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Changes in the phytoplankton-bacteria coupling triggered by joint action of UVR, nutrients, and warming in Mediterranean high-mountain lakes

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Abstract

From an extensive study, we determined that heterotrophic bacterial production (HBP) variance in Sierra Nevada (Spain) lakes was explained mainly by excretion of organic carbon by algae (EOC), underlining a bacterial dependence on algal carbon. Subsequently, we studied how the interaction among global change factors such as ultraviolet radiation (UVR), nutrient inputs, and increased temperature affected this phytoplankton-bacteria coupling through in situ factorial experiments in two model high-mountain lakes, La Caldera, and Las Yeguas. Bacterioplankton were more sensitive than phytoplankton because the joint action of increased temperature and nutrient-addition unmasked an inhibitory UVR effect on HBP while reducing the inhibitory UVR effect on primary production (PP) (in La Caldera) or augmenting the net PP values (in Las Yeguas). The interaction among the three factors had a different effect on phytoplankton-bacteria coupling depending on the lake. Thus, in the colder lake (La Caldera), EOC was not adequate to meet the bacterial carbon demand (BCD), leading to a mismatch in phytoplankton-bacteria coupling. Contrarily, in the warmer lake (Las Yeguas), the phytoplankton-bacteria coupling was accentuated by the interaction among the three factors, with EOC exceeding BCD. These contrasting responses of phytoplankton-bacteria coupling may affect the microbial loop development, becoming reinforced in warmer and less UVR-transparent high-mountain lakes, but weakened in colder and more UVR-transparent high-mountain lakes, with implications in the C-flux of these sentinel ecosystems in a scenario of global change.

High-mountain lakes are oligotrophic ecosystems naturally exposed to extreme conditions (e.g., high UVR fluxes, low temperature) but highly sensitive to anthropogenic global change despite their relative isolation from human populations (Psenner 2003; Nelson and Carlson 2011). Hence, due to their particular geographic characteristics (e.g., high elevation, small catchment areas, etc.), high-mountain lakes are increasingly being considered as suitable indicators of environmental change at the local and global scales (Catalán et al. 2006; Parker et al. 2008; Rose et al. 2009). However, high-mountain lakes may respond differently to environmental changes depending on catchment-area traits that can shape inherent physical and biological differences among lakes (Rose et al. 2009).

Organisms in high-mountain lakes are subjected to a higher flux of ultraviolet radiation (UVR) than other aquatic ecosystems because UVR increases with altitude (Blumthaler et al. 1997). Although the Montreal Protocol has successfully banned ozone-depleting substances, a thin ozone layer persists in the Arctic (Strahan et al. 2013). The recovery of the ozone layer is not expected until 2025–2040 at mid-latitudes (World Meteorological Organization, WMO/UNEP, 2010). Therefore, the UVR effect on organisms might be accentuated in high-mountain lakes (Tucker and Williamson 2011). Responses of planktonic organisms to the UVR range from inhibition to increased growth, and the intensity of the effects might also differ depending on an organisms' metabolism (Jeffrey et al. 1996; Ogbebo and Ochs 2008; Medina-Sánchez et al. 2013). Thus, the UVR effect has been reported in some studies to be inhibitory on primary production (PP) and heterotrophic bacterial production (HBP) (Carrillo et al. 2002; Conan et al. 2008; Carrillo et al. 2015) in the upper water column, as well as damage to particulate alkaline phosphatase activity (Tank et al. 2005; Korbee et al. 2012) and cell viability (Helbling et al. 1995; Llabres and Agustí 2010). However, a negative or stimulatory effect, or no effect at all, of UVR on PP and HBP has been also described

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(Aas et al. 1996; Helbling et al. 2001; Medina-Sánchez et al. 2002). These contrasting results might derive from the UVR interaction with other environmental factors such as nutrient availability, temperature, and vertical mixing (Harrison and Smith 2009; Ruíz-González et al. 2013).

Due to the evidence of current global warming (IPCC 2013), the influence of temperature (T) on planktonic response to UVR has not received attention until recently, with studies focused on the direct effects of warming on organisms exposed to UVR (Bullock and Jeffrey 2010; Domaizon et al. 2012; Fouilland et al. 2013) and the indirect effects through an increase in the stability of thermal stratification or changes in the water-mixing regime (Helbling et al. 2013; Carrillo et al. 2015). The fact that temperature, contrary to UVR, decreases with elevation makes high-mountain lakes likely to undergo some of the highest UVR:T ratios of any aquatic ecosystems (Williamson and Zagarase 2003). The stimulatory effect of increased temperature on metabolic activity is well known (Sarmento et al. 2010 and references therein), including molecular repair mechanisms for UVR damage (Hoffman et al. 2003). However, although higher T has been found to alleviate photoinhibition of bacterial (Bullock and Jeffrey 2010) and phytoplanktonic community (Roos and Vincent 1998; Doyle et al. 2005), a rise in T in some oligotrophic ecosystems can prove negative for growth if basal metabolism costs augment whereas the energy or nutrient are unable to satisfy the demands (Degerman et al. 2013).

In the Mediterranean area, one of the most sensitive regions in the world to the effects of climate change (Giorgi 2006; Giorgi and Lionello 2008; IPCC 2013), the expected rise in T and higher frequency of extreme weather events, such as heat waves (Giorgi and Lionello 2008), might have major consequences on UVR effects by changing the UVR:T ratio and thereby altering the ecosystem functioning. In addition, high-mountain lakes from the Mediterranean basin, due to their proximity to Saharan desert, are exposed to atmospheric inputs of mineral nutrient (Morales-Baquero et al. 2006). The inorganic nutrient influence altering the phytoplankton and bacterioplankton response to UVR has previously been reported (Carrillo et al. 2008; Ogbebo and Ochs 2008; Medina-Sánchez et al. 2013).

However, beyond the effects of environmental changes on phytoplanktonic and bacterial metabolisms, a more relevant issue is how those changes affect the relationship between these organisms, which is the basis of carbon flux in oligotrophic aquatic ecosystems (Medina-Sánchez et al. 2002, 2004; Morán and Alonso-Sáez 2011). A general bacterial dependence on phytoplankton through the excretion of photosynthetic carbon (EOC) has been described (Baines and Pace 1991; Pugnetti et al. 2010; Carrillo et al. 2015), this carbon source being preferred by bacteria even in lakes with considerable input of terrestrial carbon to subsidize their growth (Kritzberg et al. 2005; Kritzberg 2006). As UVR has been evidenced as a stimulator of EOC (Carrillo et al. 2008, 2015; Korbee et al. 2012), UVR might act by reinforcing the phytoplankton-bacteria coupling (Carrillo et al. 2002; Medina-Sánchez et al. 2002) through the increase in the availability of the carbon released by phytoplankton to meet the bacterial carbon requirements (Morán et al. 2002). The strength of phytoplankton-bacteria coupling is also related to other environmental factors, such as inorganic nutrient availability (Aota and Nakajima 2001) or the vertical mixing regime (Durán et al. 2014; Carrillo et al. 2015). Thus, a higher nutrient availability might improve the coupling between photosynthesis and the algal growth, reducing algal C excretion (Berman-Frank and Dubinsky 1999), which might constrain the C supply to satisfy the bacterial carbon demand (BCD). In this study, we tested the hypothesis that an increase in T and nutrient availability will mitigate photoinhibition on phytoplanktonic and bacterial metabolisms and will weaken the phytoplankton-bacteria coupling through the shortage of organic C release required to satisfy the BCD.

To do so, first, we establish the factors controlling the HBP in high-mountain lakes of Sierra Nevada (Spain). Second, we quantify the photoinhibition induced on PP and HBP by natural fluxes of solar UVR at different depths of the water column in two selected lakes sharing physical traits such as depth (>5 m) and high transparency to solar radiation. Third, we evaluate whether bacterial responses to UVR depend on the presence of phytoplankton. Ultimately, our goal was to identify experimentally how the interaction among global-change factors (UVR, nutrient-addition, and increased T) act on the phytoplankton-bacterial coupling.

