Microbial Ecology

Effects of Dissolved Organic Matter Photoproducts and Mineral Nutrient Supply on Bacterial Growth in Mediterranean Inland Waters

Eva Ortega-Retuerta[1], Elvira Pulido-Villena[1,2] and Isabel Reche[1,3]

(1) Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, Granada 18071, Spain
(2) Laboratoire d'Oceanographie de Villefranche-LOV (UMR-CNRS 7093), Villefranche-sur-Mer 06238, France
(3) Instituto del Agua, Universidad de Granada, Granada 18071, Spain

Received: 25 October 2006 / Accepted: 28 November 2006 / Online publication: 13 March 2007

Abstract

Sunlight reacts with dissolved organic matter (DOM) modifying its availability as bacterial substrate. We assessed the impact of DOM photoproducts and mineral nutrient supply on bacterial growth in seven inland waters from the South of Spain, where DOM is characterized by low chromophoric content and long residence time. Factorial experiments were performed with presence vs absence of DOM photoproducts and mineral nutrient supply. In six of the seven experiments, we found a significant and negative effect of DOM photoproducts on bacterial growth and a significant and positive effect of mineral nutrient supply. The interaction of these two factors leaded to a compensation of negative effects of photoproducts by availability of mineral nutrients. Dissolved organic matter diagenetic status and the ionic environment where organic carbon is dissolved can be influencing bacterial DOM processing.

Introduction

Dissolved organic matter (DOM) is the primary substrate for bacterial growth in most aquatic ecosystems [3, 39]. Dissolved organic matter is a heterogeneous pool of autochthonous (from phytoplankton excretion or cellular lysis) and allochthonous (from terrestrial inputs) molecules covering a wide range of size, diagenetic status, and composition that finally determine its bioavailability [2, 37, 39, 43]. Usually, autochthonous DOM is composed of highly bioavailable molecules, whereas allochthonous DOM is enriched in aromatic and aliphatic compounds (humic and fulvic acids) highly colored and slowly assimilable by bacteria [37]. In addition, mineral nutrient availability has an important influence on DOM processing by bacteria [7], enhancing DOM assimilation to generate new biomass vs respiration [17, 27, 35].

Solar radiation reacts with DOM in different processes such as photobleaching and photohumification. Photobleaching involves the transformation of chromophoric dissolved organic matter (CDOM) into smaller and less colored molecules, whereas photohumification is the opposite reaction, that is, it is a sunlight-mediated condensation from smaller molecules into polymers with higher absorption properties. Several studies have demonstrated that bacterial growth can be stimulated by CDOM photobleaching [16, 20, 27]. In contrast, photohumification of algal-derived compounds [14] appears to reduce its bioavailability [4, 23]. Consequently, the net effect of DOM photoproducts to support bacterial growth is the balance between these two contrasting processes [40].

Environmental factors such as incident solar radiation and rainfall (controlling watershed exports) are major drivers of DOM concentration and optical properties in lakes [21, 26]. The significance of watershed exports is tightly related to regional vegetation and its relative proportion of wetlands [15, 44]. Most studies on DOM photoproducts were performed in boreal and northern temperate regions, with Mediterranean inland waters being underrepresented. Mediterranean aquatic ecosystems are submitted to an intense and frequent solar radiation and scarce rainfall and are located in watersheds with poorly developed soils. These factors lead to limited watershed exports, intense evaporation rates, and consequently, long water residence times.

Correspondence to: Eva Ortega-Retuerta; E-mail: evaor@ugr.es

Under this scenario, where terrestrial inputs are restricted to short rainy periods and lake DOM is submitted to intense photoalteration, the nature of DOM and its bacterial processing has been scarcely studied [25, 41].

The main goal of this study was to experimentally determine the net effects of DOM photoproducts on bacterial growth in typical Mediterranean inland waters including karstic lakes, reservoirs, and peaty ponds.

Material and Methods

Study Sites and Sampling. We selected seven aquatic ecosystems located in the Southeastern Spain, an area with annual cumulative rainfalls ranging from 354 to 628 mm and an incident solar radiation (visible) during July from 25.4 to 27.5 MJ m^{-2}. These ecosystems were located in diverse landscapes: agricultural (Bermejales reservoir), mountain (Quéntar and Canales reservoirs), coastal (Nueva lagoon), karstic (Grande and Salada lakes), and alkaline–peaty (Padul P0 pond) (Table 1).

Each lake was sampled once at a central station during spring or summer of 1999. Underwater photosynthetically active radiation (PAR) was measured with a LiCor (LI 193SA) quantum sensor, and conductivity and pH with a multiparametric probe at 0.2-m depth intervals. Water samples were collected at a depth of 50% PAR (to avoid intense DOM photoalteration) for analysis of dissolved organic carbon (DOC) concentration, DOM optical properties, oxygen stable isotope signature ($\delta^{18}O$), chlorophyll *a* (chl *a*) concentration, *in situ* bacterial abundance, and for setting up the bacterial regrowth cultures. Water for the experiments was stored at approx. 4°C in the dark for 2 to 3 h until its processing at the laboratory.

Chemical and Biological Analyses. The significance of evapoconcentration in the study ecosystems was determined from conductivity and the oxygen stable isotope signature ($\delta^{18}O$). $\delta^{18}O$ was determined using a Finnigan-MAT 251 mass spectrometer and isotopic values are reported using the δ^{18} notation in parts per mil (‰) relative to the international standard V-SMOW. The $\delta^{18}O$ can be considered as a surrogate of water residence time in freshwater ecosystems [12].

