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Interactions of Photobleaching and Inorganic Nutrients in Determining Bacterial Growth on Colored Dissolved Organic Carbon

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Abstract

Bacteria are key organisms in the processing of dissolved organic carbon (DOC) in aquatic ecosystems. Their growth depends on both organic substrates and inorganic nutrients. The importance of allochthonous DOC, usually highly colored, as bacterial substrate can be modified by photobleaching. In this study, we examined how colored DOC (CDOC) photobleaching, and phosphorus (P) and nitrogen (N) availability, affect bacterial growth. Five experiments were conducted, manipulating nutrients (P and N) and sunlight exposure. In almost every case, nutrient additions had a significant, positive effect on bacterial abundance, production, and growth efficiency. Sunlight exposure (CDOC photobleaching) had a significant, positive effect on bacterial abundance and growth efficiency. We also found a significant, positive interaction between these two factors. Thus, bacterial use of CDOC was accelerated under sunlight exposure and enhanced P and N concentrations. In addition, the accumulation of cells in sunlight treatments was dependent on nutrient availability. More photobleached substrate was converted into bacterial cells in P- and N-enriched treatments. These results suggest nutrient availability may affect the biologically-mediated fate (new biomass vs respiration) of CDOC.

Introduction

Bacteria are key organisms in carbon cycling in aquatic ecosystems. They can directly oxidize dissolved organic carbon (DOC) to CO_2 and can link carbon transfer through food webs [3]. These organisms are controlled by a number of different factors, including environmental conditions, resource limitation, and predation [22, 44, 59, 68]. The proximate resources for bacterial growth are dissolved organic carbon compounds and organic and inorganic forms of nitrogen (N), phosphorus (P), sulfur (S), vitamins, and trace metals.

Until recently, labile organic carbon sources for bacteria were considered derived primarily from recent photosynthesis of phytoplankton [8, 11, 12]. This paradigm is being modified. The allochthonous fraction of DOC constitutes a

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large pool of relatively refractory compounds, but some of this material becomes labile under appropriate environmental conditions [5, 29, 39, 60, 65]. In comparison with DOC of phytoplankton origin, allochthonous DOC is enriched in complex structures of aromatic and aliphatic groups (mainly humic and fulvic acids) [57]. Therefore, this DOC is highly colored and strongly light-absorbing [42]. Colored dissolved organic carbon (CDOC) not only absorbs light, it is also modified by it. Early in this century, Whipple [69] showed that the color of CDOC (e.g., its ability to absorb light) is actually reduced by exposure to sunlight. This reduction in color was termed photobleaching. Photobleaching promotes a breakdown of high-molecular-weight humic carbon to lower-molecular-weight compounds, increasing bioavailability [56]. Bioassays have clearly documented stimulation of bacterial growth by photobleaching [7, 25, 35, 36, 67]. Nevertheless, the potential role of photoproducts as bacterial carbon substrates remains poorly studied, in comparison with DOC provided from algal metabolism. Besides these transformations in C bioavailability, photobleaching can also change mineral nutrient conditions. Photochemical production of ammonium [9] and the sunlight-mediated release of orthophosphate [23] from dissolved organic matter have been demonstrated. These positive and indirect effects of sunlight on bacterial growth may, however, be counterbalanced in natural systems by other sunlight-driven, negative effects. For instance, ultraviolet-B radiation induces direct DNA damage in bacterioplankton [28, 32]; algal photosynthesis can be inhibited, causing a reduction of DOC excretion; DOC can be directly photooxidated, resulting in a loss of organic resources; CDOC sunlight absorption can also produce a series of reactive toxic by-products, such as superoxide and hydrogen peroxide [14, 49], that can inhibit bacterial activity.

Several studies have demonstrated that bacteria in many natural systems are P or N limited, instead of C limited [17, 18, 43, 47]. Bacterial cells have low C:P and C:N ratios, and high demands for P and/or N, relative to other organisms [46, 62]. This limitation may have an important effect on bacterial dissolved organic carbon processing, as observed by some authors [47, 70]. A number of researchers have also shown that aquatic bacterial production can be stimulated simply by adding inorganic N and P, by adding readily-utilizable organic carbon compounds, or by these factors in combination [16, 43, 58, 64].

It is reasonable, therefore, to hypothesize that CDOC photobleaching will provide bioavailable organic carbon (and maybe inorganic nutrients) that could increase bacterial biomass, and that inorganic nutrient availability will increase bacterial growth (when C is not limiting). In this study, CDOC photobleaching and nutrient interactions were examined for contributions to bacteria growth. This question was addressed experimentally by manipulating inorganic nutrients and photobleaching.

Methods

Five experiments were performed using water from a humic lake (Old Man McMullen Pond, Connecticut; 41° 57′N 73° 15′ W) to assess the bacterial responses to CDOC photobleaching and mineral nutrients. This lake was selected because of its seasonal variability in color, DOC, and color:DOC ratio (Reche et al., in prep.). Water color was measured by filtering lake water through Whatman GF/F glass fiber filters, and measuring absorbance of the filtrate at 440 nm in 10 cm cuvettes. Absorbance is expressed as a coefficient in units of m^{-1} [19]. Color ranged, seasonally, from 3.36 to 14.76 m^{-1} in the study system. Experiments exploited this natural tendency to simulate gradients in the relative contribution of humic compounds to the total DOC pool.