Methods

Extensive study

An extensive study was carried out in 13 high-mountain lakes (2786–3067 m. a. s. l.) in the National Park of Sierra Nevada (Spain) in August of 2005. These lakes are small, shallow, and highly transparent, with very scarce littoral vegetation. Morphometric (depth), physical (temperature, radiation), chemical [total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), dissolved organic carbon (DOC)], and biological measurements [chlorophyll *a* concentration, (Chl *a*), HBP, PP, excreted organic carbon (EOC), phytoplankton abundance (PA), bacterial abundance (BA)] were collected for each lake in middle of ice free period. The water samples were taken with an acid-cleaned 6-L horizontal Van Dorn sampler at the deepest point of the lake. When possible, water from four depths (0.5 m below surface, 0.5 m above the bottom, and two intermediate depths) was mixed in a 5-L bucket. Then, subsamples were taken in triplicate for each variable.

Selected lakes and experimental setups

The experiments were performed in two model high-mountain lakes situated above the tree-line in Sierra Nevada National Park (Spain): La Caldera lake and Las Yeguas lake (Table 1). Both ecosystems have low catchment areas (La Caldera = 23.5 ha; Las Yeguas = 50 ha) (Morales-Baquero et al. 1999), are the deepest (>5 m) lakes in the study area, are oligotrophic [total phosphorus (TP) < 0.32 μmol P L^{-1}, Chl $a < 5$ μg L^{-1}; Reche et al. (2001), Helbling et al. (2013)], and highly transparent to PAR (Medina-Sánchez et al. 2010). These ecosystems experience frequent inputs of atmospheric Saharan dust containing high P levels, with a mean molar TN:TP ratio in total dust deposition ranging from 10 to 50 (Morales-Baquero et al. 2006). However, the mean temperature of the water column is about 8°C in La Caldera but >15°C in Las Yeguas (Bullejos et al. 2014). Water samples from four depths (0.5 m below surface, 0.5 m above the bottom, and two intermediate depths) of the water column in both lakes were collected in triplicate with an acid-cleaned 6-L horizontal Van Dorn sampler to determine abiotic and biotic structural variables

UVR effect on PP at different depths

An experimental setup consisting of a 2 × 2 matrix (in La Caldera) or 2 × 3 matrix (in Las Yeguas) was designed to assess a potential shift in the UVR effect on phytoplankton with depth, i.e., the effect of the differential radiation reaching different layers of the lake. We performed an experiment in Las Yeguas and La Caldera in August and September 2010, respectively. Two radiation treatments, full sunlight (UVB + UVA + PAR, > 290 nm; "UVR + PAR" treatment) and exclusion of UVR (>400 nm; "PAR" treatment), were implemented at two lake depths (surface and bottom) in La Caldera and at three lake depths (surface, middle and bottom) in Las Yeguas. Quartz flasks were used for the UVR + PAR treatment, whereas PAR treatments were applied using glass flasks covered with UV Opak 395 filter (Ultraphan, Difegra; the spectral characteristics of this filter are published elsewhere, e.g., Figueroa et al. 1997). Flasks were filled with 45-μm filtered water (for zooplankton removal) from each depth and incubated at the corresponding experimental water-column depth.

UVR effect on HBP with or without phytoplankton at different depths

For HBP, we also studied the bacterial response to UVR at different depths both in the presence and absence of phytoplankton with an experimental factorial design 2 × 2 × 2 and 2 × 3 × 2 in La Caldera and Las Yeguas, respectively. These experiments were performed at the same dates as in the previously described experiment for phytoplankton (*see* above). Quartz or glass flasks were used depending on the radiation treatment (*see* above). The treatments with the presence of phytoplankton were made by filtering water from the corresponding layer through 45-μm mesh, whereas the treatments with the absence of phytoplankton were made by subsequently filtering water through 1-μm pore-size filter (Nucleopore filters; 25 mm diameter). This separation was possible due to the absence of overlapping among the different biotic fractions and of autotrophic picoplankton in these lakes (Medina-Sánchez et al. 2002, 2013; Dorado-García et al. 2014, this study). Water was collected from and incubated at the corresponding experimental water-column depth.

Joint effects of UVR, nutrients, and temperature on carbon metabolism

Experiments to assess the combined effects of UVR, nutrient-addition, and T increase in the upper layers on PP, HBP, and bacterial respiration (BR) were conducted in situ in La Caldera and Las Yeguas in 2010 (August in Las Yeguas and September in La Caldera). The experiment in both ecosystems had a 2 × 2 × 2 factorial design (in triplicate for each treatment). Two nutrient conditions were implemented: (1) ambient nutrient concentration (NP-ambient) and (2) nutrient addition (NP-added). For each treatment, an integrated water sample was composed from equal volumes of water samples taken with an acid-cleaned 6-L horizontal Van Dorn sampler at three depths: upper, middle, and bottom layers. The composite samples were prescreened through a 45-μm mesh to remove zooplankton and then mixed in two acid-cleaned containers (6 L). A container with no added nutrients served as control (NP-ambient) whereas the other one was nutrient-added with phosphorus (as Na_2HPO_4, to a final concentration of 30 μgP L^{-1}) and nitrogen ([N] as NO_3NH_4), to a final N: P molar ratio of 30. In this way, we simulated the proportion of macronutrient input caused by pulses of Saharan dust, as previously shown by Morales-Baquero et al. (2006). After nutrient addition, the subsamples were shaken and left for an acclimation period of 120 min exposed to full sunlight in the lake (up-opened containers) before being used to fill the experimental flasks assigned for each enrichment treatment.

Subsamples from each nutrient treatment were exposed to the two radiation treatments (UVR + PAR vs. PAR) specified above and to two T treatments: ambient temperature ($T_=$) vs. 5°C above ambient temperature (T_+) to resemble the predicted increase of temperature within the range from 1.5°C (scenario B1) to 6.4°C (scenario A1FI) by the end of the century (IPCC, 2013). For this latter purpose, the set of flasks for $T_=$ treatments (10°C in La Caldera; 15°C in Las Yeguas) were incubated in situ at 0.5 m depth, whereas the set of flasks for T_+ treatments (15°C in La Caldera; 20°C in Las Yeguas) were subjected to warmer temperature using a thermostatically controlled bath on the lake shore. Water from the thermostatically controlled bath was constantly pumped to a tank (0.5 m × 1 m) which had its interior painted black to prevent any light reflection and which was situated in a sunny location on the lake shore. All flasks within the tank were incubated at 0.5 m in depth. Our experimental set up included the maintenance of organisms within a constrained

Table 1. Characterization of high-mountain lakes in Sierra Nevada. Units are given in brackets.

Lake	Latitude	Longitude	Altitude (m)	Maximum depth* (m)	Waterbody type	$k_{d\ UVR}$* (m^{-1})	$k_{d\ PAR}$* (m^{-1})	T* (°C)	TDP (μmol P L^{-1})	TDN (μmol N L^{-1})	DOC (μmol L^{-1})	HBP (μg C L^{-1} h^{-1})	EOC (μg C L^{-1} h^{-1})	Chl *a* (μg L^{-1})	PA (cell mL^{-1} × 10^3)	BA (cell mL^{-1} × 10^5)
Caballo	37°00′53.12″N	3°26′15.71″W	2851	1.97	Permanent	5.75	0.64	15.0	0.426	30.4	77	0.025	1.09	0.66	20.5	5.9
Yeguas	37°0321.91″N	3°22′50.81″W	2886	8.06	Permanent	0.61	0.20	15.5	0.100	7.1	89	0.008	0.53	7.77	12.6	2.4
Virgen 1	37°03′02.65″N	3°22′47.91″W	2955	0.77	Temporary	1.19	0.45	11.8	0.128	9.1	65	0.021	1.86	0.54	4.7	15.7
Virgen 2	37°03′07.00″N	3°22′46.83″W	2949	0.29	Temporary	7.12	0.94	20.3	0.300	21.4	45	0.409	8.28	10.04	17.4	33.1
Aguas Verdes	37°02′54.75″N	3°22′06.15″W	3067	1.22	Permanent	7.11	1.05	16.2	0.483	34.5	123	0.228	1.94	2.03	0.8	7.3
Río Seco 1	37°03′06.81″N	3°20′53.30″W	3052	1.28	Permanent	4.35	1.82	17.1	0.325	23.2	115	0.052	1.48	0.43	3.1	2.4
Río Seco 2	37°03′07.86″N	3°20′44.49″W	3032	1.42	Permanent	4.07	2.66	15.5	0.300	21.4	98	0.045	0.44	1.39	4.2	2.3
Laguneto	37°03′36.20″N	3°20′13.07″W	2786	1.93	Permanent	0.92	0.38	12.2	0.101	7.2	76	0.067	2.87	3.60	11.1	7.7
Larga	37°03′34.45″N	3°20′03.59″W	2789	3.38	Permanent	0.37	0.20	16.1	0.116	8.3	85	0.009	0.24	0.25	4.3	3.5
Caldera	37°03′17.66″N	3°19′44.85″W	3030	6.97	Permanent	0.38	0.18	8.4	0.194	13.8	87	0.017	3.28	1.86	45.2	6.5
Caldereta	37°03′12.64″N	3°19′27.30″W	3045	0.92	Temporary	2.02	0.66	17.6	0.252	18.0	103	0.200	3.38	4.94	39.9	1.0
Borreguil	37°03′09.84″N	3°17′59.24″W	2983	1.09	Permanent	3.74	0.82	16.4	0.275	19.6	140	0.263	1.23	0.69	4.7	1.4
Hondera	37°02′52.96″N	3°17′39.55″W	2899	0.21	Permanent	3.49	1.60	14.5	0.262	18.7	150	0.378	2.76	11.85	22.2	8.5

