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Bacterial Stimulation in Mixed Cultures of Bacteria and Organic Carbon from River and Lake Waters

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ABSTRACT

Interactions between natural bacterial assemblages and dissolved organic carbon (DOC) were investigated in two complementary batch experiments. In the first, a positive relationship was found between the proportion of electron transport system (ETS) active bacteria and the diversity of DOC in microcosms enriched with an increasing number of organic substrates. In a second experiment, bacterial and nutrient dynamics were measured in microcosms with natural bacterial populations and organic matter from rivers and lakes of different trophic levels. The interactions between the bacterial assemblages and DOC from different sources was investigated using source systems (rivers or lakes) and blended (different proportions of river and lake water) batch cultures. In each experiment, the number of total and ETS-active bacteria, the fluorescein diacetate (FDA)-hydrolytic activity, and the total (DOC), biodegradable (BDOC) and refractory (RDOC) dissolved organic carbon were measured four times during 5 days. The results suggested that the temperature, more than trophic level, controlled planktonic bacterial production. Furthermore, bacterial activity was stimulated in micrososms where river and lake waters were mixed. For the oligotrophic microcosms, this observation can be explained by a greater diversity of the organic nutrients ("qualitative" stimulation of bacteria), whereas for the meso-eutrophic microcosms, the production of new pools of dissolved organic carbon (both biodegradable and total) could account for the observed "quantitative" stimulation of the bacteria. These experiments suggest that the mixing of bacteria and organic matter from two different systems can give rise to novel nutrient and bacterial dynamics that are likely similar to those that occur in river–lake ecotones.

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Introduction

Estuaries are known to play a key role in regulating nutrient inputs from rivers to marine systems [2, 26, 34]. Considerable research into the composition and dynamics of organic matter in sediments [13, 21, 23, 45] and nutrient transformation, microbial dynamics, and trophic relationships in the water column [1, 4, 10, 27, 35] has demonstrated that each of these compartments functions in a unique manner. Nonetheless, few researchers have investigated river–lake ecotones [31, 32, 48], even though the dynamics of the ecotones are likely different from those of either of the individual components and certainly important to an overall understanding of the natural system.

As in estuaries, water movement and mixing are the main factors affecting nutrient dynamics in river–lake ecotones [6, 8, 17]. Vinçon-Leite et al. [48] have noted that, during floods, the circulation of water could be the primary factor controlling bacterial growth at the river–lake interface. The simple mixing of water from different systems with the resulting blending of bacterial populations and organic matter will certainly affect nutrient and bacterial dynamics, since the utilization of carbon by heterotrophic bacteria depends on the nature of both the dissolved organic carbon (DOC) and the bacterial assemblages [46, 47]. Because the chemoorganoheterotrophic bacteria extract energy and carbon from the natural organic matter [39], the proportion of active planktonic bacteria is very different according to the aquatic system [11, 12, 18, 25, 29, 51].

Our hypothesis was that the different bacterial functional groups would become successively active according to the chemical composition, molecular weight, energizing value, and concentration of the organic matter [3, 22, 30, 46, 47]. Two complementary experiments were performed to determine the influence of the mixing of organic matter and bacterial assemblages on the nutrient and bacterial dynamics of a river–lake ecotone. The goal of the first experiment was to demonstrate the influence of an enrichment in organic substrates on the proportion of electron transport system (ETS) active bacteria. In the second experiment, mixing conditions in river–lake ecotones were simulated in artificial river-lake ecotones (ARLEs) by adding different proportions of river and lake water to microcosms. Short-term variations in both organic nutrient concentrations and bacterial activities were monitored in the ARLEs.

Materials and Methods

Design of Microcosm Experiments

Substrate Addition Microcosms. The hypothesis that different bacterial groups are activated based upon the quality of organic matter was studied here by measuring the proportion of ETS-active bacteria in a natural bacterial population following a short incubation in microcosms that were enriched with an increasing number of organic substrates. ETS active bacteria are defined as the proportion of bacteria able to reduce CTC (5-cyano-2,3-ditotyl tetrazolium chloride) to its fluorescent formazan in the electron transport system (ETS) with respect to the total bacterial number. Since the proportion of ETS-active bacteria is an indication of the proportion of active bacteria, it should be related to the diversity or number of the different available organic substrates. We set out to verify this theoretical relationship.