Triplicate samples for DOC analysis and DOM optical characterization were prepared by filtering lake water through precombusted (>2 h at 500°C) Whatman GF/F filters. The filtrates were collected in combusted flasks and stored at 4°C in the dark until analysis. The filtrates for DOC analyses were acidified with HCl (final pH <2) and measured with a Shimadzu Total Carbon Analyzer TOC-5050 after Benner and Strom [5]. The filtrates for DOM optical characterization were measured in 10-cm quartz cuvettes using a PerkinElmer Lambda 40 spectrophotometer connected to a computer equipped

Table 1. Location, chemical, and biological characteristics of the studied inland waters

Lakes	*Localization*	*Landscape type*	*pH*	*Conductivity*	$\delta^{18}O$ (‰)	*Chl a* ($\mu g\ l^{-1}$)	*BA in situ*	*DOC* (*mM*)	a_{320} (m^{-1})	a_{440} (m^{-1})	ε_{320}	ε_{440}	$F_{450:500}$	hl_{320} (*d*)	hl_{440} (*d*)
Salada	37°02′ N; 4°47′ W	Karstic	9.2	61500	12.0	0.9	13.0	3.79	18.3	1.2	27.8	0.4	1.82	0.5	0.2
Grande	37°06′ N; 4°19′ W	Karstic	8.0	3200	2.1	0.4	2.6	0.68	3.8	0.2	27.0	0.3	1.73	2.9	–1.3
Nueva	36°45′ N; 2°57′ W	Coastal	8.6	3760	–2.1	4.5	5.4	0.64	3.6	0.3	25.3	0.6	1.71	1.7	11.2
Padul P0	37°02′ N; 3°38′ W	Alkaline–peaty	7.8	4600	–2.7	1.1	3.0	3.80	116.1	11.8	33.2	3.1	1.59	1.2	0.5
Bermejales	37°09′ N; 3°28′ W	Agricultural	8.1	522	–5.2	1.6	3.6	0.36	1.1	0.2	15.7	0.7	1.79	–4.1	–0.6
Quéntar	37°13′ N; 3°25′ W	Mountain	8.2	352	–7.5	1.5	2.5	0.28	0.9	0.2	12.9	0.9	1.87	2.1	–25.3
Canales	37°09′ N; 3°28′ W	Mountain	7.9	221	–8.1	3.6	3.3	0.26	0.8	0.1	12.4	0.6	1.61	1.8	0.5

Chemical characteristics: pH, conductivity ($\mu S\ cm^{-1}$), stable isotope signature of oxygen ($\delta^{18}O$), dissolved organic carbon (DOC) concentration, absorption coefficient at 320 nm (a_{320}), absorption coefficient at 440 nm (a_{440}), molar absorption coefficient at 320 nm (ε_{320}; $m^2\ mol^{-1}$), molar absorption coefficient at 440 nm (ε_{440}; $m^2\ mol^{-1}$), fluorescence ratio of 450 to 500 nm ($F_{450:500}$), half-life of absorption coefficients at 320 nm (hl_{320}), and half-life of absorption coefficients at 440 nm (hl_{440})
Biological characteristics: concentration of chlorophyll *a* (Chl *a*), bacterial abundance (BA; cell $mL^{-1} \times 10^6$)

with UV-WINLAB software. Absorbance scans from 250 to 700 nm were performed and absorbances at the specific wavelengths of 320 nm (A_{320}) and 440 nm (A_{440}) were expressed as Napierian absorption coefficients (a_{320} and a_{440}) in per meters [19]:

$$a_{320,440} = \frac{2.303 A_{320,440}}{l} \quad (1)$$

where l is the optical path length in meters. Molar absorption coefficients at 320 nm (ε_{320}) and 440 nm (ε_{440}) in square meter per mole were also calculated as:

$$\varepsilon_{320,440} = \frac{a_{320,440}}{C} \quad (2)$$

where C is the concentration of DOC in millimolar [6].

Fluorescence emission spectra from 370 to 650 nm (excitation at 370 nm, slit width of 0.5 nm) were measured in a PerkinElmer LS50B spectrofluorometer using a 1-cm quartz cuvette (rinsed twice with the sample).

Three indexes were used as surrogates of DOM quality: ε_{320}, ε_{440}, and $F_{450:500}$. Absorption at longer wavelengths is related to compounds of high molecular weight enriched in chromophores, whereas absorption at the shorter wavelengths is related to molecules of lower weight [37]. Therefore, molar absorption coefficients at 320 nm (ε_{320}) can be considered as an indicator of the relative contribution of small molecules (absorbing at short wavelengths) to the total DOC pool. Conversely, molar absorption coefficients at 440 (ε_{440}) can be considered as the relative contribution of humic acids (big molecules enriched in chromophores) to the total DOC pool [8, 36]. The $F_{450:500}$ ratio can be considered an index of the origin of fulvic acids (terrestrial vs autochthonous) [18]. A ratio of ~1.4 is typical for terrestrially derived fulvic acids, and a ratio of ~1.9 is typical for microbially derived fulvic acids (from degradation of algae and bacteria).

