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Effects of Photochemical Transformations of Dissolved Organic Matter on Bacterial Metabolism and Diversity in Three Contrasting Coastal Sites in the Northwestern Mediterranean Sea during Summer

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Abstract The effects of phototransformation of dissolved organic matter (DOM) on bacterial growth, production, respiration, growth efficiency, and diversity were investigated during summer in two lagoons and one oligotrophic coastal water samples from the Northwestern Mediterranean Sea, differing widely in DOM and chromophoric DOM concentrations. Exposure of 0.2- μm filtered waters to full sun radiation for 1 d resulted in small changes in optical properties and concentrations of DOM, and no changes in nitrate, nitrite, and phosphate concentrations. After exposure to sunlight or dark (control) treatments, the water samples were inoculated with the original bacterial com-

munity. Phototransformation of DOM had contrasting effects on bacterial production and respiration, depending on the water's origin, resulting in an increase of bacterial growth efficiency for the oligotrophic coastal water sample (120%) and a decrease for the lagoon waters (20 to 40%) relative to that observed in dark treatments. We also observed that bacterial growth on DOM irradiated by full sun resulted in changes in community structure of total and metabolically active bacterial cells for the three locations studied when compared to the bacteria growing on un-irradiated DOM, and that changes were mainly caused by phototransformation of DOM by UV radiation for the eutrophic lagoon and the oligotrophic coastal water and by photosynthetically active radiation (PAR) for the mesoeutrophic lagoon. These initial results indicate that phototransformation of DOM significantly alters both bacterial metabolism and community structure in surface water for a variety of coastal ecosystems in the Mediterranean Sea. Further studies will be necessary to elucidate a more detailed appreciation of potential temporal and spatial variations of the effects measured.

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Introduction

Bacteria are key organisms in carbon cycling in aquatic ecosystems, acting as a sink (mineralization of dissolved organic carbon [DOC] to CO_2) or as a link (production of biomass that can be transferred through the microbial food web) [10]. Microbial metabolism and diversity are mainly driven by the concentration and quality/composition of dissolved organic matter (DOM) and dissolved inorganic nutrients (e.g., [12]). Natural solar radiation, especially

ultraviolet radiation (UV-B [280–315 nm], UV-A [315–400 nm]), has been found to induce chemical transformations of DOM with the production of a variety of photoproducts, including carbon dioxide, carbon monoxide, ammonium, phosphate, and numerous LMW organic compounds (for a review, see [30]). The origin and chemical composition of DOM strongly influences its photoreactivity. The light-absorbing fraction of DOM, chromophoric dissolved organic matter (CDOM), from both terrestrial and autochthonous origins, is the primary absorber of sunlight in aquatic ecosystems and plays an important role for most photochemically mediated processes in surface waters [30]. These phototransformations are likely to affect bacterial production (BP) by changing the quantity and bacterial availability of DOM (for a review, see [20]). Contrasting results have been observed depending on the origin of DOM, and the observed effect of the phototransformations of DOM on bacterial growth is probably the net result of two opposite processes: photochemical increase in the lability of initially refractory substances and photochemical decrease in the lability of freshly produced algal carbon [32, 33, 44].

Most previous investigations have used bacterial biomass changes and/or bacterial protein (or DNA) production as criteria to evaluate the effects of phototransformations on the availability of DOM [30]. BP, however, represents only a part of the DOC used by bacteria, the other part being consumed by respiration. Bacterial growth efficiency (BGE) is estimated by comparing gross with net production, where gross production is measured as BP plus respiration or DOM utilization [11]. Recent studies have demonstrated that phototransformations of DOM affected the BGE in different aquatic ecosystems by a change in anabolic and catabolic pathways [1, 2, 28, 38, 42]. One question not investigated in previous studies is the extent that the changes in bacterial metabolism may be accompanied by a shift in bacterial community structure. Shifts in the source (terrestrial *versus* phytoplankton) and lability of DOM can result in shifts in bacterioplankton activity and ultimately in shifts in bacterioplankton diversity. Such effects have been observed during different temporal scales including seasonally [18, 40] and short-term events such as phytoplanktonic blooms [7, 13]. Thus, it might be reasonable to assume that photochemical transformation of DOM taking place during exposure to solar radiation may result in a shift in the composition of the bacterioplankton community structure and/or activity.

The importance of photochemical transformation in aquatic ecosystems is dependent on a combination of CDOM concentrations and intensity of sunlight. Coastal lagoons are semi-enclosed systems with both marine and fluvial components. Depending on the importance of these two influences, salinity, nutrients and CDOM concentra-

tions, optical characteristics, and productivity of the lagoons may vary considerably [9]. The proportion of autochthonous CDOM (e.g., derived from algae) *versus* terrestrial CDOM also fluctuates with river discharges and exchange with the open sea [9]. The Mediterranean coast of France is bordered by a series of coastal lagoons, and some of them are associated with important economic activities (shellfish farming, tourism) and high anthropogenic pressures (nutrient loadings, pollutants). These waters contrast with the surrounding oligotrophic coastal waters in the Mediterranean Sea. Moreover, these areas are subjected to significant surface UV irradiances during spring and summer seasons (up to 43.5 KJ m² per day in UV-B range; F. Joux, unpublished data).

The aim of this study was to investigate the effects of natural solar radiation in summer on DOM originating from two lagoons and coastal waters in the Northwestern Mediterranean Sea in terms of changes in (1) chemical and optical characteristics of the waters, (2) bacterial metabolism, and (3) diversity of bacteria growing on irradiated DOM.

Methods

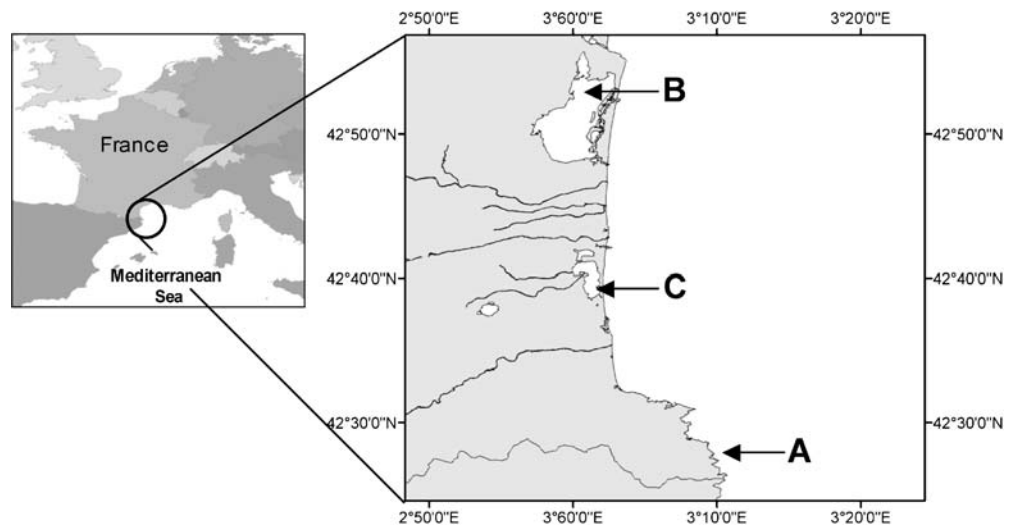
Study Sites and Sample Collection

Water samples were collected in June 2005 from two lagoons (Canet and Leucate) and one coastal station (SOLA station) of the northwestern Mediterranean Sea (Fig. 1). Canet Lagoon covers 6 km² with a mean depth of 0.2 to 0.5 m. The basin of the system is drained by three rivers and extends over 260 km². The lagoon is separated from the sea by a sand bar that opens artificially through a single channel. Leucate Lagoon covers 54 km² with a mean depth of 2 m and is heavily used for shellfish farming (oysters and mussels). There is no river input, and the lagoon connects to the sea in three places. SOLA is a shallow (maximum depth, ~26 m) station located ~500 m offshore of Banyuls-sur-Mer, France (42°29'N, 03°08'E). Surface waters were sampled by submerging an acid-washed polycarbonate bottle by hand ~30 cm below the surface and with a Niskin bottle at 5-m depth for the lagoons and the coastal station, respectively. Samples were then transferred into 20-L acid-washed polycarbonate Nalgene carboys and transported to the laboratory. Subsamples were collected for determination of salinity (Guideline 8410 portable salinometer, accuracy ±0.01), chlorophyll *a* and bacterial abundance.