Table 1. Physical and chemical characteristics of river and lake waters used in the substrate addition microcosms experiment and in the Artificial River–Lake Ecotomes (ARLEs)

	Oligotrophic		Meso-eutrophic	
	Lake Tignes	Retort brook	Lake Bourget	Leysse River
Altitude (m)	2100		231	
Surface area (ha)	60		4257	
Max Depth (m)	38		145	
Temp (°C)	2–12	2–8	5–28	5–26
PO_4^{3-} (μg P L^{-1})	<10	<10	3–100	10–200
NO_3^- (mg L^{-1})	1–1.5	1–1.5	0–5	2–7
DOC (mg C L^{-1})	0.5–2	1–1.5	4–20	3–15
Ca^{2+} (mg L^-)	200–250	150–200	35–55	50–80
SO_4^{2-} (mg L^{-1})	450–550	400–500	14–18	16–18

Five liters of water were sampled from a depth of 1 m at the deepest point of Lake Bourget, a meso-eutrophic lake (Table 1), on February 15, 1994. In this and the following experiment, no prefiltration was performed in order to maintain the natural bacterial assemblages as close as possible to their natural conditions. The influence of the particulate organic carbon (POC) on bacterial activity was considered negligible because of its very low concentration (always $\leq$0.1 mg L^{-1}) and the short incubation time (40 h) which was employed. Five 1-L microcosms were filled with 500 ml of lake water and enriched with 5 mg C L^{-1} of dissolved organic nutrients. One microcosm (microcosm C) was maintained as a control without added nutrients. An increasing number of organic compounds were added to microcosms 1 to 5 while maintaining the total carbon concentrations constant in order to represent a gradient in the diversity of biodegradable dissolved organic carbon (BDOC) (Table 2). Added substrates were selected to reflect the main metabolites involved in bacterial biosynthesis [38]: glucose for glycogen, leucine for proteins, serine for monocarbon units, alanine for peptidoglycans, and ATP for nucleotides. Microcosms were incubated for 40 h (previously determined maximum activity; data not shown) at 15°C, in the dark (to reduce phytoplankton production) and were gently shaken on a rotary shaker to improve bacterial–DOC interactions. Measurements of major ions, DOC, and the percentage of ETS-active bacteria were made at $t = 0$, $t = 16$, and $t = 40$ h. Biodegradable and refractory dissolved organic carbon (BDOC and RDOC) concentrations were measured at $t = 0$ and $t = 40$ h.

Table 2. Theoretical concentrations of BDOC supplies, DOC concentration, and BDOC concentration measured at $t = 0$ in all the microcosms in the substrate addition microcosms experiment

Number of microcosms	C	1	2	3	4	5
Glucose (mg C L^{-1})	0	5	2.5	1.66	1.25	1
Leucine (mg C L^{-1})	0	0	2.5	1.66	1.25	1
Serine (mg C L^{-1})	0	0	0	1.66	1.25	1
Alanine (mg C L^{-1})	0	0	0	0	1.25	1
ATP (mg C L^{-1})	0	0	0	0	0	1
Number of organic substrates added	0	1	2	3	4	5
Total BDOC added (mg C L^{-1})	0	5	5	5	5	5
DOC concentration (mg C L^{-1})	2.83 ± 0.06	7.76 ± 0.09	7.85 ± 0.08	7.69 ± 0.07	7.66 ± 0.09	7.57± 0.10
BDOC concentration (mg C L^{-1})	0.85 ± 0.06	5.78 ± 0.07	5.87 ± 0.05	5.71 ± 0.08	5.68 ± 0.08	5.59± 0.08

Artificial River–Lake Ecotones (ARLEs). Artificial river–lake ecotones (ARLEs) were prepared by mixing together river and lake waters into 2-L microcosms with an increasing proportion of lake water (0, 25, 50, 75, 100%). Two types of ARLEs were set up with lake waters from two different trophic levels. In the first, water from Bourget lake (meso-eutrophic) was mixed together with water from its main tributary, a fifth-order river (Leysse River). In the second, water from Tignes Lake (oligotrophic) was mixed with water from its main tributary, a first-order brook (Retort Brook). The physical and chemical characteristics of the waters are summarized in Table 1. Four experiments were performed: April and November 1994 (Lake Bourget) and August and September 1994 (Lake Tignes). From this point on, the following names will be given to the different ARLEs: (i) mesotrophic–warm ARLE (25°C, April 1994), (ii) mesotrophic–cold ARLE (10°C, November 1994), (iii) oligotrophic–warm ARLE (10°C, August 1994), (iv) oligotrophic–cold ARLE (5°C, September 1994).

Note that temperature conditions are defined here according to the prevailing temperature in the field at the sampling date (Table 1). For example, 10°C is considered to be a warm temperature for the mountain lake Tignes (2100 m) and a cold temperature for the lower elevation lake Bourget (300 m). ARLEs were incubated for 5 days, at the field temperature, in the dark and under slight rotary shaking (30 rpm). Subsamples (300 ml) of water were collected from each microcosm after 0, 1, 3, and 5 days for major ion (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{2-}, K^+, Ca^{2+}, Na^+, Mg^{2+}), organic carbon (DOC, BDOC, RDOC), and bacterial analysis (total bacterial number, ETS-active bacterial number, FDA-hydrolytic activity). Major ions were measured on day 0. BDOC and RDOC were not measured in the oligotrophic ARLEs because of their low concentrations. Microcosms containing 0% and 100% lake water are referred to as "source microcosms," the others as "blended microcosms."

