What supports the deep chlorophyll maximum in acidic lakes? The role of the bacterial CO$_2$ production in the hypolimnion

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Abstract

The interactions between phytoplankton, bacteria and resources, irradiance, and nutrients, leading to the formation of deep chlorophyll maxima (DCMs), are little understood in acid lakes. In “El Sancho” reservoir (Iberian Pyritic belt, Huelva, Spain), an acid mine drainage impacted waterbody (pH 3.5–4.0), a strong DCM forms in the metalimnion during the stratification period. The DCM was located always below the 1% irradiance level, where the decreasing irradiance profile overlapped with a dissolved inorganic carbon concentration (CO$_2$) gradient decreasing upward from the hypolimnion. The DCM was dominated by the chlorophyte Carteria sp. and showed the highest volumetric photosynthetic and dark respiration rates. The DCM, however, only contributed around 20% of water column integrated gross primary production, while it accounted for 54–66% of water column chlorophyll. The total bacterial abundance correlated significantly with the CO$_2$ concentration ($r$ = 0.74). To test the hypothesis of a possible dependence of the formation of the DCM in acid lakes on the production of CO$_2$ by heterotrophic bacteria, a one-dimensional reactive transport model (DCM-CO$_2$) was developed and tested. The DCM-CO$_2$ model simulated the vertical distribution of chlorophyll ($R^2 > 0.63$) and the vertical profile of CO$_2$ rather accurately ($R^2 > 0.79$), the position of DCM depending on both light penetration and an upward flux of CO$_2$ produced by hypolimnetic heterotrophic bacteria. Overall, the results support the hypothesis of microbial degradation of organic matter being a source of CO$_2$ for acid lake primary producers at the DCM.

Deep chlorophyll maxima (DCMs) are subsurface water layers enriched in chlorophyll commonly found in relatively nutrient-poor stratified open ocean waters (Cullen 1982; Huisman et al. 2006; Martin et al. 2010; Latasa et al. 2017) and lakes (Abbott et al. 1984; Barbiero and Tuchman 2004; Clegg et al. 2012). Generally, most DCMs result both from a certain increase in chlorophyll per cell (Latasa et al. 2017) and from the accumulation of phytoplankton cells, forming deep biomass maxima (DBMs) as well. Coinciding DCMs and DBMs can be found at the intersection of two opposite resource gradients, light from the surface, and nutrients from the bottom (Abbott et al. 1984; Durham and Stocker 2012).

Acid lakes (Nixdorf et al. 1998; Tittel et al. 2003), due to the high concentration of dissolved metals and low pH in the water column, are extreme environments with specific biogeochemical characteristics and microbiotas (Nixdorf et al. 1998; Torres et al. 2014; Corzo et al. 2018). Thus, while in typical aquatic ecosystems nitrogen and phosphorous are often considered as the primary limiting nutrients, in acid lakes, carbon and phosphorous have been suggested as the main nutrients limiting phytoplankton primary production (Nixdorf et al. 1998). In the epilimnion of these environments, dissolved inorganic carbon (DIC) is available primarily as carbon dioxide (CO$_2$), at concentrations near the equilibrium with air as determined by Henry’s law (Nixdorf et al. 1998; Tittel et al. 2005). However, although phosphates are highly soluble at low pH, the high concentrations of metals in acid lakes promote their coprecipitation with Fe(III) oxyhydroxides under aerobic conditions, reducing their bioavailability for the

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Additional Supporting Information may be found in the online version of this article.
photosynthetic organisms in the water column (Nixdorf et al. 2001). Despite these limitations under extreme conditions, DCMs have been reported in acid lakes, where chlorophyll a (Chl a) concentrations may reach about 30 μg L\(^{-1}\) similar to the values found in eutrophic lakes (Nixdorf et al. 1998). Therefore, DCMs can contribute significantly to the water column-integrated primary production in acid environments. However, the physicochemical and biological processes that determine and favor the development of DCM and whether this coincides with a DBM in different aquatic environments and especially in acid lake settings are still under debate (Fennel and Boss 2003; Cullen 2015).

El Sancho reservoir (SW Spain), located in Iberian Pyritic Belt, is a warm monomictic freshwater reservoir that has undergone an acidification process over the years (pH 3.5–4.5) due to ongoing pollution by acid mine drainage (AMD) (Torres et al. 2013, 2014). In summer, during the stratification season, a sharp DCM appears at the bottom of the photic layer; however, its ecological characteristics, including the vertical distribution of phyto- and bacterioplankton, are unknown (Torres et al. 2016). Previously, the microbial community and the limnological characteristics have been studied in small pit acidic lakes in the Iberian Pyritic Belt; however, these systems are not comparable to El Sancho reservoir due to their different origin, small dimensions, and more extreme conditions (pH < 3) (Sánchez-España et al. 2012; Santofimia et al. 2013).

Here, we present a detailed description of the conditions under which the seasonal DCM develops in El Sancho, focusing on the net metabolism of the plankton community and on the interactions between the main pelagic microbial communities: phyto- and bacterioplankton. We provide field experimental evidence supporting the hypothesis that bacterial CO\(_2\) production in the hypolimnion is an important source of inorganic carbon contributing to the formation of the DCM. This hypothesis was tested by a one-dimensional (1D) reactive transport model, which predicted the phototrophic biomass and CO\(_2\) vertical distribution as a function of irradiance and the abundance of heterotrophic bacteria.

**Materials and methods**

**Study site and sampling collection**

El Sancho Reservoir (4.27 km\(^2\), 58 hm\(^3\)) was built in 1962. Its main tributary, the River Meca (pH 2.6) is heavily contaminated by AMD with high concentrations of trace metals, iron and sulfate and is responsible for its acidification (Torres et al. 2013).

Samplings were carried out at one station (37°27'49"N, 6°59'3"W) located at the deepest part of the El Sancho reservoir (34.5 m average depth) four times during the stratification period in 2013 (12\(^{th}\), 18\(^{th}\), and 25\(^{th}\) September 2013 and 8\(^{th}\) October 2013) (Supporting Information Fig. S1). Vertical temperature (T, °C), pH, and fluorescence (relative units, r.u.) profiles were obtained using a multiparameter probe (Hydrolab MS5). Photosynthetically active irradiance profiles (PAR) (μmol photons m\(^{-2}\)s\(^{-1}\)) were obtained using a LiCor (Li-1400) radiometer equipped with a planar probe; the light extinction coefficient (k) was then calculated (Kirk 1994). Based on the fluorescence profile, water samples were collected using a 10-liter Van Dorn bottle (7–17 depths).

Sediment cores (n = 8) (Plexiglas tubes, i.d. 5.8 cm, length 60 cm) were collected using a Kajak corer (KC Denmark A/S), stored on ice (4°C) in the dark, and kept under water at 13°C overnight once in the laboratory after 4–5 h.

Two independent sets of sediment traps were deployed on September 12\(^{th}\). In each set, four traps (Plexiglas tubes, i.d. 7 cm, length 50 cm) were installed between 23 and 25 m and another four between 31 and 34 m. Traps were left in situ for 28 d.

**Water column and sediment analyses**

Samples for dissolved oxygen (O\(_2\)) determination (n = 2 per depth) were collected and fixed in 12 mL Exetainer tubes (Labco, UK) following Labasque et al. (2004), stored in darkness at 4°C, and analyzed within 24 h (limit of detection [LOD] = 3.8 μmol L\(^{-1}\)). O\(_2\) saturation was determined as a function of the water column temperature according to García and Gordon (1992). High-resolution O\(_2\) profiles were determined on the 2\(^{nd}\) and 8\(^{th}\) October with a modified MP4 Miniprofiler (UNISENSE, Supporting Information Fig. S2). CO\(_2\) samples (n = 1 per depth) were collected in 5 mL Exetainer tubes, fixed with 100 μL saturated HgCl\(_2\) and stored in darkness at 4°C until analysis. CO\(_2\) was measured following the setup of Hall and Aller 1992 on an InfraRed Gas Analyzer (Qubit systems, S151 CO2 analyzer) (LOD = 6.8 μmol L\(^{-1}\)).

