Biogeosciences Open Access
Discussions

Synergistic effects of UVR and simulated stratification on commensalistic algal-bacterial relationship in two optically contrasting oligotrophic Mediterranean lakes

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Abstract

An indirect effect of global warming is the shallowing epilimnion, causing organisms to be exposed to higher levels of ultraviolet (UVR, 280–400 nm) and photosynthetically active radiation (PAR, 400–700 nm), which could affect primary and bacterial production as well as the commensalistic algal-bacterial relationship. The combined effects of UVR and reduction in the depth of the upper mixed layer (UML) were assessed on variables related to the metabolism of algae and bacteria, during in situ experiments performed with natural microplanktonic communities from two oligotrophic lakes with contrasting UVR-transparency (clear vs. opaque) of southern Spain. The negative UVR effects on epilimnetic primary production (PP) and on heterotrophic bacterial production (HBP), intensified by high mean irradiances, were higher in the UVR-opaque than in the UVR-clear lake, and stronger on the algae than on the heterotrophic bacterial communities. Under UVR and high mean irradiance, the algal-bacterial relationship was strengthened in the UVR-clear lake, where excreted organic carbon (EOC) rates exceeded the bacterial carbon demand (BCD). This did not occur in the UVR-opaque lake. The greater UVR damage to algae and bacteria and the weakening of their commensalistic interaction found in the UVR-opaque lake indicates that these ecosystems would be especially vulnerable to stressors related to global change. Thus, our findings may have important implications for the carbon cycle in oligotrophic lakes of the Mediterranean region.

1 Introduction

Rising levels of greenhouse gases (mainly CO_2), attributed to human activities, have led to an increase of 0.56 °C in the Earth's surface temperature over the past 150 yr (IPCC, 2013). Model predictions indicate greater temperature increases, ranging from 1.5 °C (under the CO_2 scenario B1) to 6.4 °C (under the scenario A1FI high CO_2 emissions) by the end of the century. Major changes in precipitation have accompanied

these temperature variations and are expected to become more pronounced (IPCC, 2013). These climate changes affect aquatic ecosystems by increasing water temperature, altering mixing regimes, shortening the thaw time and the duration of ice cover, and/or strengthening water-column stratification (de Senerpont Domis et al., 2013).

5 These alterations in physical conditions have different effects on primary and bacterial production, plankton growth, nutrient supply, and trophic interactions, among other ecological processes (de Senerpont Domis et al., 2013). In addition, variations in stratification patterns are known to strongly affect biogeochemical cycles (van de Waal et al., 2009).

10 Higher temperatures in the upper layers of freshwater bodies increase density differences between the upper mixed layer (UML), or epilimnion, and deeper waters, augmenting the vertical temperature gradient, and thus the stratification. This process has contrasting effects on nutrient and light availability for organisms' growth. On the one hand, stratification reduces the flow of nutrients from deep and nutrient-rich ar-

15 eas into the UML, limiting their availability for growth (Huisman et al., 2006). On the other hand, stratification traps phytoplankton populations in surface layers, increasing the light availability for growth but also exposing them to higher levels of ultraviolet radiation (UVR, 280–400 nm). In this regard, it has been widely reported that greater exposure to UVR exerts an inhibitory effect on autotrophic and heterotrophic organisms

20 (Häder et al., 2011), and that UV-B (280–315 nm) in particular, harms primary and bacterial production (Carrillo et al., 2002), enzymatic activity (Korbee et al., 2012), and cell viability (Helbling et al., 1995), among other effects. However, it has been also reported (Aas et al., 1996; Medina-Sánchez et al., 2002; Gao et al., 2007) that UVR does not produce negative effects and it can even stimulate bacterial production and photosyn-

25 thetic activity. These opposite effects may be attributable to the differential acclimation capacity of organisms in severely UVR-stressed ecosystems (Medina-Sánchez et al., 2002; Ruiz-González et al., 2013) or to differences in physical-chemical factors (e.g. temperature or nutrient content) among ecosystems (Harrison and Smith, 2009).

With respect to physical factors, it has been experimentally demonstrated (Helbling et al., 1994) that vertical mixing can alter UVR-induced effects on planktonic organisms by generating a regime of fluctuating irradiance, with high values near the surface and low at the bottom of the UML. The depth of the UML also influences the mean UVR and PAR irradiance received by organisms and the duration of their residence in the photoactive zone (Neale et al., 2003). Studies on the interactive effects of UVR and vertical mixing on algae (Helbling et al., 1995; Neale et al., 2003) and bacteria (Bertoni et al., 2011) have shown that these organisms can recover from UVR-induced damage when UVR exposure is subsequently reduced or avoided. The outcome of damage vs. repair depends not only on the amount of damaging UVR received, but also on photo-repair wavelengths (UV-A, PAR) to which organisms are subsequently exposed during the fluctuating radiation regime. Moreover, the effects of different mixing depths, and thus of different mean irradiances (MIR), can act synergistically or antagonistically with UVR, depending on the composition, structure, and size of the species as well as on the environmental conditions (Villafañe et al., 2007). For instance, Barbieri et al. (2002) found that the impact of UVR in Patagonian coastal waters was negative or positive depending on the fraction of the euphotic zone (Z_{eu}) that was mixed; thus, UVR was used for photosynthesis when vertical mixing reached $\sim 90\%$ of the Z_{eu}, but carbon fixation was reduced by UVR when the UML was shallow ($\sim 60\%$ of the Z_{eu}).

Besides increased stratification of the water column, more extreme rainfall events and storms are predicted in many parts of the World in the global-change scenario (IPCC, 2013). This would increase the amount of allochthonous dissolved organic matter (DOM) reaching inland and coastal aquatic ecosystems, reducing the penetration of incident UVR (Rose et al., 2009). The UVR filtering characteristics of coloured DOM (CDOM) result in a more effective attenuation of shorter (UV-B) than longer (UV-A, 315–400 nm) wavelengths, as also observed for stratospheric ozone. These changes would modulate the exposure of aquatic organisms to UVR (Williamson and Rose, 2010), making it more complex to predict the interactive effects of UVR and stratification on the planktonic community.

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Interactive Discussion

Recent experiments carried out by our group have demonstrated that fluctuating irradiance increases the harmful UVR effects on primary producers in oligotrophic mountain lakes with high DOM, whereas the opposite effects were detected in those with low DOM content (Helbling et al., 2013). Several authors have highlighted the importance of the quality of the radiation, which can interact with DOM and either increase or decrease the availability of organic carbon for bacteria (Pérez and Sommaruga, 2007). However, despite the key role of phytoplankton and heterotrophic bacteria production as a link between the microbial and grazing food webs, no comparative studies on commensalistic algal-bacterial relationship have been done in ecosystems with high- and low-CDOM contents. Thus, at present, the information available concerning the interactive effects of radiation quality and increased MIR as a consequence of stratification on algal-bacterial interactions so far does not exist.

A growing body of literature supports the strong dependence of planktonic heterotrophic prokaryotes on organic matter released in situ by phytoplankton in the upper layers of aquatic ecosystems (Baines and Pace, 1991). It has also been demonstrated that UVR exposure in the upper layers of the water column can rise the proportion of photosynthate released as exudates (Carrillo et al., 2008; Korbee al., 2012), which would stimulate the growth of UVR-resistant bacteria (Xenopoulos and Schindler, 2003) and give rise to a coupled algal-bacterial relationship in clear oligotrophic lakes (Carrillo et al., 2002). Coupling between phytoplankton and bacterioplankton has been defined as the capacity of the carbon (C) released by algae to support the bacterial carbon requirement (Morán et al., 2002) and will therefore differ depending on: (i) the availability of alternative (allochthonous or autochthonous) carbon sources (Gasol et al., 2009), and (ii) the limitation of inorganic nutrients (Medina-Sánchez et al., 2010, 2013; López-Sandoval et al., 2011). Although the bacterial dependence on C released by phytoplankton is a well established aquatic microbiological paradigm, it is currently under renewed debate because the application of different conversion factors to raw data and modelled rates could substantially alter this paradigm (Morán et al., 2011). Further-

more, few data are available on the possible effects of indirect effect of global warming (stratification) on this relationship or on C flux into aquatic food webs.