Major Ions

Concentrations of major ions (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{2-}, K^+, Ca^{2+}, Na^+, Mg^{2+}) were measured by capillary electrophoresis (Waters Quanta 4000) fitted with a 100 μm × 100 cm capillary (Chromate OFM-OH^-, 20 kV, 60 s) for anions and a 75 μm × 60 cm capillary (UV-CAT2, 20 kV, 10 s) for cations.

Organic Nutrients

Dissolved organic carbon (DOC) was measured with a Dohrman DC80 "Total Carbon Analyser" based on a UV promoted potassium persulfate oxidation (precision 1%) following the elimination of inorganic carbon by the addition of orthophosphoric acid (final concentration 1 μl ml^{-1}) and a 10-min degassing with O_2. Biodegradable (BDOC) and refractory (RDOC) fractions of the dissolved organic carbon were assessed using the method of Servais et al. [43, 44]. In this technique, BDOC is the fraction of DOC that is metabolized by autochthonous bacteria during a short-term (~30 day) incubation. The principle of this method is to filter sterilize the water sample containing the organic matter to be tested, to inoculate it with an autochthonous bacterial population, and to measure the decrease in the DOC concentration due to the oxidation of organic matter by the bacteria. In this manner, a 130-ml water sample was sterilized by filtration through a 0.2 μm cellulose acetate membrane (Millipore) that had been rinsed with 100 ml of organic-C free distilled water and 50 ml of the water sample. Ten ml of filtrate was kept for the measurement of the initial DOC concentration and the remaining 120 ml transferred into a 150 ml precombusted (550°C, 4 h) glass bottle protected with an aluminum cap. The material collected on the filters (e.g., bacteria, protozoa, nonliving particles) was washed out and concentrated into a few milliliters of distilled water. Protozoa and nonliving particles were removed from this suspension by filtration on a Whatman GF/A filter. Two ml of the filtrate containing the autochthonous bacteria were inoculated into the 150 ml bottles, which were then incubated in the dark at 15°C for 35 days. Measurements of DOC concentrations were systematically performed after 30 and 35 days. The DOC concentration generally decreased quickly (within 4 to 5 days) to attain a near-constant value until the end of the incubation [5, 43]. The DOC measured at the end of the incubation was considered to be as the biologically refractory organic carbon (RDOC), and the difference between initial and final values of DOC as the biodegradable organic carbon (BDOC).

Bacterial Biomass and Activity

Bacterial counts were performed using epifluorescence microscopy. Total bacterial number were estimated after DAPI staining [16, 40]

Table 3. Chemical and bacterial characteristics in the microcosms at $t = 0$ for the substrate addition microcosms experiment[a]

	Cl^- (mg L^{-1})	SO_4^{2-} (mg L^{-1})	Na^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	K^+ (mg L^{-1})	Mg^{2+} (mg L^{-1})	NO_3^- (mg N L^{-1})	PO_4^{3-} (μg P L^{-1})	ETS-active bacteria (10^4 cells ml^{-1})	Total bacteria (10^6 cells ml^{-1})	ETS-active bacteria (%)
Mean ± SD	7.33 ± 0.15	14.47 ± 0.07	4.92 ± 0.02	42.98 ± 0.54	1.64 ± 0.01	5.13 ± 0.05	1.88 ± 0.01	19 ± 7	3.8 ± 0.1	2.21 ± 0.16	1.7 ± 0.3
p	0.856	0.912	0.632	0.852	0.693	0.523	0.892	0.421	0.865	0.652	0.562

[a] *p* values are given for a one-way ANOVA between the different microcosms.

subsequent to the filtration of 1 to 4 ml of water through a black polycarbonate membrane (0.2 μm pore size, GTBP, Millipore). Bacteria were stained with a 40 mg L^{-1} (final concentration) DAPI solution for 10 min at room temperature, then washed and counted under oil immersion. Active bacteria were measured using a method based on CTC staining [41]: a stock solution of CTC was added to 3 to 6 ml of water to give a final concentration of 1.48 mg L^{-1}. The mixture was incubated for 2 h at the temperature of the microcosm and then filtered through a GTBP membrane. Active bacteria labeled by the fluorescent red reduced CTC were counted using an epifluorescence microscope. Results were expressed as the proportion of ETS-active bacteria (ETS-active bacteria/total bacteria).

The enzymatic activity of the samples (two replicates) was estimated using the fluorescein diacetate (FDA) hydrolysis method [15, 42] in which 0.1 ml of a FDA solution was added to 10 ml of water prior to a 3–6 h incubation in the dark. Biological activity was stopped by freezing the sample after the addition of 3 ml of a 400 mg L^{-1} mercury choride solution. The fluorescein concentration was estimated from the absorbance of the filtrate (0.45 μm cellulose membrane) measured at 490 nm (Shimadzu UV spectrophotometer). The incubation time was adjusted so that the FDA concentration was not limiting for the enzymatic processes.