Inorganic nutrients were analyzed in filtered water samples (MF 300, 0.7 μm, 47 mm, Fisherbrand\(^{TM}\)), stored on ice and frozen at −20°C upon return to the laboratory. Ammonium (NH\(_4^+\)) (Bower and Holm-Hansen 1980), phosphate (PO\(_4^{3-}\)) (Grasshoff et al. 1999), and nitrate (NO\(_3^-\)) and nitrite (NO\(_2^-\)) (García-Robledo et al. 2014) were measured with LOD between 0.1 and 0.5 μmol L\(^{-1}\).

For determination of Chl a, water samples (1 L) were filtered in situ through precombusted filters (GF/F glass fiber filters, 0.7 μm, 47 mm, Whatman\(^{®}\)), stored on ice in darkness, and frozen at −20°C upon return to the laboratory. Chl a was extracted at 4°C for 12 h with 4 mL of acetone 90%, tubes centrifuged (2200 × g, 5 min) and the absorbance of the extracts measured on a UV 1700 Pharmaspec Shimadzu spectrophotometer. Chl a concentration was calculated according to Ritchie (2008).

Dissolved organic carbon (DOC) was measured in water samples (approximately 20 mL), filtered through nylon filters (Nylon Syringe filters, 0.2 μm, 25 mm, Fisher Scientific\(^{TM}\)) in acid-washed glass vials (n = 1), and stored at 4°C. DOC contents were determined on a Shimadzu TOC-5050 analyzer on acidified samples (1 mL of phosphoric acid 1:3) (ICMAN-CSIC external services). Particulate organic carbon (POC) and total nitrogen (PTN) samples were collected similarly to chlorophyll on preweighed filters and determined on a FlashEA1112 (ThermoFinnigan) elemental analyzer (University of A Coruña external services).
Sediment cores (two replicates per sampling) were sliced at a 1-cm interval for the first 6 cm and 2-cm intervals down to 18 cm depth within 24 h of collection under a flow of N₂, and slices from the same depth pooled. Pore water was extracted by centrifugation from each layer, filtered through nylon filters (Nylon Syringe filters, 0.2 µm, 25 mm, Fisher Scientific™), and stored at −20°C until analysis of NH₄⁺ and NO₃⁻ as described previously. Total organic carbon (Corg) and total nitrogen contents (Ng) were analyzed on a FlashEA1112 (ThermoFinnigan) elemental analyzer using standard protocols (University of A Coruña external services) on sediment samples dried at 60°C for 24 h (expressed as g [g dry sediment]⁻¹ × 100).

For pore-water CO₂ determination, sediment cores (three replicates) were sliced every 2 cm for the first 6 cm and every 4 cm down to 18 cm depth within 24 h. Extracted pore water was fixed with 100 µL saturated HgCl₂ and stored in darkness at 4°C until analysis as described previously.

Collected sediment traps, once in the lab, were left undisturbed at 4°C to allow the particles to settle, supernatant removed and particulate material dried at 60°C and analyzed for Corg and Ng as described previously.

**Fluxes calculation through water column and sediment**

Assuming steady state conditions, the net rates of O₂, CO₂, NH₄⁺, and NO₃⁻ production and consumption were calculated for the epilimnion, metalimnion, and hypolimnion layers according to Fick’s first law applied to turbulent diffusion (Okubo and Levin 2001).

\[
J_C = -K_d \frac{dC}{dz}
\]

(1)

where \(J_C\) is the net flux of substance C, \(K_d\) is the vertical turbulent diffusion coefficient, and \(dC/dz\) is the concentration gradient. Here, \(dC/dz\) was calculated for each layer from the measured concentration profiles and \(K_d\) (m² s⁻¹) was calculated by Eq. 2 (Osborn 1980):

\[
K_d = \frac{\gamma \varepsilon}{N^2}
\]

(2)

where \(\gamma\) is the mixing coefficient and \(\varepsilon\) is the dissipation rate of turbulent kinetic energy. Values of 0.15 for \(\varepsilon\) and 9 × 10⁻⁹ W kg⁻¹ s⁻¹ were used (Wuest et al. 2000). The frequency of Brunt-Väisälä (N, s⁻¹), which measures the stability of the water column, was calculated according to Eq. 3 from the density gradient in depth (dp/dz) in each layer (Gargett 1984):

\[
N^2 = \frac{g dp}{\rho dz}
\]

(3)

where \(g\) is the gravitational acceleration and \(\rho\) is the mean water density for each layer.

CO₂ and NH₄⁺ fluxes at the sediment–water interface were calculated using Fick’s law applied to molecular diffusion and the concentration gradient with depth from 2 cm above the sediment surface to 5.5 cm below the sediment surface (maximum linearity). The apparent molecular diffusion coefficient (Ds, m² s⁻¹) for each substance was calculated with Marelac R package (version 2.1.9) (Soetaert et al. 2010) the specific in situ T and pressure, and taking into account the average porosity (0.88) of the sediment (0–5.5 cm) (Torres et al. 2014).

**Microbial community analyses**

Water samples were fixed in cryotubes (4.5 mL) using glutaraldehyde (1% final concentration) and frozen at −80°C until analysis by flow cytometry. Prior to the analyses, autofluorescent beads (1.1 µm diameter, Ex/Em: 430/465 nm, FluoSpheres® Molecular Probes™) were added to each sample (1 mL) as an internal standard. Phytoplankton was identified based on autofluorescence. For bacterioplankton, 10 µL of SYBR® Green-I (Molecular Probes #S7563) (2.5 µmol L⁻¹ final concentration) was added to each sample and incubated for 10 min at room temperature in darkness before analysis. Samples were analyzed on a Dako CyAnTM ADP (Beckman Coulter®) flow cytometer. Further methodological details can be found in Corzo et al. (1999), Gasol and del Giorgio (2000), and Corzo et al. (2005). Biomass of each group (in µg C L⁻¹) was estimated from cell diameter and cell abundance using published empirical equations (Supporting Information).

Taxonomic identification of phytoplankton was carried on an inverted light microscope (Nikon Eclipse Ti-U) in water samples collected in polyethylene bottles (100 mL), stored in vivo at 4°C in dark, and analyzed within 24 h after collection.

**Photosynthesis-irradiance curves**

Water samples from specific depths (0, 5, 16, 22.5, and 30 m depth) were collected using a 10-liter Van Dorn bottle (September 25th) and transported to the laboratory. Incubations were performed in special bottles under continuous stirring at 17°C under increasing irradiances (0, 50, 100, 200, 400, and 600 µmol photons m⁻² s⁻¹). Changes in O₂ concentration to determine net primary production (Pn) and dark respiration (Rd) rates were measured with STOX sensors (Revsbech et al. 2011), with a resolution lower than 2 nmol O₂ L⁻¹ h⁻¹ (Tiano et al. 2014). Photosynthesis-irradiance (P-E) curves for each water depth and the corresponding photosynthetic parameters were obtained after fitting the experimental data to the Jassby and Platt (1976) model (Supporting Information).

