

Increased nutrients from aeolian-dust and riverine origin decrease the CO₂-sink capacity of coastal South Atlantic waters under UVR exposure

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Abstract

Increases in ultraviolet radiation (UVR) levels due to the ongoing stratification of water bodies and higher nutrient concentrations either through riverine or aeolian-dust-inputs are expected in the near future in coastal surface waters. Here, we combined remote-sensing data of particulate organic carbon (POC; 1997–2016 period), observational data of solar radiation (1999–2015 period), and a mid-term experimental approach with coastal plankton communities from South Atlantic Ocean (SAO) to test how the interaction between increased nutrients by riverine and aeolian-dust inputs and high UVR may alter the community dynamics and the CO₂ sink capacity of these ecosystems in the future. Our results show a decline ~ 27% in the sink capacity of the coastal ecosystems regardless of the nutrient source considered and under high UVR levels. This decreased CO₂ uptake was coupled with a high dynamic photoinhibition and dark recovery of photosystem II and shifts in the community structure toward the dominance by nano-flagellates. Moreover, remote-sensing data also evidences an incipient tipping point with decreasing POC values in this area over the annual planktonic succession. Therefore, we propose that to continue this climate and human-mediated pressure, these metabolic responses could be strengthened and extended to other productive coastal areas.

The growth of the human population and activities (e.g., agriculture, herding, deforestation, industries) causes a substantial transfer of nutrients, mainly nitrogen (N) and phosphorus (P) to adjacent freshwater bodies, followed by their transport to coastal waters (Peñuelas et al. 2013). Together with these massive human-caused additions, climate variability due to global change (i.e., more severe droughts, storms, and alterations in wind patterns) is also causing increases in the atmospheric N and P deposition into coastal areas (Mahowald et al. 2008). As a result of these perturbations, coastal habitats are currently confronted with several alterations (i.e., hypoxia, reduced water transparency) which are triggering cascade effects on the ecosystem functioning (i.e., less suitable habitats for feeding and reproduction, more harmful phytoplankton blooms) (Harding et al. 2016; Maar et al. 2016). In addition to changes in nutrient concentrations, solar radiation in the water column is being greatly altered by global change. Due to global warming,

stratification of the water bodies tends to increase and, therefore, organisms are expected to receive higher solar radiation levels (particularly the ultraviolet radiation portion, UVR, 280–400 nm) than at present (Williamson et al. 2014).

Previous studies conducted with planktonic communities have shown a stimulatory individual effect of nutrient inputs linked or not to aeolian dust, increasing the PSII performance (Φ_{PSII}) (Browning et al. 2014), primary production (PP) (Marcoval et al. 2008; Ridame et al. 2014), bacterial growth (Lekunberri et al. 2010; Teira et al. 2016), and respiration (Medina-Sánchez et al. 2017). Conversely, other studies have also found inhibitory effects of these inputs on the aforementioned processes, due to the presence of toxic elements (e.g., Cu, Pb) (Hoffmann et al. 2012; Dao and Beardall 2016). Also, recent studies have shown that nutrients can trigger abrupt changes in community structure toward dominance by only one functional group (e.g., fast-growing diatoms) (Macías et al. 2010; Villafañe et al. 2017). This can promote greater heterotrophy by increased community respiration (Martínez-García et al. 2013), thus altering the energy transfer to higher trophic levels (Tsagaraki et al. 2017). On the other hand, it is known that current or enhanced levels

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of UVR inhibits the PP (Helbling et al. 2015; Villafañe et al. 2015), nutrient uptake (Hessen et al. 2012), Φ_{PSII} or DNA repair (Jeffrey et al. 2000; Harrison et al. 2015; Villafañe et al. 2017); however, although the combined impact of both global change drivers is generally considered antagonistic (e.g., nutrients attenuate the harmful effect of UVR), recent results by Carrillo et al. (2015) and Harrison et al. (2015) evidence that nutrient inputs can also unmask the harmful UVR effects on planktonic responses.

Thus, because inorganic nutrients and solar radiation are pivotal for the functioning of the global C-cycle, one of the unresolved issues in global-change research is whether rising nutrient availability, due either to riverine or aeolian-dust inputs, and high UVR fluxes could impact the carbon uptake by primary producers and the total respiration by the planktonic community. Therefore, if both processes are unbalanced in a scenario of global change, the capacity of any ecosystem as CO₂-sink of the human-induced carbon dioxide (CO₂) emissions could be greatly altered in the future. This would be especially relevant for the Southwest Atlantic Ocean (SAO), which not only is one of the most productive regions worldwide, with rich and diverse communities (Acha et al. 2004; Romero et al. 2006), but is also considered one of the most intense CO₂ sinks per unit area in the global Ocean (Bianchi et al. 2009).

To shed new light on how the metabolic functioning of coastal areas may be impacted by the ongoing environmental change, we developed a dual approach: (1) a 20-yr observational study with remote-sensing and field data through which we evaluate long-term trends in the frequency and intensity of aeolian-dust deposition (and riverine inputs) and solar radiation on SAO, and how it could be altering the production of particulate organic carbon over the seasonal phytoplankton succession; and (2) a mid-term manipulation study where we experimentally increased the nutrient concentrations, mimicking future aeolian-dust deposition and riverine inputs, under high UVR exposure to determine their effects on photosynthetic activity and metabolic responses as well as on the structure of a microplanktonic community from Patagonian coastal waters. We used this area as model ecosystem to measure variations in the net primary production (NPP), daily community respiration (CR), and gross primary production (GPP) at short- and mid-term scales. From these data, we quantified how both global-change scenarios considered could alter the capacity of SAO coastal waters to sequester atmospheric CO₂.