Results

Substrate Addition to the Microcosms

At the beginning of the experiment, the microcosms were not significantly different with respect to the major ion concentrations and bacterial parameters (Table 3). Microcosms 1 to 5 had higher DOC concentrations (mean 7.71 mg C L^{-1}) than did the control microcosm (2.83 mg C L^{-1}) because of the added 5 mg L^{-1} of organic nutrients (Table 2). During the 40-h incubation, DOC and BDOC were consumed and RDOC was produced in all of the microcosms (Figs. 1 and 2). Net DOC consumption increased between 16 and 40 h to reach 0.5 mg L^{-1} for microcosms C, 1, 2, 3, 4 and 2 mg L^{-1} for microcosm 5 (Fig. 1). Net BDOC consumption increased from microcosm C to 5 (from 0.7 to 3.1 mg L^{-1}) for the 40-h incubation (Fig. 2). Simultaneously, the proportion of ETS-active bacteria after 16 and 40 h increased from microcosms C to 5 (Fig. 1). Indeed, the proportion of ETS-active bacteria was significantly correlated with the number of added organic nutrients ($t = 16$ h, $r^2 = 0.93$, $p < 0.05$; $t = 40$ h, $r^2 = 0.90$, $p < 0.05$). Furthermore, after 40 h, the proportion of ETS-active bacteria was significantly different (one-way ANOVA, $p < 0.05$) in all the microcosms except microcosms C and 1.

Artificial River–Lake Ecotones

Initial Conditions. The river and lake waters displayed different initial DOC concentrations and bacterial numbers depending upon the source (Table 4). For example, the mesotrophic–warm ARLE had 50 times more ETS-active bacteria and 4 times more DOC in the river water than in the lake water. On the other hand, in the mesotrophic–cold ARLE, the nutrients and bacteria were more concentrated in the lake water as compared to the river water (10 times for ETS-active bacteria, 6 times for total bacteria). Such important differences were not observed in the oligotrophic ARLEs. Lake Tignes and its main tributary displayed quite similar initial nutrient and bacterial concentrations in both experiments (Table 4).

Nutrient and Bacterial Dynamics (5-Day Incubation). In order to analyze nutrient and bacterial dynamics in the microcosms, the maximum variation (MV) of each of the parameters was determined. MV was defined as the difference between the highest or lowest value of a parameter over the 5 days of incubation and its value on day 0. In this manner, MV gives an indication of both the direction and magnitude of a variation. In order to analyze the effect of mixing waters on nutrient and bacterial dynamics, theoretical and measured values of MV were compared in blended microcosms. For each parameter, theoretical values have been computed in the blended microcosms based on the fractional volumes and the MV of the source microcosms (0% and 100%). Theoretical and measured values for each parameter were then compared using a one-way ANOVA in order to deter-

