

## Comparison of solutes, nutrients, and bacteria inputs from two types of groundwater to the Rhône river during an artificial drought

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### Abstract

Solute, nutrient and bacterial inputs to the River Rhône from the interstitial habitat of a gravel bar and the floodplain aquifer were investigated during an artificial drought. Eight springs were investigated: four groundwater-fed springs in the floodplain, located at the bottom of the bank; and four interstitial-fed springs located at the downstream end of a gravel bar. During this period, the inflows of groundwater to the river represented an average input of 0.77 mg l<sup>-1</sup> of nitrogen (of which 93.3% were nitrates), 0.0187 mg l<sup>-1</sup> of total phosphorus (of which 42.2% was orthophosphate), 3.56 mg l<sup>-1</sup> of silica, 2.315 ± 0.703 mg l<sup>-1</sup> of dissolved organic carbon (DOC, of which 47% was biodegradable) and 7.3 × 10<sup>4</sup> ± 3.7 × 10<sup>4</sup> bacteria per ml (of which 8.8% were active). Silica, DOC, biodegradable DOC, and bacteria concentrations displayed temporal variations during the study, which seem to be linked to the biological activity of the groundwater biofilm. There was a strong heterogeneity between the two types of groundwater that flow to the river: concentrations of calcium and alkalinity were higher in bank springs than in gravel bars springs. In these latter, sulfate, sodium, nitrogen, phosphorus were significantly higher.

### Introduction

The exchanges between rivers, their interstitial (hyporheic) habitat, and the surrounding aquifer were recently investigated by many authors (Gibert *et al.*, 1990; Vervier *et al.*, 1992; Hendricks, 1993; Hakenkamp *et al.*, 1993). It is mostly the input of nutrients from the interstitial habitats to the river that have been investigated, such as nitrate (Triska *et al.*, 1989; Valett, 1993), phosphorous (Valett *et al.*, 1990), silica (Hendricks & White, 1991), and dissolved organic carbon (Wallis *et al.*, 1981, Crocker & Meyer, 1987). In contrast, the inputs of bacteria from interstitial habitats or from terrestrial ecosystems through soil leachates to rivers were still little studied (Lefte *et al.*, 1993; Boissier & Fontvieille, 1995). Most of these studies used indirect estimations of the fluxes: the link between hydrologic events and bacterial densities in the river (Mc Dowell, 1984; Baker & Farr, 1977), comparisons between channel productivity and standing stocks in

the water column (Edwards *et al.*, 1990), or comparisons between upstream and downstream bacterial densities (Wainright *et al.*, 1992). In contrast, the present study provides a direct estimation of microbial inputs to a river from its groundwater supply.

The aim of this work was to compare the inputs to a river of solutes, organic matter and bacteria between (1) the interstitial water of gravel bars and (2) the groundwater of the alluvial plain from a natural vegetated sector. This comparison was possible because of the maintenance work performed on an upstream dam, which induced a partial drought of the studied area.

### Study site

The study took place on the River Rhône in the Chautagne sector (Fig. 1), where the river crosses an ancient wetland that has been partially drained (Bravard, 1981). The left side of the alluvial plain is

used for silviculture (poplar) and agriculture (mainly maize). In contrast, the right side is covered by natural forest (poplar, willow, alder) and belongs to a wide limestone basin.

The banks of the study channel consisted of old gravel bars covered by a 40–50 cm thick layer of brown soil, which is a mixture of sand and silt. The channel contained several riffle pool sequences, the latter being bordered on their convex side by partially vegetated gravel bars of coarse sediment within a reduced sandy matrix (Bravard, 1981, 1985). The channel is part of a bypassed section of the River Rhône downstream of the Motz Dam (Fig. 1), which is one of several hydroelectric facilities of this type on the River Rhône. The scheme consists of a retention canal (or reservoir), a headrace, a tailrace, and a bypass section, which acts as an outlet during periods of elevated flow. The discharge in the bypass section is regulated at  $10 \text{ m}^3 \text{ s}^{-1}$  from January to June and  $20 \text{ m}^3 \text{ s}^{-1}$  from July to December.