Methods

Model ecosystem

The Chubut River estuary, together with its area of influence, is a meso-tidal and highly productive estuary in Patagonia (Piccolo and Perillo 1999) characterized by a variable range of physical (kd_{PAR} ranging $\sim 1\text{--}6 \text{ m}^{-1}$), chemical (N, P,

and silicate [Si] concentrations [see “Experimental set up”]), and biological (characteristic seasonal succession with a pre-bloom, bloom, and post-bloom period) conditions due to the interaction between the SAO and the mouth of Chubut River (Helbling et al. 2010). The study area has intense horticultural production, animal-breeding farms, and different cities ($\sim 140,000$ inhabitants in total; Censo Nacional de Población, Hogares y Viviendas, 2010; INDEC - DGEyC) located along the riverbanks in the last 100 km before reaching the sea. All this implies a continuous supply of nutrients to this marine area. Also, this ecosystem is characterized by high-speed ($\sim 25 \text{ m s}^{-1}$) and frequent (10^4 min) south-western winds throughout the year except in winter, where they are minimum ($\sim 3.5\text{--}4.5 \text{ m s}^{-1}$) (Helbling et al. 2005). Due to these intense and frequent winds, Patagonia is currently considered one of the high-latitude areas ($> 50^\circ\text{N}$ and $> 40^\circ\text{S}$) most active in terms of aeolian-dust deposition, particularly during the spring (October–November) and late summer (March–April), which provide nutrients (mainly P and N) to the surrounding marine system (Bullard et al. 2016). Additionally, the high wind speeds registered in this area cause a constant mixing of the water column in surface layers, and thus organisms undergo highly variable light at short and mid-term scales over the year (Helbling et al. 2005).

Sampling and experimental set up

Surface seawater (0.5 m depth and salinity > 33) was collected at the mouth of the Chubut River estuary (Egi station, $43^\circ 18.8'\text{S}$, $65^\circ 02.0'\text{W}$) during high tide on the afternoon of 19th October 2014 using an acid-cleaned (1N HCl) bucket. The sample (200 L in total) was pre-screened through a 180 μm Nitex mesh to eliminate mesozooplankton, placed in 25-L opaque acid-washed containers and transported immediately to the EFP (10–15 min away from the sampling site) where the experiment was set as follows: the set-up consisted on 18 microcosms (10L-UVR-transparent bags, Alpax Trade Lab, São Paulo, Brazil, 72% transmission at 280 nm) where seawater samples were held. A 2×3 factorial design (in triplicate) was implemented with: (a) Two solar radiation treatments, (1) PAB (PAR + UV-A + UV-B, $> 280 \text{ nm}$; uncovered microcosms), and (2) P (PAR $> 400 \text{ nm}$; microcosms covered with Ultraphan 395 nm filter); and (b) three nutrient treatments, (1) ambient (amb), where non-manipulated nutrient concentrations were nitrite + nitrate (N) = $2.4 \mu\text{M}$, phosphate (P) = $1.76 \mu\text{M}$ and silicate (Si) = $1.7 \mu\text{M}$, (2) riverine inputs (riv) through the addition of inorganic nutrients, i.e., NaNO₃, NaH₂PO₄, and Na₂SiO₃ as majority sources of N, P, and Si, respectively, which increased the nutrient concentrations with respect to ambient conditions in $61.98 \mu\text{M}$ (N), $30.84 \mu\text{M}$ (P), and $135.37 \mu\text{M}$ (Si), and (3) aeolian-dust inputs, whose addition increased the nutrient concentrations in $0.31 \mu\text{M}$ (N), $1.15 \mu\text{M}$ (P), and $4.90 \mu\text{M}$ (Si).

The experimental addition of inorganic nutrients used in this study simulated increases of ca. 8 and ca. sixfold for N and P, respectively, with respect to the historic maximal concentrations (1986–2013) registered in the study area over an annual cycle (2–8 μM and 2–5 μM for N and P, respectively; see Bermejo et al. 2018 for a detailed analysis). Si concentrations, however, were in the mean range of those received over the year as their concentrations had remained stable over the last three decades (100–200 μM ; Bermejo et al. 2018). The dust added (size ranging between 1–10 μm) was collected in situ in Merzouga (Tafilalet, Morocco, 31° 6'.00 N, 3° 59'.24 W) and fractioned as described in Cabrerizo et al. (2016). Thus, the dust was broadly comparable with that used in previous reports showing that those particles larger than this size are rapidly removed during the atmospheric transport (Guieu et al. 2010). The rationale behind adding a single pulse of Saharan dust as a model continental dust source lies in the fact that: (1) the Sahara desert is responsible for 58% of the global dust emissions each year (Tanaka and Chiba 2006), and (2) these kinds of atmospheric inputs are significantly increasing the N and P concentrations in aquatic ecosystems, particularly in the Southern Hemisphere (Brahney et al. 2015).

The amount of dust added in the experiment (4.1 mg L^{-1}) simulates a deposition scenario of 61.5 mg m^{-2} into a 15-m-deep water layer, which constitutes the water layer potentially affected after intense dust-deposition events (Pulido-Villena et al. 2008; Marañón et al. 2010). Therefore, if we consider that: (1) the mean number of dust-deposition events per year in the area during the last two decades \sim 100, (2) the annual dust-deposition rates on South Atlantic Ocean is \sim 500 mg m^{-2} yr^{-1} (Jickells and Moore 2015) and (3) that most of aeolian-dust deposition occurs in single and strong pulses (Guerzoni et al. 1997), we simulate a future scenario of increases of up to 12-fold in dust inputs through a single deposition event.

All microcosms were placed in 200-L tanks with running water to maintain the in situ temperature (14.5°C) and exposed to natural solar radiation for 5 d. The thin water layer (\sim 0.5 m) that covered the microcosms simulated an extreme scenario with a shallow upper mixed layer (UML). The microcosms were shaken manually several times a day so that plankton received homogeneous irradiance during the exposure by preventing organisms from settling to the bottom.

Sub-samples for different measurements/analyses were taken daily [for chlorophyll *a* (Chl *a*) determinations, oxygen (O_2) concentration, and effective photochemical quantum yield (Φ_{PSII}) measurements and for changes in the picoplanktonic fraction] or every other day (taxonomic composition of nanoplanktonic fraction and nutrient analyses) using a syringe attached to a silicone tube inserted into each microcosm to prevent their tampering.