With this background, the aim of the present study was to improve our understanding about the interactive effects of UVR exposure and increased MIR, as a consequence of increased stratification, on (i) phytoplanktonic and heterotrophic bacterial production and (ii) the commensalistic relationship between them in lakes with different transparency to UVR. We hypothesised that the interactive effects of UVR and increased MIR will accentuate the harmful UVR effects on primary production (PP) and heterotrophic bacterial production (HBP), thus resulting in a greater C release by algae, which will strengthen the commensalistic algal-bacterial relationship. These effects will be more acute in UVR-opaque than in UVR-clear lakes, where UVR resistant populations are likely not selected for.

To test our hypothesis, we carried out in situ experiments to assess the combined impact of solar radiation (i.e., quality) and increased MIR by stratification on metabolism of algae and bacteria, and their commensalistic relationship in two oligotrophic lakes with contrasting transparency to UVR in the Mediterranean region.

2 Methods

2.1 Model ecosystems

The study was performed during September 2011 in two Spanish oligotrophic lakes: La Caldera Lake in Sierra Nevada National Park (37°03′ N; 3°19′ W, 3050 m a.s.l.) (Granada) and La Conceja Lake in Ruidera Natural Park (38°55′ N; 2°47′ W, 850 m a.s.l.) (Ciudad Real). La Caldera is a mixed oligotrophic (total phosphorus (TP) $< 0.3\,\mu$M and chlorophyll a (Chl a) $< 5\,\mu$g L^{-1}) high-mountain lake above the treeline on a siliceous bedrock in a glacial cirque (Carrillo et al., 2006). This lake has a surface area of 2 ha, a mean depth of 4.3 m, with a maximum depth inter-annually variable from 2 to 14 m. UVR of considerable intensity penetrates deeply in the lake (Fig. 1) due to

the high transparency of the water and low values of dissolved organic carbon (DOC) < 0.08 mM as reported in Carrillo et al. (2008), Helbling et al. (2013). Therefore, this lake is called hereafter the "UVR-clear" lake. The pelagic community is relatively simple, and characterized by the absence of autotrophic picoplankton and size overlap between algae and heterotrophic bacteria (Medina-Sánchez et al., 2002). La Conceja is a stratified oligotrophic lake (TP < 0.03 µM and Chl a < 5 µg L^{-1}), although it has an elevated nitrate concentration which can exceed 800 µM due to agricultural use of the land. This lake has a surface area of 29 ha and maximum depth of 14 m. The DOC content ranges from 0.15 to 0.25 mM. Therefore, this lake is called hereafter the "UVR-opaque" lake. The autotrophic community is composed by pico- and nanoplankton (Rojo et al., 2012).

2.2 Experimental setup

To assess the interactive effects of solar radiation quality ("UVR" factor) and increased mean irradiance (by simulating stratification conditions, "MIR" factor) on PP, HBP, TPR (total planktonic respiration, < 45 µm fraction) and BR (bacterial respiration, < 1 µm fraction in the UVR-clear lake alone), samples were collected from the surface (0–0.5 m) epilimnetic water. An acid-cleaned 6 L horizontal Van Dorn sampler was used to collect the water that was pre-screened through a 45 µm mesh to remove large zooplankton prior to the experiments. Samples for PP were placed in 50 mL quartz flasks and those for HBP, TPR, and BR in 25 mL quartz flasks. In the UVR-opaque lake, samples for PP, HBP and TPR analyses were also gathered from the hypolimnetic water below the thermocline at 6 m depth, where UV-B did not reach the cells. The idea behind sampling two communities in the UVR-opaque lake was to compare the natural algal and bacterial communities that had different light histories and acclimation to solar radiation when exposed to similar light quality treatments and irradiance conditions. Since this sharp contrast did not occur in the clear lake, only samples from the 0–0.5 m were used in these experiments.

The experimental design consisted of three (for TPR and BR), four (for PP, HBP) or two (for TPR in the UVR-opaque lake) "UVR" treatments combined with the two

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Interactive Discussion

MIR conditions: (1) the UVR treatments (triplicates for each condition) were: (i) PAB: full solar radiation, uncovered quartz flasks; (ii) PA: exclusion of UV-B (280–320 nm), wrapping the flasks with Folex 320 film (Folex, Germany); (iii) P, control: exclusion of UVR (280–400 nm), wrapping the flasks with Ultraphan UV Opak395 film (Digrefa, Germany); and (iv) dark: wrapping the flasks with black tape. The optical properties of the filters used for the radiation treatments have been published elsewhere (Villafañe et al., 2003); the filters were replaced before each experiment and tested using a double-beam spectrophotometer (Perkin-Elmer Lambda 40). (2) The MIR treatments were: (i) high MIR, samples incubated at 0.5 m depth; and (ii) low MIR, samples subjected to vertical mixing from 0 to 5 m depth. To simulate these reductions in the depth of the UML (i.e. from 5 m to near the surface) two round trays containing the samples were exposed in situ to solar radiation. One tray was placed at 0.5 m depth (high MIR) subjected to irradiance oscillations associated to waves at the surface. Transient thermoclines trapping phytoplankton very close to the surface have previously been detected in aquatic environments (Neale et al., 2003); in the present study, this high irradiance condition simulates a worst-case stratification scenario. The second tray was vertically moved between the surface and 5 m depth to simulate the irradiance changes in the upper 5 m of the water column (low MIR). The speed of movement was 1 m every 2 min, achieved by a custom-made mixing simulator, using a frequency-controlled DC motor (Maxon motor, Switzerland) to impose a linear transport rate on the vessels from the surface to the mixing depth and back. The tray was placed on a boat anchored in a deep area of each lake in such a manner as to avoid shadows or any type of interference from the shoreline or boat. All incubations lasted for 3.5 h centered on local noon, and a total of 10 cycles (from the surface to 5 m depth to the surface again) were completed for the low MIR condition.

Unfortunately, space restrictions within the trays prevented the performance of all experimental treatments in the UVR-opaque lake for TPR, which was measured only in samples exposed to PAB and P in the high and low MIR treatments. The overlapping

between autotrophic and heterotrophic picoplankton precluded the measurements of BR in the UVR-opaque lake.

2.3 Physical measurements

Incident solar radiation was continuously monitored by means of a BIC radiometer (deck unit, Biospherical Instruments Inc., CA, USA) that has three channels in the UVR region of the spectra (305, 320, and 380 nm) and one broad-band channel for PAR (400–700 nm). Vertical profiles of solar radiation in the water column were performed at noon using a BIC radiometer (underwater unit) with temperature and depth sensors, in addition to the aforementioned channels. Vertical profiles of temperature and pH in the water column were measured using a multiparameter probe (Turo Water Quality Analysis T-611 Sandy Bay, Tasmania, Australia). These profiles were done daily at noon, and the temperature data were used to estimate the strength and depth of the epilimnion in the water column.

2.4 Chemical analyses

Chemical and biological variables were sampled with a 6 L Van Dorn sampler at the deepest central station at four depths in the UVR-clear lake (surface, 5, 8, and 10 m) and six in the UVR-opaque lake (surface, 2, 4, 6, 8, and 10 m). Water samples were taken to determine the bacterial abundance (BA, 20 mL), phytoplankton species composition and abundance (250 mL), and Chl a (1 L). Samples were also collected for the chemical determination of total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrate (NO_3^-), and soluble reactive phosphorus (SRP). The samples for TDN, TDP, nitrate, and SRP analyses were filtered through GF/F Whatman filters (47 mm in diameter) before the determinations. Samples for TP and TDP were persulfate-digested at 120 °C for 30 min and determined (as for SRP) using 10 cm quartz cuvettes (following the acid molybdate technique, APHA 1992). TN and TDN samples were also persulfate-digested and measured as NO_3^- by

means of the ultraviolet spectrophotometric screening method (APHA, 1992). Blanks and standards were run in all procedures. DOC values were determined by filtering the samples through pre combusted (2 h at 500 °C) glass fiber filters (Whatman GF/F) and acidifying them with HCl. Samples were then measured in a total organic carbon analyzer (TOC V CSH/CSN Shimadzu).