DOM photoreactivity rates were calculated for each study site using the experimental protocol described elsewhere [30]. Briefly, quartz bottles were filled with 0.2-μm-filtered waters from all systems and simultaneously incubated under natural sunlight (from sunrise to sunset). Dissolved organic matter photoreactivity (k_b) was calculated determining the changes over time of the absorption coefficients at two specific wavelengths (a_{320} and a_{440}) using the following equation:

$$k_{b\lambda}\left(d^{-1}\right) = \frac{\ln \frac{a_{\lambda n}}{a_{\lambda 0}}}{t} \quad (3)$$

where t is time in days. From Eq. 3, absorption half-life (the time to reduce 50% of initial absorption coefficients) was calculated as:

$$hl_\lambda = \frac{\ln(0.5)}{k_{b\lambda}\left(d^{-1}\right)} \quad (4)$$

Chlorophyll *a* concentration was determined by filtering 0.5–1.5 L of water through a GF/C filter immediately after collection. The filter was placed in a glass vial, 5 mL of 95% methanol was added, and the vial was stored in the dark at 4°C for 24 h. The extract was measured and corrected for pheopigments using a PerkinElmer Lambda 40 spectrophotometer connected to a computer equipped with UV-WINLAB software.

Bacterial abundance was determined in three replicates by epifluorescence microscopy using 4′,6-diamidino-2-phenylindole, dihydrochloride fluorochrome stain [24]. At least 350 cells in 20 random fields were counted per replicate.

Experimental Design. To assess bacterial responses to the presence of DOM photoproducts and mineral nutrient supply, seven factorial experiments were performed using water from each lake. Lake water was initially filtered through Whatman GF/F filters (nominal pore size ca. 0.5 μm). This procedure removed a variable fraction of *in situ* bacteria (5–82%, mean value 63%) and all bacterivores and phytoplankton (examination by epifluorescence microscopy).

Each experiment consisted in four treatments: control (unamended conditions, no photoproducts, No Ph), with presence of photoproducts (Ph), control plus mineral nutrients (No Ph + NP) and presence of photoproducts and mineral nutrients (Ph + NP). Each treatment was conducted in duplicate or triplicate. NH_4Cl and KH_2PO_4 were added to nutrient-enriched treatments to a final concentration of 10 and 1 μmol l^{-1}, respectively. To set up the photoproducts treatments (Ph and Ph + NP) GF/F-filtered water was previously submitted to natural solar radiation for about 10–12 h in borosilicate (negligible UVB transmission, 80% UVA transmission) uncapped bottles. These bottles minimize UVB bacterial damage and permit the photoreactions because UVA is the most contributing band of the solar spectrum to photobleaching [29]. All regrowth cultures were running in parallel for about 100 h at 25°C in dark conditions and aliquots from each treatment and replicate were sequentially taken approximately every 24 h to determine bacterial abundance. Bacterial abundance was determined by epifluorescence microscopy as lake samples and two or three replicates were counted for each treatment and incubation time.

To compare among experiments, we calculated normalized net growth (NNG), which is an index of the net growth in cellular terms normalized by the initial abundance in the culture (Eq. 5):

$$NNG\left(h^{-1}\right) = \frac{BA_{tn} - BA_{to}}{BA_{to} t} \quad (5)$$

where BA_{tn} is the final bacterial abundance (cel mL^{-1}), BA_{to} is the initial bacterial abundance (cel mL^{-1}), and t is the incubation time in hours.

Statistical Analysis. To test the significance of the differences in bacterial abundance among treatments, two-way repeated measures ANOVAs were used. These analyses are recommended when using repeated measures designs (time) with replicate treatments to avoid temporal pseudoreplication [13]. The comparison between control and photoproducts treatments, throughout incubation time in each experiment, indicates the significance of photoproducts. The comparison between the unsupplemented and nutrient-supplemented treatments indicates the significance of inorganic nutrient supply. The combination of both factors (DOM photoproducts 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.

To compare among experiments and determine the influence of different *in situ* variables on the experimental NNG values, regression analysis were performed between NNG values and the chemical and biological parameters were studied ($\delta^{18}O$, conductivity, pH, chl *a*, DOC, and DOM optical properties). The NNG values were merged in two ways: (1) presence (Ph; Ph + NP) vs absence (no Ph; no Ph + NP) of photoproducts and (2) presence (no Ph + NP; Ph + NP) vs absence (no Ph; Ph) of mineral nutrient supply. Data were $\log_{10}$-transformed to fit regression analyses assumptions when it was necessary. To explore the effects of colinearity among the independent variables we calculated partial correlations.

Results

Conductivity and $\delta^{18}O$ values varied from 221 μS cm^{-1} and −8.1‰ in the Canales reservoir to 61500 μS cm^{-1} and 12.1‰ in the Salada lake, respectively. The study ecosystems showed moderate concentrations of chl *a*, in all cases below 5 μg l^{-1}, low ε_{440}, and DOC concentration was the highest in the Salada lake and in the Padul-P0 pond (Table 1). Dissolved organic matter photoreactions led in most cases, except in Bermejales, to losses of DOM absorption (i.e., photobleaching) at both 320 and at 440 nm. At this last wavelength, Grande and Quéntar did not show losses of DOM absorption either (Table 1). In the cases where clear photobleaching was observed, the DOM absorption half-life ranged from 0.5 to 2.9 days at 320 nm and from 0.2 to 11 days at 440 nm.