Measurements and analyses

Long-term remote-sensing and observational data

Data from the daily aerosol index (AI) and monthly particulate organic carbon (POC) for the Egi station were downloaded from Giovanni v 4. 18. 3 Earth database of the National Aeronautics and Space Administration (NASA) (Acker and Leptoukh 2007). For the period 1996–2015, AI data were provided by the Total Ozone Mapping Spectrometer-Earth Probe (TOMS-EP) (22 July 1996 to 31 December 2003) and by the Ozone Monitoring Instrument (OMI) (01 January 2004 to 31 December 2015) satellites, respectively. As previous studies (Bullejos et al. 2010; Cabrerizo et al. 2016) established that an $\text{AI} > 0.5$ constitutes a deposition event, we also considered the annual dust deposition events as the number of days per year affected by events of $\text{AI} > 0.5$. For monthly POC, data were provided by sea-viewing wide field-of-view sensor (SeaWiFS, 04 September 1997 to 31 December 2010) and by moderate-resolution imaging spectroradiometer (MODIS, 01 January 2011 to 31 October 2016).

Solar-radiation measurements: long-term and experimental data

We monitored the incident solar radiation during the experiments using an ELDONET (Real Time Computers, Germany) broadband-filter radiometer that measures UV-B, UV-A, and PAR (400–700 nm) every second, averages the data over a 1-min interval, and stores them in a computer. The radiometer is routinely calibrated (once a year) using a solar calibration procedure. For this calibration, the irradiance data during clear-sky conditions are compared with the output of models for transfer of atmospheric radiation (Björn and Murphy 1985). Also, daily PAR doses over the study area for the 1999–2015 period were taken from the Estación de Fotobiología Playa Unión (EFPU) online database (see <http://www.efpu.org.ar>).

Chl *a* measurements

Water samples (100 mL) to determine Chl *a* concentration were taken from each microcosm every day early in the morning and filtered onto M-GF (25 mm) filters (Muntzell, Sweden). Afterwards, photosynthetic pigments were extracted in absolute methanol as described in Holm-Hansen and Riemann (1978) and a scan between 250 nm and 750 nm using a spectrophotometer (Hewlett Packard, model HP 8453E). Chl *a* concentrations were calculated using the equations of Porra (2002).

Nutrient analyses

Samples from each microcosm were placed in 125 mL HDPE bottles and frozen (-20°C) until analyses, to determine nitrogen, phosphorus, and silicate concentrations using spectrophotometric techniques, as described in Strickland and Parsons (1972).

Taxonomic analyses

Samples for the identification and/or counting of autotrophic nanoplankton (ANP) under inverted microscope were taken at the first and at the last day of exposure to solar radiation, whereas

samples for autotrophic picoplankton (APP) and heterotrophic picoplankton (HPP) counting through flow cytometry were taken on a daily basis due to their high division rates. For ANP, samples were placed in 125 mL brown glass bottles and fixed with buffered formalin (final concentration 0.4% of formaldehyde in the sample). Aliquots of 25 mL were settled for 24 h in an Utermöhl chamber (Hydro-Bios GmbH, Germany) and species were identified and enumerated using an inverted microscope (Leica, model DM IL, Germany) following the technique described by Villafañe and Reid (1995). The biovolumes of the phytoplankton species were estimated by adjusting their shape to known geometric forms following Hillebrand et al. (1999), and by measuring the main cell dimensions of at least 10 cells per species. From these biovolumes, C biomass was calculated with the equations of Strathmann (1967).

For APP and HPP, 1.5 mL of sample was fixed with 75 μL of particle-free 20% (w/v) paraformaldehyde (1% final concentration) and stored at 4°C until analysis to quantify cell abundance using a flow cytometer (FACSCanto II, Becton Dickinson Biosciences, Oxford, UK). Prior to analysis, all samples were stained with Syber Green I DNA (Sigma-Aldrich Co Ltd) 1 : 5000 final dilution (Gasol and Del Giorgio 2000; Zubkov et al. 2007). After this, yellow-green 1- μm beads at standard concentration (Fluoresbrite Microparticles, Polysciences, Pennsylvania, U.S.A.) were also added to determine the absolute cellular concentration (Zubkov and Burkill 2006; Zubkov et al. 2007). As with the same samples we quantified APP and HPP, we used the phycoerythrin and Chl *a* signals to distinguish between autotrophic and heterotrophic picoplankton groups (Mercado et al. 2006). From cell abundance values we estimated the biovolumes of both picoplanktonic groups following Zubkov et al. (1998), which were converted into C biomass by using conversion factors of 0.22 (Booth 1988) and 0.35 $\text{pg C } \mu\text{m}^{-3}$ (Bjørnsen 1986) for the APP and HPP fractions, respectively.

Growth rates

The specific growth rates of ANP, APP, and HPP (μ , in d^{-1}) under the different experimental conditions were calculated from cell concentration as:

$$\mu = \ln (N_5/N_1)/(t_5-t_1) \quad (1)$$

where N_5 is the cellular concentration (in cells $\text{mL}^{-1} \times 10^3$) the last day of the experiment (t_5) and N_1 is the cell concentration at the first day of the experiment (t_1).

O₂ concentration measurements

Samples from each microcosm were taken daily (before sunrise) and placed into Teflon FEP narrow-mouth bottles (Nalgene®-35 mL) without bubbles. Each Teflon bottle (18 in total), equipped with an O₂ sensor-spot (SP-PSt3-NAU-D5-YOP, PreSens GmbH, Germany), was exposed to the same radiation treatment as that imposed on the corresponding mesocosm from which it came and were placed inside a water bath to maintain the in situ temperature. The O₂

concentrations were measured during 24 daily cycles using a Fibox 3 optode-probe oxygen transmitter (PreSens GmbH, Germany) furnished with the Oxyview 6.02 software and a fibre-optic. Measurements started at dawn and were made hourly until dusk (seven measurements), and then every 4–6 h during the night (4–5 measurements) until completing the daily cycle. Each sample was measured for 30 s, collecting one datum per second. Every day, before the measurements, the probe was calibrated using a two-point (0% and 100% saturation) calibration procedure, at the in situ temperature and taking into account the atmospheric pressure.

