototrophic eukaryotes (Yamamoto et al., 2001). The aim of this study was to determine the temporal dynamics of N_2O, CH_4 and DMSP in an open pond dominated by a *Nannochloris* monoculture, in order to evaluate potential trace gas emissions and their consequences, which are even more uncertain in small-scale aquaculture systems.

2 Materials and methods

2.1 Field measurements in a mesocosm

A massive culture of *Nannochloris* was conducted in a mesocosm system consisting of a $48\,m^3$ open pond ($6\,m$ length $\times$ $4\,m$ width $\times$ $2\,m$ depth) filled with seawater pumped from the nearby coast. The mesocosm received constant aeration and natural illumination. Several biological and physio-chemical parameters were monitored every 2–3 days, including tracers such as N_2O, CH_4 and DMSP in the open pond before and after its inoculation with *Nannochloris* and nutrient fertilization. The nutrients were added on day 5 and consisted of a mix of phosphates and urea. A continuous airlift recirculation system maintained a well-mixed open pond throughout the study period. Since the beginning of the experiment, representative discrete water samples were collected to determine dissolved O_2, N_2O, CH_4, total and dissolved DMSP, nitrogen nutrient (NO_3^-, NO_2^-) concentrations, chlorophyll *a* (Chl-*a*) and cell abundance. Water temperature, salinity and pH were recorded with various calibrated sensors.

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2.2 Chemical analysis

Dissolved O_2 (125 mL sample) was analyzed with an automatic Winkler method (AULOX Measurement System) developed at PROFC-Universidad de Concepción. N_2O (20 mL sample) was analyzed by gas chromatography (Varian 3380) with an elec-
tron capture detector maintained at 350 °C connected to an autosampler device (for more details, see Cornejo et al., 2007) using the He-headspace equilibration technique (MacAuliffe, 1971). CH_4 (20 mL sample) was manually analyzed by gas chromatography with a flame ionization detector (Schimadzu 17A) through a capillary column GS-Q (J&W, 0.53 mm × 30 m) with 60 °C oven temperature and 4 mL min^{-1} column flow. Pre-
viously, the seawater sample was equilibrated to 40 °C within 5 mL He headspace (see Farias et al., 2009). Water for DMSPd analysis was previously filtrated (0.7 µm GF.F filter) and acidified with 5 µL of 50% H_2SO_4. Samples for total DMSP consist in 15 mL unfiltered seawater were preserved in the same way. Thus, acidified DMSP samples are stable for months (Curran et al., 1999). The strong base quantitatively cleaved
DMSP into DMS, and the reaction was complete within 15 min. The DMS released was quantified by a purge and trap-gas chromatography, the DMSP detection limits as low as 0.17 nM in a 3-mL sample. These samples were analyzed at the Dauphin Island Laboratory of the University of Alabama, USA (for more details see Kiene and Slezak, 2006).
Samples for NO_3^- and NO_2^- and Chl-a were filtered (0.7 µm filter GFF) and frozen until later analysis, nutrients were analysed by colorimetric methods for small volumes (Grasshoff, 1983) while Chl-a by fluorometry of thawed samples (Parson et al., 1985). Cell abundances were counted in a Fuchs-Rosental chamber haemocytometer; samples were preserved in Lugol's iodine and counted through a microscope. Five fields
were counted for samples, with four readings per subsample at a magnification of 40 times.

2.3   Data analysis

The correlations between climate active tracers nd biological variables (e.g., *Nannochloris* cell abundance or Chl-*a*) were examined using Spearman correlation analyses. Data of tracer in the well-mixed open pond were plotted against time or Chl-*a* and fitted to a linear $[A(t) = A_0 \pm m \cdot t]$ model using the least squares method; where t is the incubation time, A_0 is the gas concentration at $t = 0$ (after nutrient addition), and m is the slope ratio. Slope was calculated from the linear regression. The DMS concentration was estimated from the degradation of DMSP assuming that DMSP degradation contributing only 3% to atmosphere (Vila-costa et al., 2006).

3   Results

Temporal variability of the physical-chemical variables of the open pond mesocosm during the experiment is shown in Fig. 1a. Temperature (8.2–13.4 °C) and salinity (31.8–34.5) varied according to natural external fluctuations (e.g., precipitation) during the six-week monitoring period. Conversely, pH values and oxygen levels reflected the exponential growth of algae after the addition of nutrients, observed as Chl-*a* levels and cell abundance (Fig. 1b). According to the temporal distribution of both Chl-*a* and cell abundance, as well as pH and oxygen levels (Fig. 1a,b), the monitoring period was separated into two periods: (i) a 16-day growth phase (phase I), where the Chl-*a* levels increased from 1.92 to 96 mg Chl-*a* m^{-3} and (ii) a senescence phase (phase II). Thus, pH and dissolved oxygen increased during the growth phase, from day 5 to day 17 as nutrients decreased due to the predominance of photosynthetic activity. From day 18 onwards, the senescence phase, algae showed a marked drop in cell abundance and Chl-*a* biomass, accompanied by decreasing oxygen concentrations and pH values due to the predominance of aerobic respiration of organic matter.