T, temperature; $k_{d\ UVR}$, mean extinction coefficient for UVR of 305 nm; $k_{d\ PAR}$, extinction coefficient for photosynthetic active radiation (PAR); TDP, total dissolved phosphorus; TDN, total dissolved nitrogen; DOC, dissolved organic carbon; HBP, heterotrophic bacterial production; EOC, excreted organic carbon from algal origin; Chl *a*, chlorophyll *a*; PA, phytoplankton abundance; BA, bacterial abundance.
*Data from Bullejos et al. (2014).

depth subjected to high solar radiation simulating that received by organisms trapped within a shallow upper mixed layer mimicking the daily events of microstratification that occur in Sierra Nevada lakes (Rodríguez-Rodríguez et al. 2004).

Physical analyses

Vertical profiles of solar radiation and temperature in the water column were determined at noon with a BIC compact four-channel radiometer (Biospherical Instruments, California, U.S.A.), which had three channels in the UVR region of the spectra (305 nm, 320 nm, and 380 nm) and one broadband channel for PAR (400–700 nm). Diffuse attenuation coefficients for downward irradiance (k_d) were calculated from the slope of the linear regression of natural logarithm of downwelling irradiance vs. depth for each wavelength range considered. A large sample size (pairs irradiance-depth data, $n > 400$) was used and a good fit ($R^2 > 0.95$) was found for all regressions. The mean UVR_{320nm} received at 0.5 m depth of the water column ($I_{m(320nm)}$) was calculated as:

$$I_{m(320)} = I_{0(320)}[1-\exp(-k_{d(320)}z)]/-k_{d(320)}z \quad (1)$$

where $I_{0(320)}$ is the mean incident surface irradiance, $k_{d(320)}$ is the mean attenuation coefficient for PAR, and z is the depth to where samples were incubated.

Analysis of structural variables

Dissolved inorganic nitrogen (DIN) was considered the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), which were determined by UV-spectrophotometric techniques, sulfanilamide and phenol-hypochlorite techniques, respectively (APHA 1992). TP and soluble reactive phosphorus were measured by analysing 50 mL aliquots with the acid molybdate technique after persulfate digestion (APHA 1992). To determine sestonic N and sestonic P, 500 mL or 1 L, respectively, were filtered through precombusted (1 h at 550°C) 1.0-μm glass-fiber filters (Whatman GF/B) at low pressure (< 100 mm Hg). Filters containing sestonic N were desiccated (24 h at 60°C) and kept dry until N analysis using a Perkin-Elmer model 2400 CHN elemental analyzer (Perkin-Elmer Corporation, Waltham, Massachusetts, U.S.A.). Filters for sestonic P were analysed following the method described for TP. Blanks and standards were performed in all procedures. The sestonic N: P ratio was calculated on a molar basis.

DOC values were determined by filtering the samples through precombusted (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 analyser (TOC-V CSH/CSN Shimadzu).

For the measurement of the Chl *a* concentration, 0.5–1 L of water from each layer of the water column considered were filtered onto Whatman GF/F filters (25 mm in diameter) and frozen at −20°C until analysed. Subsequently, filters were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone (90%) for 24 h in darkness at 4°C. Then, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorimeter (LS 55 Perkin Elmer, U.S.A.). A Chl *a* standard (Chl *a* from spinach, Sigma) was used to transform the fluorescence data into Chl *a* concentrations.

Samples for identification and enumeration of phytoplankton were preserved in 250-mL brown glass bottles containing Lugol alkaline solution (1% vol vol^{-1}). A volume of 50 mL was allowed to settle for 48 h in Uthermöl chambers (Hydro-Bios GmbH, Germany) and species were counted and identified using an inverted microscope (Axio Observer A1, Carl Zeiss, Germany).

BA was determined by a flow-cytometry technique (FACScanto II, Becton Dickinson Biosciences, Oxford, UK) from samples of water (three replicates and two controls for each considered stratum of the water column) fixed with 1% paraformaldehyde and stained with SYBR Green I DNA stain (Sigma-Aldrich) to a 1:5000 final dilution of initial stock (Zubkov et al. 2007). Stained microbial cells were discriminated on bivariate plots of particle side scatter vs. green fluorescence. Yellow-green 1-μm beads (Fluoresbrite Microparticles, Polysciences, Warrington, Pennsylvania, U.S.A.) were used as an internal standard of particle concentration and fluorescence (Zubkov and Burkill 2006; Zubkov et al. 2007).

Analyses of functional variables

For PP measurements, sets of 50-mL flasks (three clear and one dark for each experimental treatment) received 0.37 MBq of $NaH^{14}CO_3$ (SA: 310.8 MBq $mmol^{-1}$, DHI) and incubated in situ for 4 h at midday (10:00 to 14:00 h), at the same depth where the water had been collected. All flask sets were horizontally held during the incubations. PP calculations were based on the ^{14}C method (Lignell 1992). In brief, total organic carbon (TOC) produced was measured in 4-mL subsamples collected before filtration. To determine the ^{14}C retained in phytoplankton (PP), we filtered the samples through 1-μm Nucleopore filters (25 mm diameter). Low pressure (<100 mm Hg) was applied to minimize cell breakage [more details on laboratory procedure in (Carrillo et al. 2002)]. EOC was measured in 4-mL subsamples collected from the filtrates < 1 μm. The 4-mL aliquots for TOC and EOC determination, as well as filters for PP determination, were put into scintillation vials, and inorganic carbon was removed by adding 100 μL of 1 N HCl and allowing the vial to stand open in a hood for 24 h. After acidification, scintillation cocktail (Ecoscint A) was added to all the samples. The amount of carbon was determined from the disintegrations per min (dpm), counting with a scintillation counter equipped with autocalibration (Beckman LS 6000TA). In all calculations, dark values were subtracted from corresponding light values. The percentage of excreted organic carbon (%EOC) was calculated as:

$$\%EOC = EOC \times TOC^{-1} \times 100 \quad (2)$$

Samples for HBP measurements were placed in 10-mL flasks (three replicates and two blanks for each experimental treatment). The flasks for UVR × nutrients × T experiment were pre-exposed in situ at 0.5 m for 3 h under the corresponding treatment prior to the radiotracer addition. HBP was determined by incorporating ^{3}H-thymidine (S.A = 46.5 Ci mmol^{-1}, Amersham Pharmacia) into the bacterial DNA (Fuhrman and Azam 1982). ^{3}H-thymidine was added to each experimental flask to a final saturating concentration of 16.6 nmol L^{-1}. Flasks with the radiotracer, incubated for 1 h in situ at the same depth where the water was collected, were subjected to the different treatments. All flask sets were horizontally held during the entire exposure period symmetrically distributed around noon. After incubation, the incorporation of ^{3}H-thymidine was stopped with 5% (final concentration, f.c.) tricholoracetic acid (TCA). Likewise, blanks were TCA-killed before the radiotracer was added. In the laboratory, 1.5 mL from each flask was transferred to sterile microcentrifuge tubes where extraction was performed by cold TCA (5% f.c.) keeping the vials in ice for 20 min, after which the precipitate was collected by centrifugation at 16,000 × *g* for 10 min (Smith and Azam 1992). Then, vials were rinsed twice with 1.5 mL of TCA (5% f.c.) to remove any residual unincorporated radioactivity. Finally, scintillation liquid (Ecoscint A) was added for subsequent measurement in an autocalibrated scintillation counter (Beckman LS 6000TA). The conversion factor 1 × 10^{18} cell mol^{-1} (Bell 1993) was used to estimate the number of bacteria produced per mol of incorporated thymidine. The factor 2 × 10^{-14} g C cell^{-1} (Lee and Fuhrman 1987) was applied to estimate the amount of bacterial carbon produced.