**Numerical modeling of the Chl a and CO₂ vertical distributions**

A 1D reactive transport model was developed to predict the spatial distribution of the phototrophic biomass (Chl a, mg m⁻³) for the four sampling dates. The basic equations of this model were based on pre-existing literature about DCM (Fennel and Boss 2003; Huisman et al. 2006; Gong et al. 2015) and coupled the light and inorganic carbon dependency of growth with both turbulent diffusion (mixing) and a sinking term along the vertical axis, according to Eq. 4:
\[
\frac{\partial B}{\partial t} = (\mu - l)B + K_d \left( \frac{\partial^2 B}{\partial z^2} \right) - W_s \left( \frac{\partial B}{\partial z} \right)
\]

where \( z \) is depth (m, in positive values), \( B \) is the Chl \( a \) concentration (mg m\(^{-3} \)), \( \mu \) is the specific growth rate (d\(^{-1} \)), \( l \) is the natural mortality (d\(^{-1} \)), \( K_d \) is the turbulent diffusion coefficient (m\(^2\) d\(^{-1} \)), and \( W_s \) is the bulk sinking velocity of phytoplankton (m d\(^{-1} \)). Biomass production was set by the variable \( \mu \), which depended on the maximum growth rate \( \mu_{max} \) and a minimum law (Liebig’s law) applied to the two limiting factors, light and inorganic carbon by two respective dependent functions (i.e., \( f(E) \) and \( f(CO_2) \)), according to Eq. 5:

\[
\mu(E(z), CO_2(z)) = \mu_{max} \min( f(E(z)), f(CO_2(z)) )
\]

The \( f(E(z)) \) was modeled as a hyperbolic light-production curve (Jassby and Platt 1976), according to the Eq. 6:

\[
f(E(z)) = \tanh \left( \frac{E(z)}{E_k(z)} \right)
\]

where \( E(z) \) is the light irradiance at depth \( z \) (which obeys to an exponential attenuation) and \( E_k \) is the light-saturation of photosynthesis (\( \mu \)mol photons m\(^{-2}\) s\(^{-1} \)). To account for the photoclaimation of cells, a critical process in oligotrophic lakes where \( CO_2 \) (i.e., at 0, 5, 16, 22.5, and 30 m depth, exponential attenuation) and \( \mu \) is the specific growth rate (d\(^{-1} \)), \( l \) is the natural mortality (d\(^{-1} \)), \( K_d \) is the turbulent diffusion coefficient (m\(^2\) d\(^{-1} \)), and \( W_s \) is the bulk sinking velocity of phytoplankton (m d\(^{-1} \)). Biomass production was set by the variable \( \mu \), which depended on the maximum growth rate \( \mu_{max} \) and a minimum law (Liebig’s law) applied to the two limiting factors, light and inorganic carbon by two respective dependent functions (i.e., \( f(E) \) and \( f(CO_2) \)), according to Eq. 5:

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\[
f(CO_2(z)) = \frac{CO_2(z)}{CO_2 \text{half} + CO_2(z)}
\]

where \( CO_2(z) \) is the inorganic carbon concentration at depth \( z \) (\( \mu \)mol L\(^{-1} \)) and \( CO_2 \text{ half} \) is the half-saturation constant (\( \mu \)mol L\(^{-1} \), Reynolds and Irish 1997). The observed values of \( CO_2 \) concentrations at the epilimnion layer were assumed to be close to 0 \( \mu \)mol L\(^{-1} \) since concentrations were below the detection limit (i.e., < 6.85 \( \mu \)mol L\(^{-1} \)).

In addition to modeling the DCM, the profiles of \( CO_2 \) were modeled as a function of two reaction terms (i.e., \( CO_2 \) production by heterotrophic bacteria - \( CO_2 \) consumption by primary producers), according to Eq. 8:

\[
\frac{\partial CO_2}{\partial t} = K_d \left( \frac{\partial^2 CO_2}{\partial z^2} \right) + (R-P)
\]

where \( R \) is the \( CO_2 \) production due to the mineralization of organic matter (OM) (mmol C m\(^{-3}\) d\(^{-1} \)) and \( P \) is the \( CO_2 \) consumption rate due to the net photosynthesis of phytoplankton (mmol C m\(^{-3}\) d\(^{-1} \)).

The term \( P \) was estimated as a function of growth rate, biomass abundance, and the C:Chl \( a \) and represent the \( CO_2 \) demand at a given depth (Eq. 9; Cloern et al. 1995)

\[
P(z) = \mu(z)B(z)(C:Chl a)\left( \frac{1}{12} \right)
\]

where \( \mu \) was calculated from Eq. 8 and C:Chl \( a \) is the mg C: mg Chl \( a \) ratio. Two different values of the C:Chl \( a \) were used by the epi- and metalimnion layers since experimental POC:Chl \( a \) showed a wide range throughout the corresponding depths.

Rates of \( Pg \) were also extracted from the model, in order to compare with the experimental values calculated from the \( P-E \) curves. The conversion of \( P \) values to \( Pg \) rates was addressed by considering two respiratory terms, that is, a basal respiration of phytoplankton (\( R_{min} \)) which is independent on the photosynthetic gross production rate, and a second respiration term which increase linearly with photosynthesis (\( R_{ph} \), Eq. 10). These parameters were initially obtained from the universal relationship between growth rate and respiration reported by Cloern et al. (1995), but recalibrated to better fit with our \( Pg \) and Chl \( a \) data.

\[
P_g(z) = \frac{1}{(1-R_{ph})} (\mu(z) + R_{min})B(z)(C:Chl a)\left( \frac{1}{12} \right)
\]

where \( R_{min} \) is the minimum respiration rate (\( \approx 0.015 \) d\(^{-1} \)) and \( R_{ph} \) is the fraction of \( Pg \) respired (\( \approx 0.15 \), Cloern et al. 1995).

Bacterial respiration was considered to be the result of both aerobic and anaerobic respiration since \( O_2 \) levels decreased from 400 \( \mu \)mol L\(^{-1} \) to nearly 0 \( \mu \)mol L\(^{-1} \) through the water column depth. Therefore, \( R \) was a function of \( T \), bacterial abundance, and \( O_2 \) availability (Eq. 11, Grégoire et al. 2008)

\[
R(z) = \left( \frac{Bact(z)}{R_s} \right) \left( \frac{O_2(z)}{O_2(z) + K_s} \right) + R_\lambda \left( \frac{1 - O_2(z)}{O_2(z) + K_s} \right) \left( \frac{1}{f(T)} \right)
\]

where \( Bact(z) \) is the measured bacterial abundance (cell m\(^{-3} \)), \( R_\lambda \) is the maximal specific rate of aerobic respiration (mmol C cell\(^{-1}\) d\(^{-1} \)), \( R_\lambda \) is the maximal anaerobic respiration (Soetaert et al. 1996), \( O_2(z) \) is oxygen concentration at depth \( z \) (\( \mu \)mol L\(^{-1} \)), \( K_s \) is the half-saturation constant for aerobic respiration (\( \mu \)mol L\(^{-1} \)), \( K_s \) is the half-inhibitory constant for anaerobic respiration (\( \mu \)mol L\(^{-1} \)), and \( f(T) \) is a correction factor of \( R \) as a function of \( T \) (Eq. 12)

\[
f(T) = Q_{10} \left( \frac{T}{T_{ref}} \right)^{0.5}
\]

where \( Q_{10} \) is the metabolic enhancement factor, \( T(z) \) is \( T \) at depth \( z \) (\( ^\circ \)C), and \( T_{ref} \) is the superficial temperature for each
sampling period (°C). Note that Eq. 11 allows a clear vertical structure of metabolisms for extremely high or extremely low values of $O_2$, but also a coexistence of both anaerobic and aerobic activities for depths with low $O_2$ concentrations, as other authors confirmed in marine ecosystems (Grgoire et al. 2008; García-Robledo et al. 2017).