The radiatively active trace gases and precursor of aerosols fluctuated widely during the monitoring period (Fig. 2). Temporal variation of DMSP (both dissolved and total)

showed high variability. DMSPd fluctuated between 5.85 and 137 µmol m^{-3}, while DM-SPt varied between 6.31 and 142 µmol m^{-3}. Both DMSPd and DMSPt exhibited a conspicuous maximum at the end of the experiment (senescence phase). Methane concentration varied from 3.7 µmol m^{-3} (153% saturation) to 12 µmol m^{-3} (450% saturation) in phase I and decreased in phase II until levels approached background levels for seawater at around 2.3 µmol m^{-3} (89.2% saturation). The temporal distribution of CH$_4$ was closely associated with temporal distribution of Chl-a, indicating that CH$_4$ cycling could be related with photoautotrophic production. The N$_2$O concentration during the study period increased from 10 µmol m^{-3} (111% saturation) to 580 µmol m^{-3} (5500% saturation) in phase I, whereas after the first few days of phase II, N$_2$O levels decreased to near seawater background levels (see Fig. 2).

In order to discern the potential mechanisms of recycling of tracers in both well-defined phases, linear correlation between Chl-content and N$_2$O, CH$_4$ and DMSPt and DMSPd were performed (Table 1). During the growth phase, N$_2$O, CH$_4$, DMSPd concentrations were strongly correlated with Chl-a, being positive in the case of greenhouse gases and negative for DMSP (both dissolved and total). In senescence phase, DMSP and N$_2$O were negatively correlated with Chl-a although the only one significant correlation was between N$_2$O/Chl-a. It not was the case with CH$_4$ that showed a positive correlation in phase II (Table 1).

4   Discussion

The oceans play a crucial role in the dynamics of active trace gases including greenhouse gases (CO$_2$, N$_2$O and CH$_4$) and climate cooling gas (DMS) in the atmosphere. The oceans take up more than a quarter of the CO$_2$ released in natural and anthropogenic emissions from land, and produce and release N$_2$O and CH$_4$ to the atmosphere, making up about one third of the atmospheric total in the case of N$_2$O, and minor percentages for CH$_4$. DMS is estimated to make up nearly half of natural emissions of biogenic sulfur, approximately equivalent to anthropogenic sources of gaseous

sulfur oxides (Taylor and Kiene, 1989). Currently, there is an increased accumulation of the main greenhouse gases in the atmosphere, thereby increasing the global warming effect. Efforts have been made to compile accurate inventories of most of the gases associated with anthropogenic activities in order to improve efficiency and reduce these emissions.

The ocean recycles active trace gases to and from the atmosphere, which can have a positive (DMS and CO$_2$ in the case of net autotrophic systems) or negative (N$_2$O and CH$_4$) effect on the atmospheric radiation balance (Wuebbles, 2009). The rationale for using microalgae is its conversion efficiency of CO$_2$ into biomass through photosynthesis in dense algal cultures that exceed natural marine environment and terrestrial crop efficiencies. However, there are numerous indirect environmental effects to be considered, which could be potentially severe and disruptive and require the evaluation of ocean-wide unknowns. This is the case of massive algal culture systems, which efficiently sequestrate certain amounts of CO$_2$, but simultaneously produce others greenhouse gases, whose relative contributions still remain to be quantified (Williams and Crutzen, 2010).

Unicellular marine algae are being used as food in the hatchery seed production of commercially valuable shellfish, like mussels and clams. In our experimental culture system, Chl-a biomass reached values near $100\,\mathrm{mg\,m^{-3}}$ after the addition of nutrients, being almost ten times higher than most naturally eutrophic ecosystems (e.g., the coastal upwelling system off central Chile; Anabalón et al., 2007). Subsequently, this biomass dropped to nearly $10\,\mathrm{mg\,m^{-3}}$ due to senescence of the microalgae, with consequent organic matter degradation. Photosynthetic activity in *Nannochloris* is demonstrated by the increase of O$_2$ levels and pH values during the growth phase, whereas an increase of aerobic respiration of organic matter (much of it from the senescence phase) is evidenced by a decrease in pH values and O$_2$ concentrations (see Fig. 1).