Samples for BR (<1 μm fraction) measurements were placed in 25-mL flasks and pre-exposed in situ for 3 h under UVR × nutrients × T conditions described above. BR rates were measured using optode sensor-spots (SP PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fiber 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 immediately after the pre-exposure period and then every hour for 8 h. Every oxygen measurement was made for 30 sec with a frequency of 1 datum per sec; 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. The slope of the regressions provided the oxygen-consumption rates (Warkentin et al. 2007). These rates (μmol O_2 L^{-1} h^{-1}) were converted into carbon units using a respiratory quotient of 1 (Del Giorgio and Cole 1998). In turn, we used the BR values to calculate %BCD:EOC. BCD was determined as the sum of HBP plus BR. Propagation errors was used to calculate the variance for %BCD:EOC.

Statistical analyses

Stepwise multiple regression analysis was performed to assess the relative strength of abiotic (i.e., DOC, TDP, TDN, k_d, T) and biotic variables (i.e., EOC, PA, Chl *a*) in regulating the HBP in lakes of Sierra Nevada in the extensive study. Linearity and orthogonality among independent variables were confirmed by a previous correlation analysis and controlled by specifying 0.6 as the minimum acceptable tolerance (Stat Soft, 2005). The *F*-values entering the multiple regression model were established on the basis of the number of independent variables and cases.

The inhibition by UVR on the different functional variables was calculated as:

$$\%UVR_{inh} = ((PAR - UVR)/PAR) \times 100 \quad (3)$$

where PAR and UVR represent the mean values of PP, EOC, %EOC, HBP, and BR in the absence (PAR treatments) or presence (UVR + PAR treatments) of UVR for each nutrient, temperature or nutrient × temperature treatment. We used propagation errors to calculate the variance for $\%UVR_{inh}$, and the differences among treatments were evaluated by a *t*-test.

The UVR effect on PP with depth was tested using a two-way analysis of variance (ANOVA). The effect of phytoplankton removal on HBP, under both light treatments at different depths, as well as the interactive effect of UVR × nutrient × T on all functional variables, was tested by three-way ANOVA. LSD post hoc test was used to determine significant differences between treatments. Data were checked for normal distribution with Kolmorov-Smirnov's test and homoscedasticity was verified with Levene's tests. The data were log-transformed to meet assumptions of the parametric tests. Statistica 7.0 for Windows (Statsoft 2001) was used for the statistical analysis.

Results

Extensive study

Lakes of Sierra Nevada showed a wide heterogeneity in UVR transparency, with k_{dUVR} values ranging from 5.75 m^{-1} (Caballo) to 0.37 m^{-1} (Larga), and also in mean temperature, from 8.4°C (La Caldera) to 20.1°C (Virgen 2; Table 1). Stepwise multiple regression analysis indicated that HBP of Sierra Nevada lakes was dependent mainly on EOC, which explained ca. 60% of its variance, and secondarily on DOC, which contributed an additional 18%, and on temperature, with a 9% (Table 2). La Caldera and Las Yeguas were two of the most transparent lakes of Sierra Nevada to UVR and PAR, although they differed in the mean water temperature, La Caldera being colder than Las Yeguas (Table 1). Moreover, these were the lakes with the lowest HBP values (Table 1).

Table 2. Results of multiple stepwise regression analysis between heterotrophic bacterial production (HBP, dependent variable) and the set of independent variables entered in the analysis. Numbers in bold indicate significant effect on the considered variable.

Dependent variable	Independent vars. entered	β	Multiple R^2	R^2 change	df 1	df 2	$F_{df1, df2}$	p
HBP	EOC	0.81	0.60	0.60	1	11	16.805	**0.001**
	DOC	0.45	0.78	0.18	1	10	8.509	**0.015**
	T	0.31	0.88	0.09	1	9	6.743	**0.028**

EOC, excreted organic carbon; DOC, dissolved organic carbon; T, temperature; β, standardized regression coefficient; multiple R^2, coefficient of multiple determination; R^2 change, change in multiple R^2 caused by entering a new variable in a single step; $F_{df1, df2}$, *F*-test results of the relationship between the dependent variable and the set of independent variables entered in the analysis.

Based on these traits, both lakes were selected as model ecosystems to experimentally assess the interactive effect of UVR, nutrients, and temperature on primary and bacterial production and on phytoplankton-bacteria relationship.

Physical, chemical, and biological characterization of the water column in the selected lakes

Both lakes received a high flux of incident UVR (clear sky), although mean surface UVR irradiance (320 nm; 0.5 m depth) was higher in Las Yeguas (0.149 W m^{-2} nm^{-1}) than in La Caldera (0.051 W m^{-2} nm^{-1}). Despite that UVR reached the bottom in both lakes, k_{d320} values in Las Yeguas doubled those of La Caldera (Fig. 1 Supporting Information). Water temperature showed a relatively homogeneous vertical profile in both lakes, although La Caldera was a colder lake than Las Yeguas (~10°C in La Caldera and ~15°C in Las Yeguas, Table 1 Supporting Information). Also, sestonic N:P and DIN:TP ratios showed a moderately uniform vertical profile in both lakes, yielding high values (DIN:TP>12 by weight, sensu Morris and Lewis 1988) although higher in La Caldera than Las Yeguas (Table 1 Supporting Information). BA and PA showed roughly similar values throughout the water column (Supporting Information Table 1). In both lakes, Chlorophyta was the dominant group (≈60–70% of total PA in La Caldera and >60% in Las Yeguas), followed by Chrysophyta in La Caldera (≈30–40% of total PA) whereas, in Las Yeguas the remaining groups (Chrysophyta, Cryptophyta, Bacillariophyta) showed similar abundance.

UVR effect on PP at different depths

UVR had different effects on PP, %EOC, and HBP depending on the lake and depth (Fig. 1). Thus, in La Caldera, there was interactive UVR × depth effect on PP but not on %EOC (Table 3), with an inhibitory UVR effect on PP only in the surface (Fig. 1a), whereas %EOC was higher under UVR + PAR than the PAR treatment regardless the depth (Fig. 1c). In Las Yeguas, there was no interactive UVR × depth effect on PP (Table 3), nor did UVR significantly affect PP at any depth (Table 3). However, UVR and depth interacted significantly on %EOC (Table 3) and UVR diminished the %EOC at all the depths (Fig. 1d).

UVR effect on HBP with or without phytoplankton at different depths

In both lakes, UVR × depth × phytoplankton exerted a significant effect on HBP (Table 3). In the presence of phytoplankton, UVR decreased HBP in the surface in La Caldera, and in the surface and middle layers in Las Yeguas (Fig. 2a,b). In the absence of phytoplankton, HBP values decreased in both ecosystems regardless of the light treatments and depth, indicating a bacterial dependence on phytoplankton. In the treatments without phytoplankton, the negative UVR effect on HBP was suppressed only in surface layer of Las Yeguas (Fig. 2a,b).

Joint effects of UVR, nutrients, and temperature on carbon metabolism

In both lakes, a significant interactive (UVR × nutrients × T) effect on PP, EOC, and %EOC was found (Table 4). In La Caldera, UVR under $T_=$ exerted an inhibitory effect on PP regardless nutrient conditions (Fig. 3a; Table 5). Under UVR, T_+ stimulated PP, regardless the nutrient treatment (Fig. 3a), resulting in a decrease in $\%UVR_{inh}$ (Table 5). In Las Yeguas, UVR negatively affected PP under all experimental conditions (Fig. 3b; Table 5). Under ambient nutrient conditions, T_+ increased PP in the PAR treatment (Fig. 3b), accentuating the inhibitory effect of UVR (Table 5). With T_+ and UVR, nutrient addition stimulated PP (Fig. 3b).