Numerical simulations of Chl a and CO$_2$ profiles were carried out by solving the steady state of both Eqs. 4, 8 across a

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**Fig. 1.** Depth profiles of *water column* variables during the sampling period in El Sancho reservoir. (a) Chl a (---), POC (- - -), fluorescence (■), and irradiance expressed as percent of photosynthetically active radiation at the surface (PAR, ---); (b) PTN (---), temperature ($T$, - - -), and pH (---); (c) dissolved oxygen ($O_2$, - - ), percent saturation of dissolved oxygen ($O_2$, ---), and carbon dioxide (CO$_2$, - - -); (d) ammonium (NH$_4^+$, - - -), nitrate (NO$_3^-$, - - -), and DOC (---). The bottom of reservoir is shown with a horizontal black line.
$10^{-2}$ m resolution grid, that is, with a linear system of $2 \times 3300$ equations for a maximal depth of $\approx 33$ m. Spatial discretization was performed with a second-order approximation of derivatives, but a purely upwind scheme for the sinking term to avoid numerical instabilities (Soetaert and Herman 2009). Boundary conditions for Chl $a$ were set as zero-flux at $z = 0$ (Huisman et al. 2006) and as known Chl $a$ values (measured in every sampling date) at the maximum depth, that is, biomass export toward the sediment was allowed (Torres et al. 2013). Boundary conditions for CO$_2$ at the water-atmosphere interface followed a piston-based flux according to Eq. 13 (Schindler 1975; Cole and Caraco 1998; Cole et al. 2002):

$$ F^\text{atm}_{\text{CO}_2}(z=0) = K_{\text{piston}}^* (C_{\text{eq}} - \text{CO}_2(z=0)) $$

where $F^\text{atm}_{\text{CO}_2}(z=0)$ (mmol C m$^{-2}$ d$^{-1}$) and CO$_2$ ($z = 0$) ($\mu$mol L$^{-1}$) are respectively the imposed flux and concentration at the upper boundary of the profile, $K_{\text{piston}} = 10^{-5}$ m s$^{-1}$ is the piston velocity of CO$_2$ with no wind and with no chemical enhancement (Jørgensen 1979a,b), and $C_{\text{eq}}$ is the equilibrium concentration according to Henry’s law ($\mu$mol L$^{-1}$, Soetaert et al. 2010). At the bottom boundary, sediment was a net source of CO$_2$ due to mineralization of OM. Values of this flux were estimated by inverse modeling considering a range between the zero-flux of CO$_2$ from the sediment as the “minimum flux” and the flux of CO$_2$ in the hypolimnion as the “maximum flux,” which was determined for each sampling. Sediment CO$_2$ fluxes were similar to the experimentally determined CO$_2$ efflux obtained on the September 25th and to previous studies (Torres et al. 2014; Corzo et al. 2018).

The coupled DCM-CO$_2$ model included a total of 14 parameters that were calibrated simultaneously to fit the experimental information on each sampling date. The minimum and maximum limits for calibrated parameters were taken from the literature (see references from Table 3). In addition, $W_x$ was determined from the dominant (in biomass) phytoplankton at the DCM, Carteria sp. (ca. $1.9 \times 10^{-5}$ m s$^{-1}$, calculated by Stoke’s law). Since the error threshold for growth rates according to Cloern et al. (1995) were up to 35%, a reasonable uncertainty in the respiration of phytoplankton was considered accordingly, and the upper and lower limits for $R_{\text{min}}$ and $R_{\text{phot}}$ were set as $\pm 20\%$ of the original parameters calculated by these authors. Calibrations were made with ReacTran R package (version 1.4.3.1) by adequately arranging model parameters to the spatial grid (Soetaert and Meysman 2012). Whereas $K_d$ and $E_k$ were measured, the remaining parameters were calibrated by the pseudo-random algorithm of Price until the predicted Chl $a$

Table 1. Mean turbulent diffusion coefficient ($K_d$, m$^2$ s$^{-1}$) and mean net fluxes of O$_2$, CO$_2$, NH$_4^+$, and NO$_3^-$ (mmol m$^{-2}$ d$^{-1}$) within every layer, that is, epilimnion, metalimnion, hypolimnion, and sediment and their thickness (m for water column layers and cm for sediment layer) calculated from the corresponding observed vertical profiles. Fluxes within the sediment were calculated using different apparent molecular diffusion coefficients ($D_s$, m$^2$ d$^{-1}$) for every substance and taking into account the sediment porosity. Mean molecular diffusion coefficients: CO$_2$: $1.17 \times 10^{-4}$, NH$_4^+$: $1.31 \times 10^{-4}$. Positive and negative signs are net production and net consumption rates, respectively, within the corresponding layer. Data are $n = 4 \pm SE$.

| Layer     | Thickness | $K_d$ | O$_2$ | CO$_2$ | NH$_4^+$ | NO$_3^-$ |
|-----------|-----------|-------|-------|--------|----------|----------|
| Epilimnion| 0–15      | $7.3 \times 10^{-5} \pm 4 \times 10^{-5}$ | $2.7 \pm 1$ | $-0.5 \pm 0.4$ | $0.12 \pm 0.11$ |
| Metalimnion| 15–24     | $5.2 \times 10^{-7} \pm 1.4 \times 10^{-8}$ | $6.2 \pm 0.4$ | $0.8 \pm 0.2$ | $0.1 \pm 0.02$ | $-0.004 \pm 0.001$ |
| Hypolimnion| 24–33     | $8.6 \times 10^{-8} \pm 5.4 \times 10^{-7}$ | $-7.3 \pm 0.5$ | $34 \pm 5.6$ | $4 \pm 0.3$ | $-0.08 \pm 0.01$ |
| Sediment  | –2 to 5.5 | –     | 0     | 2.62   | 1.2 $\pm 0.3$ | —        |
and the CO₂ profiles fitted with the field observations (EcolMod R package version 1.2.6, Soetaert and Herman 2009). Further details of the model, as well as a description of the R code, are included within Supporting Information.

Statistical analyses
Simple linear correlation (Spearman correlation) and regression analyses were used to test statistical significance of variation between different variables through both spatial and temporal scales. A correlation of observed vs. predicted values was used to check the goodness of fit of the model outputs. Percentage of variation explained by the ecological model was calculated by the R² coefficients, testing first that the intercept and the slope were not statistically different from zero and from 1 (t-test), respectively. All analyses were made with Stats R package (version 3.5.2) (R Core Team 2014).

Results
Water column structure
The photic layer extended down to 18–22 m depth (1% of surface irradiance) depending on sampling date (Fig. 1a). The k value for light attenuation increased considerably at 18–19 m, from 0.12 to 0.18 m⁻¹ in the epilimnion to very high values at the bottom of the thermocline (0.71–0.97 m⁻¹). Fluorescence and Chl a showed a similar vertical distribution (r = 0.65, p < 0.001, n = 45), showing a pronounced DCM at the bottom of the photic layer at irradiances between 1.3–11.1 μmol photons m⁻² s⁻¹. Chl a concentration was 1–2 orders of magnitude higher at the DCM peak compared to surface waters.

POC showed a vertical distribution similar to Chl a (r = 0.94, p < 0.001, n = 45), including a peak in POC at the same depth as Chl a. POC:Chl a ratio (mg C:mg Chl a⁻¹) at the epilimnion changed considerably between samplings but decreased with depth showing a minimum (72.4 ± 4.4) at the DCM. From the DCM toward the bottom, POC:Chl a increased due to a relative

![Fig. 3. Sediment depth profiles of (a) percent of organic carbon (Corg), percent of total nitrogen (NT), and carbon to nitrogen ratio (Corg:NT), (b) carbon dioxide (CO₂) (n = 3) and ammonium (NH₄⁺) during the sampling period: 12th September (→), 18th September (→), 25th September (→), and 8th October (←) of 2013. CO₂ data correspond to a single sampling (16th September, ←). The bottom of reservoir is shown with a horizontal black line.](image-url)
The POC:PTN ratio decreased from the DCM depth toward the bottom due to the relatively higher decrease of POC with respect to PTN (Fig. 2).

The water column was stratified showing a marked thermocline between 15 and 24 m depth (Fig. 1b). Temperature decreased from 25.5°C on average in the epilimnion to less than 13.5°C in the hypolimnion. pH was rather constant in the epilimnion and metalimnion (around 3.5), increasing linearly with depth in the hypolimnion up to 4.5 (Fig. 1b).