The Motz Dam is located 23 km downstream the Genissiat Dam (Fig. 1), where fine sediments washed down from the Alps accumulate (Bravard, 1987). Thus, the Genissiat Dam must be flushed out every 3 years. During this process, the water of the River Rhône is highly polluted by the suspended sediments and ammonia (Bravard, 1987). To preserve the aquatic organisms from this pollution, the bypass sections downstream of Genissiat are isolated from the main channel's flow and thus suffer partial temporary drought during the flushing process.

The study was performed on 6, 7 and 8 June 1993 during one such artificial drought, which took place from 5 to 8 June. During this period, river discharge decreased from  $10 \text{ m}^3 \text{ s}^{-1}$  (on the 4 June) to  $0.48 \pm 0.08 \text{ m}^3 \text{ s}^{-1}$  (6 June),  $0.37 \pm 0.05 \text{ m}^3 \text{ s}^{-1}$  (7 June), and  $0.33 \pm 0.02 \text{ m}^3 \text{ s}^{-1}$  (8 June). This lower discharge correspond to a 60 cm decrease of the river water level, which affect the groundwater piezometric level for a long distance: 18 cm at 1.2 km of the river on the right side (unpublished data from the Compagnie Nationale du Rhône). Eight springs were studied. Four were located at the bottom of the right river bank, where they represented groundwater outlets from the alluvial plain covered by natural forest. The other four were located downstream of a large gravel bar, located on the left side of the river, partially covered by sand deposits, and poorly vegetated. Interstitial water of the gravel bar and left bank groundwater are significantly different for most of the chemical characteristics, such as conductivity (394 and  $662 \mu\text{S}$  respectively), alkali-

linity (3.03 and  $6.88 \text{ meq l}^{-1}$ ), and calcium (60.6 and  $123.6 \text{ mg l}^{-1}$ ). The interstitial water of this gravel bar is not influenced by alluvial plain groundwater, but mainly consists of river water which enters at the upstream end and discharged at the downstream end.

## Methods

On the first day of the experiment, one hour before sampling, sediment was dug out from below all the spring (5 cm deep) to avoid any disturbance of the water chemistry during sampling. No gravel removal was necessary for subsequent sampling dates. Temperature, conductivity, and oxygen were measured *in situ* with a thermoconductimeter (WTW LF 92) and an oxymeter (WTW OXI 92).

Spring water needed for laboratory measurements was sampled in 500 ml pre-washed polyethylene bottles using a peristaltic pump (Willy A. Bachofen type), and then kept in an isotherm box for the 30 minutes journey back to the laboratory. Suspended organic matter was measured by filtering a known volume of water (between 200 and 300 ml) through pre-combusted glass fiber filters (Whatman GF/F). Calculations were made from the weight of the filters before and after drying at  $105 \text{ }^\circ\text{C}$  for 24 hours, and after ignition at  $550 \text{ }^\circ\text{C}$  for 4 hours.

Dissolved organic carbon (DOC) was measured with a Dohrman DC80 'Total Carbon Analyser' based on u.v.-promoted potassium persulfate oxidation (precision 1%) after elimination of mineral carbon by orthophosphoric acid (final concentration  $1 \mu\text{l ml}^{-1}$ ) and  $\text{CO}_2$  stripping for 10 minutes with  $\text{O}_2$ . Biodegradable (BDOC) and refractory (RDOC) fractions of the dissolved organic carbon were assessed using the method of Servais *et al.* (1987), and Servais *et al.* (1989). BDOC is that part of the initial DOC concentration which is assimilated by autochthonous bacteria during a short-term incubation. A  $0.2 \mu\text{m}$  cellulose acetate membrane (Millipore) was washed 3 times with 20 ml of organic-C free distilled water and then used to filter 130 ml of the water being investigated. 10 ml were kept for the initial DOC measurement. The remaining 120 ml were then transferred into a 250 ml pre-combusted ( $550 \text{ }^\circ\text{C}$ , 4 h) glass bottle with an aluminium closure. The bottles were inoculated with a suspension of autochthonous bacteria (removed from the cellulose acetate filter), and incubated in the dark, for 30 and 35 days, at  $15 \text{ }^\circ\text{C}$ . The decrease of DOC concentration is rapid (around 4 or 5 days) and is fol-