Fluorescence measurements

To determine the dynamics of Φ_{PSII} over the experimental period, aliquots of 3-mL were taken daily from each microcosm at dawn, noon and dusk (18 samples and three times per day = 54 samples in total/day) to measure the in vivo PSII photochemical parameters using a pulse amplitude modulated (PAM) fluorometer (Walz, Water PAM, Effeltrich, Germany). Each sample was measured six times immediately after sampling, without any dark-adaptation, with each measurement lasting 10 s; thus the total time for measuring each sample was 1 min. The effective photochemical quantum yield of PSII was calculated using the equations of Genty et al. (1989) and Maxwell and Johnson (2000) as:

$$\Phi_{\text{PSII}} = \Delta F/F'_m = (F'_m - F_t)/F'_m \quad (2)$$

where F'_m is the maximum fluorescence induced by a saturating light pulse (ca. 5300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 0.8 s) and F_t the current steady-state fluorescence induced by a red actinic light pulse (492.2 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ – peak at 660 nm) in light-adapted cells.

Data and statistical analyses

From monthly POC data, we fitted a linear regression model POC vs. time for the 1997–2014 and 2015–2016 period to evaluate long-term trends over the time before and after the experimental study in SAO. In addition, from daily light-darkness Φ_{PSII} measurements, we fitted a polynomial regression model to the values at noon and dawn vs. time for each radiation and nutrient treatment to assess: (1) the Φ_{PSII} inhibition undergone by the communities at noon when received maximal irradiances; and (2) the dark recovery capacity of these communities from the dusk to the following dawn to counteract the potential photodamage experienced during the previous day.

Likewise, we also fitted a polynomial regression model with oxygen concentration values at noon vs. time for each radiation and nutrient treatment to calculate the changes in the net primary production (NPP, in $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$) throughout the experiment as:

$$\text{NPP} = [\text{O}_2]_{t_5} - [\text{O}_2]_{t_1}/t_5 - t_1 \quad (3)$$

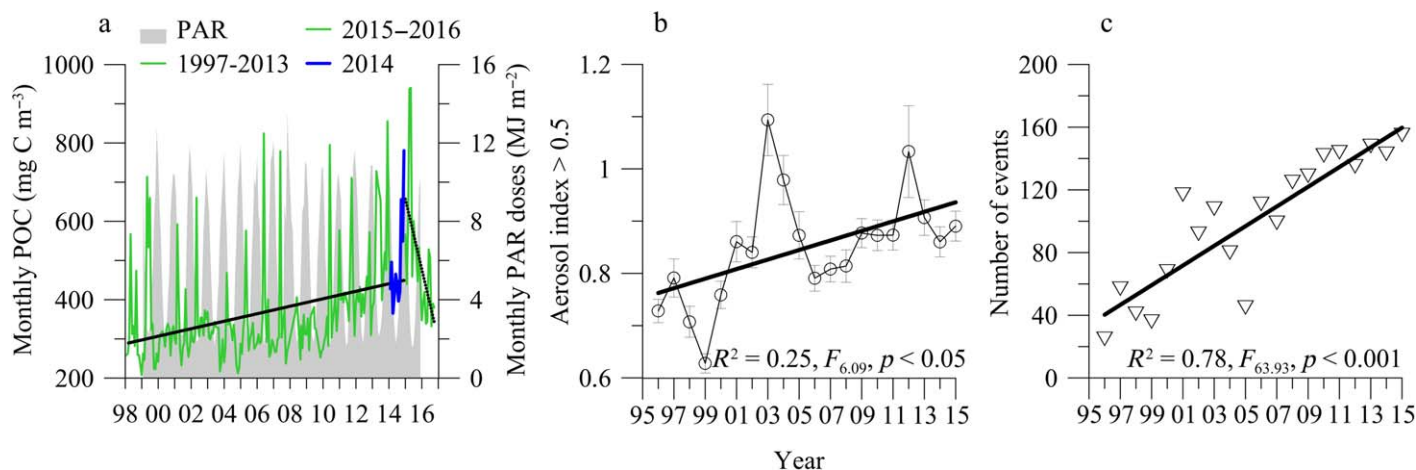


Fig. 1. (a) Mean (\pm SD) monthly particulate organic carbon (POC, in mg C m^{-3} ; green line) for from 1997 to 2016 and monthly photosynthetically active radiation doses (PAR, in MJ m^{-2} ; gray areas) from 1999 to 2015 period. Solid blue line represent the experimental year (2014). (b) Mean (\pm SD) annual aerosol index > 0.5 (AI, relative units) and (c) total number of AI > 0.5 events from 1995 to 2015 period on Egi station ($43^{\circ} 18.8' \text{S}$, $65^{\circ} 02.0' \text{W}$). Solid (1997–2014) and dashed (2015–2016) lines represent the linear regression fits. [Color figure can be viewed at wileyonlinelibrary.com]

$[\text{O}_2]_{t_5}$ being the O_2 concentrations modeled on the last day of the experiment (t_5) and $[\text{O}_2]_{t_1}$ the concentrations for modeled the first day (t_1) of exposure.

From the O_2 concentrations measured during the night, we calculated the dark community respiration (CR_{dark} , in $\text{mmol m}^{-3} \text{d}^{-1}$) for each radiation and nutrient treatment throughout the experiment as the difference between the O_2 concentrations measured before dawn of the following experimental day and those measured during the dusk of the experimental day considered. From daily data, we integrated over the week at the same way than for NPP.

Then, we assessed the effect size of UVR on NPP and CR_{dark} under ambient, dust or riverine treatments as:

$$\text{Effect size of UVR (\%)} = (X_P - X_{\text{PAB}}) / X_P \times 100 \quad (4)$$

with X being the NPP or CR_{dark} under UVR + PAR (PAB) and only PAR (P) treatments, respectively. Note that positive values denote an inhibitory effect, whereas negative values a stimulation of the considered process.

The daily CR rates ($\text{mmol O}_2 \text{ m}^{-3} \text{d}^{-1}$) for each nutrients treatment and day were calculated as:

$$\text{Daily CR} = \text{CR}_{\text{darkPAB}} + \text{light CR} \quad (5)$$

where the $\text{CR}_{\text{darkPAB}}$, represents the respiration values measured during the night in samples previously exposed to full solar radiation. The light CR was estimated after obtaining the effect size of UVR (Eq. 4) on respiration and applying this factor to the $\text{CR}_{\text{darkPAB}}$ as:

$$\text{Light CR} = \text{CR}_{\text{darkPAB}} \times (1 - \text{Effect size of UVR}) \quad (6)$$

By doing so, we had more realistic measurements of daily CR, as other experimental studies (Agustí et al. 2014; Medina-Sánchez et al. 2017) have shown that the respiration rates of

planktonic communities can be enhanced or inhibited under UVR exposure. Finally, we estimated the gross primary production (GPP) under ambient, dust, and riverine treatments as:

$$\text{GPP} = \text{NPP} + (\text{daily}_{\text{CRPAB}}) \quad (7)$$

From the GPP and daily CR_{PAB} (see Supporting Information Table S2) data we evaluated the CO_2 -sink capacity of our model coastal ecosystem, as the ratio between the two parameters, under ambient, dust, and riverine treatments after an acclimation period of 5 d.

