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Abstract: We evaluated the effect of photochemical alterations of chromophoric dissolved organic matter (CDOM) on bacterial abundance, activity and community composition in a coastal lagoon with high dissolved organic carbon concentration. On two occasions during the austral summer, filtered lagoon water was exposed to different regions of the solar spectrum (full solar radiation, UV-A + PAR, PAR) or kept in the dark. Subsequently, dilution cultures were established with bacterioplankton from the lagoon that were incubated in the pre-exposed water for 5 hours in the dark. Cell abundance, activity, and community composition of bacterioplankton were assessed before and after incubation in the different treatments. Changes in absorption, fluorescence, and DOC concentration were used as proxies for CDOM photoalteration. We found a clear CDOM photobleaching signal, as well as a stimulation of bacterial activity in the treatments pre-exposed to UV radiation, suggesting increased bioavailability of DOM. Bacterial community analysis by fluorescence in situ hybridization revealed that this stimulation was mainly accompanied by the specific enrichment of Alpha- and Betaproteobacteria. Thus, our results suggest that CDOM photoalteration not only stimulates bacterioplankton growth, but also induces rapid changes in bacterioplankton composition, which can be of relevance for ecosystem functioning, particularly considering present and future changes in the input of terrestrial CDOM to aquatic systems.

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Alteration of chromophoric dissolved organic matter by solar UV radiation causes rapid changes in bacterial community composition

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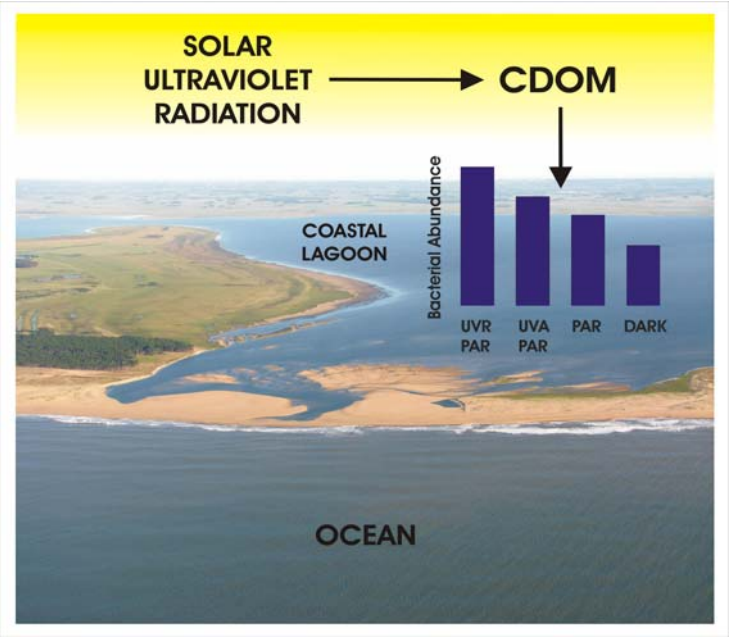

Photoalteration of chromophoric dissolved organic matter (CDOM) in a highly productive coastal lagoon of the Atlantic Ocean causes shifts in bacterial abundance and community composition

We evaluated the effect of photochemical alterations of chromophoric dissolved organic matter (CDOM) on bacterial abundance, activity and community composition in a coastal lagoon with high dissolved organic carbon concentration. On two occasions during the austral summer, filtered lagoon water was exposed to different regions of the solar spectrum (full solar radiation, UV-A + PAR, PAR) or kept in the dark. Subsequently, dilution cultures were established with bacterioplankton from the lagoon that were incubated in the pre-exposed water for 5 hours in the dark. Cell abundance, activity, and community composition of bacterioplankton were assessed before and after incubation in the different treatments. Changes in absorption, fluorescence, and DOC concentration were used as proxies for CDOM photoalteration. We found a clear CDOM photobleaching signal, as well as a stimulation of bacterial activity in the treatments pre-exposed to UV radiation, suggesting increased bioavailability of DOM. Bacterial community analysis by fluorescence in situ hybridization revealed that this stimulation was mainly accompanied by the specific enrichment of Alpha- and Betaproteobacteria. Thus, our results suggest that CDOM photoalteration not only stimulates bacterioplankton growth, but also induces rapid changes in bacterioplankton composition, which can be of relevance for ecosystem functioning, particularly considering present and future changes in the input of terrestrial CDOM to aquatic systems.

Introduction

Dissolved organic matter (DOM) is composed by different classes of carbon moieties, some of which strongly absorb solar radiation (i.e., chromophoric DOM or CDOM) and influence the transmission of ultraviolet radiation (UVR, 280-400 nm) and photosynthetically active radiation (PAR, 400-700 nm) into the water column. The absorption of UVR by CDOM produces structural changes to this pool that reduce its ability to further absorb, a process known as photobleaching (see reference 1 for a review). Furthermore, the sunlight-mediated conversion of CDOM into inorganic carbon, organic substrates (low-molecular-weight carbonyl compounds), and inorganic nutrients increases the turnover rates of CDOM in surface waters[2-5].

In coastal ecosystems, a large fraction of CDOM is derived from the decomposition of terrestrial vegetation and microbial degradation is accelerated when this pool is exposed to UVR[6]. In these ecosystems, the coupled photochemical and microbial degradation of DOM is thought to be an important sink for CDOM carried by rivers[7,8]. Several studies have shown that bacterial activity usually increases significantly after exposure of CDOM to solar radiation[4,9,10,11-13]. This increase in bacterial activity is driven by the formation of readily available low molecular weight organic photoproducts [1,9,14].