DOC was analyzed by filtering lake water through precombusted Whatman GF/F glass fiber filters, collecting the filtrate in a clean flask, and acidifying (final pH 2) the sample until analysis. DOC concentrations were measured with a Shimadzu TOC-5050 Total Carbon Analyzer.

Lake water was initially filtered through Whatman GF/F filters (~0.7 µm pore size). This reduced bacterial density to ~50% of its natural abundance [60]. The filtration procedure also removed bacterivores and phytoplankton (new DOC inputs from algal photosynthesis were excluded). Therefore, bacterial growth was based on the existing DOC pool. Each experiment consisted of four treatments: transparent (*T*), opaque (*O*), transparent plus inorganic nutrient (*T*+), and opaque plus inorganic nutrient (*O*+). Filtered water was added to 12 borosilicate-bottles. Six bottles were enriched with P and N (*T*+ and *O*+ treatments), as KH_2PO_4 and NH_4Cl, to a final concentration of 1 and 10 µM, respectively. Three of the P- and N-enriched bottles (*O*+ treatment) and three of the unenriched bottles (*O* treatment) were covered with aluminum foil, to eliminate photobleaching. Experimental bottles were incubated on a platform, under natural sunlight, at ambient air temperatures. Samples for DOC, water color, inorganic nutrients, and bacterial abundance and production were taken at least twice (initial and final conditions), from each culture, during incubation. Incubations ranged from 32 to 192 h in length, depending on the experiment. Sunlight doses were measured using a quantum sensor (LiCor model LI-190 SB). The energy transmission through the experimental bottles was: 90% for PAR (>400 nm), 65% for UVAR (400–320), and <5% for UVBR (<320) (see Reche et al., submitted, for transmittance spectrum). Therefore, the direct bacterial DNA damage by UVBR was minimized in these experiments.

DOC concentrations and water color were measured, using methods described above. Ammonium and phosphate concentra-

tions were analyzed, using the phenate and phosphomolybdate techniques [2], respectively, in an Alpkem Enviroflow Analyzer Model 3590.

Bacterial abundance was determined by acridine orange epifluorescence microscopy [30]. Three replicates were counted for each treatment; at least 350 cells per replicate were counted. Bacterial production in two of the experiments (first and fourth) was determined from the incorporation of [methyl-^{3}H]thymidine (S.A: 80.4 Ci mmol^{-1}) into macromolecules [24], at an added concentration of 25 nM. Samples were incubated for 1 h in the dark, at 20°C. Moles of [^{3}H]thymidine incorporated were converted to the number of cells produced, assuming 2×10^{18} cell mol^{-1} [6]. Bacterial carbon produced was estimated by using a conversion value of 20 fg C cell^{-1} [34]. In experiments #2, 3, and 5, bacterial production was determined from [^{3}H]leucine incorporation into proteins [52]. Three replicates and one blank (TCA added before isotope) of each treatment were incubated for 1 h in dark, 20°C, with L-[4,5 ^{3}H]leucine (S.A: 52 Ci mmol^{-1}) at 22 nm final concentration. [^{3}H]Leucine incorporation was converted to carbon units, following Simon and Azam [51]. Cultures were examined for heterotrophic nanoflagellates and photosynthetic organisms (autotrophic picoplankton or nanoplankton), using epifluorescence and autofluorescence techniques. Significant abundances of either group were not observed during the experiments.

In the last two experiments, for each treatment, bacterial growth efficiency (*BGE*) on DOC, was calculated, using the following equation [37]:

$$BGE = \frac{C\ incorporated\ into\ bacterial\ biomass}{DOC\ removed} \times 100$$

The numerator was obtained from the increase in bacterial biomass during the incubation. Bacterial biomass was estimated from bacterial abundance. The denominator was estimated from the losses of DOC in transparent treatments, corrected for abiotic DOC photooxidation.

To estimate DOC photooxidation, DOC losses were quantified in the last two experiments (4 and 5) by incubating sterile (0.2 µm-filtered) lake water in transparent and dark control test tubes. During the experiments, DOC was periodically measured in these tubes along with the cumulative sunlight dose. Since test tubes and experimental bottles had different volumes, the photooxidation rates obtained in the sterile test tubes were corrected for the attenuation of the sunlight energy inside the experimental bottles. The spectrum of energy transmitted through the two kinds of glass (experimental bottles vs test tubes) was similar. Therefore, in *T* and *T*+ treatments:

$$DOC_{removed} = DOC_{t0} - DOC_{tn} - DOC_{photooxidized}$$

and in *O* and *O*+ treatments:

$$DOC_{removed} = DOC_{t0} - DOC_{tn}$$

where $DOC_{removed}$ is the dissolved organic carbon taken up by bacteria, DOC_{t0} is its concentration at the beginning of the incubation, DOC_{tn} is the concentration at time *n*, and $DOC_{photooxidized}$ is the loss of dissolved organic carbon due to direct photooxidation.