We used a two-way analysis of the variance (ANOVA) to test the interactions between UVR and nutrient (amb, dust, and riv) treatments on NPP, CR_{dark} , cell abundances, and growth rates of ANP, APP, and HPP. We also used a one-way ANOVA to test significant differences between nutrient treatments on the effect size of UVR (%) on NPP and CR_{dark} , on daily CR and on CO_2 -sink capacity. Normality (by Shapiro-Wilk's test) and homoscedasticity (by Levene's test) or sphericity (by Mauchly's test) was checked for each variable to verify the ANOVA and RM-ANOVA assumptions (Zar 1999). When interactive effects were significant, least significant differences (LSD) Fisher post hoc tests were performed. Student's tests were used to test significant differences between the slopes of the polynomial regression fits of Φ_{PSII} . All data are reported as mean and standard deviation, whereas error propagation was used to calculate the error for the effect size of UVR (%) on NPP and CR_{dark} .

Results

Long-term trends in POC and AI on coastal South Atlantic Ocean waters

The monthly POC exhibited a characteristic response pattern throughout the 1997–2016 period (Fig. 1a), with increasing

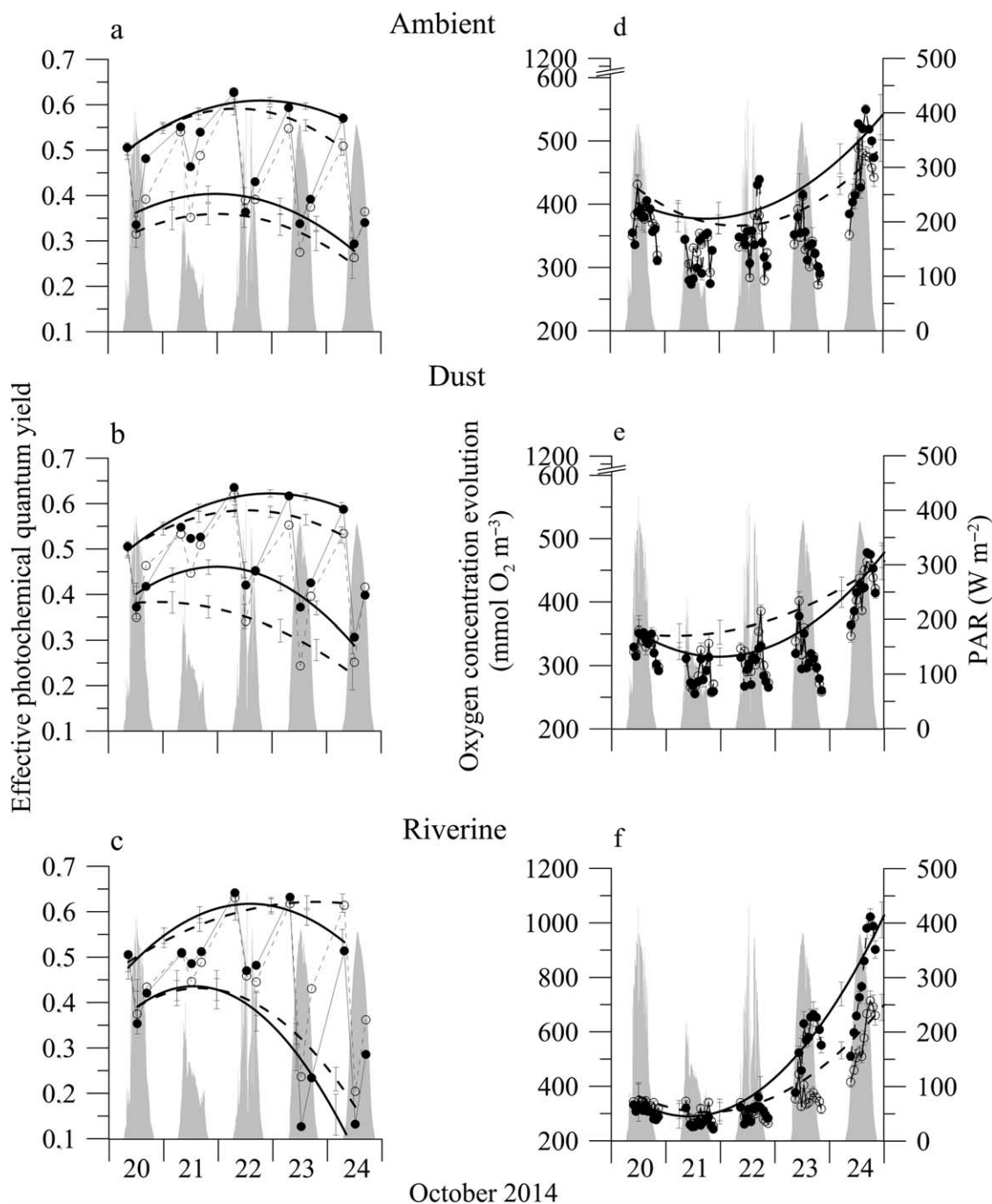


Fig. 2. Daily cycles of mean values (\pm SD) of effective photochemical quantum yield (a–c) and oxygen concentration evolution (d–f) in planktonic communities exposed to two radiation treatments, PAB (> 280 nm, open circles) and P (PAR, > 400 nm, solid circles) and three nutrient treatments, ambient, dust, and riverine inputs during the experimental period (20–24th October). Gray areas represent daily irradiances of photosynthetically active radiation (PAR, in $W m^{-2}$). Dashed (samples under PAB) and solid (samples under P) lines represent the polynomial regression fit at during the incubation period whereas the vertical lines represent the 95% confidence intervals. Note that for panels (a–c) the polynomial regression fits were done at dawn and at noon.