Oxygen concentration was constant in the epilimnion (270 ± 5 μmol L⁻¹), increasing sharply right below the beginning of the thermocline where it ranged from 362 to 439 μmol L⁻¹ (Fig. 1c) (129–158% saturation) (Fig. 1c). From this peak, O₂ decreased down to 20–25 m depth to concentrations below 10 μmol L⁻¹ (often below the LOD) until the bottom (Fig. 1c). CO₂ concentrations were below the LOD in the epilimnion and in the upper part of the thermocline, but increased linearly with the depth up to 670 μmol L⁻¹ near the bottom (r = 0.94, p < 0.001, n = 43) (Fig. 1c). CO₂ and O₂ concentrations showed a strong inverse correlation in the hypolimnion (r = −0.97, p < 0.001, n = 15).

NH₄⁺ concentrations were constant in the epilimnion and increased linearly from the thermocline downward, reaching concentrations > 100 μmol L⁻¹ close to the sediment surface (Fig. 1d). In the hypolimnion, NH₄⁺ tended to increase slightly during successive samplings and showed a high positive correlation with CO₂ (r = 0.93, p < 0.001, n = 43). NO₃⁻ concentrations were much lower (< 3 μmol L⁻¹) and showed an inversed vertical distribution to those of NH₄⁺ (Fig. 1d). PO₄³⁻ and NO₂⁻ concentrations were constant in the epilimnion and metalimnion (around 3.5), increasing linearly with depth in the hypolimnion up to 4.5 (Fig. 1b).
were always below the LOD. DOC concentration did not show any consistent vertical pattern (Fig. 1d). DOC:POC in the epilimnion decreased toward the DCM layer and remained constant with depth in the hypolimnion (Fig. 2).

**Water column net fluxes**

The shape of the vertical profiles of O$_2$, CO$_2$, NH$_4^+$, and NO$_3^-$ indicated the existence of strong gradients associated with the presence of the DCM (Fig. 1c,d). $K_d$ values were higher and more variable between samplings in the epilimnion, particularly due to a very high value on September 25th, while the values at the hypolimnion and metalimnion were one-order and three-orders of magnitude lower, respectively, and more constant (Table 1).

Despite the relatively high homogeneity of O$_2$ concentration in the epilimnion, we calculated a relatively high O$_2$ production rate and net flux toward the atmosphere due to the high $K_d$ estimated for the epilimnion (Table 1). The O$_2$ concentration peak in the metalimnion was associated with high O$_2$ production rates (6.2 $\pm$ 0.4 mmol O$_2$ m$^{-2}$ d$^{-1}$) in this layer (15-24 m). O$_2$ net consumption rate in the hypolimnion was very low, mainly due to the very low O$_2$ available in this lake compartment (Fig. 1; Table 1).

Vertical profiles of CO$_2$ clearly indicated a strong gradient from the bottom of the reservoir to the metalimnion where it was consumed. While in the hypolimnion’s CO$_2$ presented an upward flux ranging from 19.8 to 46.7 mmol CO$_2$ m$^{-2}$ d$^{-1}$, the net flux in the metalimnion was considerably lower due to both a decrease in the concentration gradient and a much lower $K_d$ than in the hypolimnion (Table 1).

Ammonium net fluxes at the epilimnion were very variable due to the variability of concentrations and the value of $K_d$ across samplings (Table 1). In contrast, NH$_4^+$ was always consumed in the metalimnion, whereas a high net upward flux in the hypolimnion toward the DCM was detected in all samplings (4 $\pm$ 0.3 mmol m$^{-2}$ d$^{-1}$). CO$_2$:NH$_4^+$ upward flux stoichiometry was about 8.5 $\pm$ 0.9. Nitrate net fluxes were always one to several orders of magnitude lower than those calculated for NH$_4^+$ (Table 1). In the epilimnion, NO$_3^-$ was generally produced, while it was consumed in the meta- and hypolimnion.

Sedimentation rate of OM from the DCM, measured with sediment traps, was 19.9 $\pm$ 3.2 mmol C$_{org}$ m$^{-2}$ d$^{-1}$ with a C:N stoichiometry of 9.6 $\pm$ 0.3, similar to the CO$_2$:NH$_4^+$ upward flux stoichiometry. An estimate for the average sedimentation velocity ($v$) of POC, calculated from POC sedimentation rates (traps) and POC concentration at the DCM peak ($v = POC$ downward flux/POC concentration at DCM), gives values between 0.15 and 0.23 m d$^{-1}$. These values are much lower than those calculated using the cell size of the main contributor to the phytoplanktonic biomass (*Carteria* sp., see below) using the Stokes equation (0.78–1.68 m d$^{-1}$).

**Fig. 6.** Water column depth profiles of (a) photosynthetic parameters and respiration rate (volume units) and (b) photosynthetic parameters and respiration rate normalized to Chl a (volume units) on 25th September at 0, 5, 16, 22.5, and 30 m depth. $P_{g}$ is the maximum gross production, $R$ is the respiration rate in the dark, $\alpha$ is the photosynthetic efficiency, $E_c$ is the light compensation point, and $E_k$ is the light saturation point.
Sediment concentrations and net fluxes to the water column

C$_{org}$ and N$_T$ decreased considerably from ~ 12% to 3% and from ~ 1.4% to 0.3%, respectively, in upper 7.5 cm sediment layer, remaining constant with depth below (Fig. 3a). C$_{org}$N$_T$ was 10.1 ± 0.1 at the sediment surface and changed very little with depth (Fig. 3a). The CO$_2$ profile indicated net production in the uppermost sediment layers increasing from 640 μmol L$^{-1}$ in the bottom water to about 2500 μmol L$^{-1}$ at 5 cm depth within the sediment, remaining constant or even decreasing slightly at higher depths (Fig. 3b). CO$_2$ production rate and subsequent efflux to the water column was 2.6 mmol CO$_2$ m$^{-2}$ d$^{-1}$ (Table 1).

### Table 3. Parameter values of the model and their description.

| Simulations | Parameter | Description | Min | Max | Units | Range values (Reference) |
|-------------|-----------|-------------|-----|-----|-------|--------------------------|
| Chl a (µg L$^{-1}$) | $\mu_{\text{max}}$ | Maximal specific growth rate of phytoplankton | 1.1 | 3 | d$^{-1}$ | 0.44–3 (Fennel and Boss 2003; Titel et al. 2005) |
| | $K_{CO2}$ | Half-saturation constant for CO$_2$ uptake | 16.3 | 208.6 | μmol C L$^{-1}$ | 0.1–200 (Hein 1997; Reynolds and Irish 1997; Monorey 2001) |
| | $l$ | Natural mortality rate of phytoplankton | 0.02 | 0.1 | d$^{-1}$ | 0.03–0.14 (Soetaert et al. 2001; Huisman et al. 2004; Torres et al. 2016) |
| | $W_s$ | Bulk sinking velocity of phytoplankton | 0.1 | 0.2 | m d$^{-1}$ | 0.1–7.7 (Gálvez et al. 1993, Present study, calculated from Stokes equation) |
| | $R^2$ | | 0.63 | 0.77 | | |
| | $p$ | | $1.3 \times 10^{-11}$ | $6.6 \times 10^{-8}$ | | |
| CO$_2$ (µmol L$^{-1}$) | $R_A$ | Maximal specific rate of aerobic respiration | $1.3 \times 10^{-12}$ | $3 \times 10^{-12}$ | mmol C cell$^{-1}$ d$^{-1}$ | 8.2 $\times 10^{-13}$ to 1.4 $\times 10^{-11}$ (Smith and Prairie 2004) |
| | $K_i$ | Half-inhibitory constant for anaerobic respiration | 1.3 | 4.7 | μmol O$_2$ L$^{-1}$ | 1–30 (Soetaert et al. 1996; Grégoire et al. 2008) |
| | $K_S$ | Half-saturation constant for aerobic respiration | 2.2 | 5 | μmol O$_2$ L$^{-1}$ | 0.1–5 (Grégoire et al. 2008) |
| | Flux$_{\text{sed CO}_2}$ | Flux of CO$_2$ from the sediment | 2.9 | 4.2 | mmol C m$^{-2}$ d$^{-1}$ | 0.02–7 (Torres et al. 2015; Corzo et al. 2018) |
| | Flux$_{\text{atm CO}_2}$ | Flux of CO$_2$ from the atmosphere | 4.3 | 7.6 | mmol C m$^{-2}$ d$^{-1}$ | (–40 to 8) (Emerson and Broecker 1973; Kelly et al. 2001; Morales-Pineda et al. 2016) |
| | C:Chl$_{\text{epi}}$ | POC-to-Chl a in epilimnion | 95.6 | 149.2 | mg C mg Chl a$^{-1}$ | 10–333 (Falkowski and Kiefer 1985; Geider 1987; Yacobi and Zohary 2010; Clegg et al. 2012) |
| | C:Chl$_{\text{meta}}$ | POC-to-Chl a in metalimnion | 15.1 | 34.4 | mg C mg Chl a$^{-1}$ | 10–333 (Falkowski and Kiefer 1985; Geider 1987; Yacobi and Zohary 2010; Clegg et al. 2012) |
| | $Q_{10}$ | Temperature coefficient | 1.91 | 2 | | 1–3 (Jørgensen 1979b) |
| | $R_{\text{min}}$ | Minimum basal respiration rate | 0.012 | 0.012 | d$^{-1}$ | 0.01–0.2 (Geider and Osborne 1989; Van den Meersche et al. 2004; Clegg et al. 2012) |
| | $R_{\text{phot}}$ | Part of photosynthesis used for respiration | 0.12 | 0.16 | | 0.1–0.25 (Cloern et al. 1995; Van den Meersche et al. 2004; Gal et al. 2009) |
| | $R^2$ | | 0.79 | 0.91 | | |
| | $p$ | | $2 \times 10^{-6}$ | $7.2 \times 10^{-3}$ | | |
Pore-water NH$_4^+$ profiles indicated a net production of NH$_4^+$ within the sediment (Fig. 3b) and a net efflux of NH$_4^+$ to the water column of $1.19 \pm 0.3$ mmol NH$_4^+$ m$^{-2}$ d$^{-1}$ (Table 1). Interestingly, CO$_2$:NH$_4^+$ stoichiometric efflux from the sediment was about ~2, much lower than the stoichiometry of the upward flux in the hypolimnion and the downward flux of OM.