Sample Preparation and Irradiation of DOM

Samples were prefiltered onto precombusted (450°C, 24 h) 142-mm GF/A filters (1.6-µm size particle retention,

Figure 1 Location of the three stations studied in the coastal northwestern Mediterranean Sea. *A* SOLA station, *B* Leucate Sea, *C* Canet Lagoon



Whatman) and then filtered through a prerinsed 0.2- μm pore-size Nuclepore polycarbonate membrane (142 mm). Filtered samples were stored at 4°C in the dark for no more 15 h before irradiation. Filtered seawater was then distributed into three experimental treatments chosen to alter the spectral characteristics of solar radiation. Dark and photosynthetically active radiation (PAR) treatments were established by placing filtered seawater in four 1-L Pyrex tubes (7-cm diameter) covered with aluminum foil and Courtgard film (50% cut-off at 400 nm), respectively. Full sun treatment was established by placing filtered seawater in four 1-L quartz tubes (7-cm diameter). All glassware was precombusted (450°C, 24 h) before use. The different tubes and bottles were placed in a recirculating water bath (0.1-m depth) maintained at *in situ* temperature ($19 \pm 1^\circ\text{C}$) and were exposed to natural solar radiation during a sunny day 8 h. Incident irradiance was measured in the PAR (400–700 nm), UV-A (315–400 nm), and UV-B (280–315 nm) using a broad band ELDONET radiometer (Real Time Computer, Inc., Ger.). Over the course of the incubation, PAR, UV-A, and UV-B doses were 11,000, 1700 and 37 KJ m^{-2} , respectively. Before and after sunlight exposure, subsamples were collected for DOC, dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP), nutrients (NH_4^+ , NO_3^- , NO_2^- , PO_4^{3-}) and DOM optical properties. NH_4^+ , DON, and DOP were measured only before irradiation. The remaining water was used as media for biodegradation experiments (see below).

Experimental Set-up for Biodegradation

Quartz and Pyrex tubes containing filtered water from both irradiated (full sun and PAR exposures) and dark treatments were inoculated (10% final volume) with a bacterial community from the same origin (un-irradiated 0.8- μm

filtered water). A portion of the sample water mixture from each light treatment was then dispensed into six 100-mL biological oxygen demand (BOD) bottles. All BOD bottles were washed with 10% HCl and rinsed with Milli-Q water before use. The BOD bottles were allowed to overflow ~ 3 times the volume of the bottle, ensuring that no bubbles remained in the bottles, and capped free of headspace with ground glass stoppers. The remaining fraction of the water (~ 2 -L for each light treatment) was then transferred into a 3-L glass bottle. The BOD bottles and the glass bottles were incubated in the dark at $18 \pm 1^\circ\text{C}$ in a water bath (to prevent gas exchange) and in an incubation chamber, respectively. At time zero and then at 39 h, three BOD bottles from each replicate incubation were fixed with Winkler reagents for the determination of O_2 concentration. Subsamples were drawn from each glass bottle at time zero and then at different intervals up to ~ 120 h for the determination of bacterial abundance, production, and community structure.

Analytical Methods

Chlorophyll *a* (Chl *a*) concentrations were obtained from water samples (50–200 mL in duplicate) filtered through 47-mm GF/F filters (Whatman) and frozen in liquid nitrogen until analysis. Pigments were extracted in 90% acetone for 12 h in the dark at 4°C, and the concentration of Chl *a* was measured by fluorometry using a Turner-Design 10-AU [26].

Sixty-milliliter samples for nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}) were stored at -20°C and analyzed within 1 mo of collection according to Tréguer and Le Corre [45] on a Skalar autoanalyzer. Measurement accuracy was ± 0.1 , ± 0.02 , and ± 0.02 μM , respectively for NO_3^- , NO_2^- and PO_4^{3-} . Samples (100 mL in duplicate) for ammonium (NH_4^+) were analyzed immediately according to the Koroleff's

manual method [22] with a spectrophotometer (Spectronic 401). Measurement accuracy was $\pm 0.05 \mu\text{M}$.

Twenty-milliliter duplicate samples for DOC were filtered through two precombusted (450°C , 24 h) 25-mm GF/F filters, transferred into precombusted glass tubes, poisoned with 85% H_3PO_4 (final $\text{pH}=2$), closed with Teflon lined screw caps, and were stored in the dark at room temperature until analysis. DOC was analyzed using the high temperature catalytic oxidation (HTCO) technique [8, 43] using a Shimadzu TOC-V analyzer. Before starting analyses and between sets of samples, an international certified reference sample for DOC concentration (available for the international community) was analyzed to check the calibration of the analyzer and its stability over time. Analytical accuracy of measurements gave a coefficient of variation (CV) of less than 2%.

Samples for DON and DOP were filtered through two precombusted (450°C , 24 h) 25-mm GF/F filters (Whatman). Samples were collected directly in Teflon bottles and immediately frozen (-20°C) and stored for later analyses. DON and DOP were simultaneously determined by the wet oxidation procedure [36, 37]. DON ($\pm 0.1 \mu\text{M}$) and DOP ($\pm 0.02 \mu\text{M}$) concentrations were determined by sample oxidation (30 min, 120°C) corrected for NH_4^+ , $\text{NO}_3^- + \text{NO}_2^-$, and PO_4^{3-} concentrations, respectively.

DOM fluorescence was determined with a PerkinElmer LS55 spectrofluorometer using a 1-cm quartz cuvette (excitation wavelength, 350 nm; emission wavelength, 450 nm) standardized with a quinine sulfate solution (1 QSU=1 ppb quinine sulfate in 0.05 M H_2SO_4) [33]. DOM absorption at 350-nm wavelength was determined with a Hitachi U-3010 spectrophotometer using a 10-cm cuvette. Absorbance was measured against Milli-Q water as blank. Absorption coefficients $a_{350} \text{ (m}^{-1}\text{)}$ were calculated as $a_{350}=2.303 D/L$, where D is the absorbance at 350-nm wavelength and L is the pathlength of the absorbance cell in meters. The $a_{350} \text{ (m}^{-1}\text{)}$ has been used to quantify CDOM because it represents the middle of the UV-A region, where the overlap of the solar irradiance spectrum and a_{CDOM} yields the highest absorption rates relative to the photobleaching [24].