In both lakes, under $T_=$, UVR resulted in lower EOC values than PAR both under NP-ambient and NP-added treatments (Fig. 3c,d). In La Caldera, under $T_=$, nutrient addition reduced EOC regardless the radiation treatment, whereas under T_+, nutrient addition decreased EOC under UVR but increased it under PAR (Fig. 3c). Thus, under T_+, nutrient addition resulted in an inhibitory UVR effect on EOC (Table 5). In Las Yeguas, T_+ increased EOC under UVR regardless of the nutrient treatment (Fig. 3d), causing either a stimulatory UVR effect under ambient nutrient conditions or eliminating the UVR inhibitory effect generated by nutrient addition under $T_=$ (Table 5).

In relation to %EOC, higher values were found in La Caldera than in Las Yeguas, particularly under ambient nutrient conditions where samples exposed to UVR reached values of

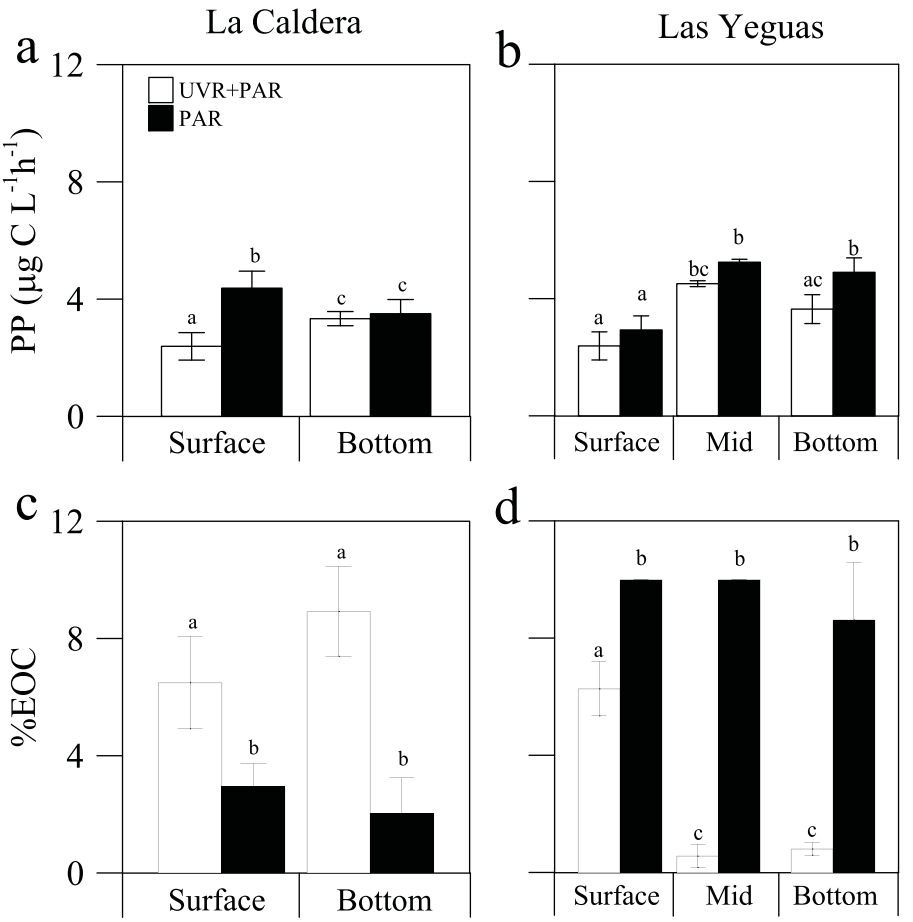

Fig. 1. Primary production (PP; in μg C L^{-1} h^{-1}) and percentage of excreted organic carbon (%EOC) under different sunlight quality (UVR + PAR vs. PAR) at different depths of water column in La Caldera (a, c) and Las Yeguas (b, d). Bars represent the mean values and error bars represent the standard deviation (SD; $n = 3$). Significant differences among treatments are denoted by different lower case letters.

62% or 97% under $T_=$ and T_+, respectively (Fig. 3e). In La Caldera, under $T_=$ and ambient nutrient conditions, UVR augmented %EOC values (Fig. 3e). Under UVR, nutrient addition decreased %EOC in both temperature treatments (Fig. 3e), whereas T_+ increased %EOC under ambient nutrient conditions (Fig. 3e). In Las Yeguas, under T_+ and nutrient-added conditions, UVR lowered %EOC (Fig. 3f), resulting in an inhibitory UVR effect (Table 5).

In contrast to PP, UVR, nutrients, and temperature were found not to have an interactive effect on HBP in any ecosystem (Table 4). Under $T_=$ and ambient nutrient conditions, UVR did not significantly affect HBP in any ecosystem (Fig. 4a,b). In La Caldera, nutrient addition reduced HBP values under UVR and $T_=$ but increased them under PAR and T_+ (Fig. 4a). Hence, nutrient addition resulted in an inhibitory UVR effect on HBP under both T treatments (Table 5). In Las Yeguas, under $T_=$, UVR slightly reduced HBP after nutrient addition whereas this effect was highly significant under T_+ (Fig. 4b).This, as in La Caldera, led to a inhibitory UVR effect under nutrient addition (Table 5).

With regard to BR, no significant interaction was found among radiation, nutrients, and temperature in any ecosystem (Table 4). In addition, no differences were found between treatments in the two ecosystems (Supporting Information Fig. 2).

Interactive effects of UVR, nutrient addition, and increased temperature on the commensalistic phytoplankton-bacteria relationship

The decrease of HBP in absence of algae proved indicative of bacterial dependence on algae in both lakes (*see* above); accordingly, the strength of phytoplankton-bacteria coupling, i.e., the capacity of the carbon released by algae to meet BCD is quantified through the BCD:EOC ratio (as a percentage). Our findings show that phytoplankton provided enough EOC to meet the bacterial demands, i.e., %BCD:EOC ratio <100 (Fig. 5a) in all treatments except those that were subjected to joint UVR and nutrient addition regardless of T in La Caldera or that represent the ambient conditions (UVR, NP-ambient, $T_=$) in Las Yeguas (Fig. 5b).

Discussion

Our results consistently showed that the bacterial production in the Sierra Nevada lakes is controlled mainly by the EOC release by algae on local and regional scales. These findings agree with the established paradigm of bacterial dependence on C supplied by phytoplankton in aquatic ecosystems (Cole et al. 1988), a topic currently under debate (*see* Fouilland and Mostajir 2010; Morán and Alonso-Sáez 2011). Furthermore, our results agree with the patterns established by Medina-Sánchez et al. (2010) of bacterial limitation mainly by carbon in Mediterranean oligotrophic lakes, and with previous observational (Carrillo et al. 2002) and experimental results (Medina-Sánchez et al. 2002, 2006) on seasonal and interannual scales showing a bacterial dependence on algal carbon in La Caldera lake. Based on these findings, we investigated how the strength of phytoplankton-bacteria coupling responded to the joint impact of UVR, nutrients, and temperature in current and expected future scenarios of global change. For this, we selected from our extensive study, two model lakes (La Caldera and Las Yeguas) which, being similar in maximum depth and transparency to PAR, differed in the mean temperature of the water column and their transparency to UVR.

Our first step was to evaluate the sensitivity of phytoplankton and bacteria to UVR and to establish the role of C release by phytoplankton modulating this bacterial sensitivity in the selected lakes. Phytoplankton, under ambient conditions (depth profile experiments) was more susceptible to UVR in La Caldera than in Las Yeguas despite that the mean irradiance during the experiment was higher in the Yeguas than in La Caldera. This higher sensitivity was reflected in higher %EOC values under UVR, supporting %EOC as a physiological stress indicator in ecosystems with high transparency to UVR (Carrillo et al. 2002, 2008). Nevertheless, we cannot exclude the possibility of an increase in %EOC due to an effect of UVR on phytoplankton C release associated with cell mortality (Agustí and Duarte 2013). However, bacterioplankton did not differ between lakes in their response

Table 3. Results from the two-way ANOVA of the interactive effect of UVR and depth for primary production (PP) and percentage of EOC (%) and from the three-way ANOVA of the interactive effect of radiation, depth, and presence of phytoplankton for heterotrophic bacterial production (HBP). Numbers in bold indicate significant effect on the variable considered.