Statistical Analysis

To test whether observed differences in bacterial abundance or production among treatments were significant, analysis of variance (ANOVA) was used. Comparison between transparent (*T* or *T*+) and opaque (*O* and *O*+) treatments, at the end of each experiment, indicates significance of sunlight exposure. Comparison between the unenriched (*T* and *O*) and P- and N-enriched (*T*+ and *O*+) treatments indicates the significance of inorganic nutrient supply. Combinations of both factors (DOM photobleaching and nutrient additions) were examined to test whether bacteria were enhanced (positive interaction) or suppressed (negative interaction) more than expected from the factors in isolation.

Results

Experimental Conditions

The sunlight dose and relative humic-acid contribution to the total DOC pool (color:DOC ratio) varied seasonally among experiments (Table 1). Color:DOC ratio ranged approximately threefold, while the sunlight dose varied approximately tenfold. Color:DOC ratios were highest in experiments 1 and 4, in late fall and summer, respectively. Lowest humic contribution to the total DOC pool (the lowest color:DOC ratio) was found in experiment 2, when the lake was ice-covered (March). Soluble reactive phosphate generally decreased during incubations, in both unenriched and enriched treatments. In enriched treatments, however, P depletion never reached background levels (unenriched treatment). Ammonium concentrations also decreased in almost all treatments and experiments, except in the third. Here the concentration increased. Initial water color varied more than four-fold among experiments. Water color was lost in *T* and *T*+ treatments. It depended on the sunlight dose, and the losses fit negative exponential functions (see Reche et al., submitted). The relative loss of color ranged, among experiments, from 5.2 to 7.7% day 1. DOC concentration remained constant in the first experiment; it decreased in all others. Significant differences ($p < 0.05$) in DOC concentration among treatments at the end of incubations, however, were only found in experiments 4 and 5.

Bacterial Responses

Bacterial production increased under most conditions (Fig. 1). Inorganic nutrient additions (*T*+ and *O*+) stimulated bacterial production in all experiments, except 4. The effect of sunlight on bacterial production varied, but exhibited a negative trend.

Table 1. Values of inorganic nutrients, water color, dissolved organic carbon and color:DOC ratio at the beginning (t_0) and at the end of incubations (t_n) for each treatment (*T*, *O*, *T*+, and *O*+)

Exp. #		Dose (E m^{-2})	Time (h)	Phosphates (µg P l^{-1})		Ammonium (µg N l^{-1})		Absorption coefficients (m^{-1})		DOC (mg l^{-1})		color: DOC
				t_0	t_n	t_0	t_n	t_0	t_n	t_0	t_n	t_0
1	*T*	27	32	n.d.	n.d.	n.d.	n.d.	8.29 ± 0.09	7.55 ± 0.02	10.2 ± 0.6	10.4 ± 0.0	0.81
	O	0	32	n.d.	n.d.	n.d.	n.d.	8.59 ± 0.21	8.43 ± 0.12	11.0 ± 0.1	10.5 ± 0.2	0.78
	T+	27	32	n.d.	n.d.	n.d.	n.d.	7.99 ± 0.09	6.96 ± 0.14	11.2 ± 0.2	10.0 ± 0.0	0.71
	O+	0	32	n.d.	n.d.	n.d.	n.d.	8.20 ± 0.14	8.01 ± 0.21	11.3 ± 0.3	10.6 ± 0.1	0.73
2	*T*	103	100	14 ± 2	9 ± 1	110 ± 28	73 ± 21	3.36 ± 0.09	2.86 ± 0.25	9.6 ± 0.7	6.7 ± 0.2	0.35
	O	0	100	14 ± 2	9 ± 1	110 ± 28	103 ± 25	3.32 ± 0.09	3.16 ± 0.14	10.7 ± 0.9	7.1 ± 0.5	0.31
	T+	103	100	99 ± 1	45 ± 8	240 ± 37	90 ± 10	2.76 ± 0.09	1.87 ± 0.09	10.5 ± 0.1	6.9 ± 0.2	0.26
	O+	0	100	99 ± 1	44 ± 4	240 ± 37	223 ± 53	2.67 ± 0.18	2.56 ± 0.07	10.7 ± 0.3	6.8 ± 0.1	0.25
3	*T*	94	99	9 ± 1	10 ± 1	20 ± 0	67 ± 5	5.60 ± 0.02	3.87 ± 0.05	9.1 ± 0.7	7.6 ± 0.1	0.62
	O	0	99	9 ± 1	9 ± 1	20 ± 0	80 ± 0	5.55 ± 0.05	4.38 ± 0.21	8.8 ± 0.2	7.9 ± 0.4	0.63
	T+	94	99	130 ± 11	91 ± 21	177 ± 12	153 ± 6	5.14 ± 0.18	3.41 ± 0.14	9.8 ± 1.4	7.3 ± 0.1	0.52
	O+	0	99	130 ± 11	79 ± 3	177 ± 12	237 ± 30	4.79 ± 0.09	3.82 ± 0.07	9.6 ± 0.7	7.3 ± 0.1	0.50
4	*T*	279	187	28 ± 1	22 ± 2	30 ± 14	33 ± 10	14.74 ± 0.28	8.61 ± 0.41	16.5 ± 0.5	11.8 ± 0.4	0.89
	O	0	187	28 ± 1	24 ± 0	30 ± 14	20 ± 7	14.81 ± 0.05	14.21 ± 0.21	16.5 ± 0.2	16.1 ± 0.1	0.90
	T+	279	187	88 ± 7	25 ± 3	320 ± 10	213 ± 30	14.21 ± 0.05	7.51 ± 0.23	16.6 ± 0.2	11.7 ± 0.4	0.86
	O+	0	187	88 ± 7	60 ± 6	320 ± 10	260 ± 10	14.32 ± 0.05	13.56 ± 0.16	16.7 ± 0.2	15.9 ± 0.2	0.86
5	*T*	97	172	22 ± 1	21 ± 1	30 ± 0	30 ± 10	11.86 ± 0.07	9.23 ± 0.39	20.8 ± 0.5	12.5 ± 1.2	0.57
	O	0	172	22 ± 1	21 ± 1	30 ± 0	40 ± 8	12.14 ± 0.18	11.63 ± 0.16	17.4 ± 1.5	13.7 ± 0.1	0.70
	T+	97	172	95 ± 1	83 ± 1	610 ± 10	575 ± 20	11.42 ± 0.14	8.48 ± 0.18	17.4 ± 2.4	12.7 ± 0.8	0.66
	O+	0	172	95 ± 1	93 ± 1	610 ± 10	580 ± 28	10.19 ± 0.05	10.89 ± 0.28	17.4 ± 1.0	14.9 ± 1.0	0.59