**Microbial community structure**

Three main phytoplankton populations were discriminated in El Sancho by their flow cytometry signatures (result not shown). Two of them were in the pico-size fraction, identified as PicoEukaryotes (PEuk) and *Synechococcus*–like cells (Synech), while the other one was in the nano-size fraction. These cells were identified by optical microscopy as the Chlorophyte *Carteria* sp.

Phytoplanktonic groups presented a clear niche separation. PEuk ($1.4 \times 10^6$ to $1.5 \times 10^7$ cells L$^{-1}$) was the only fraction observed in the epilimnion, whereas the DCM was formed by the accumulation of Synech ($3.3 \times 10^6$ to $7.2 \times 10^7$ cells L$^{-1}$) and *Carteria* sp. ($1.7 \times 10^5$ to $3.2 \times 10^6$ cells L$^{-1}$). Despite being less abundant, *Carteria* represented most of the phytoplankton biomass (> 99%) at the DCM and in the hypolimnion due to their much higher cell size (Table 2). In addition, of the three phytoplanktonic groups, *Carteria* abundance showed the highest correlation with Chl a ($r = 0.79, p < 0.001, n = 43$). The coincidence of vertical distributions of total phytoplankton biomass, Chl a concentration, and fluorescence clearly confirmed that the DCM was a biomass maximum as well (Fig. 4).

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**Fig. 7.** Comparison of Chl a (■) and CO$_2$ (△) depth profiles data against model prediction values with (—) and without sediment CO$_2$ fluxes (—−) during the sampling period in El Sancho reservoir. The bottom of reservoir is shown with a horizontal black line.

**Fig. 8.** (a) Comparisons of modeled (■) and observed (△) gross production rates (Pg, mmol C m$^{-3}$ d$^{-1}$) on 25th September. Observed Pg rates were calculated from experimentally determined $P$–$E$ curves at 0, 5, 16, 22.5, and 30 m depth and the irradiance corresponding to each depth in situ. (b) Modeled vertical profiles of phytoplankton net growth rate (d$^{-1}$) assuming saturating irradiance (—), therefore microalgae are limited only by CO$_2$, and limited by light (PAR) at saturating CO$_2$ concentrations (—−). The bottom of reservoir is shown with a horizontal black line.
Bacterioplankton abundance ranged between $4 \times 10^7$ and $5.2 \times 10^8$ cells L$^{-1}$ depending on sampling date and depth. Bacterial biomass was low and rather constant in the epilimnion as observed previously for the phytoplanktonic biomass. It increased with depth from the thermocline to the hypolimnion, especially in the last sampling (Fig. 4) and was clearly associated with the DCM’s position. From the thermocline down to the bottom of the hypolimnion, total bacterial biomass was highly correlated with CO$_2$ concentration ($r = 0.68, p < 0.001, n = 31$).

**P–E curves**

P–E curves showed marked difference in the photosynthetic characteristics of the phytoplankton communities at the DCM and other depths (Fig. 5). Surprisingly, there was no evidence of photoinhibition (up to 600 µmol photons m$^{-2}$ s$^{-1}$ tested) at any depth, despite the much lower in situ irradiance observed at increasing depths (Fig. 1a). Highest values of $\alpha_{\text{max}}$ and $\sigma$ were observed at the DCM (22.5 m depth), being much lower in the rest of the water column (Fig. 6a). The lowest $E_c$ was determined for the DCM ($19 \pm 5$ µmol photons m$^{-2}$ s$^{-1}$), while the lowest $E_d$ was at 16 m depth (Fig. 6a). Rd rates were less variable along the water column, showing a minimum at 30 m depth (Fig. 6a). Rates normalized to Chl $\alpha$ showed a different pattern. Maxima of $P_{\text{gmax}}$, Chl $\alpha$, and RdChl $\alpha$ were at the surface and the highest $\alpha_{\text{Chl}}$ was determined at 16 m depth (Fig. 6b). $E_c$, $E_{\text{Chl}}$, and $E_{\text{KChl}}$ had similar values and vertical pattern to those obtained when $P_{\text{g}}$ was expressed by volume (Fig. 6b).

**Numerical simulation of the coupled Chl $\alpha$ and CO$_2$ profiles**

We tested through the DCM-CO$_2$ model whether the CO$_2$ produced from the mineralization of the OM in the hypolimnion and in the sediments can be the main source for photosynthetic carbon fixation at the DCM. The values obtained for the calibrated parameters were consistent with values reported in the literature (Table 3).

The modeled Chl $\alpha$, CO$_2$, and $P_{\text{g}}$ values agreed well with the experimental data (Figs. 7, 8a). Correlation between modeled and observed data yielded values of $R^2$ between 0.63 and 0.77 for Chl $\alpha$ ($p < 0.001$), between 0.79 and 0.91 for CO$_2$ ($p < 0.001$), and 0.65 for $P_{\text{g}}$ ($p = 0.1$). The model reproduced rather accurately the DCM in the last two samplings in which the spatial resolution of the experimental data was higher. In addition, the model also reproduced well the temporal evolution of the DCM toward shallower peaks along the studied period (Fig. 7). However, the model estimates of Chl $\alpha$ at the DCM were always lower than the observed values. When the contribution of the sediment mineralization to the DCM was analyzed by running the model assuming zero CO$_2$ release from the sediment, we observed a general but very variable decrease of the DCM peak. The model reproduced well the changes in the observed CO$_2$ vertical profiles, except in the abrupt change of slope in the metalimnion–hypolimnion transition, likely due to the imposed abrupt changes in $K_d$ that likely did not exist in situ (Table 1, Fig. 7).