In shallow coastal lagoons, the zone exposed to solar UVR can constitute an important fraction of the water column and negative effects of UVR on planktonic and benthic communities have been reported[11,15]. Estuarine systems are characterized by the intense exchange of waters with different concentrations of CDOM[16]. The change in dominance of freshwater and marine water masses with high and low CDOM concentration, respectively, is the main process governing changes in underwater UV attenuation in those systems[17,18].

Several studies have addressed the effect of DOM photodegradation on bacterial activity and growth efficiency[14,19,20], however, very little is known about whether the composition of the bacterial community is also affected by this process. For example, Abboudi *et al*.[21] using 16S rDNA and rRNA-based capillary electrophoresis single strand conformational polymorphism

(CE-SSCP) assessed changes in bacterial diversity due to phototransformation of DOM and found differences in the pattern and number of ribotypes. However, they did not identify the affiliation of the bacteria in the different light treatments[21].

In the present study, we tested the idea that CDOM photoalteration favors specific bacterial taxa that are able to respond rapidly to changes in the bioavailability of organic substrates. Thus, we assessed how CDOM exposure to different regions of the solar spectrum affects bacterioplankton activity and community composition in the freshwater zone of a very productive coastal lagoon with high dissolved organic carbon (DOC) concentration. Our strategy was to assess those potential changes in bacterial community composition in the short-term (hours) by a quantitative approach (fluorescent in-situ hybridization with specific probes that target the most common bacterial groups in aquatic ecosystems) that allows for establishing how significant those changes are.

Materials and Methods

Study site

Laguna de Rocha is a highly productive shallow coastal lagoon (mean depth = 0.6 m, area = 72 km^2) influenced by freshwater and marine intrusions. It is located on the south-eastern coast of South America (34° 33' S, 54° 22' W). The system communicates with the sea through a single-mouth inlet on a sand barrier that opens naturally (occasionally by human action) when the water depth is higher than ~1.4 m depth. When the sand bar is opened, the marine intrusion progress gradually and segregates a zone with brackish water and high PAR penetration, and a zone that usually remains limnic and is characterized by its low transparency and high nutrient concentration[18].

Sampling and water analyses

The northern zone of the lagoon was sampled in January and February 2004. Water samples from the upper 50 cm were collected with a 5 L sampler and basic parameters such as

conductivity and temperature were measured *in situ*. For the analysis of DOC concentration and optical properties of CDOM, samples were filtered on the same day (within 6 h) through 0.2 μm polycarbonate filters (Millipore filters), previously soaked overnight in 10% HCl and rinsed with 20 ml of Milli-Q water. Filtered water was stored in clean, precombusted glass bottles (Teflon-capped) and kept in the dark at -20 ºC for DOC analysis or at 4 °C for the characterization of CDOM (absorption and fluorescence).

The concentration of DOC was measured using a high temperature catalytic oxidation method (Shimadzu TOC analyzer model 5000). Previously to the analysis, the samples were thawed at room temperature and sonicated for 1 min at 13 W. The instrument was equipped with a Shimadzu platinized-quartz catalyst for high sensitivity analysis. Vials for DOC estimation were also precombusted as described above for other glass material. Three to five injections were analyzed for each sample and blank (Milli-Q water). Blank values (0.03-0.05 mg DOC L^{-1}) were substracted from the average DOC values of the samples. Calibration of the instrument was done with potassium hydrogen phthalate (four points calibration curve). A consensus reference material (CRM) for DOC (batch 5 FS-2005: 0.57 mg C L^{-1}) provided by RSMAS/MAC, University of Miami) was run in parallel. Results differed from the CRM given value by 5% and the coefficient of variation was better than 2%.

CDOM absorbance was measured at room temperature in a double-beam spectrophotometer (Shimadzu UV1603) between 280 and 750 nm using acid-cleaned 5 cm fused silica cuvettes (Suprasil I) previously rinsed with Milli-Q water and twice with the filtered sample. Milli-Q water of low DOC concentration (0.05 mg L^{-1}) was used as a reference. Absorption coefficients (a) were calculated as: $a = 2.303\ D/l$, where D is absorbance and l is the pathlength of the cuvette in meters. Correction for the scattering caused by particles was done by subtracting the absorbance value at 690 nm. For comparative purposes, we used the absorption value at 320 nm (a_{320}). The CDOM absorption at 320 nm was also normalized to the DOC concentration (a^*_{320}).

Samples for fluorescence measurements were filtered as for DOC and the filtrates collected in 20 mL vials (acid-washed, gently rinsed with Milli-Q, and pre-combusted as above). Fluorescence was measured at room temperature in an AMINCO Bowman Serie 2 spectrofluorometer (excitation at 355 nm, emission at 450, slit width of 5 nm) using a 1 cm quartz cuvette, pre-rinsed gently with Milli-Q water and with the filtered sample. Fluorescence standardization was done by normalizing the signal (main peak height at 430–450 nm) to the fluorescence peak height of a solution of quinine sulphate 0.01 mg L^{-1} in 1 N H_2SO_4 defined as 10 standardized fluorescence units (S.FL.U.): (Fs/Fqs) x 10 = $Fs(\lambda)$, where Fs is the standardized fluorescence, Fs is the fluorescence of the sample, Fqs is the fluorescence of the quinine sulphate, and λ is the excitation wavelength at 355 nm. To account for potential instrumental drift, separate quinine sulphate spectra were acquired on each day of measurement.