For determination of bacterial abundance by flow cytometry, duplicate 3-mL samples in cryovials were preserved in buffered formalin (2% final concentration). The samples were gently mixed and left in the dark at room temperature for 10 min before quick-freezing in liquid nitrogen and storage at -80°C . The samples were later thawed at room temperature, stained with SYBR Green I [final concentration 0.01% (v/v) of the commercial solution; Molecular Probes Inc., OR] for at least 15 min at 20°C in the dark and analyzed on a flow cytometer (FACScan, Becton Dickinson, San Jose, CA) equipped with a 488 nm, 15 mW argon laser. Bacteria were detected on a plot of

green fluorescence (515–545 nm) *versus* right angle light scatter (SSC), using the green fluorescence as threshold parameter. Fluorescent beads (1.0 μm ; Polysciences Inc., Warrington, PA) were added to each sample analyzed to normalize SSC and green fluorescence. Analytical accuracy of measurements gave a CV of less than 5%.

BP was measured by ^3H -leucine incorporation [21] as modified by Smith and Azam [41]. Samples (2.5 mL in triplicate) were added to a sterile polystyrene snap cap tube (5 mL), containing 8 nM [4,5- ^3H]-leucine (specific activity 117 Ci mmole^{-1} , PerkinElmer) and 32 nM of unlabelled leucine. One killed control was prepared for each assay by addition of 250 μl 50% trichloroacetic acid (TCA), 15 min before the addition of leucine. Tubes were incubated in the dark at 18°C for 1 h and then incorporation terminated by transferring replicate 1-mL samples from each tube in microcentrifuge tubes containing 100 μl of 50% TCA. Samples were stored for at least 1 h at 4°C and then centrifuged for 15 min at $12,000\times g$. The precipitate was rinsed once with 5% TCA and once with 70% ethanol. The precipitates were resuspended in 1.0 mL of liquid scintillation cocktail (FilterCount, PerkinElmer) and radioactivity determined by liquid scintillation counter (LS 5000CE Beckman). Leucine incorporation rates were converted into carbon production using the conversion factor of 1.55 kg C produced by mole of leucine incorporated [21]. Analytical accuracy of measurements gave a CV of less than 4%.

Bacterial respiration (BR) rates were determined by following the decrease in dissolved oxygen (DO) during incubation experiments. Concentrations of DO were measured *via* an automated titration procedure using a Metrohm 716 equipped with a potentiometric electrode. At 0 and 39 h, three BOD bottles were fixed with Winkler reagents for determination of DO concentrations [6]. The whole BOD bottle was titrated to increase sensitivity of the rate estimates. Analytical accuracy of measurements gave a CV of less than 0.5%.

Data Calculation for Bacterial Growth

Bacterial carbon demand (BCD) was calculated in the first 39 h during the biodegradation experiments as the sum of the bacterial biomass production and the decrease in oxygen expressed in carbon using a respiratory quotient of 0.8. Bacterial biomass production was calculated from the geometric trapezoidal integration of leucine incorporation expressed in carbon, rather than from bacterial concentration, because changes in biovolume during growth were not measured. BGE was calculated as the ratio between bacterial biomass production and BCD [11]. BCD and BGE were the average of all possible combinations between the independent measurements of BP and the BR.

DNA and RNA Extractions for CE-SSCP Analysis

Community structure of total and metabolically active bacterial cells was analyzed at the start of the biodegradation experiments and after 111 h of incubation for SOLA and Canet Lagoon and 87 h for Leucate Lagoon (i.e., when cells were in stationary phase). Samples (500 mL) were filtered onto 0.22- μm pore size polycarbonate filters (Nuclepore) and stored at -20°C . For analysis, frozen filters were cut with sterilized scissors into small strips and vortexed briefly in 840 μl of alkaline lysis buffer (50 mM Tris hydrochloride pH 8.3, 40 mM EDTA, and 0.75 M sucrose). Cell lysis was accomplished by an initial incubation for 45 min at 37°C after adding 50 μl of freshly prepared lysosyme solution (20 mg mL^{-1}), and a second incubation at 55°C for 1 h after adding 100 μl of 10% sodium dodecyl sulfate and 10 μl of proteinase K (20 mg mL^{-1}). Six hundred microliters of lysate was treated with 10 μl of a 100-mg mL^{-1} RNase A solution (Qiagen) before DNA extraction with the DNeasy Tissue kit (Qiagen). DNA was used as a template for PCR amplification of the variable V3 region of the 16S rDNA (*Escherichia coli* positions 329–533), as previously described [18]. Four hundred microliters of lysate was treated with DNase I for RNA extraction using an SV Total RNA Isolation kit (Promega). The efficiency of the DNA removal from RNA was checked by amplifying each RNA extract by PCR (conditions as indicated below), and PCR reactions that did not give a product were used for complementary (cDNA) synthesis. RNA was reverse transcribed (RT) into first-strand cDNA using Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega), according to manufacturer instructions. PCR amplification of cDNA was performed with the same conditions as for DNA. Capillary electrophoresis SSCP-based single strand conformational polymorphism (CE-SSCP) was performed as previously described [18] using the ABI 310 Genetic Analyzer (Applied Biosystems), equipped with a capillary tube (47 cm \times 50 μm) filled with a polymer composed of GeneScan polymer (Applied Biosystems) 5.6%, glycerol 10% and Tris/borate/EDTA (TBE) 1 \times . Analysis of the electropherograms was performed by using the software SAFUM (<http://www.montpellier.inra.fr/narbonne/francais/equipes/equipe-em/safumaccueil.htm>).

Results

Initial Characteristics and Light-induced Changes in Chemical and Optical Characteristics of Waters

There were marked differences in the initial characteristics of the samples from the two lagoons and from the coastal

water (Table 1). Canet Lagoon is a eutrophic lagoon characterized by high DOC and Chl *a* concentrations (892 μM and 70 $\mu\text{g L}^{-1}$, respectively) and a low salinity (7.5; Table 1). Leucate Lagoon is characterized by moderate DOC and Chl *a* concentrations (234 μM and 2.2 $\mu\text{g L}^{-1}$, respectively) and a high salinity (34.5; Table 1). SOLA is an oligotrophic marine coastal station with low DOC and Chl *a* concentrations (80 μM and 0.3 $\mu\text{g L}^{-1}$, respectively; Table 1). The elemental ratio of DOC/DON ranged from ~ 12 (SOLA and Leucate Lagoon) to ~ 18 (Canet Lagoon; Table 1). DOP concentrations were undetectable for all the water types (Table 1). Inorganic nitrogen and phosphorus concentrations were very low for Leucate Lagoon and SOLA station, whereas high concentration of inorganic phosphorus was measured for the Canet Lagoon (Table 1). The DOC concentrations in the lagoons sustain higher bacterial biomass (4.39×10^9 and 23.6×10^9 bacteria per liter for Leucate and Canet Lagoons, respectively) compared to the coastal station (0.62×10^9 bacteria per liter). CDOM fluorescence and absorbance at 350-nm wavelength (a_{350}) varied widely between the three systems with higher values measured at Canet, then Leucate, and then SOLA (Table 1). Fluorescence and absorbance of CDOM were linearly correlated to DOC ($r^2=1.00$ for both parameters) for the three locations (Fig. 2). When CDOM fluorescence and absorbance were normalized to DOC concentrations, values were comparable between the two lagoons (40.8–50.9 and 8.67–9.14 for normalized fluorescence and absorbance, respectively) and closer to those measured at SOLA (14.5 and 5.75 for normalized fluorescence and absorbance, respectively) than for absolute values (Table 1). This