			La Caldera		Las Yeguas	
	df1	df2	$F_{df1, df2}$	*p*	$F_{df1, df2}$	*p*
PP						
Depth	1	8	0.02	0.892	24.56	**0.000**
UVR	1	8	16.44	**0.004**	15.27	**0.001**
Depth × UVR	1	8	11.67	**0.009**	0.54	0.664
%EOC						
Depth	1	8	0.97	0.354	42.06	**0.000**
UVR	1	8	46.76	**0.000**	436.00	**0.000**
Depth × UVR	1	8	4.84	0.059	25.76	**0.000**
HBP						
Depth	1	16	22.68	**0.000**	285.27	**0.000**
Phytoplankton	1	16	26.07	**0.000**	2078.97	**0.000**
UVR	1	16	12.92	**0.002**	111.68	**0.000**
Depth × Phytoplankton	1	16	0.53	0.477	169.46	**0.000**
Depth × UVR	1	16	27.09	**0.000**	37.13	**0.000**
Phytoplankton × UVR	1	16	1.13	0.304	11.18	**0.003**
Depth × UVR × Phytoplankton	1	16	6.15	**0.025**	114.80	**0.000**

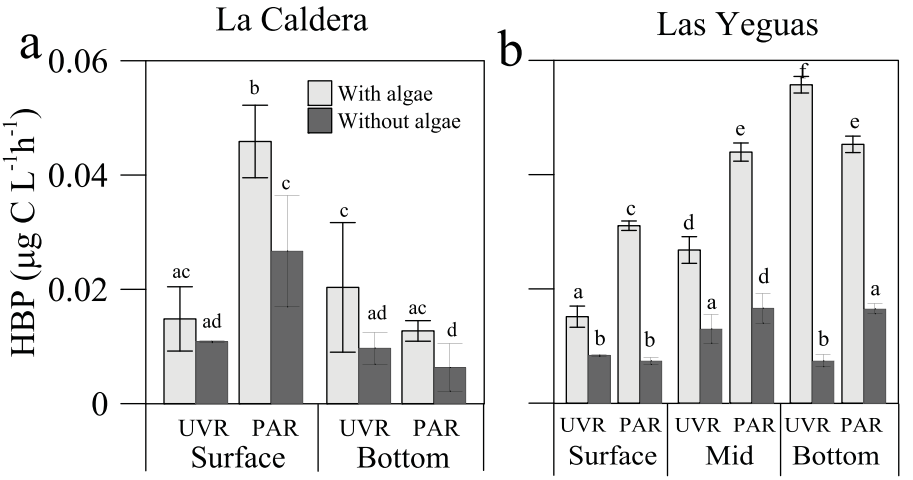

Fig. 2. Heterotrophic bacterial production (HBP; in μg C L^{-1} h^{-1}) under different sunlight quality (UVR + PAR vs. PAR) at different depths of water column in La Caldera (a) and Las Yeguas (b) in presence vs. absence of phytoplankton. Bars represent the mean values and error bars represent the standard deviation (SD; $n = 3$). Significant differences among treatments are denoted by different lower-case letters.

Table 4. Result of the three-way ANOVA of the interactive effect of radiation, nutrient-addition, and temperature (*T*). Numbers in bold indicate significant effect on the considered variable.

	PP		EOC		%EOC		HBP		BR	
	$F_{1,16}$	*p*	$F_{1,16}$	*p*	$F_{1,16}$	*p*	$F_{1,16}$	*p*	$F_{1,16}$	*p*
La Caldera										
UVR	278.18	**0.000**	94.61	**0.000**	47.96	**0.000**	10.68	**0.004**	1.09	0.313
Nutrients	7.58	**0.010**	19.23	**0.000**	93.08	**0.000**	0.17	0.683	0.01	0.929
T	44.73	**0.000**	24.79	**0.000**	1.68	0.214	4.39	0.052	0.38	0.547
T × UVR	29.34	**0.000**	11.21	**0.004**	0.31	0.588	1.25	0.279	0.00	0.983
T × Nutrients	2.35	0.145	4.38	0.053	17.40	**0.001**	14.51	**0.002**	0.16	0.699
UVR × Nutrients	4.03	0.062	34.70	**0.000**	89.43	**0.000**	3.66	0.074	2.63	0.124
UVR × Nutrients × *T*	5.91	**0.020**	31.20	**0.000**	33.01	**0.000**	0.12	0.736	0.47	0.502
Las Yeguas										
UVR	92.83	**0.000**	3.59	0.076	0.03	0.875	15.59	**0.001**	0.00	0.970
Nutrients	23.41	**0.000**	0.26	0.619	3.56	0.077	5.82	**0.028**	0.07	0.793
T	35.17	**0.000**	78.74	**0.000**	2.23	0.155	17.60	**0.001**	5.65	**0.030**
T × UVR	3.54	0.078	55.72	**0.000**	0.65	0.433	0.31	0.588	0.60	0.451
T × Nutrients	1.16	0.297	10.18	**0.006**	3.21	0.092	4.83	**0.043**	1.73	0.207
UVR × Nutrients	0.00	0.976	42.04	**0.000**	4.07	0.061	5.71	**0.029**	0.00	0.972
UVR × Nutrients × *T*	8.19	**0.011**	39.08	**0.000**	7.34	**0.015**	3.56	0.077	0.23	0.636

PP, primary production; EOC, excreted organic carbon; %EOC, percentage of excreted organic carbon; HBP, heterotrophic bacterial production; BR, bacterial respiration.

to UVR, and the removal of phytoplankton resulted in a noteworthy decrease in HBP, this being consistent with the findings of Aas et al. (1996) and Sommaruga et al. (1997), and supporting a bacterial dependence on C released by phytoplankton (Carrillo et al. 2002; Medina-Sánchez et al. 2002). Notably, in Las Yeguas in absence of phytoplankton, the bacterial sensitivity to UVR was masked in the surface layer, probably as result of a stronger C limitation to growth than in La Caldera, in line with the %BCD:EOC ratio (further discussion below).

In relation to the interactive effects of UVR, nutrients, and temperature on phytoplankton-bacteria coupling, our results show a decrease of the magnitude of response of PP and %EOC to UVR under increased nutrient availability and temperature in the colder and higher UVR-transparent lake (La Caldera) than in the warmer and less UVR-transparent lake (Las Yeguas). Thus, in La Caldera, the joint action of nutrients and warming alleviated the strong negative UVR effect on PP, but, contrarily, triggered a negative UVR effect on HBP (Table 5), which could be indirectly induced by competitive and commensalistic interactions with phytoplankton.