In the Fig. 1, we show the changes in bacterial abundance along the regrowth cultures for all the treatments and experiments. To determine the significance of the differences in bacterial abundance among treatments, we performed the two-way repeated measures ANOVAs showed in the Table 2. In six of seven experiments, the abundance was higher in control treatments than in those with photoproducts. The only exception was the experiment from Nueva lagoon, where there was a positive effect of DOM photoproducts on bacterial growth. By contrast, mineral nutrient supply had a positive effect on bacterial abundance in all the experiments except in the one from the Bermejales reservoir. We found significant interactions between the presence of DOM photoproducts and mineral nutrient availability in all the experiments (Table 2). Mineral nutrient addition compensated the negative effect of DOM photoproducts over time in six experiments and enhanced the positive effect of DOM photoproducts in the experiment from Nueva lagoon.

Normalized net growth varied from 0.06 to17 h^{-1} (Fig. 2). In general, NNG values were maxima in those treatments without photoproducts and with mineral nutrient supply. We did not obtain significant relationships between NNG and *in situ* chl *a* concentrations or the indexes of DOM quality assayed (ε_{320}, ε_{440}, and $F_{450:500}$) irrespective of how data were merged. By contrast, we found significant and positive relationships between NNG values and conductivity, pH, and $\delta^{18}O$ values for all groups (Fig. 3). In all cases, the slopes of the regression lines were higher in absence of photoproducts and under mineral nutrient additions (Fig. 3). We also found significant and positive relationships between NNG and DOC concentration, but only in the absence of photoproducts (r^2=0.37, $p<0.05$) or under nutrient-amended conditions (r^2=0.36, $p<0.05$).

The independent variable DOC was cocorrelated to $\delta^{18}O$, pH, and conductivity. Therefore, we calculated partial correlation coefficients to discriminate the relative importance of the substrate quantity (DOC), water residence time ($\delta^{18}O$ signature and conductivity), and the ionic environment where DOC is dissolved (pH). In all cases, the $\delta^{18}O$ significantly influenced the NNG values (keeping constant DOC concentration). The partial correlation coefficient value (r) was 0.89, grouping the treatments presence vs absence photoproducts ($p<0.001$), and 0.63, grouping the treatments unsupplemented vs supplemented with mineral nutrients ($p<0.001$). However, the DOC influence (keeping constant $\delta^{18}O$ values) on NNG was not significant. Similarly, pH significantly influenced the NNG values (keeping constant DOC concentration). The r value was 0.74, grouping the treatments presence vs absence photoproducts ($p<0.01$), and 0.58, grouping the treatments unsupplemented vs supplemented with mineral nutrients ($p<0.05$). In one case (in the absence of photoproducts) the DOC concentration also influenced on NNG values (keeping constant pH) (r=0.55, $p<0.05$).

Figure 1. Changes in bacterial abundance of the different treat-▶ments in the seven experiments performed.

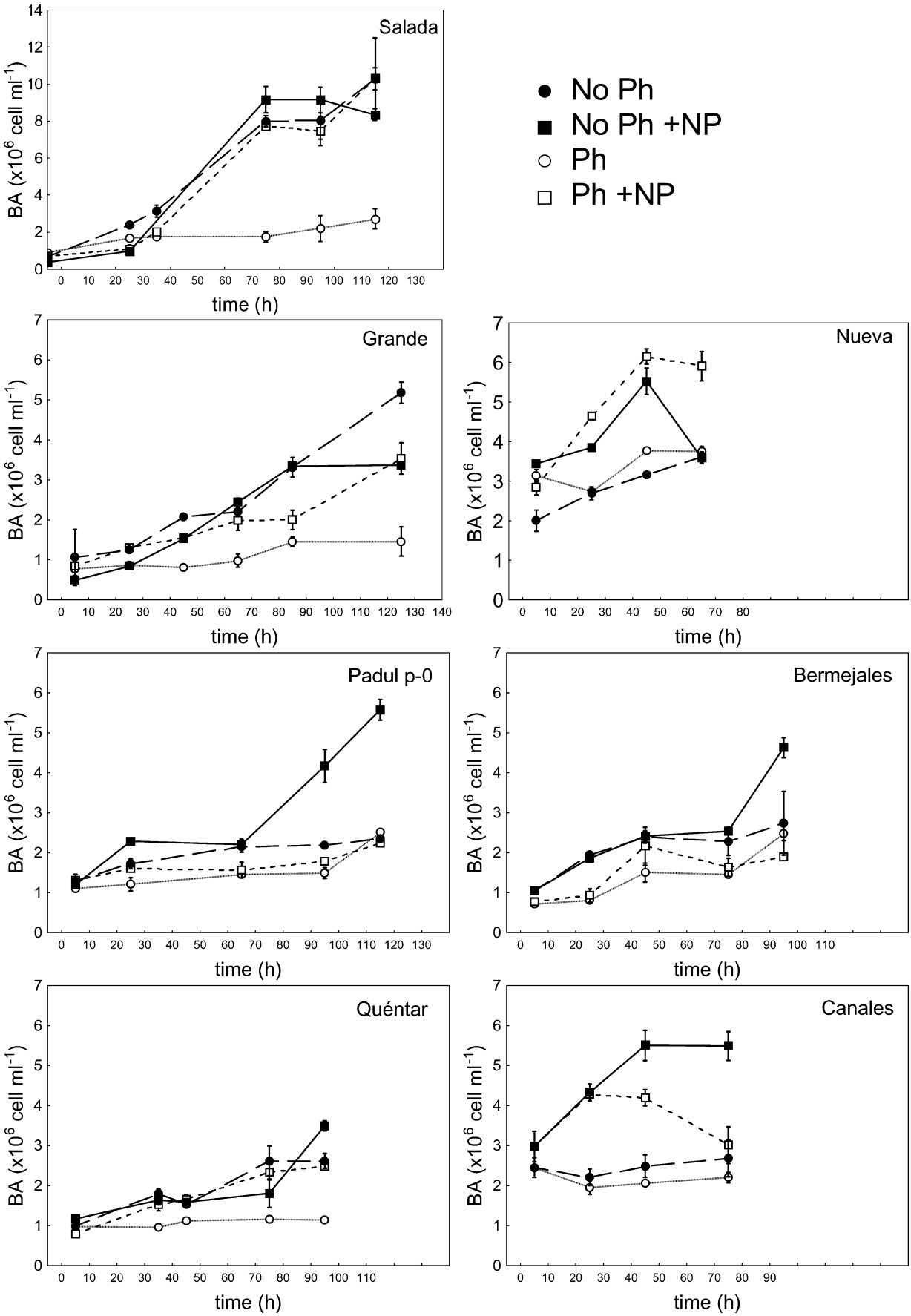
Salada
Grande
Nueva
Padul p-0
Bermejales
Quéntar
Canales
No Ph
No Ph +NP
Ph
Ph +NP
BA (x10^6 cell ml^-1)
time (h)