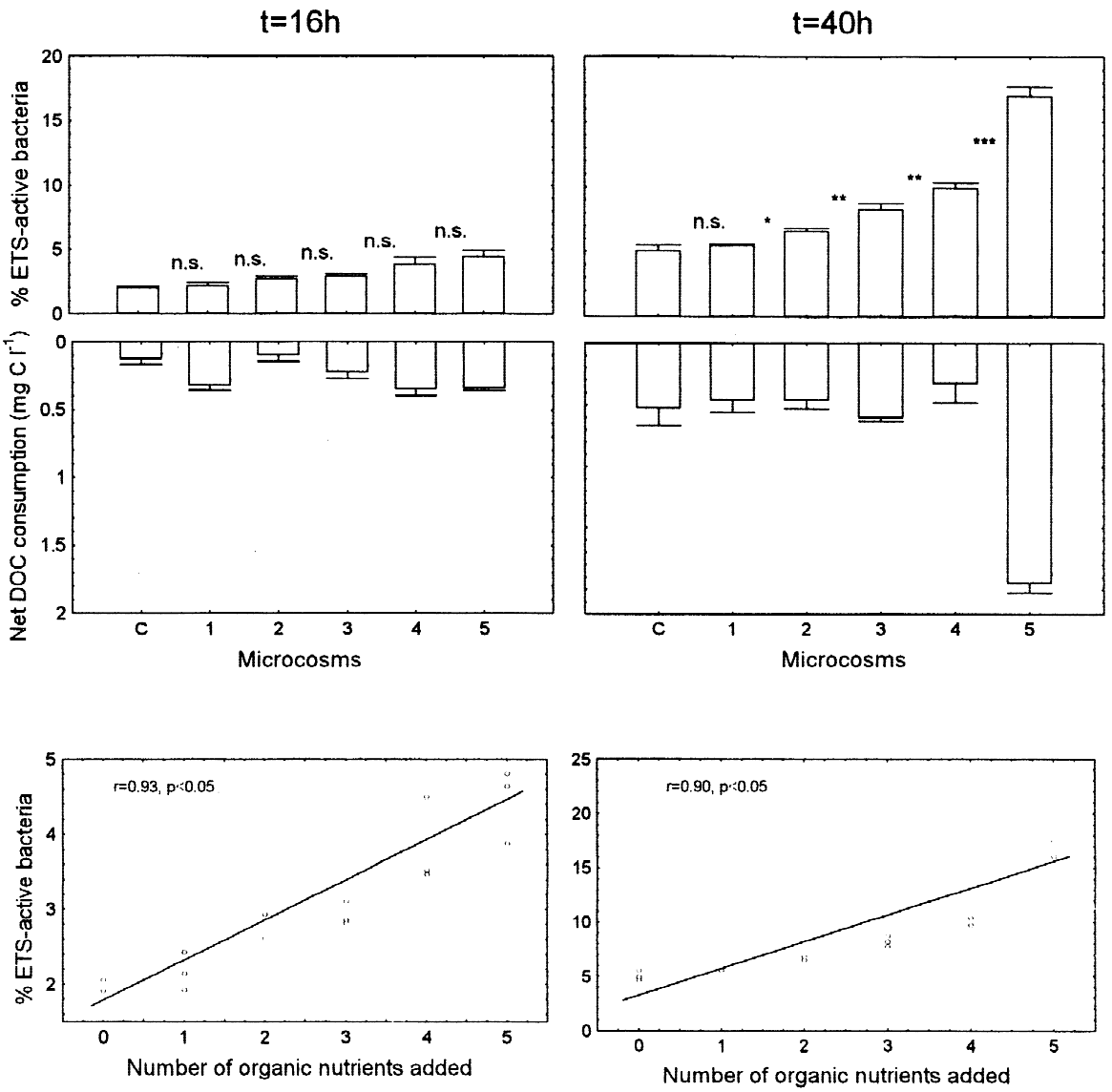

Fig. 1. Evolution (means and standard deviations) of net DOC consumption and percentage of CTC-active bacteria in substrate addition experiment. Results of the one-way ANOVA for the percentage of CTC-active bacteria among neighboring microcosms are indicated in the figures. Correlations between DOC diversity in each microcosm and the percentage of CTC-active bacteria (r values) are given in the graphs. The left graphs give results at t = 16 h and the right ones at t = 40 h.

mine the effect of mixing waters on nutrient and bacterial dynamics in the blended microcosms (Table 7). Bacterial stimulation or repression and differences in nutrient production were then determined based on observed differences between the measured and theoretical MV values. An increase in the total number of bacteria was observed in all ARLEs for all dilutions (Fig. 3). The MV of total bacterial number over the five day incubation was calculated for each dilution and each ARLE (Table 5). Net bacterial production was significantly higher in the warm microcosms in the two systems (Table 6). There was no significant difference between the two trophic levels for the same temperature condition (warm or cold). Theoretical and observed values of bacterial number in blended microcosms were not significantly different, i.e., the mixing of organic matter and bacteria did not lead to a stimulation or a repression of the net bacterial production in any of the ARLEs except for the mesotrophic–cold system (Table 7). In this ARLE, the observed net bacterial production was significantly higher than the calculated production (p = 0.076), indicating a stimulation in the blended microcosms (Table 7). This stimulation was accompanied by the stimulation of the FDA-hydrolytic