2.5 Analysis biological variables

Chl a fluorescence: Chl *a* fluorescence parameters of the photosystem II were measured at different depths in the water column by using a pulse-amplitude-modulated fluorometer (Water PAM, Walz, Germany). Samples were gently pumped from each depth (using an aquarium pump) into a custom-made darkened flow-through measuring quartz cuvette (5 mL) connected to the pump via a dark silicon tube (5 mm diameter). The flow rate was ca. 250 mL per min, i.e. sufficient to minimize the time spent by cells (< 1 min) in the silicon tube before the measurement. The intrinsic photochemical quantum yield (Y) was calculated with the equations of Genty et al. (1989):

$$Y = \Delta F : F_m' = F_m' F_t' : F_m' \tag{1}$$

where F_m' is the instantaneous maximum intensity of Chl *a* fluorescence in an irradiated cell induced by a saturating white-light pulse ($\sim 5300 \, \mu mol$ photons $m^{-2} \, s^{-1}$ in 0.8 s) in the presence of a weak actinic light, and F_t' the steady-state fluorescence induced by a weak actinic light in light-adapted cells. These fluorescence measurements were made every 10 s, with at least 6 measurements per depth. Comparisons with samples from the Van Dorn bottle showed that the measurements were not affected by pumping the phytoplankton into the cuvette.

Chl a concentration: For measurements of the Chl *a* concentration, water samples from different depths in the water column were filtered onto Whatman GF/F filters (25 mm in diameter), which were frozen at −20 °C until their analyses. For Chl *a* analysis, samples were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone

(90 %) for 24 h in the dark at 4 °C. Next, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorometer (APHA, 1992) (LS 55 Perkin Elmer, USA).

Identification and cell counting: Samples for the identification and counting of phytoplankton were placed in 250 mL brown glass bottles and fixed with Lugol's reagent (approx. 1 % vol/vol). Sub-samples (100 mL) were settled for 48 h in Utermöhl chambers (Hydro-Bios GmbH), and species were then identified and counted using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany). BA was determined by the 4′,6-diamidino-2-phenylindole (DAPI) direct-count method described by Porter and Feig (1980). Water samples were fixed with neutralized formaldehyde (2 %), stained with DAPI to a final concentration of 2.5 μg m L^{-1}, and then filtered through a 0.2 μm pore-size polycarbonate black Nucleopore filter. At least 400 cells per sample were counted by epifluorescence microscopy (Karl Zeiss AX10).

2.6 Analysis of biotic functional variables

Primary production and excreted organic carbon: For PP measurements, samples of phytoplankton communities were placed in 50 mL round quartz flasks (three clear and one dark per radiation treatment), inoculated with 0.37 MBq of NaH^{14}CO$_3$ (specific activity: 310.8 MBq mmol^{-1}, DHI Water and Environment, Germany), and exposed to solar radiation in situ, as described above. The samples for PP were filtered through 0.2 μm Nuclepore filters (25 mm diameter), under low vacuum (< 100 mm Hg) to minimize cell breakage. Excreted organic carbon (EOC) was measured on 4 mL aliquots from the filtrates (< 0.2 μm). Both filters and filtrates were placed in 20 mL scintillation vials and acidified with 100 μL of 1 N HCl for 24 h (no bubbling) to remove inorganic radiocarbon before the addition of a liquid scintillation cocktail (Ecoscint A) to the vials. The amount of organic carbon was obtained by counting of disintegration per minute (dpm), using an autocalibrated scintillation counter (Beckman LS 6000 TA). The total CO$_2$ in the lake water was calculated from alkalinity and pH measurements (APHA,

1992). In all calculations, dark values were subtracted from the corresponding light values (more details in Carrillo et al., 2002).

Heterotrophic Bacterial production: Samples for HBP measurements were placed in 25 mL quartz flasks and exposed in situ for 3.5 h under the radiation and MIR conditions as described above. Then, the HBP was determined in the dark by incorporation of ^{3}H-thymidine (S.A = 52 Ci mmol^{-1}, Amersham Pharmacia) into the bacterial DNA. Briefly, ^{3}H-thymidine was added to independent sets of five (three replicates + two blanks per treatment) sterile microcentrifuge tubes filled with 1.5 mL of the pre-exposed samples to a final (saturating) concentration of 15.2 nM. The vials were then incubated at in situ temperature in the dark for 1 h. After incubation, the incorporation of ^{3}H-thymidine was stopped by adding (6 % final concentration) of trichloroacetic acid (TCA). Likewise, blanks were TCA-killed before the radiotracer was added. After the cold TCA extraction, the precipitate was collected by centrifugation at 14 000 rpm for 10 min. The conversion factor 1.5×10^{18} cell mol^{-1} was used to estimate the number of bacteria produced per mol of incorporated ^{3}H-thymidine (Bell, 1993). The factor 20 fg C cell^{-1} was applied to convert bacterial production into C (Lee and Fuhrman, 1987).

Respiration rates: Samples for TPR (< 45 µm fraction) and BR (< 1 µm fraction) measurements were placed in 25 mL quartz flasks and exposed in situ for 3.5 h under the radiation and MIR conditions described above. TPR and BR rates were measured using optode sensor-spots (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fibre oxygen transmitter (Fibox 3; PreSens GmbH, Germany) connected to a computer. Data were recorded using the OxyView 3.51 software (PreSens GmbH). The system was calibrated by a two-point calibration, together with data of atmospheric pressure and temperature before each experiment, following the manufacturer's recommendations. Measurements were made at the initial time (t_0) and then every hour during 8 h. Every oxygen measurement was done during 30 s with a frequency of 1 datum per s; only the last 10 data points of each measurement were used in our analysis to ensure the stability of the data. Oxygen data were then adjusted to a linear model via

least-squares regression. Slope of the regressions provided the oxygen consumption rates (μM O$_2$ h^{-1}) (Warkentin et al., 2007).

Oxygen was converted into carbon units using a respiratory quotient of 1 (del Giorgio and Cole, 1998). The bacterial carbon demand (BCD) is the HBP plus BR. The bacterial growth efficiency (BGE) is the proportion of C entering the bacterial pool that is incorporated into the biomass and was calculated as BGE = HBP/BCD. The absence of size-overlapping between algae and bacteria in the UVR-clear lake (Medina-Sánchez et al., 2002) allowed for a direct measurement of BR. This, however, was not possible in the UVR-opaque lake, where picoplankton autotroph and bacteria coexisted in the $< 3\,\mu$m fraction. Therefore, BCD in this lake was estimated by assuming that BR values lies within two limits: (i) a conservative value of 75 % of TPR, which is an average value based on data reported for oligotrophic waters (Lemeé et al., 2002); and (ii) a potential minimum value of 50 % of TPR (Robinson, 2008), comparable with direct measurements made in this study on the TPR *vs.* BR in La Caldera lake (Herrera et al., unpubl. data).

2.7 Data calculation and statistical analysis

The effect size of the UVR was quantified as:

$$\text{Effect size of UVB (\%)} = 100 \times [((C_\text{P} - C_\text{PAB})/C_\text{P}) - ((C_\text{P} - C_\text{PA})/C_\text{P})] \tag{2}$$

$$\text{Effect size of UVA (\%)} = 100 \times [(C_\text{P} - C_\text{PA})/C_\text{P}] \tag{3}$$

where C_P, C_PA, and C_PAB represent the carbon production by algae or bacteria in samples under the P, PA and PAB treatments, respectively. We used propagation errors to calculate the variance of the effect-size (as percentage) due to UV-B and UV-A. The change (Δ) in the effect size of UV-B and UV-A, between the high and MIR treatments, was calculated as the difference of the effect size for each radiation band.