Table 1 Surface water characteristics of the different stations studied

	SOLA	Leucate	Canet
Salinity	37.7	34.5	7.5
DOC (μM)	80	234	892
DON (μM)	6.5	21.3	49.9
DOP (μM)	<dl	<dl	<dl
C/N (mol/mol)	12.3	11.0	17.9
<i>F</i> (QSU)	1.16	9.55	45.4
a_{350} (m^{-1})	0.46	2.14	7.73
<i>F</i> /DOC (QSU mM^{-1})	14.5	40.8	50.9
a_{350} /DOC (m^{-1} mM^{-1})	5.75	9.14	8.67
NO_3^- (μM)	<dl	0.14	0.17
NO_2^- (μM)	<dl	0.07	0.02
NH_4^+ (μM)	0.08	1.52	2.15
PO_4^{3-} (μM)	<dl	<dl	22.4
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.32	2.17	69.9
Bacteria (10^9 L^{-1})	0.62	4.39	23.6

DOC Dissolved organic carbon; *F* fluorescence of chromophoric dissolved organic matter (excitation 350 nm, emission at 450 nm, given in quinine sulfate units); a_{350} absorption coefficient at 350 nm. *F*/DOC and a_{350} /DOC: dissolved organic carbon-normalized fluorescence and absorption, respectively; <dl below detection limit

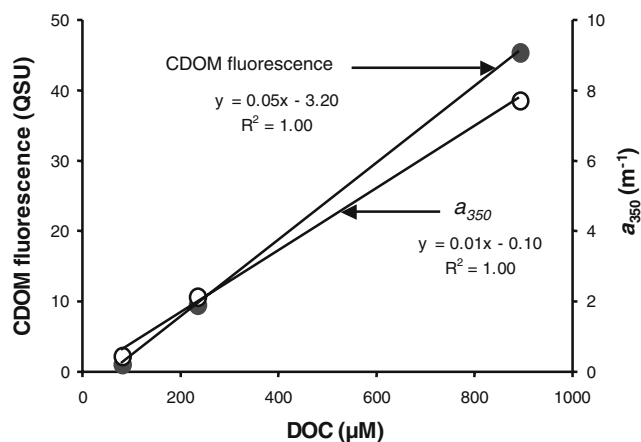


Figure 2 CDOM fluorescence and absorbance at 350 nm (a_{350}) of the natural water samples (lagoons and coastal water) as a function of DOC concentration. Coastal water has the lowest CDOM fluorescence and absorbance, Leucate Lagoon intermediate, and Canet Lagoon highest

indicates that the concentration of CDOM is highly variable between the three systems but that contribution of CDOM to the total DOM is more consistent.

Using the absorbance at 305, 360, and 550 nm measured in 0.2- μm filtered waters before exposure (data not shown), we estimated the transmission of UV-B, UV-A, and PAR, respectively, across the quartz tube (full sun condition; Table 2). UV transmission differed noticeably between the three locations. Whereas $\sim 95\%$ of UV-B penetrated the sample from the oligotrophic SOLA coastal station, only ~ 71 and $\sim 29\%$ was transmitted for the Leucate and Canet Lagoons, respectively.

A loss of 27 μM in DOC (3% of the initial value) was observed for Canet Lagoon after full sun treatment (Table 3). Effects of photobleaching on CDOM fluorescence were greater than changes in absorbance (Table 3). Whereas a significant loss of fluorescence was measurable for Leucate and Canet Lagoons (22 and 33%, respectively), we only observed a significant decrease in a_{350} by 10% for Canet Lagoon (Table 3). Loss of CDOM fluorescence and absorbance was attributable to UV radiation in the lagoon waters (i.e., no effects were measurable in the PAR treatment). No significant changes were observed in nutrient concentrations (NO_3^- , NO_2^- , PO_4^{3-}) after irradiation (Table 3).

Effect of Photochemical Transformations on Bacterial Growth and Diversity During Biodegradation Experiments

After the inoculation of the original bacterial community to each of three water types after the light treatments, an exponential bacterial growth was observed until ~ 63 h for Leucate Lagoon and ~ 87 h for SOLA and Canet Lagoon (Fig. 3). The different light treatments on 0.2- μm filtered

samples modified neither the bacterial growth rate nor the bacterial concentration in stationary phase for SOLA and Canet samples. For Leucate samples, a decrease in growth rate and maximum bacterial concentration was observed in the full sun treatment compared to dark and PAR treatments. Flow cytometry analyses also gave information on the cell size (using the SSC as proxy) and the cellular DNA content (using the FL1 fluorescence as proxy) of the bacteria during the experimental cultures. The data clearly show that bacteria growing in the full sun treatment were larger and contained more DNA than bacteria growing in the dark and PAR treatments for all the water types studied (Fig. 3).

CE-SSCP fingerprinting revealed different patterns of bacterial community structure for total (16S rDNA) and active (16S rRNA) depending on light treatment and sample location. The initial community structure of total and metabolically active bacterial cells of each environment differed markedly from those obtained at the end of the growth for the different light treatments and the dark control (Fig. 4). The unweighted pair group method with arithmetic mean (UPGMA) dendrogram of the CE-SSCP profiles showed that dark and PAR treatments clustered together but to a lower extent with FS for SOLA and Canet (Fig. 4A and C), whereas dark, PAR, and FS clustered together for Leucate (Fig. 4B). Detailed analysis of the CE-SSCP profiles for SOLA shows that not all the population were able to respond during the regrowth experiment to DOM bioavailability changes, as revealed by the decrease of the observed number of 16S rDNA ribotypes (14) for the full sun compared to the dark and the PAR treatments (19 and 18 ribotypes, respectively; Figs. 4A and 5). In contrast, the modifications to the total community structure (16S rDNA) observed with the light treatments did not change the number of ribotypes compared to the dark treatment for Canet and Leucate Lagoons (Fig. 4B and C). The same trends were observed when 16S rRNA was used as a template, with a more pronounced difference between dark and PAR clusters compared to full sun at SOLA and Canet Lagoon, and between the three treatments for Leucate Lagoon, as determined by the higher Euclidean distance between samples (Fig. 4). In this case, full sun treatment resulted in

Table 2 Percent transmission of UV-B, UV-A, and PAR through the 0.2- μm filtered water contained in the quartz tubes (full sun condition)

	UV-B (%)	UV-A (%)	PAR (%)
SOLA	94.8	97.4	99.8
Leucate	71.1	87.9	99.6
Canet	28.6	64.2	98.8

See the text for the detail of calculations.

Table 3 Changes on carbon, nitrogen, and phosphorus concentrations and optical properties in 0.2- μm filtered water samples in the different light treatments; 8 h darkness (dark treatment) or 8 h to PAR (PAR treatment) or 8 h to natural sunlight radiation (full sun)

Station	Light treatment	DOC (μM)	F (QSU)	a_{350} (m^{-1})	NO_3^- (μM)	NO_2^- (μM)	PO_4^- (μM)
SOLA	Dark	80.5 (0.2)	1.25 (0.44)	0.41 (0.12)	<dl	<dl	<dl
	PAR	80.0 (0.7)	0.75 (0.22)	0.44 (0.22)	<dl	<dl	<dl
	Full sun	82.8 ^a	0.77 (0.05)	0.47 (0.03)	<dl	<dl	<dl
Leucate	Dark	233 (0)	9.59 (0.03)	2.10 (0.03)	0.11 (0.01)	0.08 (0.00)	<dl
	PAR	229 (6)	9.56 (0.63)	2.02 (0.08)	0.12 (0.00)	0.08 (0.00)	<dl
	Full sun	228 (2)	8.17 (0.17) ^b	1.96 (0.09)	0.16 (0.05)	0.07 (0.00)	<dl
Canet	Dark	877 ^a	44.7 (2.0)	7.73 (0.33)	0.14 (0.00)	0.03 (0.02)	22.3 (0.2)
	PAR	870 ^a	44.2 (0.5)	7.82 (0.34)	0.14 (0.03)	0.02 (0.00)	22.5 (0.1)
	Full sun	850 ^a	35.5 (0.5) ^b	7.34 (0.10) ^b	0.13 (0.02)	0.02 (0.01)	21.5 (0.6)

All data are means from duplicate samples (range).