concentrations coupled with decreasing monthly PAR doses within each year (maximal peaks ranging between $\sim 600 mg C m^{-3}$ and $1000 mg C m^{-3}$) followed by decreasing POC values that matched increasing monthly PAR doses. Despite these

variable inter-annual trends, our results showed a steady increase in POC over the time which lasted up to 2014 ($R = 0.41, F_{5,11}, p < 0.001$). Noticeably, this trend was significantly inverted ($R = -0.59, F_{0,01}, p < 0.001$) in the last 2 yr

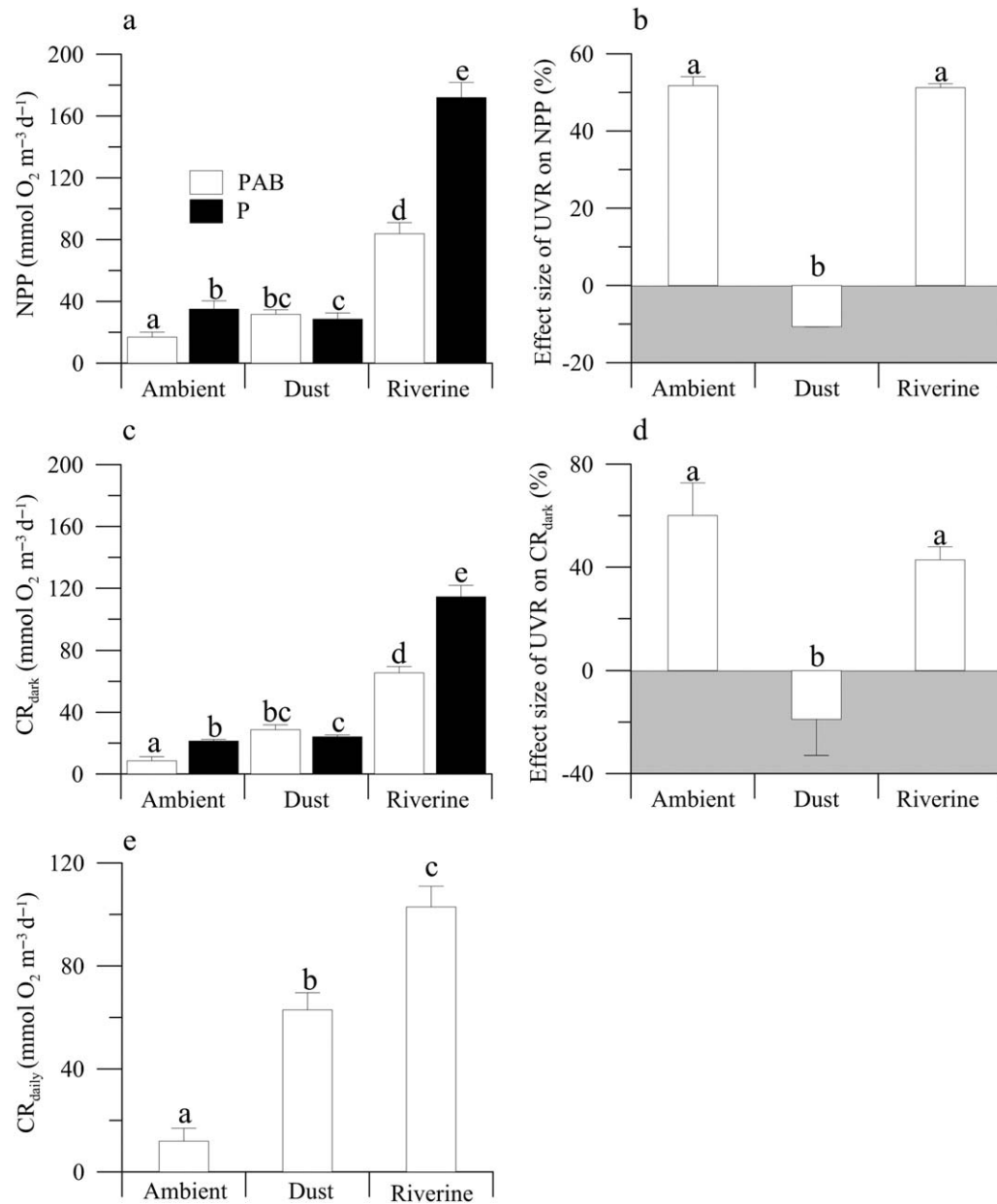


Fig. 3. (a) Mean (\pm SD) net primary production (NPP, in $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$) and (c) dark community respiration (CR_{dark} , in $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$) rates in samples exposed to two radiation treatments, PAB ($> 280 \text{ nm}$) and P (PAR, $> 400 \text{ nm}$), and three nutrient treatments, ambient, dust, and riverine over the experimental period (20–24th October). Mean (\pm SD) effect size of UVR (%) on the total NPP (b) and CR_{dark} (d) under ambient, dust, and riverine treatments. (e) Daily CR rates (in $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$) under ambient dust and riverine treatments over the experimental period. The letters on the top of bars indicate significant differences by the least significant differences post hoc test.

(2015–2016). Likewise, the AI values showed both the intensity and the frequency of these events significantly increased from 1997 until present on this coastal area (AI > 0.5 , Fig. 1b,c).