The boost in metabolic algal activity by the combined effects of nutrient and temperature under UVR was evidenced by the increase in the PP value and the reduction in EOC and %EOC values, reflecting the coupling between photosynthesis (^{14}C assimilation) and phytoplankton growth (Berman-Frank and Dubinsky 1999). The increase in *T* (*see* UVR × T_+ treatment) was the main factor that reduced the damaging UVR effect on PP in La Caldera, through the disposing of the surplus fixed C (EOC rate and %EOC). In fact, the involvement of glycolate metabolism (main constituent of EOC) has been described as mechanism to protect chloroplasts against photoinhibitory damage by the consumption of excess absorbed light energy (Kozlowska-Szerenos et al. 2000). This response could be interpreted as one possible pathway to maintain the Mehler reaction (Radmer and Kok 1976), leading to the reduction of an inhibitory UVR effect on PP. However, the nutrient addition played a crucial role in diminishing the extracellular carbon release, reaching values of lower than 20% and reflecting the balance between photosynthesis and algal growth (Berman-Frank and Dubinsky 1999). However, we cannot rule out the involvement in PP enhancement of potential repair processes, such as reparation of PSII through higher synthesis of the D1 protein, dependent on temperature (Bouchard et al. 2005; Sobrino and Neale 2007). In the warmer lake (Las Yeguas), greater PP occurred only with increased simultaneous nutrient and temperature, as reported by Degerman et al. (2013) suggesting colimitation. However, the increase in *T* alone (*see* UVR × T_+ treatment) accentuated the inhibitory UVR effect and augmented EOC rates. The contrasting responses to warming between the two ecosystems suggest that, in Las Yeguas, the rise in temperature (from 15°C to 20°C), reaching values far from those characterizing permanent high-mountain lakes of more than 5 m in depth (Bullejos et al. 2014), even in

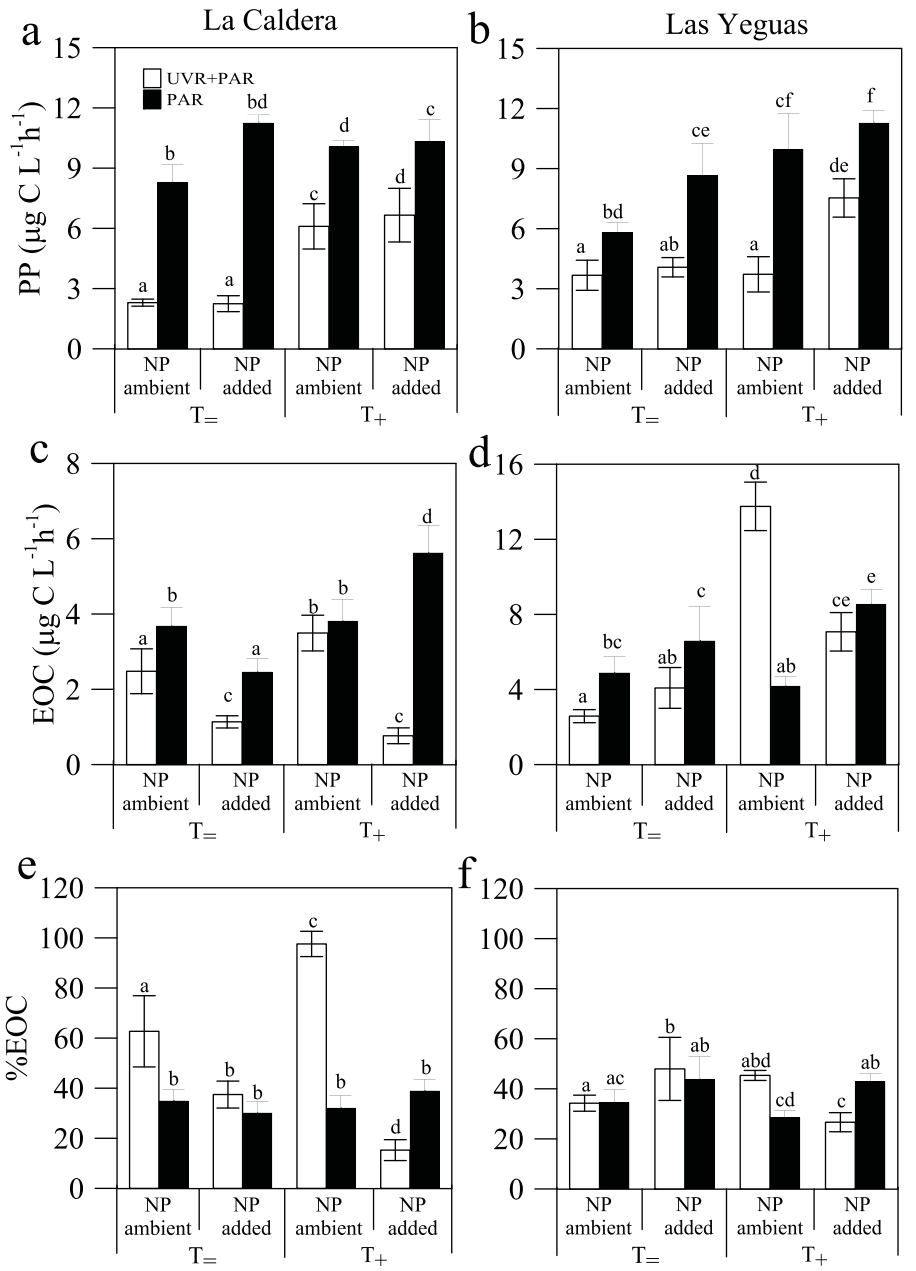

Fig. 3. Primary production (PP; in μg C L^{-1} h^{-1}), excreted organic carbon (EOC; μg C L^{-1} h^{-1}) and percentage of EOC (%EOC; as percentage) under different solar radiation (UVR + PAR vs. PAR), nutrient concentration (ambient vs. nutrient added), and temperature (ambient vs. increased) conditions in La Caldera (a, c, e) and Las Yeguas (b, d, f). Bars represent the mean values and error bars represent the standard deviation (SD; $n = 3$). Significant differences among treatments are denoted by different lower-case.

warmer dry periods (Villar-Argaiz et al. 2002), exceeded the optimum temperature for algal performance (Hoffman et al. 2003; Sobrino and Neale 2007), making the phytoplankton more susceptible to UVR.

Regarding bacterioplankton, under UVR, the generalized lack of positive response of HBP after nutrient addition and the increase in the negative UVR effect in both lakes, is consistent with the ability of phytoplankton to overcome bacteria in P-uptake (Duarte et al. 2000; Joint et al. 2002; Villar-Argaiz et al. 2002). Nevertheless, this negative phytoplankton effect on bacteria could be reinforced in La Caldera by the reported decrease in the extracellular matter released by P-enriched phytoplankton (Wang and Priscu 1994; Villar-Argaiz et al. 2002), which was not sufficient to satisfy the BCD, as indicated by the %BCD:EOC>100%.Therefore, in La Caldera, consistent with our initial hypothesis, the lower %EOC under the joint action of

Table 5. Percentage of UVR inhibition (UVR_{inh}) on phytoplanktonic and bacterial variables under the treatments indicated. Different superscript letters indicate significant differences based on *t*-test among the different treatments. ns, not significant, indicates that differences between UVR and equivalent PAR treatment for each nutrient or nutrient × temperature treatment were not found (LSD-test > 0.05).

	La Caldera					Las Yeguas				
Variable treatment	**PP**	**EOC**	**%EOC**	**HBP**	**BR**	**PP**	**EOC**	**%EOC**	**HBP**	**BR**
UVR	72^{a}	32^{a}	-79^{a}	ns	ns	36^{a}	47^{a}	ns	ns	ns
UVR × NP-added	80^{a}	53^{a}	Ns	45^{a}	ns	52^{ab}	38^{a}	ns	52^{a}	ns
UVR × T_+	39^{b}	Ns	-204^{b}	Ns	ns	62^{b}	-229^{b}	ns	ns	ns
UVR × NP-added × T_+	35^{b}	86^{b}	60^{c}	42^{a}	ns	33^{a}	ns	37^{a}	59^{a}	ns

PP, primary production; EOC, excreted organic carbon; %EOC, percentage of excreted organic carbon; HBP, heterotrophic bacterial production; BR, bacterial respiration.

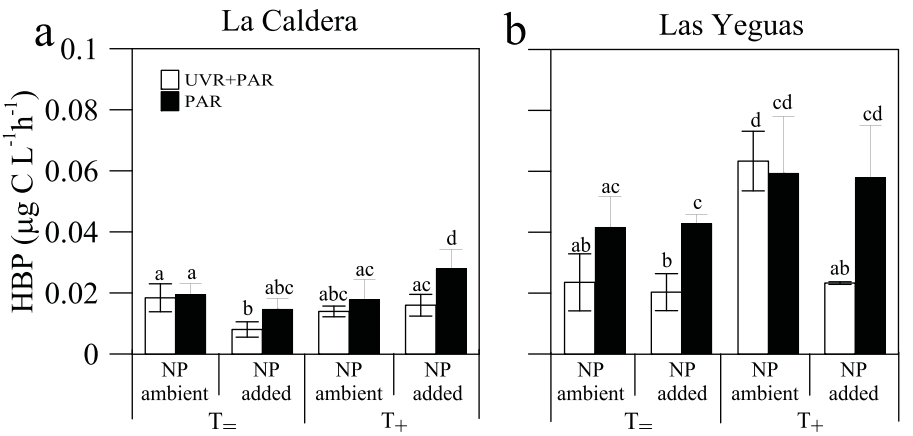

Fig. 4. Heterotrophic bacterial production (HBP; in μg C L^{-1} h^{-1}) under different solar radiation (UVR + PAR vs. PAR), nutrient concentration (ambient vs. nutrient added), and temperature (ambient vs. increased in) conditions in La Caldera (a) and Las Yeguas (b). Bars represent the mean values and error bars represent the standard deviation (SD; $n = 3$). Significant differences among treatments are denoted by different lower-case.