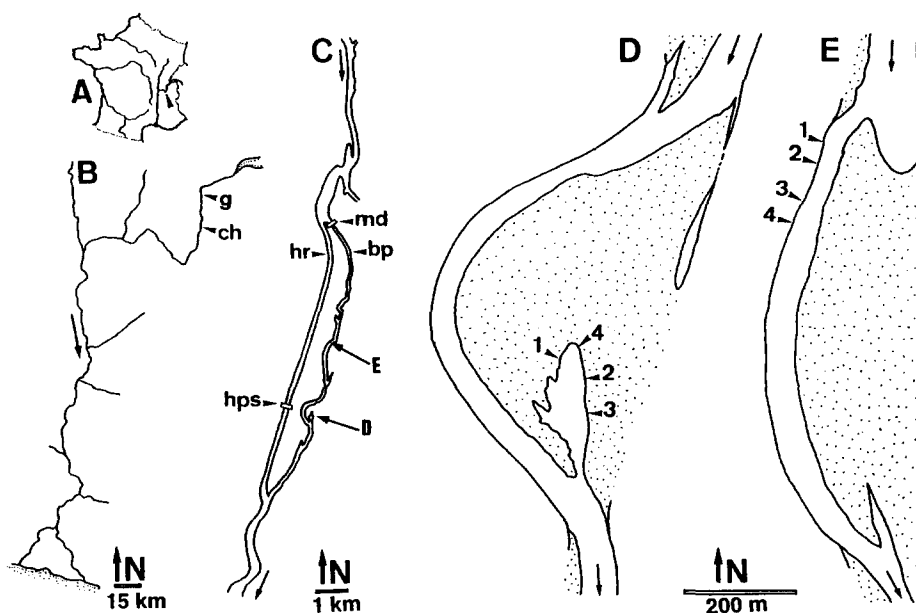


Fig. 1. A – Location of the Rhône River (arrow) in France. B – Location of the Génissiat Dam (g) and the Chautagne sector (ch) where the study took place. C – The Chautagne sector: the Motz Dam (md) diverts most of the water through a head race (hr) to the hydroelectric power station (hps), the ancient active channel is bypassed (bp). The two sampling sites are noted D and E. D – Location of the four gravel bar springs. E – Location of the four bank springs. Dotted area: gravel bars.

lowed by a stabilization at a constant value until the end of the incubation (Servais *et al.*, 1987; Boissier & Fontvieille, 1993). The DOC concentration measured at the end of the incubation was considered as the biologically refractory material (RDOC), and the difference between initial and final values of DOC was considered as the biodegradable fraction (BDOC).

Alkalinity and pH were measured using a Metrohm Titroprocessor (Type 636). Ammonia ( $\text{NH}_4$ ), nitrite ( $\text{NO}_2$ ) and soluble reactive phosphorus ( $\text{PO}_4$ ) were determined by colorimetry (indophenol blue, diazotation and the Murphy & Riley (1962) methods, respectively). Nitrate ( $\text{NO}_3$ ) and silica ( $\text{SiO}_2$ ) were determined by automatic continuous flow colorimetry (Aliance instrument) using the cadmium and silicomolybdate (aminonaphthol sulphonic acid) reduction methods, respectively. Total nitrogen and total phosphorus were measured after conversion of all forms of nitrogen (except  $\text{NO}_2$ ) to nitrate and all forms of phosphorous to  $\text{PO}_4$  by peroxodisulfate oxidation in alkaline medium for nitrogen and acidic medium for phosphorus at 130 °C (1.5 bar) in an autoclave for one hour. Ion chromatography (Dionex DX 100) was used to measure sulfate concentrations and atomic absorption spectrophotometry with an air-acetylene flame (Varian Spectra AA 400) for magnesium, sodium, and calcium

(with addition of lanthanum chloride to avoid sulfate interference for calcium).

Bacterial counts were performed using epifluorescence microscopy. The total number of bacteria was estimated after DAPI staining (Porter & Feig, 1980; Fry, 1988), 4 to 10 ml of water was filtered onto a GTBP-type membrane (Millipore), stained with a  $40 \mu\text{g ml}^{-1}$  DAPI solution (final concentration) for 10 minutes at room temperature, then washed and counted under immersion oil. The number of active bacteria was measured using a new method, CTC staining (Rodriguez *et al.*, 1992): 50 to 100 ml of water were filtered on a GTBP millipore membrane, stained with a  $1.48 \text{ mg l}^{-1}$  CTC solution (final concentration) incubated for 3 h at 20 °C.