The effects of solar radiation quality ("UVR" factor) and increased mean irradiance ("MIR" factor) on the response variables were tested using two-way ANOVA. When

the interactive effects were significant, a post hoc Bonferroni test was used to determine significant differences among treatments. The normality (by Shapiro-Wilks' W test or Kolgomorov–Smirnov) and homoscedasticity (using Cochran, Hartley & Bartlett or Levene's tests) were checked for each data group before ANOVA application. HBP data from the hypolimnetic community in the UVR-opaque lake were log-transformed to meet ANOVA assumptions. Significance of the effect size of UV-B and UV-A on PP and HBP between high- and low MIR were evaluated using t tests. Regression analyses were made to assess the dependence of EOC in controlling BGE for the experimental data in each lake. Statistica 7.1 software for Windows was used for the statistical analyses.

3   Results

3.1   Physical, chemical, and biological variables in the water column

Figure 1a and b depicts the penetration of solar radiation into the water column in both lakes. The lakes greatly differed in their transparency to UVR, but not to PAR. Thus, in the UVR-clear lake, the 1 % of the surface energy at 305 nm reached the bottom of the lake, whereas in the UVR-opaque lake most of the UVR energy was attenuated in the upper layers of the lake (1 % of the surface energy at 305 nm reached only ca. 1 m depth). This differential penetration of solar UVR resulted in two contrasting environments, with organisms being exposed to UV-B along the water column in the UVR-clear lake (Fig. 1a) but only in the upper 1–2 m of the water column in the UVR-opaque lake (Fig. 1b). This was related to the differential DOC concentrations between the lakes that reached values of 0.07 and 0.18 mM in the UVR-clear and UVR-opaque lakes, respectively (Fig. 1c and d). Vertical temperature profiles also differed between the lakes: the temperature was 14 °C, ranging only 0.4 °C between the surface and bottom in the UVR-clear lake (Fig. 1c), whereas a weak thermal stratification between

2–3 m was detected in the UVR-opaque lake, where the temperature ranged from 22 to 19.5 °C between the surface and bottom layers (Fig. 1d).

The concentrations of total dissolved and inorganic forms of N and P were homogeneous in the water column in both lakes; therefore only mean values are reported in Table 1. TN values were higher in the UVR-opaque than in the UVR-clear lake, by up to one order of magnitude, and NO_3^- constituted most of the TN (90 % in the UVR-opaque and 68 % in the UVR-clear lake). By contrast, TP values were $< 0.16\,\mu M$ and mostly in organic form in both lakes. The NO_3^- : TP ratio was > 100 in the UVR-clear lake and $> 10\,000$ in the UVR-opaque lake, indicating a strong P limitation (Table 1).

Figure 2a and b shows the vertical distribution of Chl a and Y in the two lakes. In the UVR-clear lake (Fig. 2a), Chl a concentrations had small variations with depth. However, Y had a significantly lower value at the surface (0–1 m) that steadily increased with depth. The change in Y from the surface down to 7 m was ca 0.4. In contrast, in the UVR-opaque lake (Fig. 2b), both Chl a and Y had slightly greater values at mid-water depths (4–6 m), reaching a difference between the surface and 4 m of < 0.2. The vertical distribution of phytoplankton and bacteria also differed between the lakes: in the UVR-clear lake (Fig. 2c) bacterial abundance was rather homogeneous, but phytoplankton abundance increased with depth; however, in the UVR-opaque lake (Fig. 2d) the abundances of bacteria and phytoplankton were rather uniform with depth. Mean algal and bacterial abundance values were greater in the UVR-clear than in the UVR-opaque lake (Table 1). In terms of taxonomic composition, the Chlorophyceae *Monoraphidium* sp. represented > 90 % of the total abundance of cells in the UVR-clear lake whereas the Bacillariophyceae, *Cyclotella ocellata* was the dominant species in the UVR-opaque lake (> 75 %).

3.2 Variations in solar MIR during experiments

The MIR for three wavelengths within the UVR and PAR region received by the samples under the experimental conditions are shown in Table 2. The MIR at 305 nm, 320 nm and 380 nm in the UVR-clear lake were 2.8-, 2.5-, and 1.9-folds higher, respectively,

in the high MIR than in the low MIR. The respective ratios between high and low MIR in the UVR-opaque lake were 8.7-, 7.1-, and 3.7- for the 305 nm, 320 nm, and 380 nm wavelengths, respectively. The energy ratio at 380 and 305 nm (i.e., $UVA_{380} : UVB_{305}$ ratio) had higher values in the UVR-opaque lake as compared to the UVR-clear lake, reflecting the lower penetration of UV-B in the former.

3.3 Joint effects of UVR and MIR on algal and bacterial metabolism in the UVR-clear lake

The PP values did not show significant differences between high- and low MIR in the PAB treatment, while samples under the PA and P treatments had significant higher PP values in high MIR as compared to the respective treatments at low MIR (Fig. 3a). A significant UVR × MIR effect was found for PP (Table 3) and according to our hypothesis, the high MIR resulted in higher UV-B (11.5 %) and UV-A (18.3 %) inhibition as compared to the low MIR (Table 4). Solar UVR at high MIR also significantly increased the rates of EOC, with significantly higher values in samples under the PAB and PA treatments (Fig. 3b). Like PP, HBP did not differ between PAB-high MIR and PAB-low MIR treatments. However, HBP was significantly lower under PA-high MIR than under PA-low MIR treatments (Fig. 3c) resulting in a significant UVR × MIR effect (Table 3). By contrast, only the "UVR" factor significantly affected BR (Fig. 3d, Table 3), with the lowest BR value determined in the PAB treatment at high-MIR (Fig. 3d). BGE had higher values in the PAB treatment at high MIR as compared to the other radiation treatments at high MIR; other comparisons between paired treatments did not result in significant differences of BGE (Fig. 3e). There was, nevertheless, a significant UVR × MIR interaction on BGE (Table 3). No relationship was found between EOC and BGE (R^2 = 0.149 p > 0.05). Finally, to quantify the capacity of EOC released by algae to support the bacterial C demand (BCD) in each treatment, the BCD : EOC ratio (as a percentage) was calculated (Fig. 3f). Carbon released by algae resulted in excess to meet BCD (i.e., BCD : EOC values <100 %) only in the PAB treatment at high MIR (Fig. 3f).

3.4 Joint effects of UVR and MIR on algal and bacterial metabolism in the UVR-opaque lake

UVR-fluxes exerted negative effects on both epilimnetic (Fig. 4) and hypolimnetic (Fig. 5) communities. For the epilimnetic community, PP was significantly lower in the PAB than in PA and P treatments at high MIR, while UVR did not affect PP at low MIR (Fig. 4a). A significant UVR × MIR effect on PP (Table 3) was determined, with the lowest PP at PAB-high MIR. The high MIR resulted in higher UV-B (40 %) and UV-A (27 %) inhibition (Table 4). As for PP, EOC was significantly lower in the PAB than in PA and P treatments at high MIR, but not significant differences among radiation treatments at low MIR were determined (Fig. 4b). HBP only showed differences between high MIR and low MIR treatments to dark treatments where significant higher values were found at high- than at low MIR treatments. A significant interactive effect of UVR × MIR on HBP was found (Table 3). Noticeably, a strong inhibition of HBP by UV-B and UV-A in high MIR and in low MIR conditions was found (Table 4). By contrast, the estimated BR was not significantly affected by any factor (Table 3; Fig. 4d shown $BR_{50\%}$), UVR was the only factor that significantly reduced BGE values in both low- and high MIR conditions (Fig. 4e). No relationship between EOC and BGE was found ($R^2 = 0.055$ $p > 0.05$). The BCD : EOC (%) was < 100 % for every experimental condition except for that under PAB in the high MIR, where the BCD : EOC (%) reached values from ~100 % (assuming BR = 50 % of TPR) to 145 % (assuming BR = 75 % of TPR) (Fig. 4f). So, in this latter case (PAB-high MIR) EOC was not enough to meet BCD.