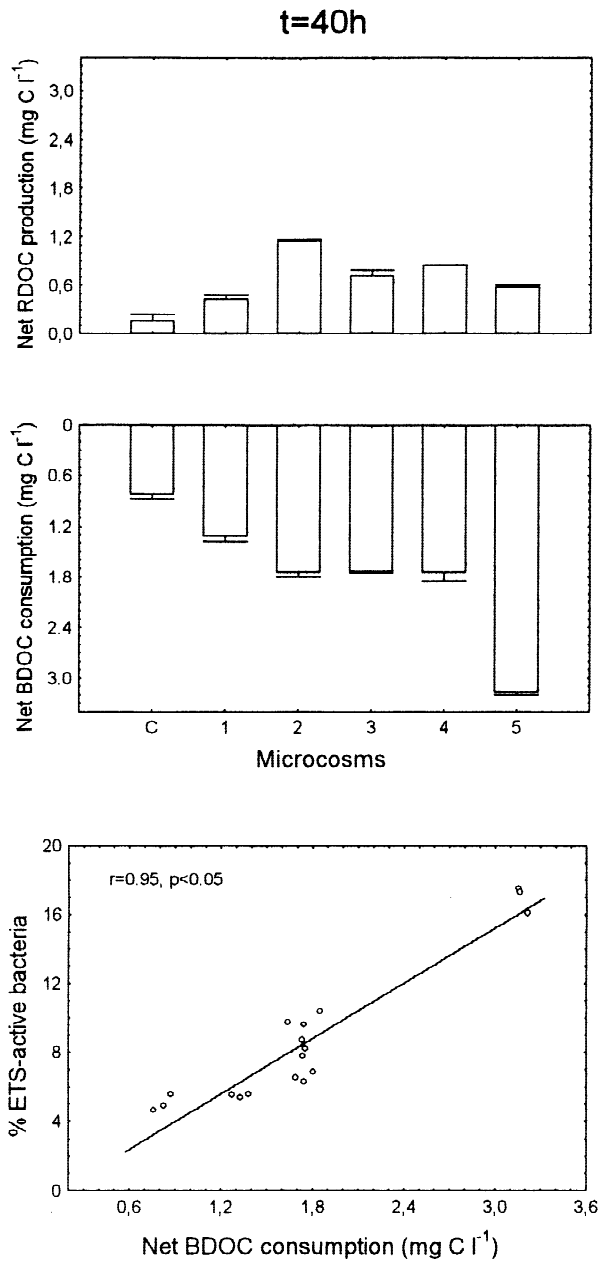

Fig. 2. Evolution (means and standard deviations) of the net BDOC consumption and net RDOC production at t = 40 h of the enrichment experiment. The bottom graph displays the between the net BDOC consumption and the percentage of CTC-active bacteria at t = 40 h. The correlation coefficient is given on the figure.

activity (calculated value > theoretical value in blended microcosms). The oligotrophic–cold ARLE did not present a similar pattern, i.e., observed values were not statistically different from calculated values in blended microcosms for any of the measured parameters (Table 7). In the mesotrophic–warm ARLE, observed values of ETS-active bacteria (p = 0.017), FDA-hydrolytic activity (p = 0.063), DOC (p = 0.098), and BDOC (p = 0.091) were higher in blended microcosms than for computed values (Table 7). In this ARLE, bacterial activity and DOC production were stimulated by the mixing of the two waters. In the oligotrophic–warm ARLE, this mixing led to a stimulation of the number (p = 0.004) and the proportion (p = 0.001) of ETS-active bacteria (Table 7).

For all parameters for which stimulation occurred (ETS-active bacteria, percentage of ETS-active bacteria, FDA-hydrolytic activity), the maximum activity was observed earlier in the warmer ARLEs than in the cooler ones (Table 8).

Table 4. Concentration of dissolved organic carbon (DOC), total bacteria, and ETS-active bacteria in both river and lake waters used to prepare the four Artificial River–Lake Ecotones (ARLEs)

		Oligotrophic		Mesotrophic	
		Cold	Warm	Cold	Warm
DOC	River	1.25	1.61	2.42	13.01
(mg C L^{-1})	Lake	1.21	1.74	5.47	3.05
Total bacteria	River	4.30	5.52	2.54	9.31
(10^5 cells ml^{-1})	Lake	3.57	9.74	17.2	5.67
ETS active bacteria	River	1.61	7.76	1.37	12.7
(10^4 cells ml^{-1})	Lake	2.38	13.4	13.4	0.25

Discussion

Relationship between the Proportion of ETS-Active Bacteria and the Number of Organic Substrates

In the substrate addition experiment, consumption of DOC in the microcosms was lower than BDOC consumption. These results have also been observed in other microcosm experiments [Gayte and Fontvieille, submitted] and are consistent with the fact that the bacteria can transform a part of the BDOC into RDOC [36]. Depending on the microcosm, the ratio of BDOC consumption to RDOC production ranged from 18% to 66%, which is in accord with recent results for which we have recorded a RDOC production equal to 40% of the glucose consumption under similar experimental conditions [Gayte and Fontvieille, submitted].

In all the microcosms, the proportion of ETS-active bacteria did not exceed 18%, even in microcosm 5, where the diversity of the DOC was at a maximum (five organic substrates added). Even for enriched batch experiments, a large majority of the heterotrophic bacterial pools was metabolically inactive. Nonetheless, the organic molecules used as