Each of the two types of spring was considered as replicate samples. Two-way ANOVA on dates and spring types were performed using the Statistica/W statistical package (StatSoft Inc.).

## Results

Some of the physical and chemical characteristics of the water were different in the two types of springs and had no significant temporal variations. The water coming from the gravel bar springs had higher sulfate

Table 1. Chemical characteristics measured in gravel bar springs (averages and standard deviations,  $n = 12$ ), bank springs ( $n = 12$ ), and in River Rhône water ( $n = 3$ ). Statistical significant differences (ANOVA  $p < 0.05$ ) between gravel bar and bank spring waters are noted with \*. Statistical significant differences (ANOVA  $p < 0.05$ ) between spring waters and Rhône River waters are noted with \*\*.

	Bank springs	Gravel bar springs	Rhône water
Nitrogen ( $\text{mg l}^{-1}$ )*	0.78±0.24	1.10±0.15	0.77±0.07
N-NO <sub>3</sub> ( $\text{mg l}^{-1}$ )*	0.723±0.245	1.103±0.157	0.693±0.066
Phosphorus ( $\text{mg l}^{-1}$ )*	0.0187±0.014	0.0374±0.0126	0.0560±0.0153
P-PO <sub>4</sub> ( $\text{mg l}^{-1}$ )*	0.0079±0.0033	0.0279±0.0118	0.0250±0.0029
Sulfate ( $\text{mg l}^{-1}$ )*	31.43±3.07	35.86±0.59	33.63±0.45
TAC ( $\text{meq l}^{-1}$ )*	3.56±0.36	3.03±0.26	2.97±0.06
Calcium ( $\text{mg l}^{-1}$ )*	68.56±5.28	60.59±3.96	59.20±1.10
Sodium ( $\text{mg l}^{-1}$ )*	4.44±0.42	4.91±0.21	4.58±0.14
Suspended Matter ( $\text{mg l}^{-1}$ )**	1.28±1.01	1.35±0.56	2.33±1.82
Suspended Organic Matter ( $\text{mg l}^{-1}$ )**	0.67±0.32	0.68±0.24	0.87±0.25
Temperature **	11.2±0.5	11.5±0.3	18.4±0.2
Oxygen ( $\text{mg l}^{-1}$ )**	6.8±3.0	6.8±3.2	11.4±2.5

Table 2. Change in various chemical and biological components of River Rhône water during the 3-day artificial drought

	Day 1	Day 2	Day 3
Oxygen ( $\text{mg l}^{-1}$ )	14.8	10.5	8.9
N-NO <sub>3</sub> ( $\text{mg l}^{-1}$ )	0.75	0.73	0.60
Phosphorus ( $\text{mg l}^{-1}$ )	0.077	0.050	0.041
P-PO <sub>4</sub> ( $\text{mg l}^{-1}$ )	0.029	0.024	0.022
pH	8.17	8.12	8.01
SiO <sub>2</sub> ( $\text{mg l}^{-1}$ )	2.67	2.13	1.97
Bactena ( $10^6 \text{ ml}^{-1}$ )	2.43	1.95	1.84
Active bacteria ( $10^5 \text{ ml}^{-1}$ )	1.28	0.74	0.06
TAC ( $\text{meq l}^{-1}$ )	2.88	2.99	3.03
Ca ( $\text{mg l}^{-1}$ )	57.7	59.6	60.3
Conductivity ( $\mu\text{S}$ )	360.0	372.0	375.0
SO <sub>4</sub> ( $\text{mg l}^{-1}$ )	33.0	33.9	34.0
DOC ( $\text{mg l}^{-1}$ )	2.624	14.174	2.000
BDOC ( $\text{mg l}^{-1}$ )	1.343	12.693	0.263
Na ( $\text{mg l}^{-1}$ )	4.48	4.78	4.48

and sodium contents ( $p < 0.05$ ; Table 1). In the same way, the concentrations of mineral nutrients (such as nitrogen and phosphorus) measured in the gravel bar springs were significantly higher ( $p < 0.05$ ) than in the bank springs. Phosphorous concentrations were generally at least two times higher in the gravel bar springs than in bank springs, and very similar to that of the Rhône water. In contrast, nitrogen concentrations in

Rhône water were low, being similar to those measured in the bank springs. Regardless of sampling site, the largest part of this nitrogen consisted of nitrate, the other nitrogenous elements (i.e. ammonia and nitrite) were poorly represented in both types of water. Other components had higher concentrations in the bank springs than in the gravel bar springs: conductivity, calcium, and alkalinity. Such high values are typical of the calcareous area and the groundwater origin of the bank spring waters.