n.d., not determined

Unlike bacterial production, bacterial abundance response was consistent among experiments (Fig. 2). The increases in abundance were generally higher in the *T*+ treatments of experiments 1, 4, and 5 (where color:DOC ratios were relatively high) and in the *O*+ treatment of experiment 2 (where color:DOC ratio was the lowest). In experiment 3 (where color:DOC ratio was relatively low), no significant differences were found between *T*+ and *O*+ treatments. Overall, the P- and N-enriched treatments had much higher final bacterial abundance than their corresponding, unenriched treatments.

P and N additions had a significant positive effect on final bacterial abundance and production (Table 2), except in the fourth experiment. The effect of sunlight on bacteria, among experiments, varied more than the effect of added nutrients. Sunlight had a significant positive effect on bacterial abundance in four of seven cases; the effect was negative in one case. However, bacterial production was negatively affected by sunlight in most cases (in five of seven) (Table 2).

The interaction between sunlight and mineral nutrient additions was significant and positive in five of seven cases for bacterial abundance and production. No significant negative interactions were found either for bacterial abundance or production (Table 2).

Light-Enhanced Cell Accumulation

To evaluate the role of CDOC photobleaching as a bacterial substrate supplier and, consequently, as a producer of new bacterial biomass, the changes in abundance were considered an integrated measure of this new biomass. The net effect of CDOC photobleaching on growth can be estimated by comparing bacterial increases in transparent bottles (where photobleaching occurs) with increases in the opaque bottles (no photobleaching). The change was calculated over time, in the transparent treatments (final abundance less initial abundance for *T* (ΔT) and for *T*+ (ΔT+)), and in the opaque treatments (final abundance less initial abundance for *O* (ΔO) and for *O*+ (ΔO+)). Light-enhanced cell accumulation (LECA) was then calculated by subtracting the change in abundance in the opaque treatments from the change in the transparent treatments ($\Delta T - \Delta O$ and ΔT+ −