Table 2. Results of two-way repeated measures ANOVAs to determine the effect of DOM photoproducts and mineral nutrient supplements on bacterial abundance

Lakes	*Treatment comparisons*	*F*	*P value*	*Effect*
Salada	Photoproducts	275	***	–
	Nutrients	155	***	+
	Time	218	***	+
	Nutrients × photoproducts	185	***	C
	Photoproducts × time	20	***	–
	Nutrients × time	33	***	+
	Nutrients × photoproducts × time	17	***	C
Grande	Photoproducts	153	***	–
	Nutrients	6	0.068	n.s.
	Time	164	***	+
	Nutrients × photoproducts	85	***	C
	Photoproducts × time	21	***	–
	Nutrients × time	4	*	+
	Nutrients × photoproducts × time	17	***	C
Nueva	Photoproducts	97	***	+
	Nutrients	469	***	+
	Time	95	***	+
	Nutrients × photoproducts	41	**	+
	Photoproducts × time	12	**	+
	Nutrients × time	29	***	+
	Nutrients × photoproducts × time	20	***	+
Padul P-0	Photoproducts	1776	***	–
	Nutrients	911	***	+
	Time	77	***	+
	Nutrients × photoproducts	707	***	C
	Photoproducts × time	14	***	–
	Nutrients × time	19	***	+
	Nutrients × photoproducts × time	33	***	C
Bermejales	Photoproducts	50	**	–
	Nutrients	5	0.093	n.s.
	Time	62	**	+
	Nutrients × photoproducts	2	0.200	n.s.
	Photoproducts × time	6	*	–
	Nutrients × time	3	0.103	n.s.
	Nutrients × photoproducts × time	19	**	C
Quéntar	Photoproducts	160	**	–
	Nutrients	87	*	+
	Time	23	**	+
	Nutrients × photoproducts	93	*	C
	Photoproducts × time	5	*	–
	Nutrients × time	5	*	+
	Nutrients × photoproducts × time	4	0.07	n.s.
Canales	Photoproducts	882	***	–
	Nutrients	120	***	+
	Time	3	0.069	n.s.
	Nutrients × photoproducts	37	***	C
	Photoproducts × time	4	*	–
	Nutrients × time	12	**	+
	Nutrients × photoproducts × time	8	**	C

F=Variance ratio, + = positive, − = negative, C = compensated, n.s. = nonsignificant
*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

Discussion

The molar absorption coefficients at 440 nm in the study ecosystems were noticeably lower than that typical for temperate and humic lakes [22, 28], indicating the low chromophoric content of the organic matter in these Mediterranean inland waters (Table 1). This low chromophoric content is likely caused by the low precipitation rates at this ecoregion, which limits external inputs of chromophoric DOM to the lakes [26, 38], and a considerable preceding photobleaching because of the intense and frequent solar radiation and the high water

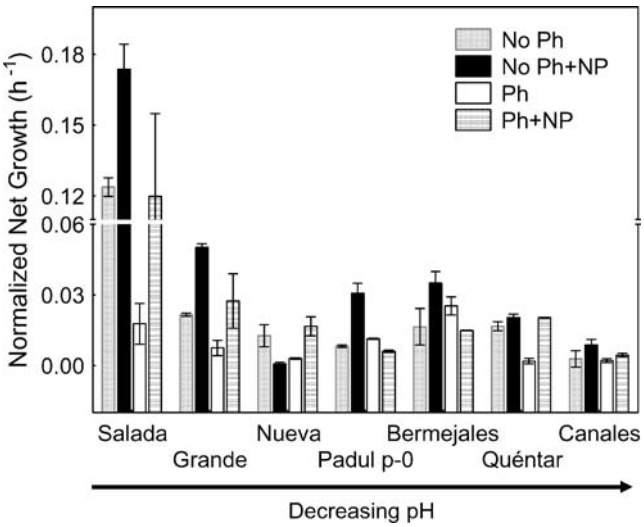

Figure 2. Values of NNG of the different treatments in the seven experiments performed. The mean and standard error (error bars) values are represented. Note the scale break for *y* axis from 0.06 to 0.12.

residence times (as indicated by the relatively heavier $\delta^{18}O$ values) with respect to temperate or boreal lakes [12]. Therefore, DOM in these ecosystems appears to be more diagenetically altered and, likely, with smaller molecular size than typical DOM from boreal and temperate lakes.