In order to compare the relative contribution of each active trace gases in the radiation balance, production (net accumulation), consumption (net decrease) and air-sea fluxes are shown in Table 2. Considering a Chl-a:C relationship of 60 and

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assuming that one mole of average marine plankton detritus requires the oxidation of 106 moles of organic carbon (molecular weight of MOP of 3555 g mol^{-1}), our open pond was consuming 12.69 mmol CO$_2$ m^{-3} d^{-1} during phase I but releasing an equivalent to 4.82 mmol of CO$_2$ m^{-3} d^{-1} during phase II. This algal culture system was also producing a huge amount of other greenhouse gases, particularly N$_2$O that was being released to the atmosphere, given its extremely high supersaturation (4819% saturation). In the same sense, net daily accumulation of active trace gases (corrected for daily output as air-sea flux, see below) was i.e. 25.385 µmol m^3 d^{-1} for CO$_2$; 27.65 µmol m^3 d^{-1} for N$_2$O; 0.12 µmol m^3 d^{-1} for CH$_4$ and −0.08 µmol m^3 d^{-1} for DMS in phase I, whereas net consumption of i.e. CO$_2$ −9.654 µmol m^{-3}; −11.70 µmol m^{-3} d^{-1} for N$_2$O; −0.61 µmol m^{-2} d^{-1} for CH$_4$ and 0.01 µmol m^{-3} for DMS was estimated during phase II.

Taking into account the measured N$_2$O and CH$_4$ concentrations and the estimated DMS (from DMSPd) concentrations in the well-mixed water column of the open pond, as well as the compiled daily mean wind velocities and the Wanninkhof (1992) equation for N$_2$O and CH$_4$, and the Nightingale et al. (2000) equation for DMS, air-water fluxes for active trace gases were estimated (see Table 2). These fluxes were very high in relation to other eutrophic systems such as estuaries (see Table 2), confirming that massive algal culture is an important source of these gases towards the atmosphere (Williams and Crutzen, 2010). In the case of N$_2$O, estimated fluxes ranged from 1.04 to 500.3 µmol m^{-2} d^{-1}, were ten times higher than those reported for coastal areas of high biological productivity, e.g. the Arabian Sea (Law and Owens, 1990) and central Chile (Cornejo et al., 2006) and off Perú (Farías et al., 2009). Estimated fluxes for CH$_4$ in both phases around −0.14 to 9.14 µmol m^{-2} d^{-1} were slightly greater that the adjacent coastal area (Farias et al., 2009; see Table 2). Sea-air exchange of DMS, calculated from DMSPd, ranged from 1.56 to 7.45 µmol m^{-2} d^{-1} for both phases. Average DMS flux were nearly in the range of other regions, such as Bay of Bengal (Shenoy et al., 2002) and 4.7° N, 108.5° W (Kiene and Bates, 1990).

The radiative forcing (RF) of each trace gas is a good tool to evaluate the relative contribution of gas emission to global radiative balance; thus, our data showed according the potential production in culture followed the order: $N_2O > CH_4 > DMS$, 104 386; 127.47; -224.75 watt m^{-2} as total RF, respectively.

Regarding the estimated relationships among Chl-*a* and tracers over monitoring time, the positive relationship indicated that net tracer accumulation depended directly on the photoautotrophic activity of *Nannochloris* whereas negative or no correlations might indicate a net tracer consumption by which other activities (e.g., bacterial or archaeal activity, micrograzing), depending indirect on photoautotrophic biomass were acting. Ratios of change over time between tracers and Chl-*a* during both phases reveal different biochemical processes involved in the cycling of these gases (see Table 1). According to current knowledge, N_2O and CH_4 are cycled by Bacteria and Archaea, which produce or consume these gases. In contrast, DMS and its precursor DMSP, are directed related to photosynthetic activity and consequently to Chl-*a* biomass (Belviso et al., 2000).

Our results turned out opposite to what we might have expected. In the case of N_2O, under oxygenated and the well-mixed water column conditions, as observed in our system, aerobic NH_4 oxidation is the most probable N_2O production pathway (Nevinson et al., 1995). The only consumption pathway for N_2O is the complete denitrification process that occurs at very low oxygen concentration, reason for which it is assumed to be insignificant in our open pond. The net N_2O accumulation (see Table 1) and significant positive correlation between N_2O and Chl-*a* in phase I seem to indicate that some phototrophic pathway triggers N_2O accumulation. This has been described before in axenic cultures of microalgae such as *Chorella* spp (Whethers, 1984), reopening the discussion about N_2O production mechanisms in surface waters. Net CH_4 cycling was also controversial, due to unexpected CH_4 production in oxygenated water (Karl et al., 2008). Despite the common fact that biological processes are responsible for its origin, the formation of CH_4 in aquaculture is poorly understood.