Temperature, oxygen content, total and suspended organic matter of the spring water were similar ( $p > 0.1$ ) in bank and gravel bar springs (Table 1). During the study period (early June), the surface waters were warmer, more oxygenated and richer in suspended matter than groundwater (Table 1). The same observation could be made for the biological variables (Fig. 2): no statistically significant differences were found between the two spring types for both forms of DOC, total number of bacteria, and the number of active bacteria. The bacterial densities were lower in spring waters than in Rhône water.

Some of the physical and chemical characteristics of the groundwaters varied significantly during the 3-day experiment, but were not significantly different between the spring types. Oxygen content, pH and silica decreased significantly ( $p < 0.05$ ) from the first to the third day (Fig. 2). In contrast, DOC, BDOC and bacterial concentrations were higher on the second day than the two others (Fig. 2). A similar increase

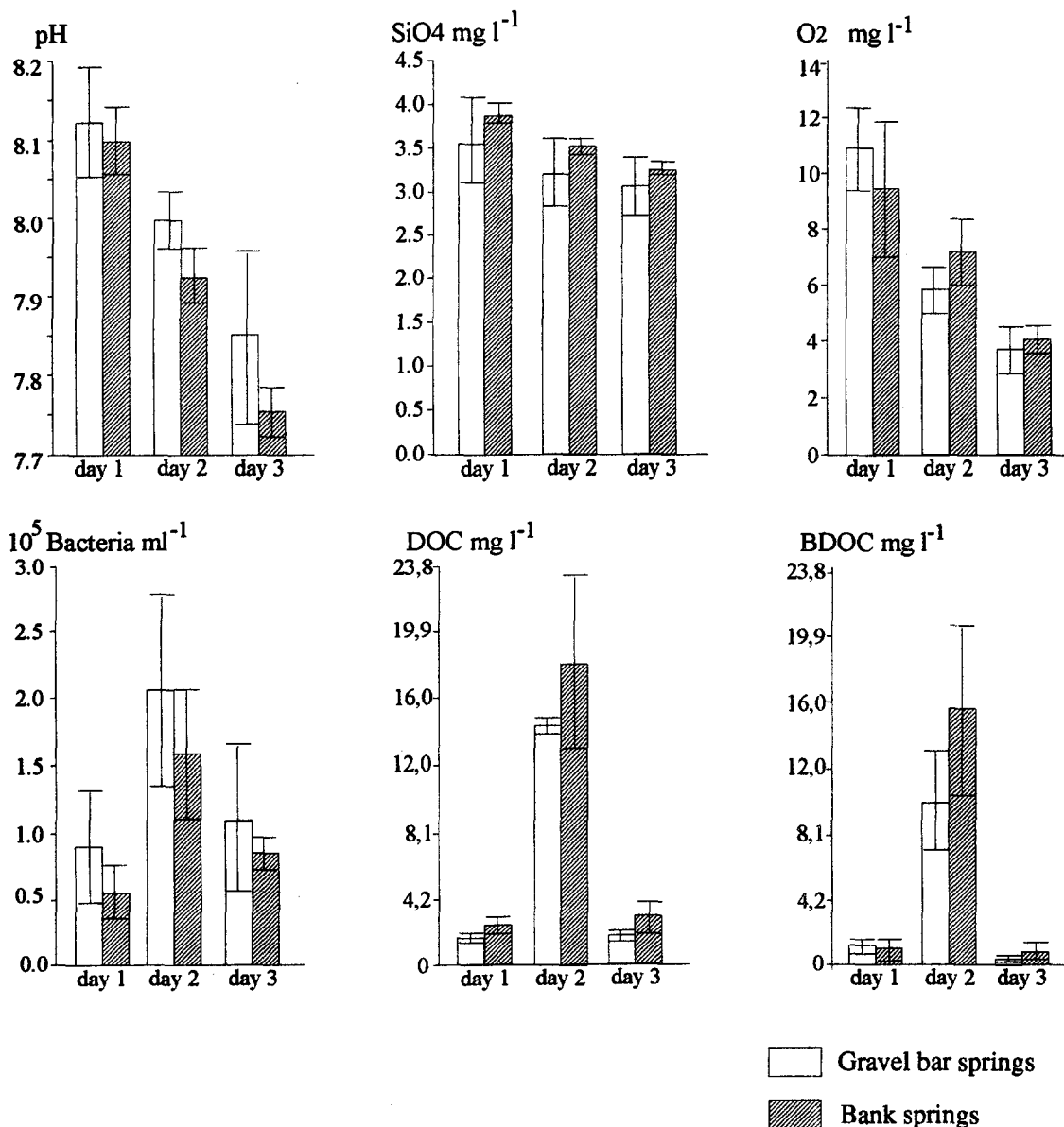


Fig. 2. Change in various chemical and biological components of water from gravel bar springs and bank springs during the 3-day artificial drought (mean values and standard deviations,  $n=4$ ).

can be observed for active bacteria, but this was only significant for the gravel bar springs ( $p<0.05$ ): they represented 8.6% on the first day, 23.6% on the second, and 21.1% on the last day (Fig. 2).

During the studied period, groundwater inputs from the plain represented  $0.77 \text{ mg l}^{-1}$  of nitrogen (93.3% of  $\text{NO}_3^-$ ),  $0.0079 \text{ mg l}^{-1}$  P- $\text{PO}_4$  and  $3.56 \text{ mg l}^{-1}$  of  $\text{SiO}_4$ . Because DOC and bacterial inputs increased greatly during the second day, it is difficult to use these results to estimate the values of the groundwater inputs

into the river. The values obtained on the first day can be considered as a rough estimation of these inputs:  $2.315 \pm 0.703 \text{ mg l}^{-1}$  of DOC (47% of which were biodegradable) and  $7.3 \times 10^4 \pm 3.7 \times 10^4$  bacteria per ml (8.8% of which were active).