Most studies reporting positive effects of DOM photobleaching on bacterial growth were performed in humic lakes with significant amounts of chromophoric DOM [9, 16, 27]. Negative effects were exclusively associated with phototransformations of algal-derived DOM [40], or condensation and transformation of existing biopolymers [14]. However, the negative effect of the photoproducts on bacterial growth found in our experiments (Table 2) can hardly be attributed to a photohumification of DOM derived from phytoplankton. This assertion is based on the absence of a negative correlation between the chl *a* concentration and the

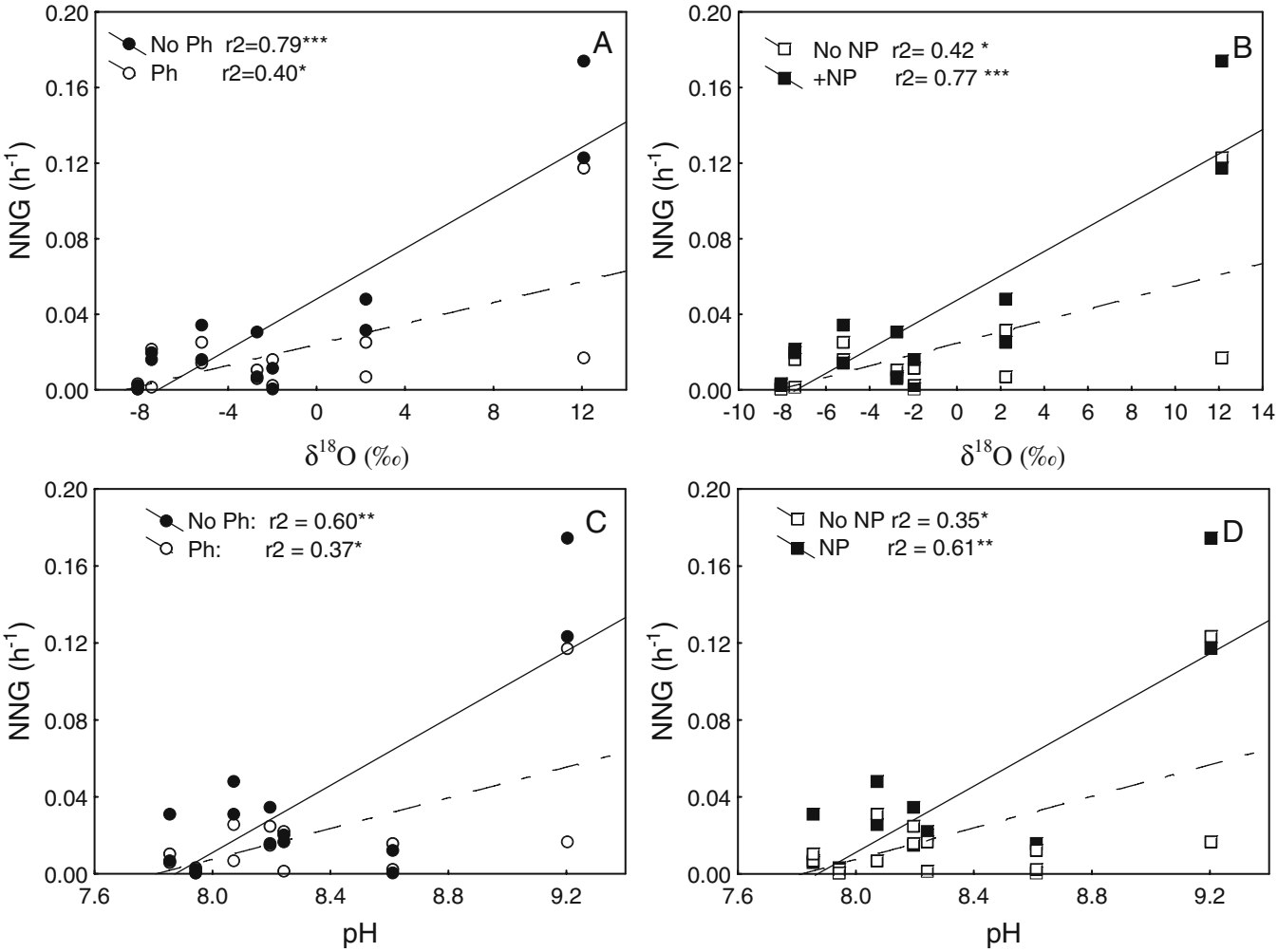

Figure 3. Regression analysis between the values of NNG, $\delta^{18}O$ (surrogate of water residence time), and pH (surrogate of ironic environment). Data were grouped by presence vs absence of photoproducts and unsupplemented vs supplemented with mineral nutrients.