Despite an evident resemblance, the correlation between modeled and experimentally determined vertical changes in $P_{\text{g}}$ rates was not statistically significant (only five pairs of points were available for the statistical correlation). Nonetheless, the model reproduced well the high rates observed in the upper part of the epilimnion and at the DCM (Fig. 8a). Phytoplankton growth rates extracted from the model (0.1–0.3 d$^{-1}$) showed a maximum at about 20.7 m depth, 2 m shallower than the peak of DCM (Fig. 8b). Phytoplankton growth was generally limited by CO$_2$ in the epilimnion, while it was light-limited in the meta- and in the hypolimnion.

**Discussion**

The ecological “niche” of the DCM in El Sancho

The DCM in El Sancho represents a DBM as observed in other freshwater (Gálvez et al. 1988; Sterner 2010; Leach et al. 2018) and marine ecosystems (Latasa et al. 2017) (Figs. 1, 4). Chl $\alpha$ at the DCM represented 54–66% of the Chl $\alpha$ integrated in the entire water column. Nonetheless, the low POC:Chl $\alpha$ ratio observed at the DCM suggests the presence of photoacclimation mechanisms (Fennel and Boss 2003; Clegg et al. 2012) and it was in the typical range of actively growing phytoplankton (Cloern et al. 1995; Wang et al. 2009; Yacobi and Zohary 2010). DCM usually develop in a stable water column at the depth where at least two opposed resource gradients, for example, light and nutrients, interact in the right proportion (Abbott et al. 1984; Camacho 2006; Martin et al. 2010; Durham and Stocker 2012). In El Sancho reservoir, due to a highly transparent epilimnion, the photic layer ($Z_{\text{eu}}$) extended down to 18–22 m, well below the bottom of the surface mixing layer ($Z_{\text{sm}}$) found at 15 m (Fig. 1). Therefore, the formation and the vertical position of the DCM (22.5 m depth) fulfilled the condition of $Z_{\text{eu}} > Z_{\text{sm}}$ (Hamilton et al. 2010; Bretrup et al. 2016).

In El Sancho, the DCM was located mainly in the metalimnion, where the low $K_d$ reduces the exchange of solutes and particles with the epilimnion and hypolimnion (Martin et al. 2010). This favors the accumulation of cells and other particles in this layer and reduces the dispersion of phytoplankton by wind-induced turbulence as might occur in the epilimnion (Abbott et al. 1984). Therefore, in addition to the $Z_{\text{eu}} > Z_{\text{sm}}$ condition, our results suggest that the formation and maintenance of the DCM is facilitated by a layer with reduced $K_d$, where the phytoplankton growth rate plus the sedimentation from the epilimnion is higher than the losses due to grazing, viral lysis, and sedimentation toward the hypolimnion (Gong et al. 2015; Leach et al. 2018).

In the epilimnion, CO$_2$ concentration was below our detection limit and below the equilibrium with the atmosphere according to Henry’s law (about $13 \mu$mol L$^{-1}$) suggesting a
net consumption by the planktonic community. This low CO₂ concentration likely limits primary production in the epilimnion of acid lakes and reservoirs (Satake and Saijo 1974; Nixdorf et al. 1998; Tittel et al. 2005). However, in the hypolimnion, the concentration of CO₂ was much higher and increased with depth. This suggests that the source of CO₂ in the hypolimnion can be the mineralization of OM, either in the water column or in the sediment (Satake and Saijo 1974). The observed strong positive correlations of CO₂ with NH₄⁺ and the bacterial abundance support the role of microbial degradation as the main source of CO₂ in the hypolimnion. Therefore, the CO₂ produced by OM microbial degradation in the hypolimnion likely fuels the photosynthetic CO₂ fixation at the DCM. Most of the CO₂ consumed at the DCM was provided by the mineralization of OM in the hypolimnion (90%), whereas the degradation of OM in the sediment contributed marginally (7%). It is interesting to note that the sediment in El Sancho released less CO₂ than expected according to the stoichiometry of the sedimenting OM, which might indicate the possibility of an important consumption of CO₂ within the sediment by methanogenesis and other chemooautotrophic metabolisms.

In addition to CO₂, phosphorus has been considered a potential limiting nutrient for primary production in acid lakes due to the precipitation of phosphate with Fe(III) oxhydroxides (Nixdorf et al. 1998, Tittel et al., 2005). Since phosphate was below our detection limit in the water column of El Sancho, even in the anoxic hypolimnion, it is a strong candidate to, at least, colimit primary production in these systems, although phosphate limitation is unlikely to be responsible for the DCM formation. Dissolved organic compounds containing phosphorous might be a potential source of this nutrient for phytoplankton in El Sancho reservoir as has been found elsewhere (Boavida and Heath 1986).

Contrary to CO₂ and phosphate, the inorganic nitrogen concentration in El Sancho was high, indicating that primary production in El Sancho was not N-limited. In the epilimnion, NH₄⁺ and NO₃⁻ remained constant with depth but presented inverse trends in the meta- and hypolimnion, NO₃⁻ presented a typical consumption profile suggesting that it was being used for phytoplankton growth at the DCM and in the oxidation of OM by dissimilatory nitrate reduction in the hypoxic hypolimnion (Tiedje 1988). In contrast, NH₄⁺ profiles showed a characteristic increase with depth in the hypolimnion, same as for CO₂, which suggests that it was being produced during the degradation of OM and consumed in the DCM as a N source for phytoplankton growth.

Epilimnetic O₂ maxima are typically associated to DCMy in lakes and the sea (Matthews and Deluna 2008; Wilkinson et al. 2015; Latasa et al. 2017) The cause of the formation and persistence of these O₂ maxima is under debate, with both physical and biological processes being likely involved. The change in O₂ solubility due to thermal difference between the warm epilimnion and the cooler metalimnion (4–5°C between the epilimnion and the O₂ maximum in the metalimnion) only represents an increase in O₂ concentration of 26 μmol L⁻¹, whereas the observed differences were between 95 and 159 μmol L⁻¹. Therefore, the O₂ maximum in El Sancho and its position above the DCM peak was not possible. However, vertical migration and the accumulation of photosynthetically produced O₂ below the metalimnetic peak, O₂ decreased quickly with depth because its consumption, in the aerobic oxidation of OM by heterotrophic bacteria and the oxidation of reduced inorganic compounds formed during the anaerobic mineralization of OM, exceeded its supply from the DCM (Friedrich et al. 2014).

Primary production and net metabolism

Production and net metabolism measurements in acidic lakes are scarce (Nixdorf et al. 2003). The phytoplankton community at the DCM in El Sancho reservoir was highly productive. Pg and PgChlα rates reached values that were 2–30 times higher than those measured in some pit lakes with lower pH (Nixdorf et al. 2003; Gerloff-Elias et al. 2005; Kamjunke et al. 2005), but were far lower than those found in DCM in circumneutral lakes (Sadro et al. 2011; Staehr et al. 2012).

Photoacclimation of the phytoplankton community to the in situ light environment in the water column was evident in the P-E curves when Pg was normalized by Chl a (Fig. 5b). PgChlα was clearly higher at the epilimnion than at the DCM for a given irradiance, suggesting an increase of Chl a per cell where irradiance is lower. Photosynthetic parameter PgmaxChlα and αChlα presented similar patterns with depth showing higher values at the surface and at 16 m depth, just above the DCM (Fig. 6b), as observed in other acidic lakes (see table 1 in Gerloff-Elias et al. 2005).