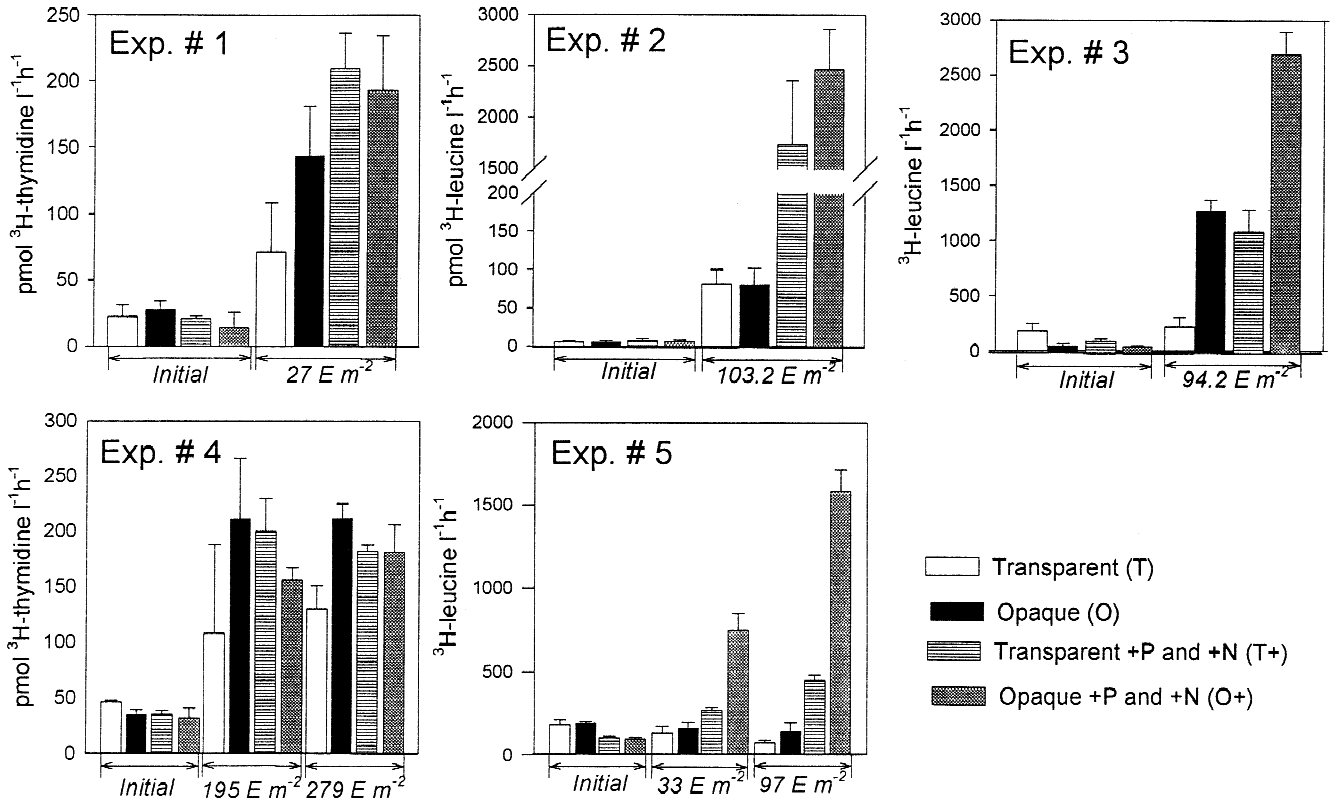

Fig. 1. Bacterial production changes during incubations. Units for experiments 1 and 4 are in pmol [^{3}H]thymidine l^{-1} h^{-1}, and units for experiments 2, 3, and 5 are in pmol [^{3}H]leucine l^{-1} h^{-1}. On the *x* axis, the sunlight dose (Em^{-2}) of each experiment was plotted. Error bars are the standard errors of the three replicates.

$\Delta O+$). This calculation of LECA provides an estimate of the net conversion of photobleached CDOC to bacterial biomass.

LECA was not related to sunlight dose (Fig. 3A). We observed positive LECA in the experiments that received both low and high light doses; generally LECA was higher under nutrient-enriched conditions (open squares). LECA in the unenriched treatments (filled squares) of the different experiments was constant (below 1×10^6 cell ml^{-1}) irrespective of water color (Fig. 3B). However, in the enriched treatments, there was a clear, positive trend between LECA and color ($n = 21$, $p < 0.001$). This indicates that, when the water was highly colored, there was an increased conversion of photobleached CDOC to bacterial biomass. The same pattern was observed when LECA was plotted against the color:DOC ratio. This indicates the relative contribution of humic compounds to the total DOC (Fig. 3C).

Bacterial Growth Efficiency

The dependence of the LECA differential response on inorganic nutrient availability suggests that the transfer of carbon from the humic fraction to bacterial biomass was higher in the enriched treatments. To test whether this conversion was actually different among treatments, bacterial growth efficiency (BGE) was determined in experiments 4 and 5. BGE was based on the net change in DOC of each treatment. These calculations were possible because the losses of DOC related to photooxidation were determined, thereby quantifying organic carbon unavailable for bacterial consumption in transparent treatments. In both experiments, DOC losses were linear functions of the sunlight dose. Dark controls did not change DOC concentration during incubation. The functions were:

$$Exp\ 4 \quad -\Delta DOC = 17.59 - 0.0185 * D \ (r^2 = 0.96,\ p < 0.001)$$

$$Exp\ 5 \quad -\Delta DOC = 14.87 - 0.0186 * D \ (r^2 = 0.86,\ p < 0.001)$$

$-\Delta DOC$ is the loss of DOC in mg l^{-1} due to sunlight-driven oxidation, and D is the sunlight dose received in E m^{-2}. Photooxidized DOC was 4.4 mg C l^{-1} in experiment 4 (approx. 0.63 mg C l^{-1} d^{-1}) and 1.5 mg C l^{-1} (approx. 0.37 mg C l^{-1} d^{-1}) in experiment 5. These values are similar to photooxidative DIC production found