<dl Below detection limit

^aNo duplicate available.

^bTreatment means are significantly different (t test, $p < 0.05$) from dark treatment, for within-site comparisons only.

a lower number of 16S rRNA ribotypes compared to the dark and PAR treatments for both SOLA and Canet Lagoon (Fig. 4A and C).

Effect of Photochemical Transformations on Bacterial Metabolism During Biodegradation Experiments

During the biodegradation experiments, we observed a net stimulating effect of the full sun treatment on the BP for SOLA and Canet Lagoon (Fig. 6a, c). This stimulatory effect was primarily attributable to phototransformations induced by UV, as PAR and dark BP were more similar. In contrast, we observed an inhibitory effect of full sun and PAR treatments on BP for Leucate Lagoon (Fig. 6b). Highest values of BP were measured after 15-h incubation for all treatments except Canet Lagoon with full sun where the BP increased until 39 h of incubation. Integrated values of BP for the first 39 h of incubation transformed into carbon are given in Table 4. Light treatments resulted in no change in BR for SOLA and Leucate Lagoon, whereas a significant increase of BR was observed with the full sun treatment for Canet Lagoon (Table 4). Changes in BCD were noticeable only in the case of the full sun treatment for Canet Lagoon (+74% relative to dark treatment; Table 4).

Values of BGE ranged from 10 to 37% in dark treatments for the different water types with higher values for lagoon waters (Table 4). Light treatments had contrasting effects on BGE derived from the BP and BR measurements. Full sun treatment had a stimulating effect on BGE for SOLA, resulting in BGE value that was 120% higher than the corresponding value for the dark treatment (Table 4). By contrast, PAR and full sun treatments had a similar and negative effect on BGE for both Leucate and Canet Lagoons, resulting in BGE values that were 20 to 40% lower than the corresponding values in the dark treatments (Table 4).

Discussion

Photochemical Transformations

DOC, CDOM fluorescence, and absorption were affected differently by exposure to sunlight. Indeed, we found that DOC concentrations decreased more slowly than absorption or fluorescence values and that CDOM fluorescence losses outpaced CDOM absorption losses during photobleaching. These patterns agree with those previously noted by others for light-induced degradation of natural organic matter (e.g., [5, 31]) and may be caused by the presence of a reservoir of DOC resistant to photodegradation in contrast to the reservoir of CDOM. We did not detect changes in NO_3^- and PO_4^- concentrations after light exposure as has been previously reported (e.g., [42]). However, we cannot conclude that there were no changes in nutrients, as we did not measure ammonium photoproduction, which has been frequently reported in photochemical studies (e.g., [5, 42]).

Bacterial Response to Photochemical Transformations

Effects of DOM phototransformations on net production may be interpreted differently depending on whether we consider bacterial cell density or BP measurements. We hypothesized that the discrepancies observed between these two measures could be partly explained by a change in bacterial biovolume, which may vary according to the different light treatments of DOM based on the observation that bacteria growing in full-sun-exposed DOM had a higher biovolume relative to other light treatments and that they incorporated more leucine for the same cells produced. Although, we did not measure the biovolume of bacteria during the experimental cultures using epifluorescence microscopy [15], flow cytometry analyses provided infor-

mation on cell size and DNA content by the mean of side scatter and SYBR-green fluorescence, respectively. Both of these parameters have been correlated to the biovolume of aquatic bacteria [16]. We observed that bacteria growing with DOM irradiated by full sun were characterized by a higher side scatter and SYBR-green fluorescence. This indicates that cell numbers alone were insufficient to determine net production and demonstrate that a single theoretical factor may not be appropriate to convert cell numbers to biomass (e.g., [1]). In contrast to our observation, however, Farjalla et al. [14] did not observe differ-

ences in biovolume for bacteria growing on irradiated and non-irradiated leachates from two aquatic macrophytes.

The experimental design used in this study allows us to determine the contribution of UV radiation and PAR to these changes. Changes in BGE seem to be due primarily to UV radiation for the coastal water and PAR for the coastal lagoons. The changes in BP and BR induced by DOM phototransformations resulted in an increase of BGE for the oligotrophic coastal station (120%) and a decrease for the coastal lagoons (20 to 40%), whereas BCD only increased for the more eutrophic lagoon (74%). However, the BGE

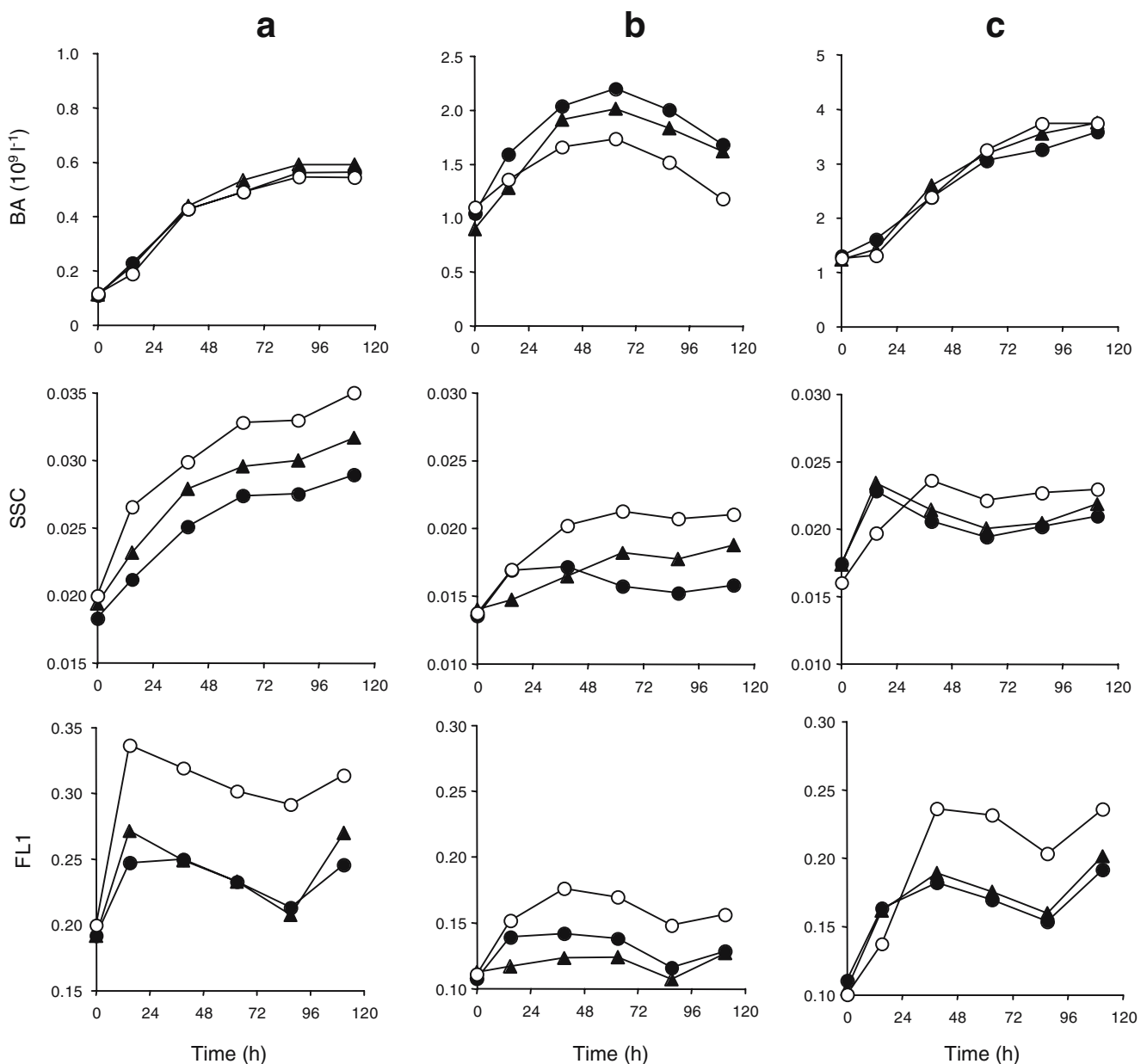
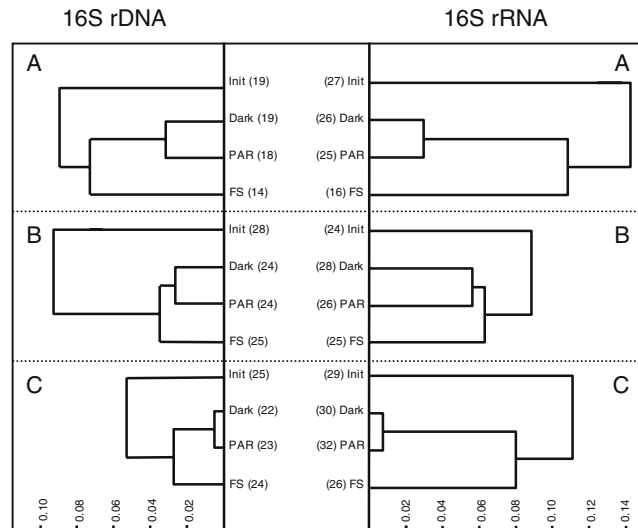