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Finally, the fact that DMSP peaked during the final part of the senescence phase and that both DMSPt and DMSPd were not correlated with Chl-*a* levels can be argued by different explanations, such as those observed by Kwint and Kramer (1995) during a *Phaeocystis* bloom. The transformations of DMSP into DMS gas require processes such as DMSP-lyase activity (Stefel and Van Boekel, 1993) and bacterial consumption (Wolfe, 1996). Cleavage of DMSP by bacterial and algal DMSP-lyases is considered to be the main source of DMS (Kiene and Bates, 1990; Simó et al., 2000). Another possibility is that DMSP is presumably released into the dissolved pool by grazing microorganisms (i.e., ciliates, flagellates; Dacey and Wakeham, 1986) or senescent microalgae (Matrai and Keller, 1993).

This report shows that active trace gases were emitted system of culture stimulated by fertilization with nutrients. Recognition of new or poorly studied biological sources of greenhouse gases from massive microalgae cultures could change the Earth's energy balance, particularly N$_2$O produced at greater rates. These data also show that the green microalgae are a previously unrecognised source of greenhouse gases. Furthermore, these results should be viewed within the context of the current projection of increasing aquaculture activity worldwide. At present, capture fisheries and aquaculture supply the world with about 110 million tonnes of biomass, with a small overall CO$_2$ carbon foot print and making up an unprecedented 47% of the total global fish harvest (FAO, 2008).

The effect of global aquaculture on climate is uncertain. Measurements are needed to quantify the climatic effect of aquaculture production systems and the of emitted gases may also need to be considered, because depending of species this may also contribute to far high value in global warming potential (Stief et al., 2009). Also, determining gas-aerosol interactions might substantially alter the relative importance of current GWP (Shindell et al., 2009). Understanding these measurements undoubtedly requires an integrated approach.

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Table 1. Correlated variables during sampling period divided by the phase I and II.

Tracers	Spearman correlation Coefficients	
	Phase I	Phase II
DMSPd/Chl-a	−0.714	−0.479
	(**0.04**)	(0.12)
DMSPt/Chl-a	−0.263	−0.377
	(0.21)	(0.42)
CH$_4$/Chl-a	0.698	0.317
	(**0.01**)	(0.16)
N$_2$0/Chl-a	0.832	−0.548
	(**0.04**)	(**0.01**)

Bold numbers in parenthesis are significant relationships.

Table 2. Estimated net accumulation or consumption rates of radiatively active trace and their air-water fluxes and forcing radiative.

Tracer	Production or consumption rate (μmol m^{-3} d^{-1})		Air-water gas flux (μmol m^{-2} d^{-1})		Forcing radiative Watt m^{-2}
	I Phase	II Phase	I Phase	II Phase	
CO$_2$	25 385	−9654	–	–	
DMSPd	−1.49 ± 1.5	−13.81 ± 9.6	–	–	
DMSPt	−1.07 ± 2.2	−12.1 ± 20.0	–	–	
CH$_4$	0.12 ± 0.5	−0.61 ± 0.35	+3.65 (0.59–9.14)	+2.42 (−0.14–6)	127.47
N$_2$O	27.65 ± 7.1	−11.70 ± 11.05	+17.93 (1.04–31.74)	+318.8 (5.79–500.3)	104 386.3
DMS	−0.08 ± 0.33	0.01 ± 0.27	+1.56 (0.4–3.7)	+7.45 (0.5–34.1)	−224.75

Numbers in parenthesis are ranges, (+) positive values are production and (−) negative are consumption.