Experimental design

Two experiments were done during the austral summer of 2004 (January and February). To assess DOM photoalteration, the lagoon water was double-filtered as described above for DOC, but under sterile conditions (i.e., in a laminar flow) and kept in the dark at 4 ºC until the experiment was set up (within 24 h). The filtered water was distributed in spherical quartz bottles of 100 mL (diameter: 5 cm) that were previously acid-washed, rinsed with Milli-Q water, and muffled at 500 ºC for 1 h. Then, the bottles were placed in a circulating water bath at *in situ* temperature (24-25 ºC) and were rotated regularly during exposure. Triplicates bottles were exposed to natural solar radiation during 6 days using different filters to exclude different regions of the solar spectrum. Quartz bottles with no additional spectral filters were used for the 'full spectrum' treatment and those used as dark control were wrapped with three layers of aluminum foil. The bottles in the PAR treatment were wrapped with one layer of a vinyl chloride foil (CI Kasei Co., Tokyo, Japan, 50% transmittance at 405 nm) to exclude the UVR. To differentiate between UV-B (280-315 nm) and UV-A (315-400 nm) effects, quartz bottles were wrapped with one layer of Mylar-D (150 µm thickness, 50% transmission at 325 nm) to exclude

the UV-B. Changes in the transmittance of the different foils were measured in a double-beam spectrophotometer, and foils were replaced, if necessary.

To prepare the bacterial inoculum, water from the same freshwater zone of the lagoon was collected on the sixth day of exposure and filtered through a glass-fiber filter with a 'pore size' of 1.2 μm (Whatman GF/C). Then, 10 ml of the filtrate were added to each bottle containing the water from the different treatments (1:10 dilution). Bacterial abundance, community composition, and activity were determined before the GF/C filtration and in the filtrate at time zero (i.e., before the inoculation into each treatment). Potential bacterial contamination in the pre-exposed filtered lagoon water proved negative as determined by inspection of samples from additional bottles (i.e., only used for this purpose) by epifluorescence microscopy (see below).

After 5 h incubation in the dark, bacterial abundance, activity, and community composition in each treatment were again assessed. This incubation time was chosen in order to minimize the effect of confinement on bacterial growth[22], and was based on previous data on bacterial activity for this lagoon, which is very high in summer[23].

Radiation measurements

Irradiances in the UV-A and UV-B wavebands were measured *in situ* with an International Light radiometer (IL-1400A) consisting of the broadband SUL033/W (UV-A) and SUL240/W (UV-B) sensors. Incident radiation for UV-B and UV-A was integrated for the time of exposure.

Bacterial abundance

Water samples were fixed with paraformaldehyde (1% final concentration), kept in the dark at room temperature for 1 h, and then overnight at 4 ºC. Total bacterial abundance was determined by epifluorescence microscopy after staining the samples with the fluorochrome 4', 6-diamino-2-phenylindole staining (DAPI, final concentration 1 μg mL^{-1}) as previously described[24]. After staining, samples were filtered onto polycarbonate filters (type GTTP; diameter, 25 mm; pore size, 0.2 μm; Millipore) using a cellulose nitrate filter (pore size, 0.45 μm; Sartorius) as support to optimize the distribution of cells on the filters. Bacterial enumeration was done by

epifluorescence microscopy using a Zeiss Axioplan II Imaging epifluorescence microscope (Carl Zeiss, Jena, Germany).

Bacterial activity

Bacterial bulk activity was estimated following the incorporation of [^{3}H]-L-leucine (Amersham, Little Chalfont, England) into bacterial biomass. Radiolabelled leucine (specific activity 173 Ci/mmol) was added at saturating concentrations (20 nM) to triplicate sub-samples and to one formaldehyde-killed control (3% final concentration) from each water treatment. The treatments were incubated in a water bath in the dark at the *in situ* temperature (24-25 ºC) for 1 h and fixed by the addition of formaldehyde (3% final concentration). Macromolecule extraction was done with cold TCA and cold ethanol[25]. Measurements of [^{3}H]-L-leucine incorporation were done in a Beckman LS5000TD liquid scintillation counter (Beckman, Fullerton, CA). Values were corrected for quenching (external standard method) and by subtraction of counts from the controls.

Bacterial community composition

Fixed bacteria were collected by filtration onto white 0.2 μm pore size polycarbonate filters, rinsed twice with 1x phosphate-buffered saline and once with deionized water, and then the filters were stored at -20 ºC. FISH with horseradish-peroxidase labelled probes and tyramide signal amplification (CARD-FISH) was performed as described previously[26] using a modified permeabilization protocol[27]. The following bacterial group-specific oligonucleotide probes (ThermoHybrid, Germany) were assayed: ALF968[28] (Alphaproteobacteria), BET42a[29] (Betaproteobacteria) GAM42a[29] (Gammaproteobacteria), CF319a[30] (originally described to target the phylum Cytophaga-Flavobacter-Bacteroides, but now only *Cytophaga*-like within the Bacteroidetes), and HGC69a[31] (Actinobacteria), as well as the probe EUB I-III for the Bacteria domain[32]. To determine the relative abundance of each targeted group, bacterial counting was done by epifluorescence microscopy and a semi-automated counting system[33].