For the hypolimnetic community (Fig. 5), UVR was the only factor that significantly inhibited PP. Samples under the PAB and PA treatments had significantly lower PP values than those under the P in both high- and low MIR (Fig. 5a). The EOC rates (Fig. 5b) were significantly lower in the PAB and PA treatments as compared to those in the P treatment at high MIR. No significant differences among MIR treatments were determined when comparing each radiation treatment (Fig. 5b). HBP was significantly inhibited only by UV-B (Fig. 5c), whereas it was stimulated by PA and P in the high MIR

(Fig. 5c). At low MIR, however, UVR did not affect HBP. Therefore, high MIR triggered the inhibition due to UV-B by 45.6 % (Table 4). Only UVR, as a single factor, significantly affected BR (Table 3), with the lowest values under the PAB-low MIR treatment (Fig. 5d), whereas only the MIR factor affected BGE, with the lowest BGE values in the PAB-high MIR treatment (Fig. 5e). The BCD : EOC was < 100 % under all conditions (assuming both BR = 50 % or 75 % of TPR) indicating the EOC was capable of supporting BCD (Fig. 5f).

Summarizing, and taking into account the changes (Δ) in the inhibitory UVR effect (UV-B and UV-A) on PP and HBP with increased MIR (Table 4), our results reveal greater UV-B sensitivity of: (i) epilimnetic algae and heterotrophic bacteria communities in the UVR-opaque lake than in the UVR-clear lake; (ii) epilimnetic algae than heterotrophic bacteria in both lakes; and (iii) hypolimnetic heterotrophic bacterial than algae community in the UVR-opaque lake. In addition, significant interactive UVR × MIR effects were observed on the BCD : EOC (%) only in the epilimnetic communities (Table 3). Thus, partially supporting our hypothesis the BCD : EOC (%) significantly decreased under PAB-high MIR treatment in the UVR-clear lake but increased in the UVR-opaque lake.

4 Discussion

The main outcome of our work was that we demonstrated that the increased MIR associated to stratification of the water column altered the commensalistic algal-bacterial relationship in oligotrophic lakes. The present study is the first, so far, directly assessing the interactive effects of UVR and changes in MIR on algae and bacteria and their commensalistic relationship in freshwater ecosystems. Furthermore, in our complex experimental approach we simulated reductions in the depth of the UML due to the stratification of the water column (one of the potential consequences of global warming) (Gao et al., 2012; de Senerpont-Domis et al., 2013); under these conditions, we measured the extracellular carbon release by algae, and directly determined the BR be-

cause these are the key variables implied in the bacterial carbon demand to C-supply ratio. Moreover, since a strong feedback between physical processes (e.g. mixing, stratification) and changes in DOC concentration in small lakes have previously been reported (Read and Rose, 2013), we further achieved an advance in our knowledge by investigating two oligotrophic ecosystems that differed in their UVR penetration in the water column due to their DOC content. This provides a framework for disentangling the complex processes that underlie biological interactions under changing physical (stratification, UVR) and chemical (DOC) conditions, which can then modify the C flux in aquatic ecosystems.

4.1 Sensitiveness of algae and bacteria to UVR with increased MIR due to stratification

Despite the physical and ecological differences between the two lakes, PP and HBP responses to the joint effect of UVR and MIR were quite similar in that the latter augmented the effect size of UVB, mainly on the epilimnetic communities in both ecosystems. This effect reached a higher magnitude in the UVR-opaque lake (Table 4), which coincided with a greater relative exposure to UV-B (9-fold) and an more accentuated decrease in the UV-A : UV-B ratio (58 %) at shallower layer in the opaque- than in the UVR-clear lake. This result agrees with the findings of higher UVR damage on primary producers in UVR-opaque lakes than in UVR-clear lakes as reported by Helbling et al. (2013), although in their study this response was found only under fluctuating irradiances. The results presented here indicate increased susceptibility to UVR of communities relatively less exposed to UV-B during their life cycles (Pakulski et al., 2007). In addition, a higher sensitivity to UVR was found for epilimnetic algae than for bacteria mainly at high MIR, suggesting that photosynthetic processes are more sensitive under extreme conditions that mimic the global-warming scenario. This result contrasts to previous reports of greater UVR damage to bacterioplankton than to phytoplankton in oligotrophic waters of the Mediterranean Sea (Bertoni et al., 2011), the northern South

China Sea (Yuan et al., 2011), high-mountain lakes (Sommaruga, et al., 1997) and boreal lakes (Xenopoulos and Schindler, 2003).

Taken all together, our results show that stratification, by trapping the cells in a shallower epilimnion, with increased UVR exposure, triggered or exacerbated the inhibitory effect of UVR on algal and bacterial metabolism quantified under mixing condition. Because this negative effect was greater in opaque ecosystem to UVR due to their DOC content, we propose that the "ideal" photoprotective DOM may become harmful in a scenario of greater stratification and high UVR irradiance induced by global warming. Furthermore, UV-B may have harmful effects due to the free radicals (O^{-2}, H_2O_2, OH^-) generated by photo-oxidation of the DOC (Banaszak, 2003; Pullin et al., 2004), exacerbating the negative UVR effect in UVR-opaque lakes.

As expected, UVR was the main factor which affected the non-acclimated hypolimnetic community, and thus PP and HBP underwent negative UV-B and UV-A effects in both high- and low MIR. Nevertheless, HBP of the hypolimnetic community was stimulated by UV-A and PAR when exposed to shallower conditions. These results suggest that the hypolimnetic bacteria possessed photorepair mechanisms, via UV-A and PAR-promoted photolyase activity (DNA repair), which may be activated after 4 h of UVR and PAR exposure (Jeffrey et al., 1996; Bertoni et al., 2012). This photorepair mechanism has a low energy cost and may be an important adaptive mechanism to attenuate the net negative effect of UVR when a non-UVR-acclimated bacterioplankton community is exposed to high PAR and UV-A intensity and harmful UV-B levels in ecosystems with low nutrient availability (Medina-Sánchez et al., 2002). Notwithstanding, in agreement with our hypothesis, photorepair mechanisms were insufficient to completely counteract UVR-induced damage, this being concordant with a sharp decrease in the UVA/UVB ratio (58 %) in the upper layers (high MIR conditions). Moreover, the increased HBP found after exposure of samples to higher PAR intensity in the upper layers is consistent with the previously reported stimulatory effect of PAR on HBP (Morán et al., 2001; Medina-Sánchez et al., 2002; Pakulski et al., 2007). Besides, a potential presence of aerobic anoxygenic phototrophic bacteria (Bertoni et al., 2011; Mašín et al., 2012; Fer-

rara et al., 2011) should not be ruled out to account for the increased HBP under high PAR in UVR-opaque lake.

4.2 UVR and increased MIR effect on commensalistic algal-bacterial relationship

As noted above, UVR and high MIR exerted an interactive effect on both the algal and bacterial communities in the epilimnetic layer. These interactive effects were also reflected in algal C availability to support the bacterial C demand in both lakes. Quantification of the dependence of heterotrophic bacteria on organic substrate released by algae requires an accurate assessment of the BCD (Morán et al., 2002). Our study offers a quite precise estimate of the BCD, because both HBP and BR were directly measured in the UVR-clear lake, due to absence of size overlap between auto- and heterotrophic organisms. In the UVR-opaque lake, where segregation between both biological fractions was not feasible, BR was estimated from direct measurements of TPR and the reported percentages of the latter variable accounted for BR (i.e. 50 and 75 %, Lemeé et al., 2002; Robinson, 2008). This procedure brought about a min–max range where the actual BR should safely fall. In addition, its reliability is supported in that our estimated mean BGE and BR values fell within the range reported for oligotrophic ecosystems (Biddanda et al., 2001; Amado et al., 2013).