Photosynthetic activity and planktonic metabolic responses

Our experimental manipulation determined a typical V-shaped pattern of Φ_{PSII} over daily light-dark periods, with decreases in Φ_{PSII} as light intensities increased during the day followed by increases in Φ_{PSII} at lower light intensities during

the evening (Fig. 2a–c; Supporting Information Table S1). Thus, the highest photoinhibition of PSII occurred at noon when the communities received maximal radiation levels. In addition, this inhibition was particularly high during the last 2 d, when the maximal mean irradiance values were registered. In fact, whereas the mean daily irradiance values were ca. 152.21 W m^{-2} and 0.50 W m^{-2} during the three first days, they reached ca. 223 W m^{-2} , 32 W m^{-2} , and 0.70 W m^{-2} during the last 2 d of the experiment, for PAR, UV-A,

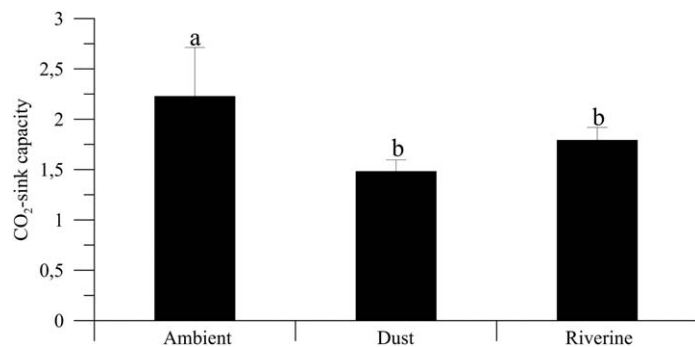


Fig. 4. Mean (\pm SD) CO₂-sink capacity in coastal waters of the South Atlantic Ocean after an acclimation period of 5 d under ambient, dust, and riverine inputs. The letters on the top of bars indicate significant differences by least significant differences post hoc test.

and UV-B, respectively. Moreover, this greater photoinhibition found toward the end of the experiment was dependent not only on the radiation treatment but also on the nutrient source considered. In this sense, we found that the noon Φ_{PSII} values decreased twofold (from ~ 0.36 to 0.17) under riverine treatments regardless of the radiation treatment considered (Fig. 2c; Supporting Information Table S1). Also, we found that the noon Φ_{PSII} values were significantly lower (twofold) under these conditions than under dust or ambient treatments (Fig. 2a,b) where they had values ~ 0.33 , on average, during the same period (t -test riv vs. amb = 6.92 , $p < 0.001$; t -test riv vs. dust = 3.62 , $p < 0.001$). Conversely, dawn Φ_{PSII} exhibited similar or even higher values (between ~ 0.5 and 0.6) over the experiment, particularly under riverine treatments (with one exception, day 24 in P_{riv}), denoting that night recovery was enough to counteract the daily inhibition, avoiding any chronic damage to the PSII.

The O₂ time course also exhibited a response modulated by the daily light-dark cycles. During the first 3 d, these O₂ cycles showed a fluctuating response due to the dominating cloudy conditions; by contrast, during the last 2 d these cycles exhibited a characteristic response pattern with increases from dawn to dusk, followed by a continuous decrease during the darkness period (Fig. 2d-f; Supporting Information Table S1). The O₂ concentrations showed an increasing trend over the experiment, which were steeper under riverine (> 500 mmol O₂ m⁻³; Fig. 2f) than under ambient or dust treatments (\sim or < 500 mmol O₂ m⁻³). Regarding to the NPP, our results showed a significant UVR \times Nut interactive effect ($F_{2424.59}$, $p < 0.001$); thus, the NPP was 2- to 4-fold higher under riverine treatments than in ambient and dust conditions (~ 40 mmol O₂ m⁻³d⁻¹; Fig. 3a). However, the UVR exerted a similar inhibitory effect ($\sim 50\%$) on NPP under ambient and riverine treatments, but it significantly augmented (ca. -10%) under dust treatments (Fig. 3b).

In the same way as for NPP, there was a significant UVR \times Nut interaction over the experiment on CR_{dark} ($F_{74.92}$, $p < 0.001$). In fact, we also found that CR_{dark} was 3- to 6-fold

higher under riverine than in ambient and dust treatments (Fig. 3c). In addition, when we evaluated how the previous UVR exposure could alter the CR_{dark} in our nutrient-enriched treatments, our results evidenced that dust addition significantly stimulated (ca. -20%), whereas that riverine inputs inhibited, reaching similar inhibition values as those found under ambient conditions ($\sim 50\%$ on average; Fig. 3d). However, when we studied how these nutrient treatments affected the daily CR rates over the experiment, we found that whereas the former significantly increased the latter (LSD post hoc, $p < 0.001$), these rates were significantly fourfold higher under riverine than under dust treatments Fig. 3e).

Plankton metabolic balance: nutrients source and UVR interaction

The CO₂-sink capacity showed values > 1 in all treatments, indicating a net autotrophic metabolism in our coastal model ecosystem (Fig. 4; Supporting Information Table S2). Nevertheless, we found significant decreases in the CO₂-sink capacity for the dust and riverine treatments as compared to ambient conditions. In fact, at the end of the experimental period these decreases in the sink capacity indicate a significant reduction of the CO₂ uptake by communities acclimated to the future conditions, by as much as $\sim 27\%$ on average, regardless of the nutrient source considered.

Plankton community structure

After 5 d of acclimation, there were significant shifts in the planktonic community structure as opposed to the interaction UVR \times dust or riverine; thus, whereas the HPP abundances markedly declined over the experiment and the APP fraction did not vary during this period, ANP abundance significantly augmented (Supporting Information Figs. S1a, S2). Moreover, ANP and HPP showed significantly higher abundance values in samples receiving only PAR, regardless the nutrient treatment, whereas APP abundance showed the opposite pattern (except under ambient nutrients; Supporting Information Fig. S1a). These shifts in the autotrophic fraction over the experiment were coupled with increasing Chl *a* concentrations, which were on average fourfold higher (~ 88 mg m⁻³ vs. ~ 20 mg m⁻³; Supporting Information Table S3) under riverine than under ambient and dust treatments.

These diverse responses of the cell abundance resulted in different nano- and picoplanktonic growth rates (Supporting Information Fig. S1b), exhibiting positive values in the ANP (~ 1.5 d⁻¹) and negative in the HPP (-0.5 d⁻¹). Notwithstanding, whereas the dust and riverine inputs counteracted the negative UVR effects on ANP growth, both nutrients accentuated them in HPP growth (Supporting Information Fig. S1b). APP did not show a clear response either to radiation or to nutrient treatments. In terms of biomass, the plankton community was co-dominated by autotrophs and heterotrophs at the beginning of the experiment; however, after 5 d of acclimation, ANP greatly increased (~ 85 – 95%) compared to the APP and HPP fractions regardless the

treatment considered (Supporting Information Fig. S1c). Within the ANP fraction, unidentified flagellates $< 10 \mu\text{M}$ dominated or co-dominated the community as opposed to diatoms (mainly pennates). The contribution of dinoflagellates was almost negligible ($< 1\%$ of the total biomass) in all treatments. These shifts in the plankton community resulted in acute drops in the nutrient concentrations throughout the experiment. In fact, under ambient and dust input treatments, such concentrations ranged from $\sim 2.60 \mu\text{M}$ and $2.34 \mu\text{M}$ at the beginning of the experiment to $0.03 \mu\text{M}$ and $0.40 \mu\text{M}$ at the end for N and P, respectively. For Si, we found a moderate consumption between ~ 25 (amb and dust) and 50% (riv) with respect to the initial conditions (data not shown).