Statistical analyses

One-way analysis of variance (ANOVA) was performed to test the significance of the differences observed in bacterial abundance, activity, and relative abundance of different phylogenetic groups among irradiation treatments after checking for homogeneity of variance and normal distribution. For all parameters, the average values were calculated from the three replicates in each treatment. Post-hoc comparisons were done with the Scheffé test. Differences were considered significant when $p < 0.05$. All statistical analyses were performed with Statistica 5.0 (StatSoft Inc.).

Results

Incident radiation and optical characteristics of CDOM

Values of incident integrated UV-B and UV-A radiation were 5.39 J cm^{-2} and 260.25 J cm^{-2}, respectively in January and 2.96 J cm^{-2} and 163.29 J cm^{-2} in February. Water temperature in both experiments was 24-25 ºC. Conductivity increased from 0.54 mS cm^{-1} in January to 13.11 mS cm^{-1} in February.

In January, the *in situ* (*i.e.,* before exposure) absorption coefficient (a) for CDOM was higher than in February (Table 1) coinciding with the lower water conductivity value. In January, a significant reduction in CDOM fluorescence (F) by 42 and 44% in the UVR+PAR ('full spectrum') and UV-A+PAR (Mylar) treatments, respectively, was observed (Table 1). Absorption at 320 nm and DOC-specific absorption at 320 nm (a^*_{320}) decreased after exposure to UVR+PAR and UV-A+PAR (Table 1). In addition, the DOC content in the UVR+PAR (17.7 mg C L^{-1}) and in the UV-A+PAR (17.9 mg C L^{-1}) treatments was significantly different from that of the dark control (21.7 mg C L^{-1}, $p < 0.05$). In addition, the DOC concentration in the UVR+PAR treatment was significantly lower than in the PAR one ($p< 0.05$), suggesting that the observed decrease in a, a^*_{320}, F, and DOC in January was mostly due to the UVR, as values for PAR were not significantly different from those in the dark control. Within the UVR, the largest photoalteration (i.e., photobleaching and DOC loss) effect on CDOM was caused by UV-A radiation.

In February, photobleaching was principally caused by UV-B radiation as suggested by the more pronounced decrease in *F* in the UVR+PAR treatment (56.3% compared to initial *F*) and the decrease in DOC-specific absorption (17.8% related to the initial) (Table 1). There was also a significantly decrease of *F* in the UVR+PAR treatment compared to the dark control (56.3%, Table 1). However, similarly to the experiment in January DOC photodegradation was mainly caused by the exposure to UV-A radiation. In this experiment, the initial a^*_{320} was only 35.9% of that estimated in January (Table 1).

Bacterial response to sunlight-induced changes in DOM

In both experiments, bacterial abundance significantly increased in all treatments after 5 h of incubation in the dark ($p < 0.05$) (Fig. 1), however, no significant differences between the three treatments were found. In January, bacterial abundance was highest in the UV-A+PAR treatment, but it was not significantly different from the dark control ($p = 0.057$), whereas in February, bacterial numbers significantly increased in the UVR+PAR treatment compared to the control ($p = 0.02$). After 5 h of incubation in the dark, bulk bacterial activity in the January experiment significantly increased in the treatment where CDOM was pre-exposed to UV-A+PAR ($p = 0.01$ compared to the dark control) (Fig. 2A). In February, bulk bacterial activity was clearly stimulated in the treatments where CDOM was pre-exposed to UVR, but it was significantly different from PAR (ANOVA, $p = 0.028$) and the dark control (ANOVA, $p = 0.020$) only in the UVR + PAR treatment, though the largest increase was observed when comparing the PAR and UV-A + PAR treatments (Fig. 2B).

A marked increase in the detection level with the Eub I-III probe (domain Bacteria) was observed in both experiments. In January, this increase ranged from 50% at the beginning to 90.9 ± 0.74% of the DAPI-stained objects after 5 h of incubation (average ± 1 SD for all treatments), whereas in February ranged from 50% to 82.1 ± 5.2% (average ± 1 SD for all treatments).

In January, Beta- and Gammaproteobacteria were significantly enriched compared to the initial abundances ($p < 0.05$) (Fig. 3A). Betaproteobacteria was the only group showing

significant differences among treatments ($p = 0.017$), specifically between UV-A+PAR and the dark control ($p = 0.025$), between UV-A+PAR and PAR ($p = 0.035$), and between both UV treatments ($p = 0.02$) (Fig. 3A). In the February experiment, the fractions of Beta- and Gammaproteobacteria again significantly increased in all treatments followed by Alphaproteobacteria that increased in the UV treatments (Fig. 3B). Significant differences among treatments were found for Alphaproteobacteria ($p = 0.002$), Betaproteobacteria ($p = 0.03$), and Gammaproteobacteria ($p = 0.02$) (Fig. 3B). The UVR+PAR treatment showed a significantly higher abundance of Alphaproteobacteria compared to PAR ($p = 0.007$) and the dark control ($p = 0.008$) (Fig. 3B). Significantly higher abundances of Betaproteobacteria were found in UVR+PAR than in the PAR ($p = 0.015$) and the dark control ($p = 0.03$) (Fig. 3B), and in UV-A+PAR compared to PAR ($p = 0.03$). For Gammaproteobacteria the trend was the opposite, so this group was extremely abundant in the PAR treatment and dark control reaching ~ 60% of the bacterial community and being significantly higher than in UVR+PAR ($p = 0.02$). The same was observed for the *Cytophaga*-like group, which showed a higher proportion of hybridized cells in the control than in the other treatments, being significantly different when compared to the PAR treatment ($p<0.05$) (Fig. 3B). Abundances of Actinobacteria were under the detection limit of CARDFISH in both experiments.