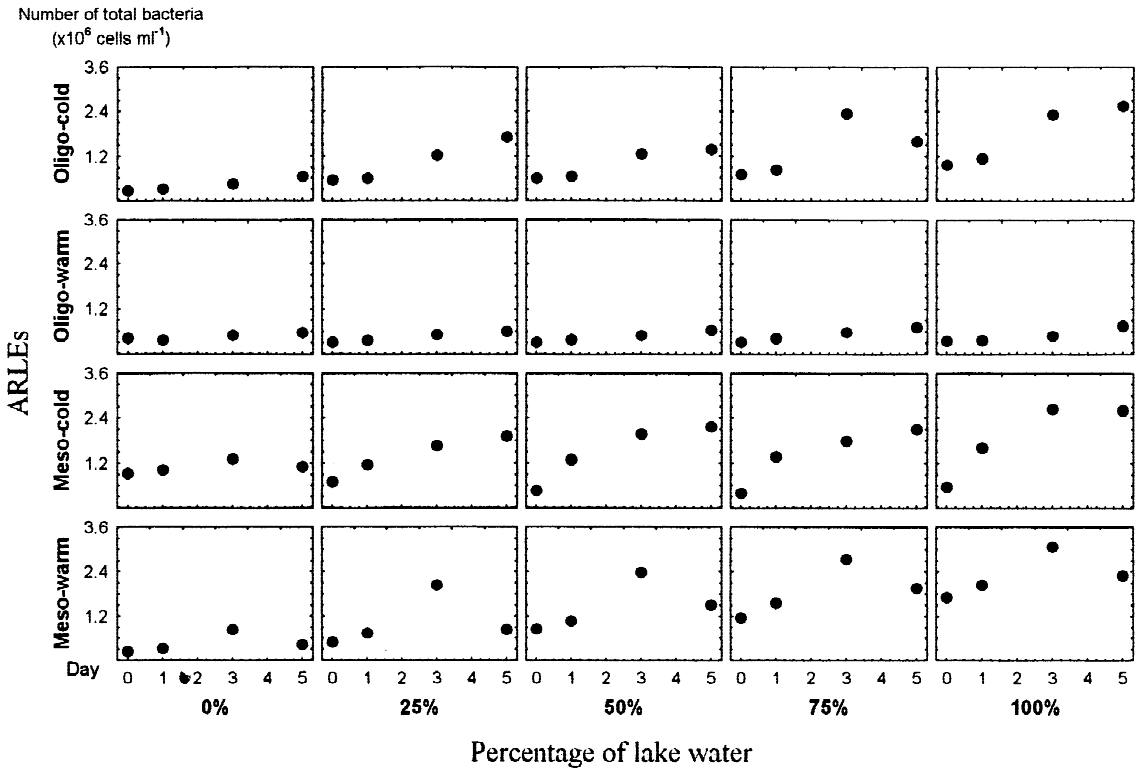

Fig. 3. Evolution of total bacterial number in the five microcosms (0, 25, 50, 100% of lake water) for the four Artificial River–Lake Ecotones (oligotrophic–cold, oligotrophic–warm, mesotrophic–cold, mesotrophic–warm) during the 5-day incubation.

BDOC in this experiment did not represent all possible types of organic compounds that are available in natural aquatic systems [30]. For example, high molecular weight and generally less labile DOC can represent a large proportion of natural DOC [7, 44] but was not present in our DOC supplies. Furthermore, in separate batch experiments [30, Gayte and Fontvieille, submitted], it was observed that the addition of phenolic compounds that are naturally present in aquatic systems and not present in this DOC supply stimulated the deshydrogenase activity in planktonic bacteria.

Nevertheless, the addition of the organic nutrients used here stimulated the activity of the chemoorganoheterotrophic bacteria and their BDOC consumption. Moreover, the proportion of CTC-active bacteria and BDOC consumption were significantly related to the number of organic substrates added to the microcosms. The number of different organic substrates available at a time (which as a first approximation reflects the diversity of the organic nutrients) is thus likely to be an important factor which can determine the proportion of metabolically active chemoorganotrophic bacteria. The increase in metabolically active bacteria could, in turn, explain the higher BDOC consumption in the microcosms with a high number of organic substrates.

If we assume that the proportion of ETS-active bacteria is related to the number of active groups, then we can conclude that the addition of organic substrates stimulated some bacterial groups that were not previously active. Should the results of this experiment be applicable to natural aquatic

Table 5. Net bacterial production (bacterial cells/ml) in each ARLE and each dilution calculated over the 5 days of incubation

	0%	25%	50%	75%	100%	Total
Oligo cold	3.63E+04	5.98E+04	6.29E+04	7.99E+04	7.92E+04	6.36E+04
Oligo warm	7.64E+04	2.48E+05	1.74E+05	2.39E+05	3.49E+05	2.17E+05
Meso cold	5.58E+04	1.22E+05	1.80E+05	1.96E+05	1.52E+05	1.41E+05
Meso warm	4.52E+04	2.37E+05	3.25E+05	3.06E+05	3.98E+05	2.62E+05

Table 6. Comparison (one-way ANOVA) of net bacterial production from the four ARLEs all dilutions pooled[a]

ARLEs	P values
Oligo–cold vs meso–warm	0.002**
Oligo–cold vs oligo–warm	0.014**
Meso–warm vs meso–cold	0.046*
Oligo–cold vs meso–cold	0.186
Oligo–warm vs meso–cold	0.193
Oligo–warm vs meso–warm	0.434

[a] Significant differences are quoted as follows: *($p < 0.05$), **($p < 0.01$).

settings, then the diversity of organic substrates could be a major factor controlling chemoorganoheterotrophic bacterial succession in pelagic areas.

Nutrients and Bacterioplankton Dynamics in ARLEs

The increase in bacterial numbers during short time incubations has been previously observed in a similar experimental design [3, 9, 20, 37]. However, in our ARLEs, net bacterial production seemed to be more dependent on temperature than on the trophic level of the ARLE. Furthermore, in addition to its control of the net bacterial production, temperature would appear to control the time delay that was necessary to reach the highest bacterial activity (Table 8). The important role of temperature to regulate bacterial production and specific growth rates has previously been reported in both *in situ* and laboratory experiments [14, 24, 28, 33, 49, 50].