NNG values and on the measured losses of DOM absorption within 10–12 h of solar exposure in the majority of the study sites (Table 1). However, this negative response of bacterioplankton growth to the presence of DOM photoproducts could be explained by other two alternative reasons. First, the reaction of DOM with sunlight also yields reactive oxygen species (ROS), which have direct negative effects on bacterial growth and/or indirect effects because of the loss of bioavailable DOM associated to ROS mineralization [33]. Second, extracellular enzymes (e.g., phosphatase and glucosidase) can be inactivated in natural waters by secondary photochemical processes [34], leading to a reduction in the substrate uptake by bacteria.

Currently, the significance of mineral nutrients in bacterial DOM processing is being notably considered [31, 35]. Bacteria also require mineral nutrients and have particularly high cellular nitrogen and phosphorus requirements relative to carbon [11]. The positive response to mineral nutrient additions found in most of the experiments (Table 2) is then sound considering this importance of mineral nutrients on bacterial growth. It is likely that the availability of mineral resources increases the bacterial efficiency to convert DOC into new bacterial biomass. More noteworthy is the interactive role of mineral nutrients in compensating or enhancing bacterial growth on DOM photoproducts. Although enhancements of the growth efficiency associated with nutrients availability have been reported [17, 27], the alleviation of the negative effects of DOM photoproducts by mineral nutrients, to our knowledge, has not been previously reported, and underlines the role of mineral nutrient availability to determine the net effect of DOM photoproducts on DOM processing.

Regression analyses indicated that NNG values were higher in waters with higher conductivity, pH, or heavier $\delta^{18}O$ signature (longer water residence time), suggesting that alkaline environments or aged (diagenetically altered) DOM can favor NNG (Fig. 3). These results seem to contradict the model proposed by Amon and Benner [1], which states that less diagenetically altered DOM is more bioreactive. The NNG increases associated with aged DOM (Fig. 3A,B) or alkaline conditions (Fig. 3C,D) have several plausible explanations. Aged DOM usually corresponds to small size molecules [2], which are apparently less bioavailable [32]. However, these colorless molecules are likely more accessible to bacterial uptake than large chromophoric molecules. In addition, alkaline waters can involve higher ectoenzymatic activity than acidic waters because of the cationic suppression of the interference of humic compounds with extracellular enzyme activities. This cationic suppression permits us to enhance the use of nutrients and/or organic substrates [42]. Recent studies [34] have also demonstrated that as pH is higher the photochemical inactivation of ectoenzymes (e.g., phosphatase and glucosidase) is mitigated. Therefore, in alkaline environments both the increase of enzymatic activity by cationic suppression and the mitigation of photochemical inactivation of ectoenzymes could lead to a higher DOM processing. In addition, the ionic environment where the organic carbon is dissolved can also affect its own bio- and/or photoreactivity [10, 28].

Overall, we document that in these Mediterranean inland waters, where DOM has been submitted for long periods to photo- and biological transformations, that the effect of photoproducts on bacterioplankton growth is generally negative and can be compensated by the availability of mineral nutrients. Bacterial uptake and processing of DOM photoproducts can be also influenced by the ionic environment, leading to more efficient DOM processing in the most alkaline ecosystems.

Acknowledgments

We thank J. Montero Meléndez, A. Castañeda, and J.A. Delgado Molina for their assistance in the field. Dissolved organic carbon samples were kindly analyzed in the laboratory of Dr. M.L. Pace. Stable isotope analyses of oxygen were carried out by the Stable Isotope Facility at Estación Experimental del Zaidín (CSIC, Granada, Spain). This research was supported by Project CGL 2005-00076 to I. Reche.

References

1. Amon, RMW, Benner, R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr 41: 41–51
2. Amon, RMW, Benner, R (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369(6481): 549–552
3. Azam, F, Fenchel, T, Field, JG, Gray, JS, Meyerreil, LA, Thingstad, F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10(3): 257–263
4. Benner, R, Biddanda, B (1998) Photochemical transformations of surface and deep marine DOM: effects on bacterial growth. Limnol Oceanogr 43: 1373–1378
5. Benner, R, Strom, M (1993) A critical evaluation of the analytic blank associated with DOC measurements by high-temperature catalytic oxidation. Mar Chem 41: 153–160
6. Braslavsky, SE, Houk, KN, Verhoeven, JW (1996) Glossary of Terms Used in Photochemistry. International Union of Pure and Applied Chemistry
7. Carlson CA, Giovannoni SJ, Hansell D, Goldberg S, Parsons R, Otero M, Vergin K, Wheeler B (2002) Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. Aquat Microb Ecol 30: 19–36
8. De Haan, H (1972) Molecule-size distribution of soluble humic compounds from different natural waters. Freshw Biol 2: 235–241
9. De Lange, HJ, Morris, DP, Williamson, CE (2003) Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. J Plankton Res 25(1): 111–117