Discussion

In this study, we found a clear photoalteration effect on CDOM when water from this coastal lagoon was exposed to solar radiation. CDOM photoalteration (i.e., DOC loss and photobleaching) was accompanied by a significant stimulation of bacterial activity. Stimulation of bacterial activity after CDOM exposure to solar UVR has been described for different aquatic ecosystems and seems to be related to the increased availability of carbon substrates[9,34]. For example, Wetzel *et al.*[10] found that plant-derived DOM exposed to natural solar radiation stimulated bacterial growth through the progressive release of several short-chain fatty acids.

One novel finding of our study, however, was the very rapid (5 h) change in bacterial community composition observed in the experiments where CDOM underwent UVR-induced transformations.

DOM photoalteration

In January, the CDOM content of the lagoon water was higher than in February (Table 1) and the reduction in DOC concentration after UVR+PAR and UV-A+PAR exposure indicated direct photodegradation. The fact that the change in absorption and fluorescence between the UVR+PAR and UV-A+PAR treatments (Table 1) was not significantly different indicates a higher contribution of UV-A radiation. This is in agreement with the photobleaching model reported by Reche *et al*.[35] for lacustrine systems. These authors reported that UV-A is the relevant waveband for photobleaching in lakes with DOC concentrations >3 mg L^{-1}, as found in Laguna de Rocha[18] (Table 1). However, in February, UV-B radiation had a stronger photobleaching effect than UV-A as indicated by the strong decrease in a_{320} in the UVR + PAR treatment though this effect was less pronounced when considering fluorescence (Table 1). Interestingly, DOC loss was again mainly caused by UV-A radiation and it was less pronounced than in January, which is coincident with the lower integrated UVR registered in February. The discrepancy between the wavebands causing the reduction in DOC concentration and the loss of chromophores and fluorophores in February may reflect a change in the CDOM pool associated to the intrusion of brackish water (conductivity: 13.11 mS cm^{-1}). This change is clearly visible when the values of a^*_{320} (Table 1) are compared and suggests that the CDOM in January (i.e., higher CDOM a_{320} and a^*_{320}) was more terrestrially-derived. It is arguable that the CDOM pool in February had different types of chromophores to that in January, but the methods used here do not allow for this comparison. Nevertheless, during marine intrusions as occurred in February in this lagoon, changes in underwater optical characteristics and in composition of CDOM are typically observed[15,18]. In fact, the brackish area of Laguna de Rocha is characterized by low DOC concentrations and high UV penetration[15,18].

Effect on bacterial abundance and activity

The observed increase in bacterial abundance and activity in all treatments and in the dark control during both experiments (Fig. 1 and 2) suggest that bacterial growth was stimulated by the dilution of bacteria into an environment enriched in bioavailable DOM, but also free of grazers. Dilution cultures have been used to study bacterial growth rates *in situ*[36,37], though there is evidence that dilution of the inoculum rapidly activates bacterial growth[22]. In addition, in both experiments, it was clear that pre-filtration of the lagoon water to prepare the inoculum (GF/C filtration) caused a strong reduction in bacterial activity suggesting a relevant role of particle-attached bacteria in this system.

The significantly higher stimulation in the incorporation of leucine observed in January in the UV-A+PAR treatment (Fig 2 A) suggests that photodegradation of CDOM by UV-A radiation caused a positive feedback on bacterial activity. Similarly, in February the strongest stimulation of bacterial activity was observed in the treatment where CDOM was pre-exposed to UV-A + PAR, though only in the UVR + PAR treatment it was significantly different from the control (Fig. 2 B). This pattern is coincident with the significant reduction in DOC concentration caused by pre-exposure to UV-A in both experiments (Table 1).

The stimulation of bacterial activity after irradiating CDOM has been described by several authors for different aquatic ecosystems [9,10,34]. For example, McAllister *et al*.[38] studying the effect of CDOM photodegradation on bacterial community along the York River estuary, USA, found a significant increase in bacterial activity in treatments with water collected from the freshwater zone of the estuary. This stimulation is attributed to the increase in the bioavailability of simple organic substrates after CDOM exposure to UVR[9,10]. However, Abboudi *et al*.[21] observed a decrease by 20 to 40% in bacterial production and respiration when they incubated a bacterial community from a eutrophic lagoon in pre-irradiated CDOM from the same lagoon. These contrasting results suggest that the change in bacterial activity by photodegraded CDOM depends on the quality and origin of this pool and may be different for different ecosystems[14,21].

In fact, Obernosterer *et al*.[19] found that seawater DOM that was initially bioreactive turned biologically more recalcitrant following exposure to solar radiation, while DOM of initially low bioavailability was rendered more bioavailable by photochemical processes[19].