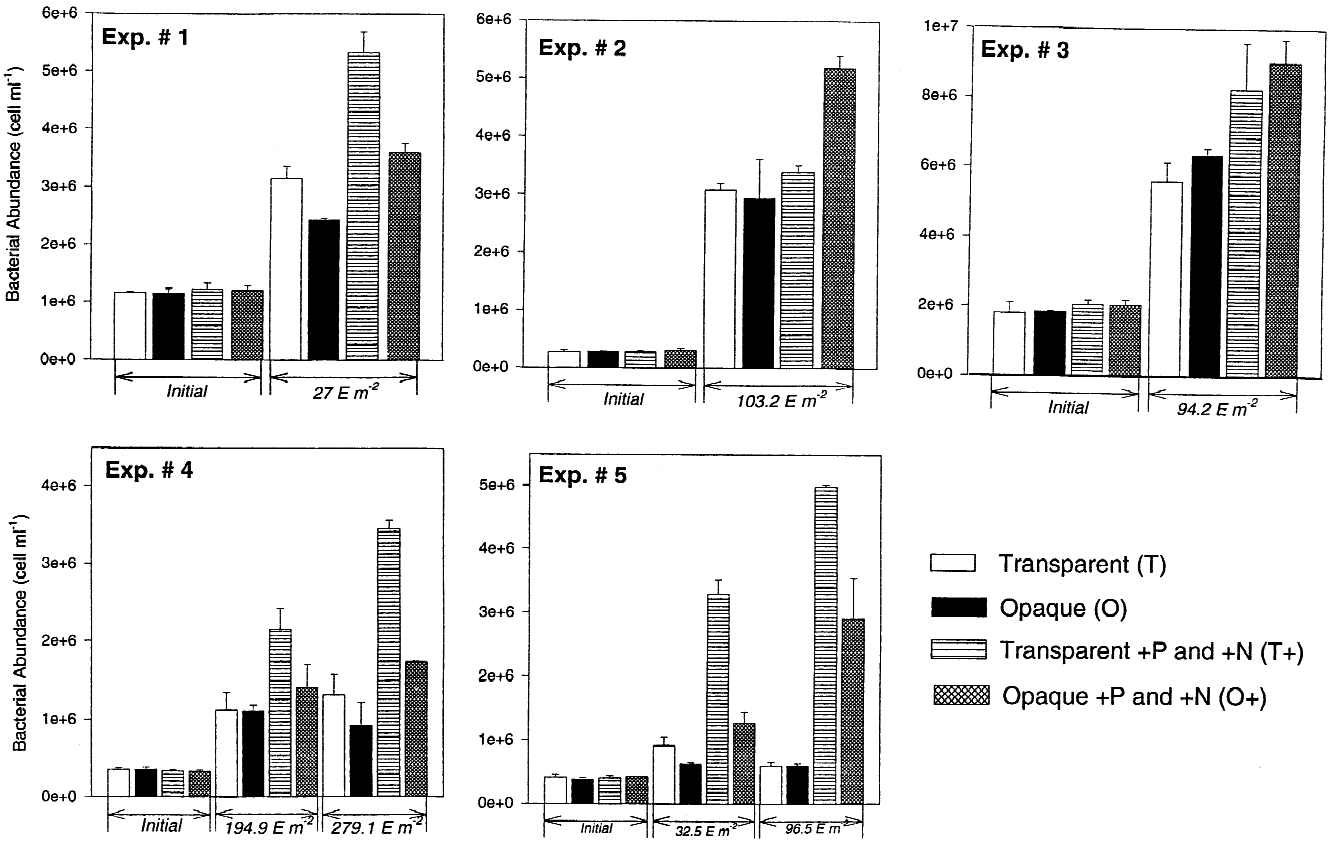

Fig. 2. Bacterial abundance changes (cell ml^{-1}) during incubations. On the *x* axis, the sunlight dose (Em^{-2}) of each experiment was plotted. Error bars are the standard errors of the three replicates.

by Graneli et al. [26] in lake surface waters. DOC removed by bacteria ranged from 0.3 to 0.8 and from 2.5 to 3.2 mg C l^{-1} in experiments 4 and 5, respectively.

BGE in the *T*+ treatments of both experiments was higher than for any of the other treatments (Table 3). In experiment 4, BGE was ~15% in *T*+ and 4% in the other treatments. In experiment 5, BGE in *T*+ was 2.5%, close to 2% in the *O*+ treatment, and <0.5% in the unenriched treatments. In both experiments, sunlight and mineral nutrient additions had a significant positive effect on BGE. These two factors also interacted significantly and positively (Table 4).

Discussion

Bacterial Response to CDOC Photobleaching

There was a positive effect of photobleaching on bacterial abundance, but not production, in our experiments (Table 2). The mostly negative response of bacterial production was not related to UV-B damage of bacteria, because this radiation did not penetrate the experimental bottles (see Methods). The photoinhibition of bacterial production was due to longer ultraviolet and visible wavelengths. Several authors have reported similar results [1, 4, 36, 50]. Bacterial inhibition by UV-A and PAR results from negative effects on biochemical and cellular processes [45, 53], or indirectly, due to toxic by-products derived from DOC reactions with sunlight [49]. Bacterial production was measured only during daylight hours. This measure may, therefore, reflect the short term (specific to sampling time) response of bacteria, rather than the overall response. Bacterial abundance, however, reflects an integrated (from the beginning of the experiment to sampling time) response, so this parameter was used as the primary index.

Bacteria increased in response to nutrients and photobleaching, except in experiment 2. In this case, color and the DOC:color ratio are relatively low. The relative clarity of the water may have allowed sufficient light penetration to inhibit the bacteria. It is also possible that DOC in this experiment was more recalcitrant. Whatever the cause, the experimental results suggest that important seasonal changes in the quality of DOC, as reflected by the color:DOC ratio, can modulate bacterial activity in natural systems.