The chemical characteristics of the river water also changed during the study (Table 2). As in the springs, oxygen, pH, and all the mineral nutrients (silica, all forms of ammonium and phosphorus) decreased from the first to the third day; whereas DOC and BDOC

increased on the second day. Those variables in low concentrations in bank springs, (phosphorus, nitrates and sodium) decreased in the river, whereas those in high concentrations in bank springs (calcium, conductivity and alkalinity) increased in the river (Table 2). Finally, the number of active bacteria showed a uniform decrease in the River Rhône between the first and third days (from 5.3% to 0.3% of the total number of bacteria).

## Discussion

In view of the unusual character of the study period, an artificial drought, which modified the dynamics of the groundwater, it should be kept in mind that the results may represent an over-estimation of the variables measured. The rapid decrease of Rhône's water level induced the formation of springs, and probably also altered the natural concentration of nutrients and bacteria in the groundwater flows. Nevertheless, direct measurements performed during this field experiment confirm previous reports that groundwater can be a source of organic and mineral nutrients for surface waters (Wallis *et al.*, 1981; Grimm & Fisher, 1984; Triska *et al.*, 1989; 1990; Hendricks & White, 1991; Hendricks, 1993; Valett, 1993). Overall, this study demonstrates that groundwater can be a source of biodegradable organic matter and bacteria for aquatic systems.

### *Organic matter and bacteria inputs*

Some authors (Crocker & Meyer, 1987; Ford & Naiman, 1989) considered that dissolved organic carbon coming from the terrestrial ecosystem through groundwater was mostly refractory to microbial degradation. The present study demonstrates that a part of the DOC (47%) is in fact biodegradable and 8.8% of the interstitial bacteria that drifted into the River Rhône were active. This last value is similar to those observed by Marxsen (1988) in groundwater (from 0.66 to 7.4%) using the INT-formazan staining method.