The planktonic community inhabiting the DCM (dominated by Carteria sp.) presented an Ec of 19 ± 3 μmol photons m⁻² s⁻¹, similar to that of the phylogenetically related Chlamydomonas acidophila (Clegg et al. 2012). Since this irradiance was higher than the irradiance measured at the peak of the DCM in situ (1.3–11.1 μmol photons m⁻² s⁻¹), net autotrophic growth at the DCM peak was not possible. However, vertical migration and mixotrophic growth have been suggested as fitness traits favoring the survival and growth at the light limiting conditions of DCM (Tittel et al. 2003, 2005; Clegg et al. 2012). Nonetheless, another explanation is possible. The peaks of net primary production and biomass are uncoupled in El Sancho reservoir. The primary production peak occurs 3–4 m above the maximum of biomass, where in situ irradiance (14–39.6 μmol photons) is generally above Ec. Therefore, net autotrophic growth is possible at that depth (Fig. 8b). The mismatch between the depth of maximum net growth rate and the DCM has been demonstrated to depend on the sinking rate and the turbulent diffusivity coefficient.
Maximum growth rate is estimated to be found at 19.3 m depth, 3–4 m above the mean depth of the DCM in El Sancho according to eq. 12 from Gong et al. (2015). This estimation is remarkably close to the maximum growth rate depth predicted by our model (20.7 m).

The $R_{\text{Chl}a}$ were higher than those reported for in situ incubations and cultures of _C. acidophila_ at different irradiances (Gerloff-Elias et al. 2005; Clegg et al. 2012) but in the low range of those measured in neutral lakes per unit of volume (Pace and Prairie 2005). The high respiration rate in the epilimnion is likely supported by the relatively high concentration of DOC, and the high POC:Chl $a$ and DOC:POC ratios observed in situ (Figs. 1, 2) that likely favored an intense heterotrophic bacterial activity. The lowest values of O$_2$ consumption rate measured at 30 m depth suggest a shift from aerobic to anaerobic pathways of OM oxidation at the bottom part of the hypolimnion and sediment.

**Coupling carbon fixation at the DCM and CO$_2$ production by heterotrophic respiration: A modeling approach**

In the DCM-CO$_2$ coupled model presented here, the vertical distribution of Chl $a$ was modeled as a function of phytoplankton net growth-dependent on the opposite vertical gradients of CO$_2$ and light -, turbulent mixing processes and sedimentation (Huisman and Sommeijer 2002; Fennel and Boss 2003; Gong et al. 2015). The bulk sinking rates obtained from the model were similar to those measured with the sedimentation traps. However, both were lower than those calculated for passively sinking _Carteria_ cells, likely due to their capacity to compensate sinking by vertical migration. In addition, the model takes into account the differences in the phytoplanktonic communities between the epilimnion and the DCM by using two different C:Chl $a$ ratios. The calibrated C:Chl $a$ ratios produced by the model were very close to those calculated from experimental data according to Geider (1987), 114 mg C mg Chl $a$$^{-1}$ and 22.05 mg C mg Chl $a$$^{-1}$ for the epilimnion and metalimnion, respectively (Supporting Information Fig. S3) and within the range of published values (Table 3). This distinction improved the simulation of the vertical profile of primary production, specifically the surprisingly high production rates measured in the epilimnion (Fig. 8a). Modeled daily Pg (0.1–1.7 mmol C m$^{-3}$ d$^{-1}$) were similar to the daily Pg at in situ irradiances determined from experimental P–E curves (0.02–1.9 mmol C m$^{-3}$ d$^{-1}$) (Fig. 8a). The model predicted the high daily Pg observed in the epilimnion and at the DCM, but slightly underestimated daily Pg in the epilimnion and overestimated it at the DCM. The fraction of Pg being consumed by respiration according to the model was about 12–16%, which is consistent with the 12% determined from the P–E curves at the DCM depth and the 13–18% value determined from _C. acidophila_ cultures (Clegg et al. 2012). Altogether, the model is able to reproduce noticeably well the Pg and the Chl $a$ profiles with a high correlation between experimental data and model estimates (Table 3). This good agreement supports our initial hypothesis, suggesting that the vertical position of the DCM and its existence in El Sancho depends on both the light penetration and the supply of CO$_2$ from the hypolimnion. The systematic underestimation of the Chl $a$ concentration measured at the peak of DCM could be due to the existence of a detrital Chl $a$ fraction at the DCM which is not considered in the present model formulation.

The DCM-CO$_2$ coupled model takes into account three CO$_2$ inputs to the water column, that is, from the atmosphere (upper boundary flux), from bacterial respiration in the water column and from the sediment (lower boundary flux). This is a major difference with other models where nutrients are only recycled from the bottom boundary (Huisman and Sommeijer 2002; Fennel and Boss 2003; Gong et al. 2015). Our model suggests that the CO$_2$ distribution through the water column can be explained basically by the bacterial respiration in the water column. Detrital OM produced in the DCM sunk through the hypolimnion and was degraded by microorganisms releasing CO$_2$ in the process. This led to an upward net CO$_2$ flux by turbulent diffusion, which is essential to support the photoautotrophic primary production and growth at the DCM. The model takes into account that the mineralization of OM in the hypolimnion occurs by aerobic and anaerobic processes. The values of the half-saturation constant for aerobic respiration $K_I$ and the half-inhibitory constant for anaerobic respiration $K_f$ and the profiles of the bacterial respiration rates extracted from the model (results not shown) suggest that in the hypoxic conditions prevailing at the bottom of the metalimnion and in the hypolimnion, both aerobic and anaerobic metabolism occur at the same time (Gerrits et al. 1990). Closer to the sediment, the O$_2$ availability decreases, and a higher fraction of the OM is probably mineralized by anaerobic respiration pathways (Torres et al. 2014; Corzo et al. 2018).

Experimental evidence and the model simulations suggest that both the input of CO$_2$ from the atmosphere and the release from the sediment might represent important contributions to the C budget in acid lakes. In the epilimnion, CO$_2$ was in equilibrium with the atmosphere through a piston flux (Grégoire et al. 2008). Due to the low pH and the efficient consumption of the CO$_2$ regenerated in the metalimnion by the DCM, the CO$_2$ demand for the primary production in the epilimnion was supported from the atmosphere (Gross 2000). The areal gross primary production in the epilimnion on September 25$^{th}$, estimated from the P–E curves and in situ irradiance and from the DCM-CO$_2$ model, was similar; 14.9 mmol CO$_2$ m$^{-2}$ d$^{-1}$ and 12 mmol CO$_2$ m$^{-2}$ d$^{-1}$, respectively. This areal Pg rates represented 69–67% of total water column integrated Pg. Although the fraction of water column integrated Chl $a$ was larger in the DCM (56%) than in the epilimnion (30%), the contribution of the DCM to the integrated areal Pg was lower; 4.3 and 5.9 mmol CO$_2$ m$^{-2}$ d$^{-1}$, 19.8% and 32.5%, respectively. The episimnetic CO$_2$ flux from the atmosphere was completely consumed by primary producers.
at the epilimnion, that is, virtually no atmospheric CO$_2$ reached the DCM layer. Using the DCM-CO$_2$ coupled model, we could test, for the first time as far as we know, the potential contribution of the CO$_2$ released from the sediment to support the primary producers’ CO$_2$ demand at the DCM. This contribution ranged between 36% and 58% of the DCM integrated Chl $a$ (Fig. 7), bearing in mind that we are using here a 1D model which does not take into account any potential lateral transport within the reservoir. The estimated contribution of the sediment using the model was higher than that calculated from the observed CO$_2$ profiles at the hypolimnion and sediment (Table 1), probably because the modeled CO$_2$ profile in the hypolimnion systematically underestimated the observed profiles. Without the contribution of the CO$_2$ from the sediment, the intensity of the DCM in El Sancho would be lower.

In conclusion, the DCM-CO$_2$ model is able to reproduce noticeably well both the Chl $a$ and CO$_2$ profiles, with a high correlation between experimental and modeled data. This good agreement supports our initial hypothesis that the vertical position of the DCM and its existence in El Sancho depends on both the light penetration and the supply of CO$_2$, which is regenerated by the hypolimnetic heterotrophic bacteria from the water column and the sediment.

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Acknowledgments

The research was funded by projects P11-RNM-7199 from the Junta de Andalucía, CTM2017-82274-R from the Spanish I+D+I Program, and 20.DG.UE.II.05 from University of Cadiz. Support for the work with microsensors was obtained from the Poul Due Jensen Foundation.

Conflict of Interest

None declared.