Table 2. Results of ANOVAs to determine the effect of presence/absence of photobleached substrates and inorganic nutrients on bacterial production and abundance[a]

	Dose ($E\ m^{-2}$)	Time (h)	Treatments	Production		Abundance	
				Effect	*p*-level	Effect	*p*-level
Exp 1 December 95	27.0	32	Sunlight exposure		n.s.	+	***
			P and N additions	+	*	+	***
			Interaction		n.s.	+	**
Exp 2 March 96	103.2	100	Sunlight exposure	−	*	−	**
			P and N additions	+	***	+	***
			Interaction	+	*	+	**
Exp 3 May 96	94.2	99	Sunlight exposure	−	***		n.s.
			P and N additions	+	***	+	***
			Interaction	+	*		n.s.
Exp 4 August 96	194.9	122	Sunlight exposure		n.s.		n.s.
			P and N additions		n.s.	+	**
			Interaction		n.s.		n.s.
	279.1	187	Sunlight exposure	−	**	+	***
			P and N additions		n.s.	+	***
			Interaction	+	**	+	**
Exp 5 October 96	32.5	96	Sunlight exposure	−	***	+	***
			P and N additions	+	***	+	***
			Interaction	+	***	+	***
	96.5	172	Sunlight exposure	−	***	+	**
			P and N additions	+	***	+	***
			Interaction	+	***	+	**

[a] Bacterial parameters were compared at the end of incubation time.
n.s., not significant; n.d., not determined; + increase; − decrease, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

The positive effect of photobleaching on bacterial abundance resulted from transformations in the quality of the substrate. Sunlight exposure involves changes in size (from high to low molecular weight), and in DOC chemical composition, that facilitate bacterial use [7, 15, 33, 38, 67]. In addition, photodegradation might accelerate carbon utilization by bacteria via cometabolism, defined as the microbial degradation of a resistant substance in the presence of a readily degradable substrate [27]. Several biodegradable photoproducts have been identified [15, 40, 67]. Most of these compounds contain three or fewer carbon atoms, and have a molecular weight of less than 100. These small organic compounds could act as cometabolites and catalyze the use of more recalcitrant organic carbon.

The positive effect of photobleaching on bacterial abundance was also evident in our measures of BGE in the last two experiments (Table 4). However, BGEs in these experiments, irrespective of treatment, were low compared to literature values [13, 21]. This difference could be related to the nature of the experimental substrates (relatively enriched in humic compounds due to the absence of DOC inputs from algae or zooplankton). Growth efficiencies based on changes in DOC tend to be low [31], probably because bacterial biomass increase represents a net measure of growth (bacterial mortality has not been included). The change in substrate, however, represents the gross consumption of DOC. Thus, our measures are a lower bound to the actual growth efficiency. The usefulness of the calculated BGEs is based on the distinctions, found among treatments, that indicate significant effects of both factors (CDOC photobleaching and P and N enrichments) on BGE (Table 4).

Bacterial Response to Inorganic Nutrient Additions

Inorganic nutrient additions also stimulated bacterial abundance and growth efficiency. The existence of nutrient limitation in the cultures was expected and confirmed by the similar effects of P- and N-additions on bacterial abundance and production (Table 2). The nutrient stimulation of BGE reflects changes in the transformation of carbon, relative to N and P requirements. It is well known that most organisms