The fast generation times of the bacterial community observed in our study, i.e., ca. 67 min in the UV-A+PAR treatment in January and 89 min in the UVR+PAR treatment in February, were similar to those observed for marine isolates grown in artificial media with low organic carbon content (<5 mg L^{-1})[39]. This suggests that the community growing in our experiments was composed by the so-called "eutrophic" bacterial species[40]. Pinhassi and Hagström[41] reported that this bacterial strategy might be dominant in some habitats such as brackish waters. Laguna de Rocha supports bacterial production rates similar to those found for eutrophic water systems[23], which together with the high DOC content measured in this study could account for the high bacterial growth rates observed. When we calculated the amount of carbon (C) incorporated per cell during 5 h, we found an average of 17.4 (± 1.9) and 11.2 (± 2.5) fg C per cell for the first and second experiment, respectively. According to Simon and Azam[42] bacteria with cell volumes between 0.026 and 0.4 μm^3 have a cell-specific C-content from 10.4 to 53.3 fg, respectively. Hence, the carbon incorporated by the bacterial community during our experiments agreed with that expected on theoretical grounds[42].

Effect on bacterial community composition

In both experiments, CARD-FISH detection levels after 5 h of incubation were higher in all treatments than in the inocula, which coincided with the observed increase in bacterial activity (Fig. 2 and 3). The proportions of cultivable cells[43] and of cells with high metabolic activity typically increase during confinement experiments[14,44,45]. Prefiltration and confinement of bacterioplankton during enrichments[46], dilutions[22], and incubation in mesocosms[47,48] can result in changes of taxonomical composition of bacterial communities[49,50]. This was also observed in our experiments, where the relative abundance of Gammaproteobacteria increased particularly in all treatments including the control (Fig. 3 A and B). Enrichment of members of

Gammaproteobacteria are usually observed during incubations of marine waters[22,48,49,51] and in our study, it was probably consequence of the exclusion of predators by filtration. However, the fact that the increase was less pronounced under UVR, particularly during the experiment in February (Fig. 3 B), suggests that members of this group were negatively affected by the photochemical alteration of CDOM or most probably, were outcompeted by other bacterial groups. In fact, similar experiments but in an alpine lake have indicated that members of Betaproteobacteria rapidly outcompete other bacterial groups[14]. Thus, it seems arguable that Gammaproteobacteria are at disadvantage when CDOM is photoaltered and changes in availability and substrate quality take place. However, the broad target of the probe used in our study does not allow for making generalizations.

Abboudi *et al*.[21] also found that CDOM exposed to sunlight caused changes in bacterial community structure in three contrasting coastal sites in the Northwestern Mediterranean Sea. However, they did not identify which bacterial groups or populations were enriched. Our experiments in Laguna de Rocha showed that the most significant change occurring within the bacterial community in relation to the pre-exposure of CDOM to UVR was that of Beta- and Alphaproteobacteria (Fig. 3). Similarly, in a study assessing the enrichment of bacterial populations in DOC-enriched water from a bog lake, stimulation of Betaproteobacteria growth was observed[52]. As suggested by several authors[14,52], it is possible that members of the Betaproteobacteria are well adapted to abrupt changes in substrate availability responding rapidly to conditions such as those we had in our experiments. In particular, this change was pronounced in February, when Betaproteobacteria represented a low percentage of the DAPI-stained cells at T_0 (Fig. 3 B). By contrast, at T_0 in the January experiment, Betaproteobacteria were dominant together with Alphaproteobacteria (Fig. 3 A). In a previous study about the microbial community composition of Laguna de Rocha[23], the dominant bacterial group in both regions (i.e., freshwater and brackish) of the lagoon during an annual study was the

Alphaproteobacteria. Our results extend these findings in that also *Betaproteobacteria* can occur in high relative abundance in this lagoon.

In summary, our results illustrate that UVR promotes the alteration of CDOM, which in turn stimulates growth of particular groups within the bacterial community such as Betaproteobacteria and Alphaproteobacteria. This suggests that photoalteration of CDOM is another factor to take into account when assesing temporal changes in bacterial community composition. The relevance of this process will depend on the hydrological regime, which also strongly influences bacterial dynamics in this type of coastal ecosystem[23]. Finally, our results are important considering present and future changes in the input of terrestrial CDOM to aquatic ecosystems. In connection to this, a major task is still the identification of the bacterial 'species' responsible for the rapid shift observed in community composition.

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Table 1 Optical characteristics of CDOM and DOC concentration in the initial sample and after exposure for 6 days to PAR, PAR + UV-A, PAR + UVR or kept in the dark. a_{320}: absorption coefficients at 320 nm; *F*: fluorescence in quinine sulfate units (QSU). DOC: dissolved organic carbon,; a^*_{320}: DOC-specific absorption at 320 nm. Values are the average of three replicates for each treatment ± 1 SD.

Experiment		**Initial**	**UVR+PAR**	**UV-A +PAR**	**PAR**	**Dark**
January	a_{320} (m^{-1})	53.7	40.4 ± 4.5	43.2 ± 3.6	53.4 ± 3.7	53.8 ± 4.6
	F (QSU)	9827	5659 ± 383	5492 ± 207	9660 ± 320	9994 ± 392
	DOC (mg C L^{-1})	21.3	17.7 ± 0.6	17.9 ± 1.4	20.4 ± 1.9	21.7 ± 0.1
	a^*_{320} (m^{-1} [mg C L^{-1}]$^{-1}$)	2.51	2.28	2.40	2.62	2.48
February	a_{320} (m^{-1})	16.5	12.9 ± 1.3	16.1 ± 2.6	16.4 ± 2.5	16.8 ± 3.0
	F (QSU)	5326	2326 ± 258	3826 ± 180	4993 ± 405	5320 ± 320
	DOC (mg C L^{-1})	18.3	16.4 ± 2.5	16.7 ± 0.4	18.4 ± 0.9	18.0 ± 1.4
	a^*_{320} (m^{-1} [mg C L^{-1}]$^{-1}$)	0.90	0.74	0.96	1.00	0.93

Legend to figures

Fig. 1 Bacterial abundance (cells mL^{-1} x 10^6; average ± 1 SD) measured at T_0 and after 5 h incubation in each treatment for January (A) and February (B). Letters over the bars indicate those treatments showing significant differences.