Figure 3 Changes in bacterial abundance (*BA*), cell size scatter (*SSC*), and DNA fluorescence (*FLI*) of bacteria during the biodegradation experiments for irradiated [full sun (*open circle*) and PAR

(*filled triangle*)] and dark (*filled circle*) treatments of the three experimental cultures. **a** SOLA station, **b** Leucate Lagoon, **c** Canet Lagoon

Figure 4 Cluster analysis dendrogram based on comparison of 16S rDNA and 16 rRNA CE-SSCP patterns obtained from the bacterial communities before (*Init*) and after growth on DOM pre-irradiated by full sun (*FS*), PAR, or maintained in the dark (*Dark*). The numbers of ribotypes identified in each bacterial community are indicated in parentheses. *A* SOLA station, *B* Leucate Lagoon, *C* Canet Lagoon



calculation is based on both BP and BR measurements, and the same BGE value may be obtained with different values of BP and BR.

We have compared our results for changes of bacterial metabolism caused by DOM phototransformations to other studies (Table 5). Most have observed an increase of the BCD accompanied by a BGE decrease after DOM photo-

transformations. Positive effect on BGE has been observed only for lake water with high humic concentrations [2, 39] or for leachates from submersed macrophytes [3]. According to Anesio et al. [3], the initial bacterial availability of DOM can partly explain the positive or negative response effect as well as the magnitude of bacterial response to the photochemical modifications of DOM. The higher the

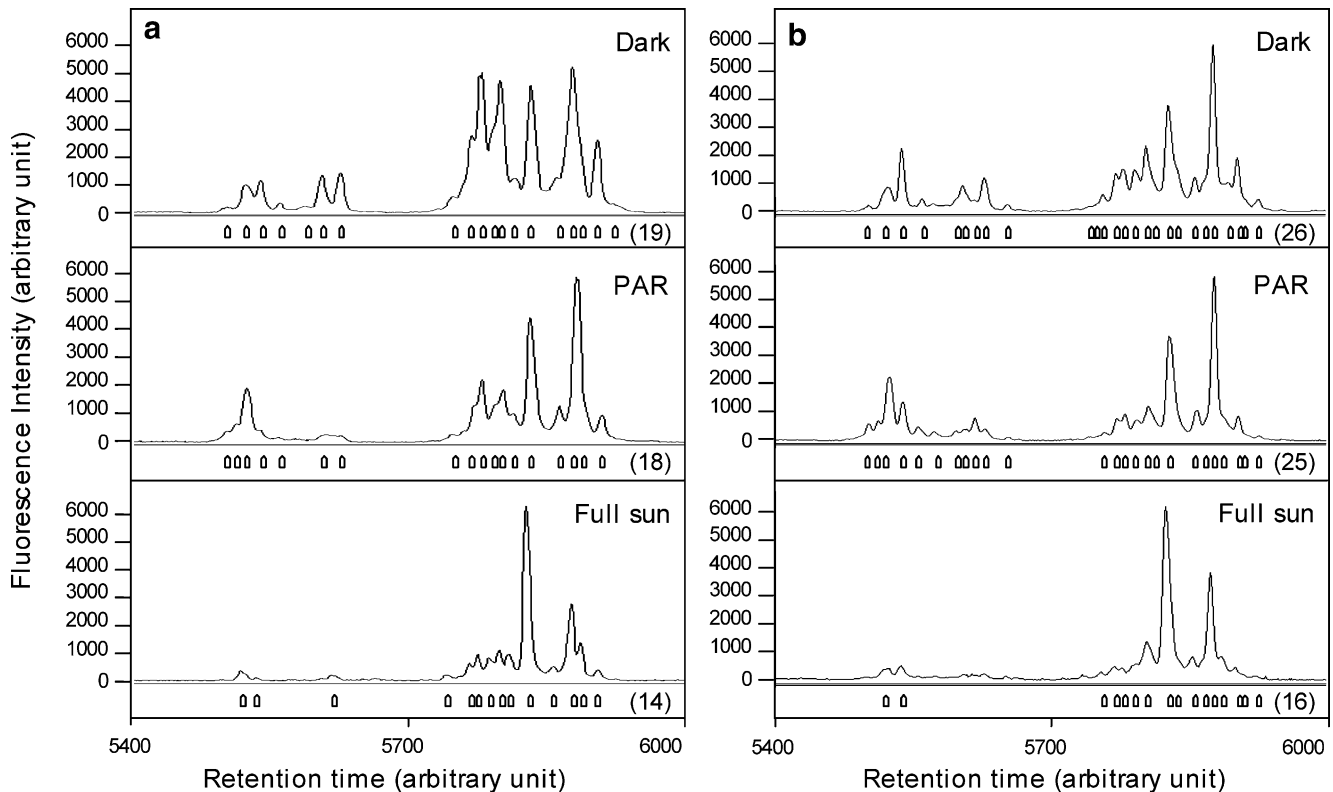


Figure 5 Example of CE-SSCP electrophoregram corresponding to a 16S rDNA or b 16S rDNA for dark, PAR, and full sun treatments after 111 h of the experimental culture using water from SOLA. The

identified ribotypes were drawn in a schematic diagram (Δ) and the number of ribotypes per sample is indicated in brackets