The increase in DOC, BDOC, and bacterial contents in groundwater on the second day of the experiment (Fig. 2) is difficult to explain. An hypothesis linked to the lowering of the water table in the subterranean environment can be proposed. The artificial drought might induce two different processes: 1) an increase in groundwater velocity, thus recruiting a high bacterial concentration, which normally live in con-

tact with the sediment particles; and 2) a decrease of the groundwater level. These two processes may have induced oxygenation of sediments located close to (or just below) the piezometric level. These sediments are known to be rich in organic matter because they are close to the soil. The increase in heterotrophic activities (linked to this better oxygenation) can be observed in the springs on the second day (increase of DOC and bacterial concentrations, modification of the dissolved carbon quality; Fig. 2). On the third day, the further lowering of the water level may have resulted in an isolation of this sediment layer, rich in organic matter, from the groundwater table, thus leading to lower DOC and bacterial concentrations in the springs.

### *Comparison between gravel bar and alluvial plain groundwater*

The present study also demonstrates the strong heterogeneity between the different types of groundwater that flow into the river (Table 1). The interstitial water that flows out from the gravel bars have higher nitrate content than groundwater from the alluvial plain. During the sampling period (growing season), the forest and the periphyton of the river assimilated nitrate to produce biomass, leading to low concentrations in the surface water as well as the groundwater of the bank. By contrast, in the interstitial habitat of the gravel bars, oxidation of the organic matter trapped in the sediment could induce nitrate formation, which flows out of the bar and is detected into the springs. The weak development of gravel bar vegetation (regularly disturbed by floods) was not sufficient to have a significant effect on nitrate concentrations.

Similarly, the phosphorous (both orthophosphate and total phosphorous concentrations) was higher in the gravel bar springs than in bank springs. This nutrient tends to be held in the soil and little is transported to groundwaters (Reddy *et al.*, 1978; Conesa *et al.*, 1979). However, higher phosphate content in groundwater than in surface water has been reported for a desert stream in Arizona (Valett *et al.*, 1990), and a Northern Michigan river (Hendricks & White, 1991). In the River Rhône, the interstitial water of the gravel bar cannot be considered as a source of phosphorous because the concentrations measured in the springs and in the surface waters did not differ significantly (Table 1). The similarity in phosphorous concentrations could be explained by a simple crossing of the Rhône water through the gravel bars without any enrichment.

The sulfate concentration of Rhône water is generally high because of Triassic deposits, rich in Gypsum, drained by the river in its Swiss reaches (Juget *et al.*, 1979). This origin also explains the high concentrations in the interstitial water of the gravel bar (Table 1). Storage of sulfate in the interstitial water of the gravel bars can be long, at least when oxygen concentrations enable aerobic processes.

#### *Consequences for surface water chemistry*

The chemical variables that had low concentrations in bank springs (phosphorus, nitrate and sodium) decreased in the river, whereas those in high concentrations in bank springs (calcium, conductivity and alkalinity) increased in the river during the 3-day study. This suggests that during the temporary drought, the surface water was mostly fed by groundwater originating from the alluvial plain and that interstitial water from the gravel bars did not represent a source large enough to influence surface water chemistry. However, surface water chemistry was not completely dependant on groundwater inputs. The decrease in mineral nutrients (i.e. silica, nitrogen and phosphorus) could be explained by microphyte assimilation.

The groundwater inputs appeared to influence the DOC and BDOC concentrations in surface water, which showed similar temporal dynamics in the springs and in the River Rhône. The number of bacteria, and above all the number of active bacteria, decreased uniformly in the River Rhône water from the first to the third day despite the input of bacteria from groundwater. This decrease in bacterial density may be a consequence of the drought on riffles. As the amount of water flowing into the channel decreases, the filtering effect of the sediment on particulate suspended matter (which decreased from 4.9 to 1.2 mg l<sup>-1</sup> during the experiment) increases. This is particularly true with respect to bacteria attached to these particules. Bacterial concentrations observed in springs during the artificial drought were quite low (Fig. 2) in comparison with Rhône water (Table 2). This bacterial input may, nevertheless, be qualitatively important for the biodiversity of microbe assemblages of surface water. These initial results bring new perspectives for future research both on species composition and on gene dynamics between terrestrial, groundwater, and running water ecosystems.

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