Fig. 2 Bacterial activity (pM [^{3}H]-L-leucine h^{-1}; average ± 1 SD) in January (A) and February (B) for each water treatment. Bacterial activity *in situ* and after GF/C filtration (T_0) are also shown (same unit as in the main graphs). Bars showing the same letters indicate those treatments where significant differences were found.

Fig. 3 Bacterial community composition (CARD-FISH) in the different treatments of both experiments (% of DAPI-stained objects; average ± 1 SD). Assayed probes: BET42a (Betaproteobacteria), Alf968 (Alphaproteobacteria), Gam42a (Gammaproteobacteria), CF319a (*Cytophaga*-like). Panel A, January; B, February. Significant differences between treatments are indicated by asterisks. Bars belonging to each probe showing the same letters indicate those treatments where significant differences were found for those bacterial groups.

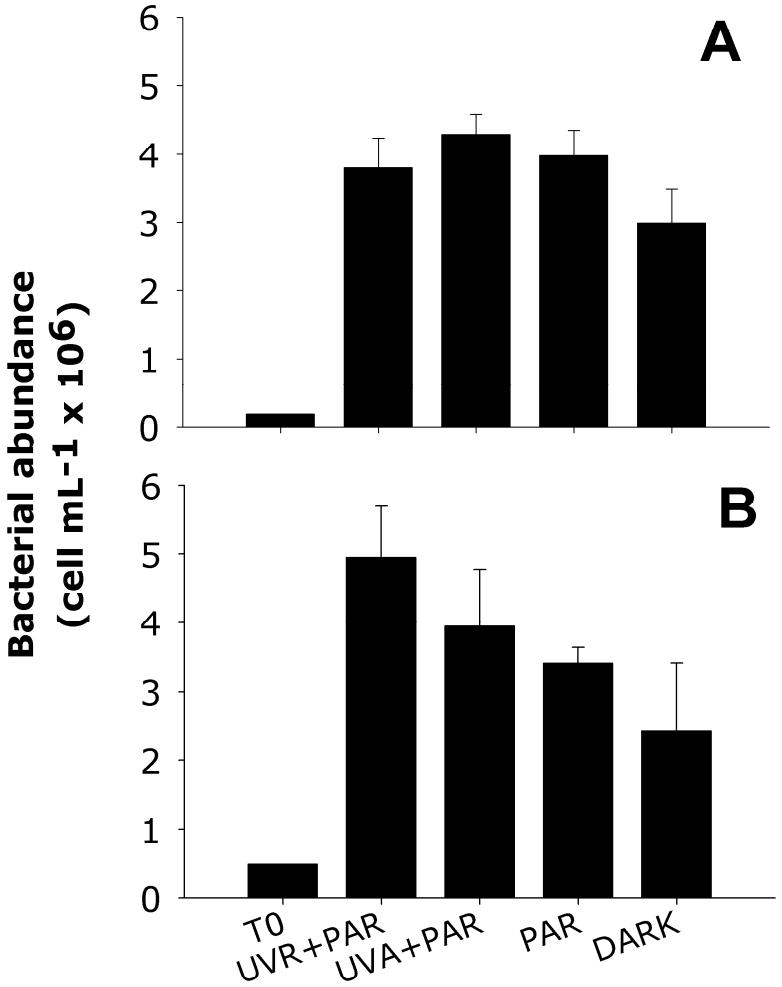
A
B
Bacterial abundance
(cell mL-1 x 10^6)
0
1
2
3
4
5
6
T0
UVR+PAR
UVA+PAR
PAR
DARK