In the UVR-clear lake, BGE was increased under full-sunlight and high MIR conditions, reflecting greater changes in bacterial respiration than production. The reduction in BR and, as a consequence, the increase in bacterial growth efficiency could be interpreted as a tolerance-related mechanism under full-sunlight exposure in accordance with the non-inhibitory effect of UV-B on HBP found under shallower conditions. By contrast, in the UVR-opaque lake, BGE values were lower under full sunlight and high MIR (stratified conditions). The lack of the inhibitory effect of full sunlight (PAB vs. P) on TPR (and hence BR) concomitantly with a strong inhibitory effect of UV-B on HBP determined a reduction in bacterial growth efficiency according to the high sensitivity of the bacterial community. The differences in the bacterial responses between the lakes

could be the outcome of specific bacterial composition inhabiting each lake. These results agree with previous laboratory findings of a negative UV-B effect on BGE or BR in some bacterial strains isolated from alpine lakes, but a positive effect on others, suggesting a strain-specific response (Hörtnagl et al., 2010). Nevertheless, changes in
5   BGE are frequently observed when bacterial growth is limited by substrate availability (del Giorgio and Cole, 1998; López-Urrutia and Morán, 2007). Although our experiments were not specifically designed to test the role of organic substrates on BGE, we did not find a significant direct relationship between EOC and BGE in either lake. Thus, our data support the view that BGE can be altered by direct solar UVR impact.
10   Regarding the algal-bacterial relationship, it was noticeable that in the UVR-clear ecosystem, EOC rates increased with full sunlight and high MIR, reaching values that exceeded the C demand of a bacterial community which seemed to have undergone an inactivation or dormancy under PAB, reflected by lower respiration. This slowing of the bacterial metabolism, concomitant with an increase in the availability C released by
15   algae, was the mechanism that determined the "coupling" algal-bacterial relationship. However, the fate of C released by algae could be transitory accumulation in lake water until its consumption by enhanced bacterial metabolic processes (growth and respiration) after an improvement in the light conditions, or could be definitively incorporated into the dissolved-C pool of the lake water.
20   In the UVR-opaque ecosystem, particularly to the epilimnetic community, the strong inhibitory effect of UV-B under high MIR on PP (i.e. decreasing C incorporation) was also reflected in a lesser C release by algae under these conditions. These decreased EOC rates did imply a change in their capability to meet the BCD, which ranged from barely sufficiency (if a 50 % loss of TPR is assumed) to non-sufficiency (if a 75 % loss
25   of TPR is assumed). Therefore, the estimated min-max interval for each experimental condition shows an unexpected trend to a weakening of the bacterial dependence on algal C under full-sunlight and high MIR in UVR-opaque lake, which may be induced by global warming. These results partially support our hypothesis because the interaction UVR × MIR strengthened the commensalistic algal-bacterial dependence in the

UVR-clear lake, but weakened this relationship in the UVR-opaque lake (Figs. 3f and 4f). Moreover, they underline the capability of UVR in altering the efficiency of algal C excretion to support bacterial demands in optically contrasting ecosystems. Since the interaction of UVR and stratification on this crucial biotic interaction in UVR-clear

5　and UVR-opaque lakes has not been previously examined, more data is needed in order to generalize these responses by microbial organisms, not only on short term (as considered in this study) but also on long term basis.

To summarize our findings, we propose a conceptual functioning model that fits both contrasting model ecosystems (Fig. 6). According to the global-warming scenario: (i)

10　the vertical stratification of aquatic ecosystems will intensify (de Senerpont Domis et al., 2013); (ii) the depth of the mixed layer will be altered (micro-stratification in shallow lakes) (van de Waal et al., 2009); and (iii) microbial communities and DOC will be confined within a highly irradiated layer. Based on our results, the interactive effect of UVR and stratification on the microbiota might strengthen the C flux through the microbial

15　loop in the UVR-clear lake (or increasing the DOC pool in the lake) but weaken it in the UVR-opaque lake. Therefore, our results showing a greater UVR damage in the UVR-opaque lake imply that these types of ecosystem might be especially vulnerable to these factors related to global change.

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Synergistic effects of UVR and simulated stratification on algal-bacterial relationship

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Table 1. Mean values of the main chemical and biological variables measured in the water column in Lake La Caldera (UVR-clear lake) and in Lake La Conceja (UVR-opaque lake). Values are mean (± SD) of concentrations for four (La Caldera lake) o six (La Conceja lake) depths of, inorganic, total and dissolved nitrogen (N) and phosphorus (P), Chlorophyll *a*, and algae and bacterial abundances. TN: Total Nitrogen; TDN: Total Dissolved Nitrogen; NO_3^-: Nitrate; TP: Total Phosphorus; TDP: Total Dissolved Phosphorus; SRP: Soluble Reactive Phosphorus; Chl *a*: Chlorophyll *a* concentration; AA: Algal Abundance; BA: Bacterial Abundance.

Variable	UVR-clear lake	UVR-opaque lake
TN (µM)	21.50 ± 1.54	787.1 ± 10.7
TDN (µM)	20.71 ± 1.46	786.4 ± 12.9
NO_3^- (µM)	14.28 ± 1.02	702.1 ± 6.7
TP (µM)	0.10 ± 0.003	0.06 ± 0.012
TDP (µM)	0.051 ± 0.002	0.038 ± 0.012
SRP (µM)	0.02 ± 0.001	0.018 ± 0.012
Chl *a* ($\mu g\,L^{-1}$)	2.02 ± 0.42	2.66 ± 0.46
AA ($cell\,mL^{-1}$) $\times 10^3$	7.03 ± 1.65	4.03 ± 0.72
BA ($cell\,mL^{-1}$) $\times 10^6$	1.94 ± 0.17	1.28 ± 0.21

Table 2. Mean irradiances (MIR) during the incubations for 305 nm, 320 nm and 380 nm within the UVR wavelengths ($\mu W\,cm^{-2}\,nm^{-1}$) and for PAR ($\mu mol\ photons\ m^{-2}\,s^{-1}$). The ratio of the mean irradiances of 380 and 305 nm is also presented.

Wavelength		305 nm	320 nm	380 nm	PAR	UV-A$_{380}$: UV-B$_{305}$
UVR-clear lake	high MIR	3.90	23.40	60.10	1480	15.41
	low MIR	1.40	9.50	31.50	900	22.50
UVR-opaque lake	high MIR	1.44	12.90	47.90	1428	33.26
	low MIR	0.16	1.80	12.80	824	80.00

Table 3. Results of the two-way ANOVA of the interactive effect of "UVR" (PAB, PA, P, Dark) and "MIR" (low-and high mean irradiance) factors on carbon incorporation of algae (PP, $\mu g\,C\,L^{-1}\,h^{-1}$), and Excreted Organic Carbon (EOC, $\mu g\,C\,L^{-1}\,h^{-1}$), Heterotrophic Bacterial Production (HBP, $\mu g\,C\,L^{-1}\,h^{-1}$), Bacterial Respiration (BR, $\mu g\,C\,L^{-1}\,h^{-1}$) was directly measured in the UVR-clear lake or it was calculated as 50 % of Total Planktonic Respiration (TPR) in the UVR-opaque lake; Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC; as a percentage). Numbers in bold indicate, $p < 0.05$. df1, df2, and df3, df4, are the degrees of freedom.

		df_1	df_2	PP $F_{df1,df2}$	P	EOC $F_{df1,df2}$	p	df_3	df_4	HBP $F_{df3,df4}$	p	df_1	df_2	BR $F_{df1,df2}$	p	BGE $F_{df1,df2}$	p	BCD:EOC (%) $F_{df1,df2}$	p
UVR-clear lake																			
Epilimnetic	MIR	1	12	42.29	**0.000**	44.00	**0.000**	1	16	6.41	**0.022**	1	12	1.07	0.321	0.26	0.619	6.15	**0.029**
	UVR	2	12	124.12	**0.000**	6.33	**0.013**	3	16	8.65	**0.001**	2	12	12.38	**0.001**	7.22	**0.009**	35.47	**0.000**
	UVR × MIR	2	12	20.90	**0.000**	0.11	0.895	3	16	5.46	**0.009**	2	12	3.71	0.056	4.80	**0.029**	14.59	**0.001**
UVR-opaque lake																			
Epilimnetic	MIR	1	12	0.61	0.450	2.46	0.143	1	16	7.37	**0.015**	1	8	5.28	**0.05**	1.45	0.263	18.76	**0.002**
	UVR	2	12	6.78	**0.011**	9.78	**0.003**	3	16	27.96	**0.000**	2	8	0.14	0.72	46.13	**0.000**	14.42	**0.005**
	UVR × MIR	2	12	16.71	**0.000**	16.51	**0.000**	3	16	6.38	**0.005**	2	8	0.63	0.45	0.06	0.810	44.86	**0.000**
Hypolimnetic	MIR	2	12	0.33	0.574	4.33	0.060	1	16	32.98	**0.000**	1	8	0.29	0.604	6.01	**0.040**	4.65	0.063
	UVR	2	12	41.58	**0.000**	52.75	**0.000**	3	16	12.05	**0.000**	1	8	8.39	**0.020**	0.15	0.711	0.81	0.394
	UVR × MIR	2	12	0.39	0.688	3.21	0.076	3	16	7.98	**0.002**	2	8	0.90	0.372	5.24	0.061	1.99	0.196

Table 4. Effect size of UV-B and UV-A on primary production (PP, $\mu g\,C\,L^{-1}\,h^{-1}$); and bacterial heterotrophic production (HBP, $\mu g\,C\,L^{-1}\,h^{-1}$) in the experimental conditions. The change (Δ) in effect size of UV-B and UV-A was calculated as difference effect size of UV-B and UV-A between high- and low MIR. Numbers in bold indicate $p < 0.05$.