Discussion

This study reports for the first time a potential reduction of the CO_2 -sink capacity of coastal waters of SAO by an increase in nutrients either through aeolian-dust deposition or riverine discharge under high UVR fluxes. Despite the consistent impact of both drivers acting together on the planktonic metabolic balance, we found that UVR exerted a stimulatory effect on NPP and CR under dust inputs but inhibitory under riverine treatments. The underlying mechanism that can explain this stimulatory effect on both metabolic processes after aeolian-dust inputs is the lower dynamic photoinhibition of phytoplankton communities found under these conditions together with an enhanced dark recovery. This latter mechanism, dark recovery, would divert part of the energy expenditure (e.g., through respiration) of planktonic organisms toward protein and ATP synthesis rather than PSII repair, and thus, it would permit an increase the photosynthetic activity (Li et al. 2016).

These findings partially contrast with recent reports showing a consistent inhibitory UVR effect on PP and CR after aeolian-dust inputs (Cabrerizo et al. 2016), and on PP after nutrient inputs (Carrillo et al. 2008, 2015). A plausible explanation to these contrasting results may lie in the different trophic state of the two marine areas considered, a highly productive and non-limited by nutrients in the case of the coastal SAO vs. an unproductive and strongly nutrient-limited (mainly P) area in the case of the south-western coast of the Mediterranean Sea.

Surprisingly, although the joint action of UVR \times dust or riverine was opposite on the metabolism of planktonic communities (see above), we found that it was translated into a similar shift in the structure of the planktonic community toward a dominance by nanoplanktonic flagellates. The specific reasons why nanoflagellates dominated the community regardless of the nutrient source considered might include the following: (1) higher metabolic demands and nutrient uptake when nutrient availability increases (Roberts and Howarth 2006; Mercado et al. 2014); (2) their active movement capacity, which could confer them a competitive advantage under

shallow and stable UML conditions, as found in our study, compared with organisms lacking this capacity (e.g., diatoms) (Striebel et al. 2009); and (3) their potential ability to combine two metabolisms, one phototrophic to obtain carbon and other phagotrophic to obtain limiting nutrients, into the same organism to grow (Raven 1997; Fischer et al. 2017). Despite that we did not quantify mixotrophy *sensu stricto*, we found a consistent decrease of the HPP compartment throughout the incubation period. This decline observed in HPP suggests potential bacterivory by flagellates. Nevertheless, we can rule out that toxic elements and heavy metals (e.g., Pb, Cu) carried by the dust may have interfered with the planktonic metabolism and growth (e.g., HPP) (Paytan et al. 2009; Jordi et al. 2012) and, consequently, may have influenced the CO_2 -sink capacity of the ecosystem with respect to the riverine treatments. In fact, we found that the concentration of such elements were below the detection limits, in agreement with recent reports showing no significant amount of these elements after dust deposition (González-Olalla et al. 2017).

The decreased sink capacity found in SAO under enriched nutrient conditions and high UVR levels is lower than those that predict reductions in the C-downward fluxes between 38% and 50% in surface waters of ecosystems in low (LNLC) and high (HNLC) latitudes, respectively (Boyd 2015). Moreover, the Boyd model simulations, which included more 10 biotic and abiotic factors, revealed that changes in the phytoplankton community structure had the greatest single effect on C fluxes in the future ocean (Boyd 2015). Thus, the consistent response pattern reported in our study between flagellate-dominated communities and decreased CO_2 -sink capacity under both global-change scenarios assayed could support the Boyd's proposal. In addition, the estimates provided here regarding the planktonic metabolic balance agree with the values reported in previous observational studies both in temperate and in tropical ecosystems of both Hemispheres (GPP/CR = ~ 1.5), although they are between 3- and 5-fold lower than those found in polar ecosystems, possibly due to the fact that these studies were performed mostly during continuous spring-summer daylight (Regaudie-de-Gioux and Duarte 2013).

Considering all the above-mentioned findings, we can postulate that the nutrient enrichment of coastal ecosystems, which comprise only 5% of the total oceanic area, could drive that a high C-fixed fraction by phytoplankton photosynthesis to be respired and released as CO_2 into the atmosphere in the future, thus weakening the current role that these key buffers possess in the global C cycle as strong CO_2 sinks (Padín et al. 2010; Bauer et al. 2013). Notwithstanding, recent results by Bermejo et al. (2018) also show that other abiotic factors such as present-day shifts in the wind patterns can influence the trends observed, delaying and weakening the timing as well as the intensity, respectively, of the phytoplankton bloom in the SAO. This likely decline in C uptake proposed in this study for coastal waters

of SAO could sustain the recent decreases that are being registered in the area in terms of POC (Fig. 1a) which subsequently are also triggering significant decreases in fishing catches (Economía and Regiones 2016).

Conclusions

Although we are fully aware that our experimental setup simulates extreme events of nutrients inputs under high UVR irradiance and it was performed at a specific time, they offer realism and ecological significance because: (1) the longer-term scales (> 1 d) avoided bias in the estimates of the metabolic balance of ecosystems which traditionally has been evaluated through short-term scales (< day, see Duarte et al. 2013; Regaudie-de-Gioux and Duarte 2013) and (2) the study combines both the plankton metabolic status (García-Corral et al. 2017) as well as the shifts in the plankton community structure (Villafañe et al. 2017), which are usually neglected in these types of metabolic studies. Therefore, as we are now at an unprecedented juncture in the field of the global-change ecology, the integration of both mid-term experimental approaches together with long-term remote-sensing and observational data implies an improvement in the understanding about how the increasing climatic variability that the marine ecosystems are undergoing could have severe impact on their capacity to sequester CO₂ in the future.

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Conflict of Interest

None declared.

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