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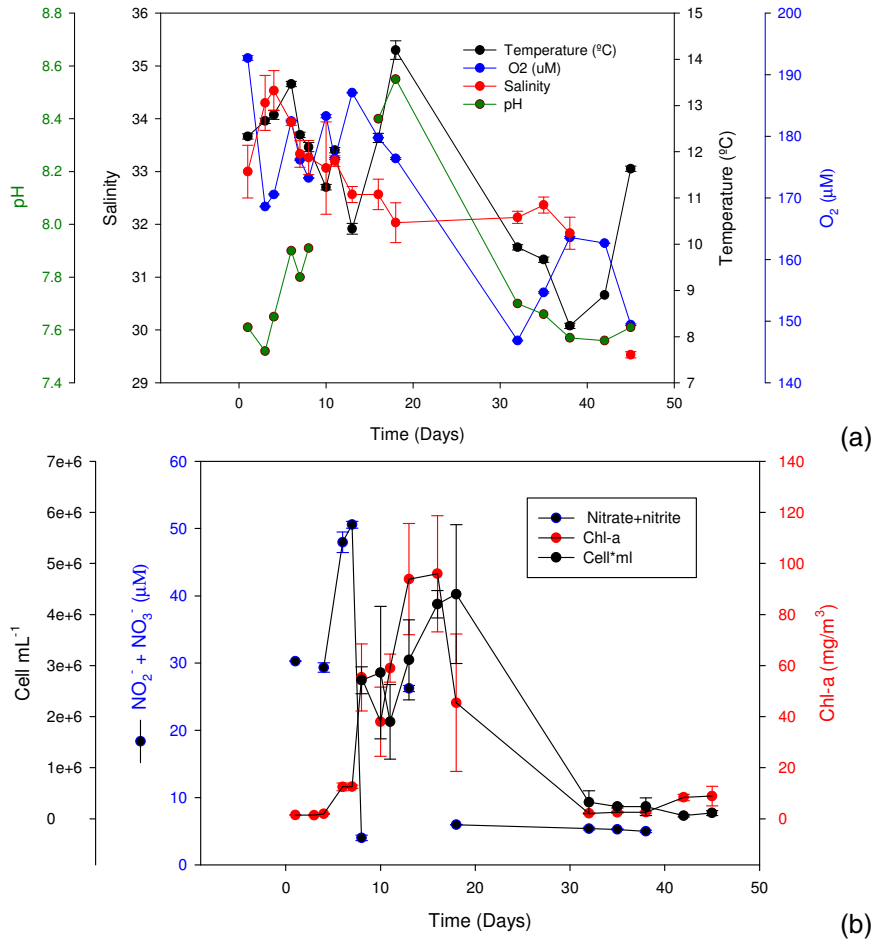
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Fig. 1. (a) Temporal variability of salinity, oxygen, temperature and pH variables in the open pond system. **(b)** Temporal variability of nitrite and nitrate, chlorophyll-*a*, and cell abundances in the open pond. Bars represent ± 1 SE ($n = 3$).

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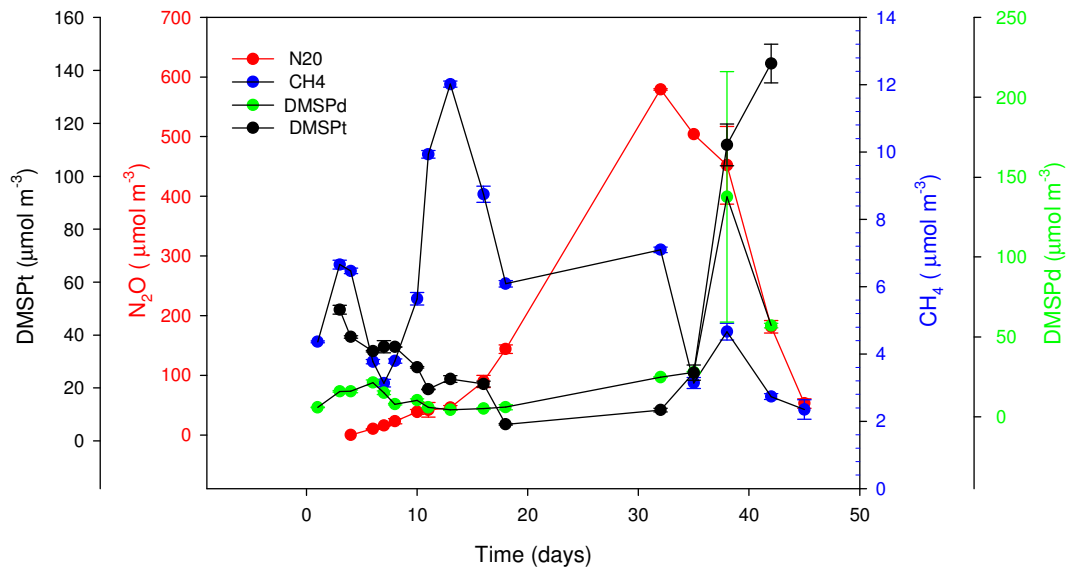Full Screen / Esc

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Fig. 2. Temporal variability of DMSP, N_2O and CH_4.

High production of N_2O, CH_4 and DMSP in phytoplankton culture

L. Florez-Leiva et al.

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