Fig. 1

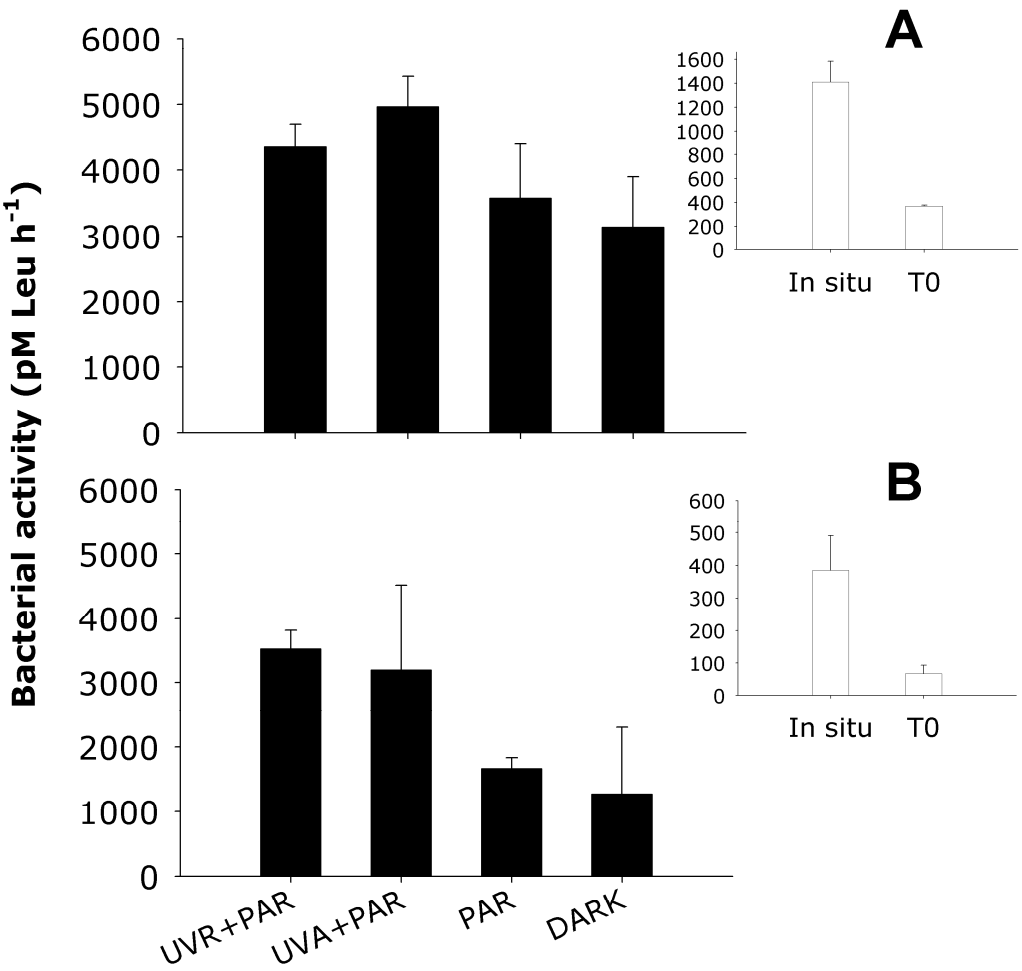
Bacterial activity (pM Leu h-1)
6000
5000
4000
3000
2000
1000
0
A
1600
1400
1200
1000
800
600
400
200
0
In situ
T0
B
600
500
400
300
200
100
0
In situ
T0
UVR+PAR
UVA+PAR
PAR
DARK

Fig. 2

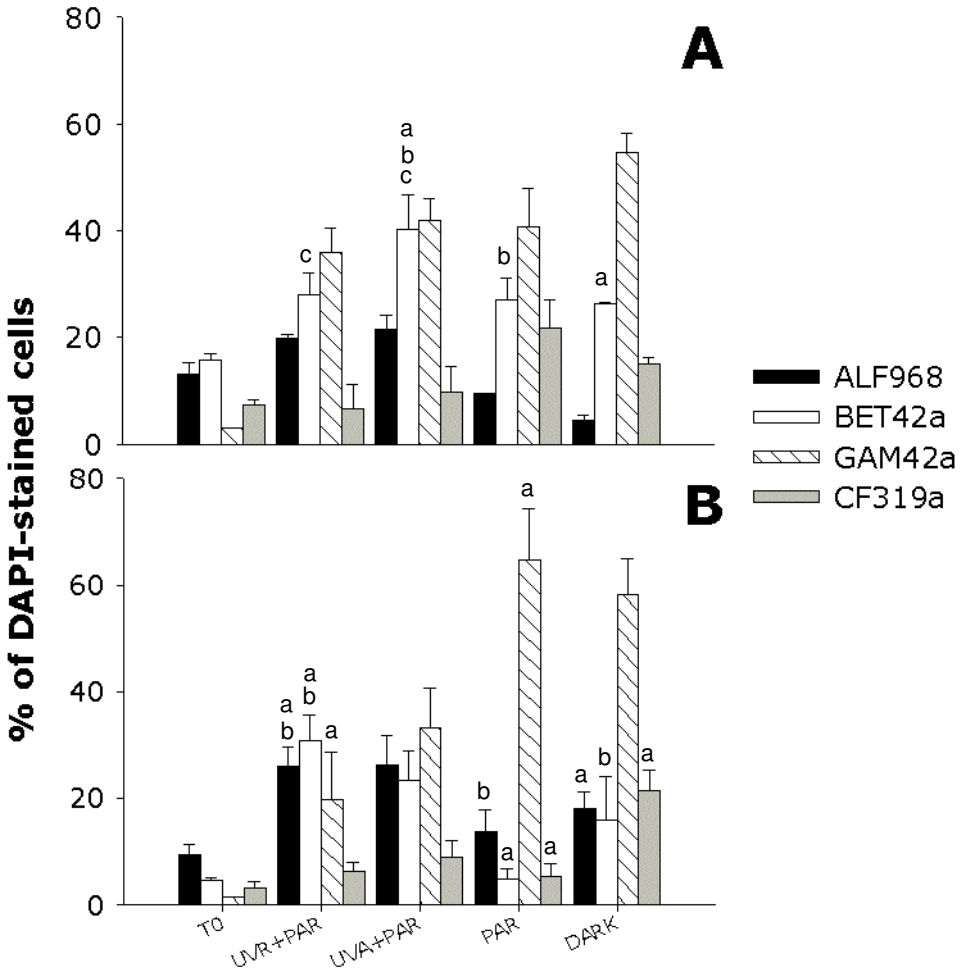
A
B
% of DAPI-stained cells
ALF968
BET42a
GAM42a
CF319a
T0
UVR+PAR
UVA+PAR
PAR
DARK

Fig. 3