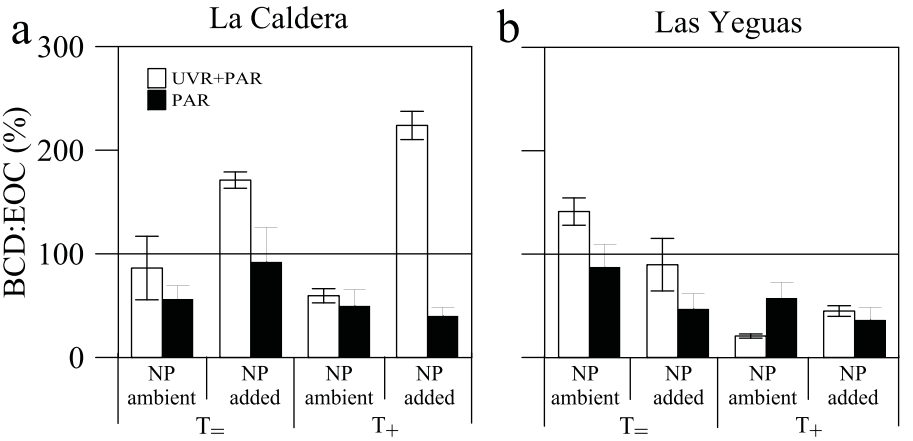

Fig. 5. Ratio (as percentage) between BCD and supply of carbon by algal excretion (EOC), measured under different solar radiation (UVR + PAR vs. PAR), nutrient concentration (ambient vs. nutrient added), and temperature (ambient vs. increased) conditions in La Caldera (a) and Las Yeguas (b). The line of 100% means carbon demand equals carbon supply. Bars represent the mean values and error bars represent the standard deviation (SD; $n = 3$) calculated with error propagation.

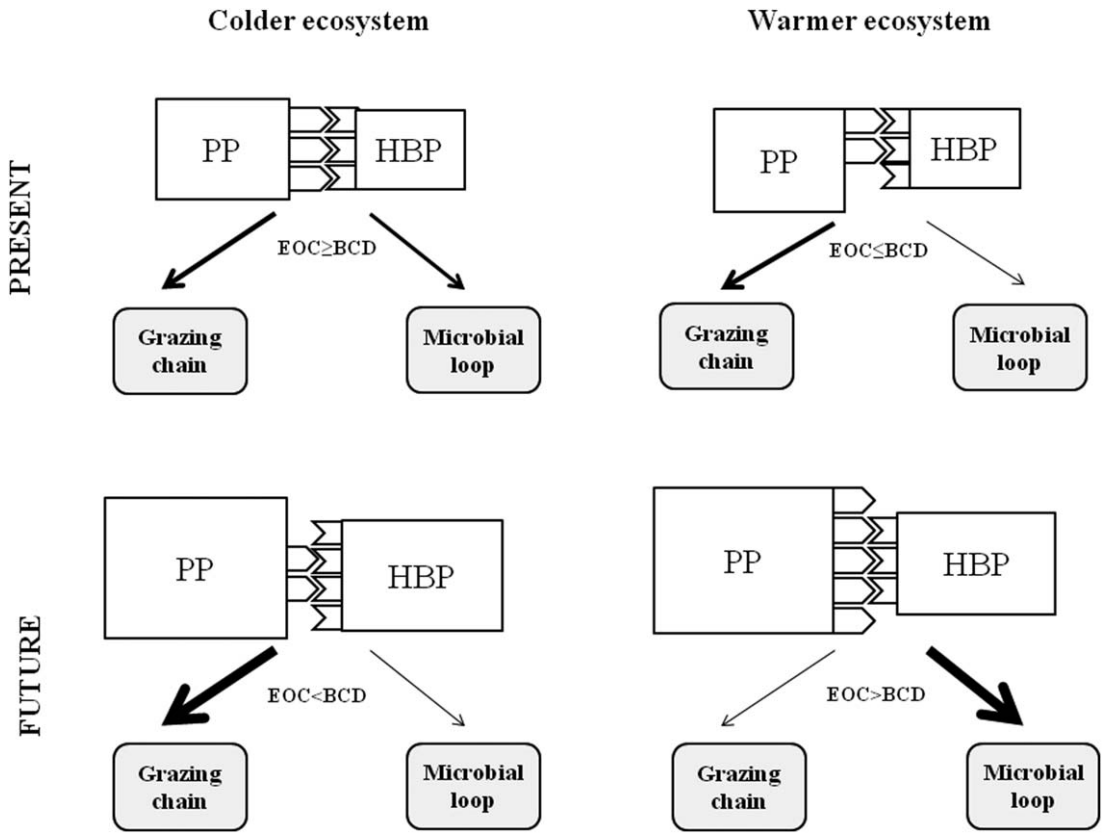

Fig. 6. Phytoplankton-bacteria coupling and development degree of microbial loop and grazing chain under current ambient and future conditions of global change (warming and nutrient loads). The set of arrows from each PP box indicates the magnitude of algae exudation (EOC) whereas the set of inverted arrows from each BP box represents the magnitude of the BCD. The sizes of the boxes and black arrows are proportional to the magnitude of the respective metabolic variables and development degree of each food web. PP: primary production; HBP: heterotrophic bacterial production.

UVR, nutrient addition, and increased *T* would promote a better coupling between photosynthesis and phytoplanktonic growth with a fall in absolute EOC values, leading to a weakened phytoplankton-bacteria coupling. By contrast, in Las Yeguas, there was enough EOC to meet BCD, suggesting that the increase in UVR_{inh} on HBP after nutrient addition might be an effect of UVR on HBP as a consequence of induced competition with phytoplankton for P, rather than through the shortage in the availability of carbon of algal origin.

Therefore, contrary to our hypothesis and regardless of the intrinsic mechanisms, in both lakes, simultaneous action of an increase in *T* and nutrients triggered a negative UVR effect, and resulted in a non-generalized stimulus of bacterial growth.

Implications

Our results indicate that in the coldest and most transparent lake, where a dependency of bacteria on EOC has been consistently evidenced (Carrillo et al. 2002; Medina-Sánchez et al. 2002, 2004), in a global-change scenario (i.e., increases in temperature and nutrient inputs), the strength of phytoplankton-bacteria coupling might be weakened. Consequently, the heterotrophic bacterial growth would be depressed, weakening the poorly developed microbial loop in this ecosystem (*see* Medina-Sánchez et al. 2004, 2013), (Fig. 6). Contrarily, in the warmer and less transparent lake, where phytoplankton-bacteria coupling is weak, warming and nutrient inputs would strengthen this coupling, leading to a higher C-flux through the microbial loop (Fig. 5), somewhat more developed in this ecosystem (Cruz-Pizarro et al. 1994). Although caution should be exercised in extrapolating results from short-term experiments to a long-term scale, our results show the initial steps of diverting trends of the commensalistic phytoplankton-bacteria coupling under changing multiple environmental conditions. Despite that high-mountain lakes have been considered rather homogeneous ecosystems subjected to extreme conditions, their trophic webs may change in response to environmental changes determined by slight differences of physical, chemical, and biological traits (Rose et al. 2009). Thus, even in nearby high-mountain lakes exposed to a similar abiotic environment and with simple planktonic communities, the joint impact of global-change stressors can lead to contrasting planktonic structure and functioning, i.e., a relative dominance of the grazing chain against the microbial loop.

This might have implications for C cycle, through the number of trophic levels involved in energy transfer from primary producers.

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