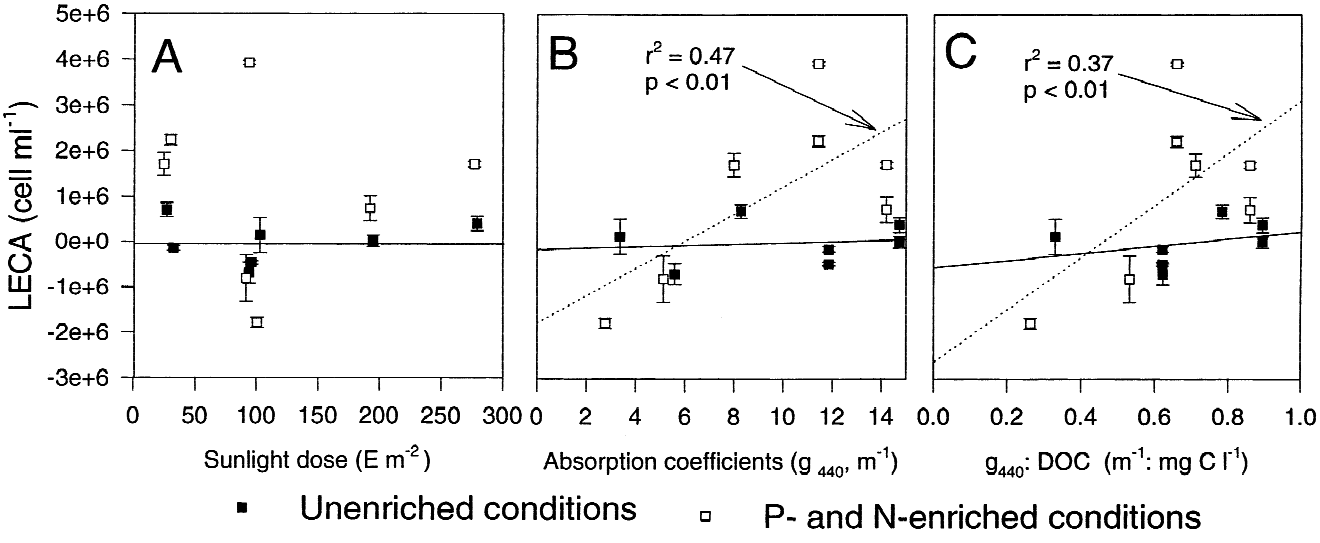

Fig. 3. Values of light-enhanced cell accumulation (LECA) in nutrient-enriched conditions (open squares), and in unenriched conditions (filled squares), vs the sunlight dose of each experiment (A), along color gradient (B), and along the relative contribution of humic compounds to the total DOC pool (C). Squares are the average values of the three experimental replicates; error bars are the standard errors of each experiment.

can independently regulate their respiration or excretion of C, N, and P, to move toward a stoichiometric optimum [10, 54, 55]. Bacteria have particularly high requirements for P and N [46, 62, 63]; reducing inorganic nutrient limitation promotes bacterial growth, relative to carbon loss via respiration. This explains the higher growth efficiencies in nutrient-enriched treatments.

Dissolved organic matter photobleaching can also release inorganic nutrients which were part of larger molecules [9, 23]. In our experiments, however, the processes of P-release or ammonification during DOM photobleaching were apparently not sufficient to overcome bacterial nutrient limitation, as indicated by the differential growth in *T* relative to *T*+ treatments.

Interaction between Photobleaching and Inorganic Nutrients

There were significant, positive interactions of CDOC photobleaching and P and N enrichments on bacterial abundance, production, and BGE. These interactions indicate that conversion of C from substrate into bacterial biomass was higher than expected from the addition of individual treatments.

The interactions of CDOC photobleaching (as a supplier of bioavailable DOC) and inorganic nutrient availability can be explained by several mechanisms. Processes such as concurrent or reciprocal limitation of C, P or N; cometabolism of different compounds; and/or biological feedbacks related, for example, to the activation of enzymatic chains [20, 25, 27, 41] are potential explanations for enhanced bacterial growth, when exposed to both light and nutrients. The actual mechanism(s) underlying this response, however, were not revealed by these experiments. They require further study.

Table 3. Results of bacterial growth efficiency (BGE), expressed as percentage, in the different treatments of experiments 4 and 5

Treatments	BGE exp 4	BGE exp 5
T	4.0 ± 0.2	0.2 ± 0.0
O	3.6 ± 0.3	0.1 ± 0.0
T+	15.4 ± 0.9	2.5 ± 0.0
O+	4.5 ± 0.5	1.7 ± 0.0

Ecological Implications

These results imply that the conversion of photobleached CDOC to bacterial biomass (LECA) is enhanced under P- and N-enrichments. This key result should be applied with caution to natural conditions. Increased nutrient loading in aquatic ecosystems could facilitate the utilization of photoproducts, and transfer of carbon, to the food web via bacteria. Bacterial utilization of this photobleached CDOC could partly explain the high biomass of zooplankton and fish in dystrophic lakes [48, 61], as well as the imbalance between bacterial respiration and phytoplankton production

Table 4. Results of ANOVAs to compare the effect of presence/absence of photobleached substrates and inorganic nutrients on bacterial growth efficiency (BGE) in the experiments 4 and 5[a]

	BGE exp 4		BGE exp 5	
	F	*p*-level	*F*	*p*-level
Photobleached vs no photobleached CDOC	16.5	0.0036**	6.4	0.0360*
P- and N- vs no P- and N-additions	16.4	0.0037**	62.6	0.0000***
Interaction	10.5	0.012*	6.4	0.0348*

[a] *F* is the variance ratio, *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$

that is found in many unproductive lakes [21]. CDOC is rich in aromatic humic compounds of allochthonous origin. These compounds have not been degraded during transport from terrestrial and littoral habitats to open waters [65, 66]. The conversion of carbon from humic compounds to bacterial biomass which occurs upon exposure to light, may operate at a low efficiency, but, given the concentration of DOC, may still represent a significant contribution to secondary production.

These experiments did not include the effects of phytoplankton, which are likely to be important given their ecological interactions with nutrients, bacteria, and DOC. The designs of experiments manipulating phytoplankton, as well as comparisons of lakes with contrasting conditions (e.g., phytoplankton and bacterial biomass, nutrients, DOC), should contribute to an accurate assessment of the interactions of photobleaching and inorganic nutrients, and how these, in turn, influence microbial processes in natural systems.

Inorganic nutrients have a clear effect on CDOC biodegradation. Therefore, the dynamics of inorganic nutrients and CDOC should not be considered independent, but, rather, interactive drivers of lake structure and function. Primary production and nutrient cycling have long been viewed as dynamically linked. The utilization of allochthonous carbon also may be linked to nutrients. We need to assess the significance of this pathway to total carbon flux, in terms of both respiration and the support of higher trophic levels in natural systems.

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