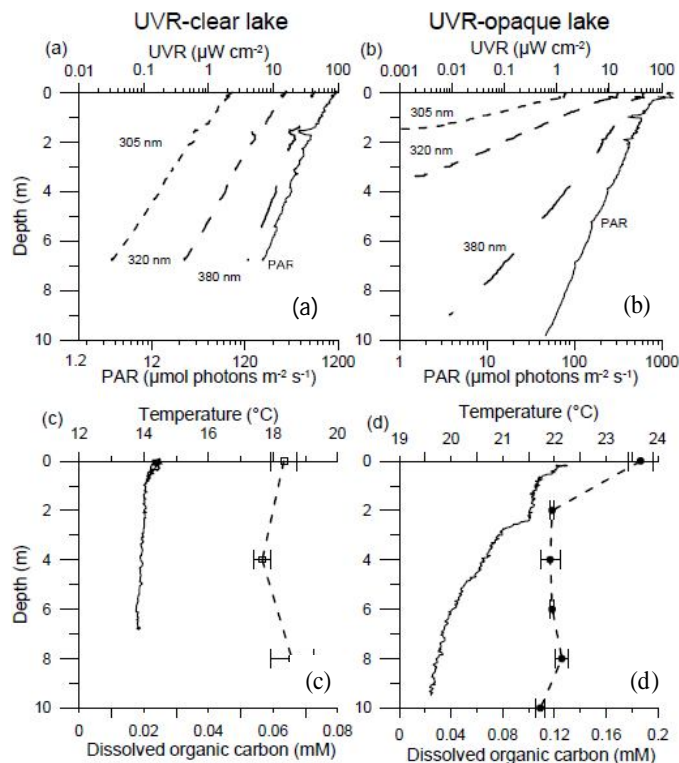|		PP				HBP			
		%UVB	Δ%UVB	%UVA	Δ%UVA	%UVB	Δ%UVB	%UVA	Δ%UVA
UVR-clear lake Epilimnetic	high MIR	37.3 ± 2.4	**11.55**	25.6 ± 7.6	**18.32**	2.7 ± 18.3	−20.3	51.9 ± 26.7	**110.2**
	low MIR	25.7 ± 5.0		7.3 ± 7.1		23.0 ± 1.5		−58.3 ± 0.2	
UVR-opaque lake Epilimnetic	high MIR	33.7 ± 4.2	**40.00**	17.4 ± 13.9	27.41	42.9 ± 6.2	−4.2	30.0 ± 8.7	1.2
	low MIR	−6.3 ± 10.9		−10.0 ± 23.5		47.1 ± 2.0		28.2 ± 6.7	
Hypolimnetic	high MIR	27.2 ± 22.5	0.09	20.8 ± 28.9	−5.98	52.1 ± 5.8	**45.6**	12.0 ± 24.4	−11.5
	low MIR	27.1 ± 5.6		26.8 ± 12.8		6.5 ± 12.2		23.6 ± 2.6	

Figure 1. Depth profiles of the irradiance at 305, 320, and 380 nm, and PAR (400–700 nm) for **(a)** UVR-clear lake (Lake La Caldera) and **(b)** UVR-opaque lake (Lake La Conceja); Dissolved Organic Carbon (DOC) concentrations (mM) and vertical profiles of temperature (°C) as a function of depth in **(c)** UVR-clear lake and **(d)** UVR-opaque lake. Values of vertical attenuation coefficients (m^{-1}) were: k_{d305} = 4.84, and 0.61; k_{d320} = 2.53, and 0.52; k_{d380} = 0.93, and 0.34, k_{dPAR} = 0.28, and 0.25, in the UVR-opaque lake and the UVR-clear lake, respectively.
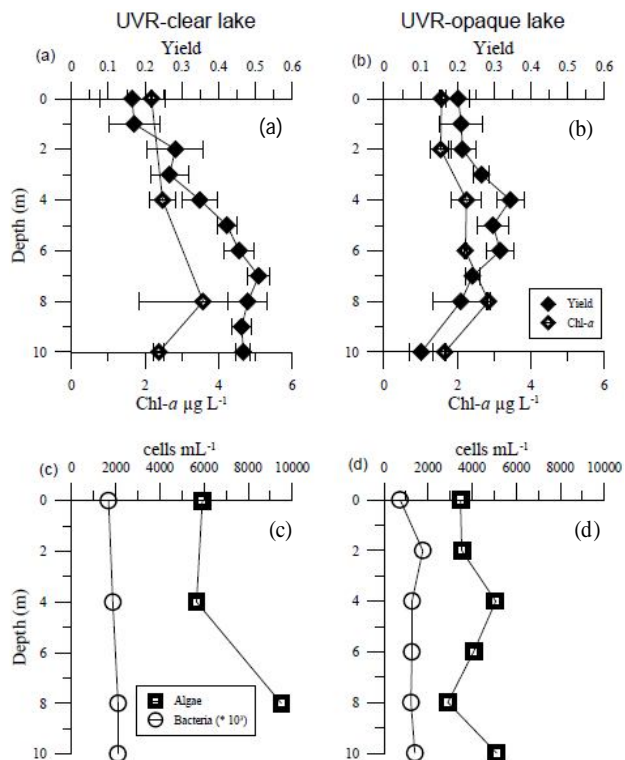
Figure 2. In situ photochemical effective quantum yield and Chl a ($\mu g \, L^{-1}$) in the UVR-clear lake **(a)** and the UVR-opaque lake **(b)**; algae and bacterial abundance (cell mL^{-1}) in the UVR-clear lake **(c)** and the UVR-opaque lake **(d)**.
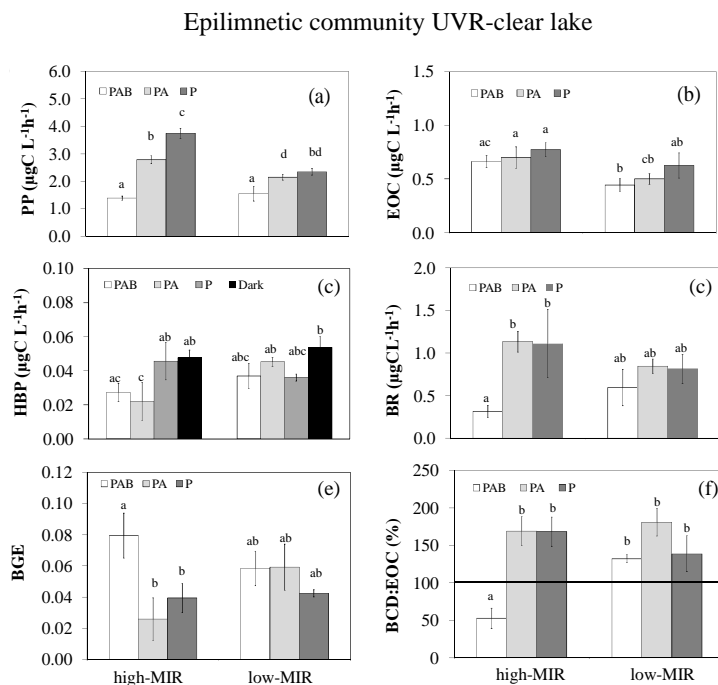
Epilimnetic community UVR-clear lake