No bacterial stimulation was observed in the blended microcosms of the oligotrophic–cold ARLE, i.e., measured values were not significantly different from theoretical values for any of the measured parameters. Mixing river and lake waters in these conditions did not significantly affect nutrient or bacterial dynamics. On the other hand, in blended microcosms from the mesotrophic–cold ARLEs, net bacterial production and FDA-hydrolytic activity were stimulated, whereas the ETS activity was not significantly different from expected values. The stimulation of FDA activity and increased production of the pelagic bacteria was observed in the absence of a significant increase in the DOC concentration.

The blended microcosms from the two warm ARLEs had higher numbers of ETS-active bacteria than predicted by calculation (mesotrophic: $p = 0.017$ and oligotrophic: $p = 0.004$), indicating that mixing the two waters stimulated the bacteria for both trophic levels of the ARLE. The stimulation occurred regardless of whether the ARLEs were made with similar river and lake waters (oligotrophic system) or with source waters that were very different (mesotrophic system). In the mesotrophic system, this stimulation occurred concurrent with the production of DOC, even though the proportion of ETS-active bacteria was not significantly different from the predicted values. This type of stimulation could be qualified as a "quantitative stimulation" because it was due to an increase of organic nutrient concentrations (both DOC and BDOC). Our study was not designed to explain the observed production of DOC, but we can hypothesize that microbial lysis may have occurred in these microcosms in response to a chemical or physical stress. The stimulation of the FDA-hydrolytic activity in the blended microcosms could indicate that a portion of the BDOC that was produced was a high molecular weight DOC fraction.

In blended microcosms from the oligotrophic–warm system, a stimulation of the number and proportion of ETS-active bacteria was observed where no obvious production of DOC had occurred. The higher percentage of ETS-active bacteria could be explained by an increase in the molecular diversity of the organic matter in blended microcosms resulting from the mixing of two different organic pools. Bacteria that were not metabolically active in one of the "source" systems may also have been stimulated by inputs of organic matter from the other system. Such a stimulation could be qualified as a "qualitative stimulation" because bacteria were stimulated in response to an increase in DOC diversity rather than DOC concentration. Neither the quantitative nor qualitative stimulation induced a significant variation of the net bacterial production ($p = 0.303$ and $p = 0.877$). Bacteria stimulated by an increase of the quantity or the diversity of DOC did not appear to use this extra energy supply to build biomass. It remains to be examined whether this "misuse" of DOC is due to "bacterial choice" or to a low energizing value of the DOC supplies.

This study demonstrated that the mixing of nutrients and bacterial assemblages from river and lake waters led to novel nutrient and bacterial dynamics that appeared to be controlled by the trophic level of the water (quantitative or qualitative stimulation) and by temperature (low or high stimulation). If these results are confirmed in natural river–lake ecotones, then they could be considered as an original functional zone from the bacterial point of view. The mixing efficiency of river and lake waters should then be considered as a primary factor controlling organic nutrient transformations from rivers to lakes.

Table 7. One-way ANOVA from the comparison of observed and calculated maximum variations each of the parameters of the blended microcosms[a]

		Total bacteria (10^5 cells ml^{-1})	ETS-active bacteria (10^5 cells ml^{-1})	ETS-active bacteria (%)	FDA-hydrolytic activity (μmol L^{-1} h^{-1})	DOC (mg C L^{-1})	BDOC (mg C L^{-1})	RDOC (mg C L^{-1})
Oligotrophic	Observed mean	0.67	1.68	22.5	3.79E-05	0.17		
Cold	Calculated mean	0.58	1.45	21.4	1.85E-05	0.21		
	p	0.329	0.557	0.877	0.125	0.872		
Oligotrophic	Observed mean	2.20	2.78	27.0	1.59E-06	−0.46		
Warm	Calculated mean	2.13	0.82	3.2	1.71E-06	−0.82		
	p	0.877	0.004 ***	0.001 ***	0.974	0.391		
Mesotrophic	Observed mean	1.66	2.04	5.4	3.20E-03	2.95	0.91	3.38
Cold	Calculated mean	1.04	1.06	9.8	1.27E-03	1.47	0.29	3.84
	p	0.076 *	0.188	0.252	0.004 ***	0.289	0.279	0.628
Mesotrophic	Observed mean	2.89	5.37	35.5	6.92E-02	17.91	2.55	5.98
Warm	Calculated mean	2.21	3.98	32.2	3.86E-02	10.13	1.22	6.92
	p	0.303	0.017 **	0.598	0.063 *	0.098 *	0.091 *	0.589

[a] Statistically significant differences are quoted as follows. * ($p < 0.1$), ** ($p < 0.05$), *** ($p < 0.01$). Theoretical values of the blended microcosms were calculated on the basis of maximum variation in pure microcosms (0% and 100%) by assigning proportional values for microcosms 25%, 50% and 75%.

Table 8. Day of maximum activity of each ARLE (all dilutions pooled)

ARLEs	Oligo warm	Oligo cold	Meso warm	Meso cold
ETS-active bacteria	1	5	1	3
FDA-hydrolytic activity	1	3	1	3
% ETS-active bacteria	1	5	1	3

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