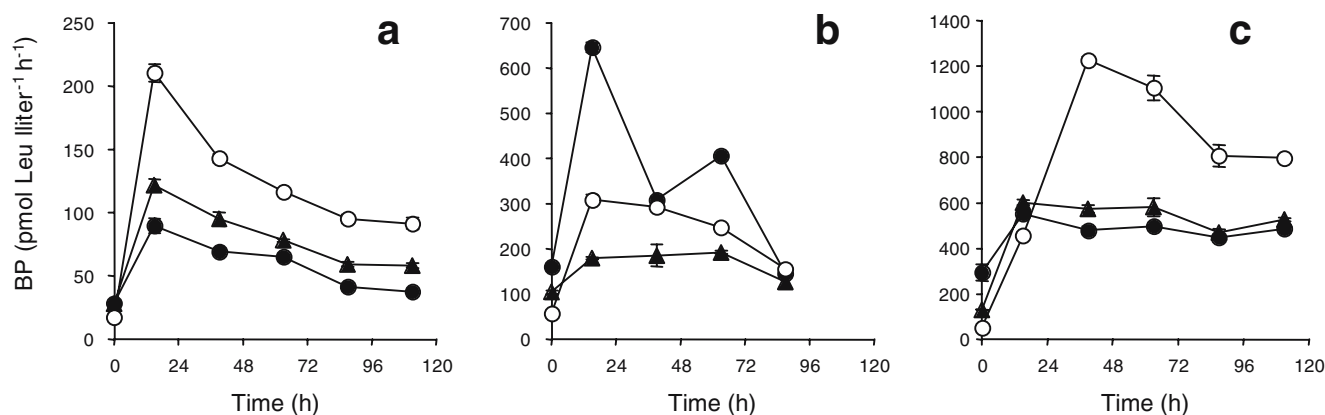


Figure 6 Changes in bacterial production (*BP*) during the biodegradation experiment for irradiated [full sun (*open circle*) and PAR (*filled triangle*)] and dark (*filled circle*) treatments. **a** SOLA station, **b** Leucate Lagoon, **c** Canet Lagoon

bioavailability of DOM before irradiation, the more negative the bacterial response to photochemical modifications of BGE. Smith and Benner [42] observed that for different highly colored ‘blackwater’ systems ($a_{350}=41$ to 117 m^{-1}), exposure of DOM to sunlight increased the BCD but decreased the BGE. The decrease in BGE appeared predictable from the initial DOM optical characteristics: The importance of the decrease in the BGE was positively correlated to the initial a_{350} value [42]. Our results agree with the main conclusions of these two relationships: (1) a negative change in BGE was observed after irradiation to full sun for the lagoons waters characterized by a higher BGE measured in the dark treatment (an index of the bacterial availability of DOM) and a higher CDOM content, and (2) a positive change in BGE was observed after irradiation to full sun for the oligotrophic coastal water characterized by a lower BGE measured in the dark treatment and a lower CDOM content. Decreasing BGE values upon exposure can be partially explained by the highly oxidized nature of several of the quantitatively important

LMW photoproducts identified in different studies [4, 38]. In general, although these oxidized molecules (e.g., organic acids) are readily available for bacterial utilization, they are expected to be utilized at a lower BGE compared with less oxygenated, more reduced substrates for energetic reasons [38]. Increasing BGE upon exposure may also be explained by the photoproduction of free dissolved sugars and amino acids [5, 23], which are expected to be used with a higher BGE [11].

Limiting nutrients during bioassays may interfere with the response of bacteria to phototransformed DOM [39]. During a set of experiments where DOM and bacteria were exposed simultaneously to natural sunlight (i.e., conditions different from those usually used herein where exposure of DOM was conducted before the addition of bacteria), Reche et al. [39] observed a positive interaction of CDOM photobleaching and N and P enrichments on bacterial abundance, production, and BGE. These interactions indicate that conversion of carbon from substrate into bacterial biomass was higher than expected from the addition of

Table 4 Bacterial production (BP), bacterial respiration (BR), and bacterial carbon demand (BCD=BP+BR) integrated for the first 39 h of experimental cultures

Station	Light treatment	BP ($\mu\text{g C L}^{-1}\text{ d}^{-1}$)	BR ($\mu\text{g C L}^{-1}\text{ d}^{-1}$)	BCD ($\mu\text{g C L}^{-1}\text{ d}^{-1}$)	BGE (%)
SOLA	Dark	2.7 (0.1)	24.4 (10.4)	27.0 (10.4)	9.9 (4.8)
	PAR	3.6 (0.1) ^a	24.7 (7.1)	28.2 (7.1)	12.6 (5.0) ^a
	Full sun	5.7 (0.1) ^a	19.5 (1.7)	25.2 (1.7) ^a	22.5 (4.4) ^a
Leucate	Dark	16.2 (1.3)	36.6 (16.6)	52.8 (16.6)	30.7 (13.0)
	PAR	6.2 (0.2) ^a	26.1 (8.0)	32.3 (8.0) ^a	19.2 (6.1) ^a
	Full sun	8.9 (1.6) ^a	40.3 (9.7)	49.2 (9.8) ^a	18.1 (6.0) ^a
Canet	Dark	17.9 (0.3)	30.9 (10.3)	48.8 (10.3)	36.6 (9.9)
	PAR	18.6 (0.3) ^a	45.0 (8.7)	63.6 (8.7) ^a	29.3 (5.1) ^a
	Full sun	22.8 (0.2) ^a	62.2 (9.5) ^a	85.0 (9.5) ^a	26.8 (3.8) ^a

Bacterial growth efficiency (BGE) was derived from BP and BR measurements ($\text{BGE}=\text{BP}/[\text{BP}+\text{BR}]$). All data are means (standard deviation).

^aTreatment means are significantly different from dark treatment, for within-site comparisons only (*t* test for BP and BR and *U* test for BCD and BGE, $p<0.05$)

Table 5 Synthesis of results on changes in bacterial abundance (BA), production (BP), respiration (BR), carbon demand (BCD), and growth efficiency (BGE) after irradiation of DOM from various aquatic systems to natural or artificial sunlight