Figure 3. Metabolic variables of epilimnetic planktonic community under different stratification treatments in the UVR-clear lake. **(a)** Primary Production (PP, $\mu g\,C\,L^{-1}\,h^{-1}$); **(b)** Excreted Organic Carbon rates (EOC, $\mu g\,C\,L^{-1}\,h^{-1}$); **(c)** Heterotrophic Bacterial Production (HBP, $\mu g\,C\,L^{-1}\,h^{-1}$), **(d)** Bacterial Respiration (BR, $\mu g\,C\,L^{-1}\,h^{-1}$); **(e)** Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC) as a percentage. The lines on top of the bars are the standard deviation.
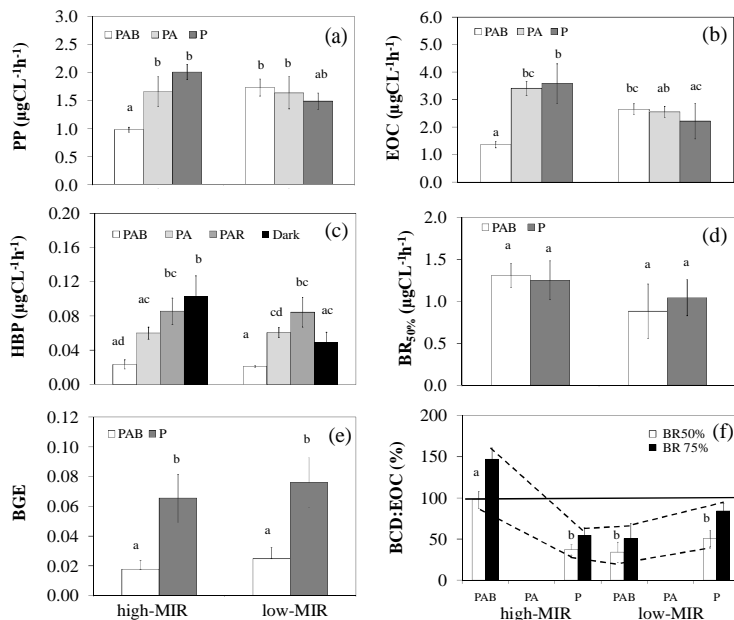
Epilimnetic community UVR-opaque lake

Figure 4. Metabolic variables of epilimnetic planktonic community under different stratification treatment in the UVR-opaque lake. **(a)** Primary Production (PP, $\mu g\, C\, L^{-1}\, h^{-1}$); **(b)** Excreted Organic Carbon rates (EOC, $\mu g\, C\, L^{-1}\, h^{-1}$); **(c)** Heterotrophic Bacterial Production (HBP, $\mu g\, C\, L^{-1}\, h^{-1}$), **(d)** Bacterial Respiration (BR$_{50\%}$ $\mu g\, C\, L^{-1}\, h^{-1}$) calculated as 50 % of Total Planktonic Respiration (TPR); **(e)** Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC) as a percentage. BCD was calculated assuming a BR as 50 % and 75 % of Total Planktonic Respiration (TPR). The lines on top of the bars are the standard deviation.

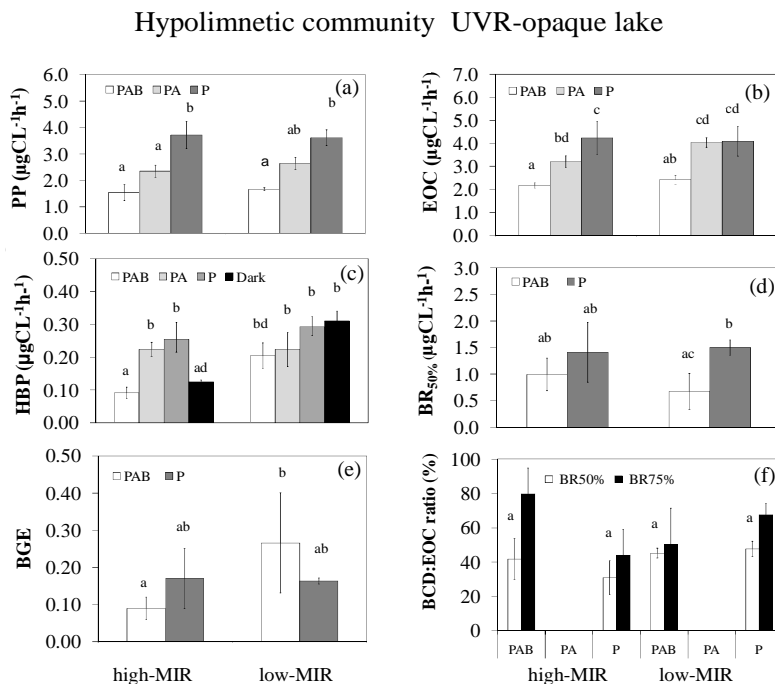Hypolimnetic community UVR-opaque lake

Figure 5. Metabolic variables of hypolimnetic community under different stratification conditions in the UVR-opaque lake. **(a)** Primary Production (PP, $\mu g\,C\,L^{-1}\,h^{-1}$); **(b)** Excreted Organic Carbon rates (EOC, $\mu g\,C\,L^{-1}\,h^{-1}$); **(c)** Heterotrophic Bacterial Production (HBP, $\mu g\,C\,L^{-1}\,h^{-1}$), **(d)** Bacterial Respiration (BR$_{50\%}$ $\mu g\,C\,L^{-1}\,h^{-1}$) calculated as 50 % of Total Planktonic Respiration (TPR); **(e)** Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD) : Excreted Organic Carbon (EOC) as percentage assuming a BR as 50 % and 75 % of Total Planktonic Respiration (TPR). The lines on top of the bars are the standard deviation.
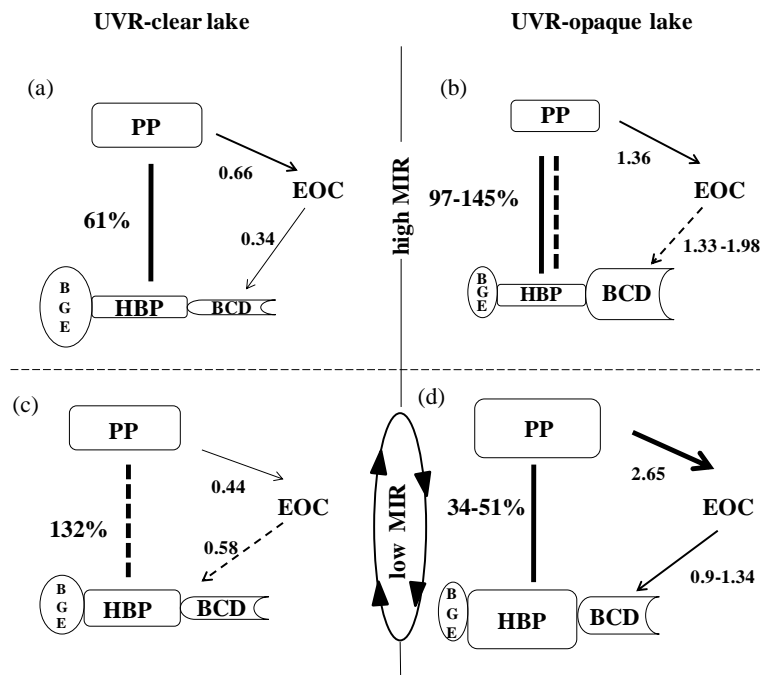
Figure 6. Changes in epilimnetic algal-bacterial relationship under PAB-high MIR and PAB-low MIR in UVR-clear lake **(a, c)** and UVR-opaque lake **(b, d)**. The sizes of the boxes are proportional to the rates (in µg C L^{-1} h^{-1}). The thicknesses of the arrows indicate the relative magnitude of a particular carbon flux. The broken-lines arrows indicate that EOC is not enough to satisfy the Bacterial Carbon Demand (BCD). Thick black lines represent the BCD : EOC (%), indicating either coupled (solid lines) or uncoupled (broken lines) algal-bacterial relationship. PP: Primary Production, HBP: Heterotrophic Bacterial Production, BGE: Bacterial Growth Efficiency. Numbers are rates of C flux (in µg C L^{-1} h^{-1}).

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