10. Edling, H, Tranvik, L (1996) Effects of pH on β-glucosidase activity and availability of DOC to bacteria in lakes. Arch Hydrobiol 48: 123–132
11. Fagerbakke, KM, Heldal, M, Norland, S (1996) Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. Aquat Microb Ecol 10(1): 15–27
12. Gibson, JJ, Prepas, EE, McEachern, P (2002) Quantitative comparison of lake throughflow, residency, and catchment runoff using stable isotopes: modelling and results from a regional survey of Boreal lakes. J Hydrol 262(1–4): 128–144
13. Hulbert, SH, White, MD (1993) Experiments with freshwater invertebrate zooplanktivores: quality of statistical analyses. Bull Mar Sci 53: 128–153
14. Kieber, R, Hydro, L, Seaton, P (1997) Photooxidation of triglycerides and fatty acids in seawater: implication toward the formation of marine humic substances. Limnol Oceanogr 42: 1454–1462
15. Kortelainen, P (1993) Content of total organic carbon in Finnish lakes and its relationship to catchment characteristics. Can J Fish Aquat Sci 50: 1477–1483
16. Lindell, MJ, Granéli, W, Tranvik, LJ (1995) Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnol Oceanogr 40: 195–199
17. McAllister, SL, Bauer, JE, Kelly, J, Ducklow, HW (2005) Effects of sunlight on decomposition of estuarine dissolved organic C, N and P and bacterial metabolism. Aquat Microb Ecol 40: 25–35
18. McKnight, DM, Boyer, EW, Westerhoff, PK, Doran, PT, Kulbe, T, Andersen, DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46: 38–48
19. Miller, WL (1998) Photochemical principles and experimental considerations. In: Hessen, DO, Tranvik, LJ (Eds.) Aquatic Humic Substances: Ecology and Biogeochemistry, Springer, Berlin Heidelberg New York, pp 125–143
20. Moran, MA, Zepp, RG (1997) Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol Oceanogr 42(6): 1307–1316
21. Morris, DP, Hargreaves, BR (1997) The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. Limnol Oceanogr 42(2): 239–249
22. Morris, DP, Zagarese, HE, Williamson, CE, Balseiro, EG, Hargreaves, BR, Modenutti, B, Moeller, RE, Queimalinos, C (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnol Oceanogr 40: 1381–1391
23. Obernosterer, I, Reitner, B, Herndl, GJ (1999) Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnol Oceanogr 44 (7): 1645–1654
24. Porter, KG, Feig, LS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25: 943–948
25. Pulido-Villena, E, Reche, I (2003) Exploring bacterioplankton growth and protein synthesis to determine conversion factors across a gradient of dissolved organic matter. Microb Ecol 46: 33–42
26. Reche, I, Pace, ML (2002) Linking dynamic of dissolved organic carbon in a forested lake with environmental factors. Biogeochemistry 61: 21–36
27. Reche, I, Pace, ML, Cole, JJ (1998) Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. Microb Ecol 36: 270–280
28. Reche, I, Pace, ML, Cole, JJ (1999) Relationship of trophic and chemical conditions to photobleaching of dissolved organic matter in lake ecosystems. Biogeochemistry 44: 259–280
29. Reche, I, Pace, ML, Cole, JJ (2000) Modeled effects of dissolved organic carbon and solar spectra on photobleaching in lake ecosystems. Ecosystems 3: 419–432
30. Reche, I, Pulido-Villena, E, Conde-Porcuna, JM, Carrillo, P (2001) Photoreactivity of dissolved organic matter from high-mountain lakes of Sierra Nevada, Spain. Arct Antarct Alp Res 33(4): 426–434
31. Rivkin, RB, Anderson, MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. Limnol Oceanogr 42: 730–740
32. Rochelle-Newall, EJ, Pizay, MD, Middelburg, JJ, Boschker, HTS, Gattuso, JP (2004) Degradation of riverine dissolved organic matter by seawater bacteria. Aquat Microb Ecol 37: 9–22
33. Scully, NM, Cooper, WJ, Tranvik, L (2003) Photochemical effects on microbial activity in natural waters: the interaction of reactive oxygen species and dissolved organic matter. FEMS Microbiol Ecol 46: 353–357
34. Scully, NM, Tranvik, LJ, Cooper, WJ (2003) Photochemical effects on the interaction of enzymes and dissolved organic matter in natural waters. Limnol Oceanogr 48(2): 1818–1824
35. Smith, EM, Prairie, YT (2004) Bacterial metabolism and growth efficiency in lakes: the importance of phosphorus availability. Limnol Oceanogr 49(1): 137–147
36. Strome, DJ, Miller, MC (1978) Photolytic changes in dissolved humic substances. Verh Int Verein Limnol 20: 1248–1254
37. Sun, L, Perdue, EM, Meyer, JL, Weis, J (1997) Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnol Oceanogr 42: 714–721
38. Tipping, E, Woof, C (1983) Seasonal-variations in the concentrations of humic substances in a soft-water lake. Limnol Oceanogr 28(1): 168–172
39. Tranvik, LJ (1988) Availability of dissolved organic-carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16(3): 311–322
40. Tranvik, L, Bertilsson, S (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. Ecol Lett 4: 458–463
41. Waiser, MJ, Robarts, RD (2000) Changes in composition and reactivity of allochthonous DOM in a prairie saline lake. Limnol Oceanogr 40: 566–574
42. Wetzel, RG (1990) Land–water interfaces: metabolic and limnological regulators. Verh Int Verein Limnol 24: 6–24
43. Wetzel, RG (1992) Gradient-dominated ecosystems—sources and regulatory functions of dissolved organic-matter in fresh-water ecosystems. Hydrobiologia 229: 181–198
44. Xenopoulos, MA, Lodge, DM, Frentress, J, Kreps, TA, Bridgham, SD, Grossman, E, Jackson, KJ (2003) Regional comparisons of watershed determinants of dissolved organic carbon in temperate lakes from the Upper Great Lakes region and selected regions globally. Limnol Oceanogr 48(6): 2321–2334