DOM origin	Irradiation conditions	Nutrients ^a	BA	BP	BR	BCD	BGE	Reference
Leachates from aquatic macrophytes								[14]
<i>Phragmites australis</i>	Artificial UVA+UVB	Yes	–	nd	= or –	=	–	
<i>Hydrocharis morsus-ranae</i>			–	nd	= or –	=	= or –	
Leachates from different primary producers								[3]
Floating-leaf macrophytes	Artificial UVA+UVB	No	–	–	–	–	–	
Submersed macrophytes			+	=	=	=	+	
Emergent macrophytes			=	+	=	=	=	
Terrestrial leaves			=	+	+	+	–	
Batata Lake (Brazil)								[1]
Drawdown period	6 h natural sunlight	Yes	+	+	=	+	–	
Low-water period			=	=	=	=	=	
Filling-up period			=	=	+	+	–	
High-water period			+	=	=	+	–	
Caraña stream (Brazil)								[1]
Dry period	6 h natural sunlight	Yes	+	=	+	+	=	
Rainy period			=	=	+	+	–	
York River (VA, USA)								[28]
Moderate streamflow (August). Salinity=0	9 h natural sunlight	No	–	nd	nd	+	–	
Salinity=10			–	nd	nd	+	–	
High streamflow (April). Salinity=0			=	=	nd	=	=	
Salinity=11			=	=	nd	+	=	
Salinity=22			=	=	nd	+	–	
Low streamflow (July). Salinity=0			+	+	nd	+	–	
Salinity=10			=	+	nd	=	=	
Salinity=20			=	+	nd	=	=	
Parker River (MA, USA)								[38]
Upstream (terrestrial DOM input)	14 h natural sunlight	Yes	+	nd	nd	+	–	
Downstream (diatom bloom)			+	nd	nd	+	–	
Estuary Satilla River (GA, USA)	Artificial PAR, UVA, UVB	Yes	nd	nd	+	+	–	[31]
Humic lake. Old Man (CO, USA)	32–192 h natural sunlight–UVB	No	–	–	nd	+	+	[39]
		Yes	+	–	nd	+	+	
Rivers ‘blackwater’ systems (SC, USA)	Artificial sunlight	No	+	nd	+	+	–	[42]
Humic substances added to lake water	Artificial UVA+UVB	Yes	+	+	+	+	+	[2]
Coastal lagoons (NW Med. coast, France)								This study
Leucate	8 h natural sunlight	No	–	–	=	=	–	
Canet			=	+	+	+	–	
Coastal waters (NW Med. coast, France)	8 h natural sunlight	No	=	+	=	=	+	This study

+, – and = denote positive, negative and no effects, respectively, after irradiation of DOM for the parameter considered

^aNutrients added at the start of the biodegradation.

individual components. Increased nutrient loading in aquatic ecosystem could facilitate the utilization of the photoproducts and transfer of carbon to the food web via bacteria. Most of the experiments conducted to measure changes in bacterial metabolism after addition of phototransformed DOM have used nutrient additions (N and P) at the start of the biodegradation to avoid any nutrient limitations during growth (Table 5). We did not add nutrients in this study to measure the response of bacteria to “natural” conditions; consequently, we are aware that a part of the photoproducts may have not been used because of limiting nutrients, in

particular for SOLA and Leucate Lagoon for which PO₄ was undetectable.

Links Between Changes in Bacterial Metabolism and Diversity

Microbial communities may respond to a varying supply of substrates either by physiological adaptation and/or by changes in the community composition. Even small additions of organic substrates may trigger a shift in the composition of the microbial community and an accompa-

nying change in the BGE [12]. To our knowledge, this is the first study comparing the changes in bacterial community structures and BGE induced by phototransformation of DOM. In this study, we used 16S rDNA and rRNA-based CE-SSCP fingerprinting to resolve the community structure of total and metabolically active bacteria, respectively, as active bacteria contain more rRNA than resting or starved cells [19, 35].

The experimental design used for the biodegradation experiments (inoculation of bacteria at 10% in water free of protozoa) resulted in an increase of bacterial abundance until a stationary phase for the three water types (Fig. 3). In parallel, both DNA- and RNA-based community structures at the beginning of the experiment differed markedly from those obtained at the stationary phase for all light and dark treatments and for all water type studied (Fig. 4). We could not exclude the possibility that growth of a few opportunistic bacteria overwhelmed the original assemblage during bottle incubations (4 to 5 d) [17, 27]. However, dominance of few bacterial species was not detected, as revealed by the number of ribotypes remaining almost identical (even if their positions were different) between the start and the end of experimental cultures (with the exception of the full sun treatment for SOLA station). This indicates that bacterial structure was altered during the regrowth but without dominance of a few bacterial species. Moreover, the interpretation of our data is mainly based on the comparison of the diversity at the end of the experimental cultures between the different treatments, and all include the same potential “bottle effect” on the bacterial diversity composition. We did not perform replicates of each experimental culture because of operational limitations. However, high similarities sometimes obtained in the community structures of total and metabolically active bacterial cells at the end of the experimental cultures for two different light treatments (i.e., full sun and PAR treatments for Leucate Lagoon, PAR, and dark treatments for Canet Lagoon) indicate that results may be considered as robust.

The influence of phototransformations of DOM to the bacterial diversity differs with the sampling location. The effect was most dramatic in the oligotrophic SOLA coastal station (Fig. 4,A). Bioavailability changes of DOM because of full sun treatment resulted in a decrease of the number of dominant ribotypes that was not recordable for the PAR treatment (Fig. 5). Along with the increase of both BP and BGE for the full sun compared to dark and PAR treatments at SOLA, the composition of the metabolically active bacteria (16S rRNA based CE-SSCP) was also more affected in the full sun treatment, with 78% of ribotypes present at the DNA level that were active at the RNA level. At the Leucate Lagoon, inhibition of BP and BGE in both full sun and PAR treatments compared to dark was related to the dissimilarity between dark, PAR, and full sun in

community structures of both total and metabolically active bacteria (Fig. 4,B). At the eutrophic Canet Lagoon, community structures of total and metabolically active bacteria were clearly influenced by DOM bioavailability changes because of UV radiation (Fig. 4, C). These changes can be related to the increase of BP measured in the full sun treatment compared to the PAR and dark treatments but not to the decrease of BGE observed for both PAR and full sun treatments compared to dark. These results corroborated several studies suggesting that the quality and supply of organic substrates affect the function as well as the composition of bacterial diversity [12, 34].

Interestingly, our results show that community changes of metabolically active bacteria enhance the differences observed at the DNA level, resulting in a closer link between rRNA-based analysis and BP changes compared to rDNA-based analysis. Recent work based on the comparison between 16S rDNA and rRNA libraries indicates that the characterization of the bacterial community based on rRNA has the potential to characterize distinct ribotypes from the marine environment, which remain undetected on the DNA level [29]. The number of ribotypes per sample can be lower, equal, or higher on the RNA level than on the DNA level depending on the detection limit of the fingerprinting technique and their relative contribution to the community in terms of cell number or metabolic activity [46]. In our study, the number of ribotypes per sample was always higher or equal on the RNA level than on the DNA level (Fig. 4). This suggests that their contribution to the community in terms of cell numbers may have been too low for detection, but because of their high metabolic activity and the accompanying 16S rRNA synthesis, these ribotypes were readily detectable at the RNA level [47]. Metabolically active bacteria that were below the detection limit at the DNA level represent from 12 to 26% of the total number of ribotypes. These bacteria may correspond to organisms present in low cellular abundance and therefore probably not detectable on the DNA level but with higher ribosome content and thus detectable at the RNA level. In addition, most of the ribotypes present at the DNA level in each of the locations studied were active (from 74 to 79%), and a few were not detectable at the RNA level (from 0 to 12%). These results suggest that the phototransformation of DOM affects both the bacterial community structure and the composition of active bacteria and that RNA-based analysis was more sensitive to point out small changes of organic substrate availability.

In conclusion, we found that BP, BR, BCD, BGE, and bacterial community structure and related metabolically active bacteria may be altered by phototransformations of DOM for different oligotrophic to eutrophic aquatic ecosystems. Changes in total and active bacterial structures

induced by phototransformations of DOM can be correlated to changes in BP. These changes may occur even after a short exposure of DOM to solar radiation and without major CDOM photobleaching and photomineralization of DOM. We encourage a conservative interpretation of our results in this initial study. Further studies will be necessary to elucidate a more detailed appreciation of potential temporal and spatial variations of the effects measured. Different results may be obtained seasonally because of changes in DOM composition, bacterial community structure, and UV intensity and penetration in the water column. Future works may also elucidate the bacterial species and functional groups most responsive to photochemically modified DOC compounds [25].

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