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# Surface and hyporheic oligochaete assemblages in a French suburban stream

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This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

## **Abstract**

The Chaudanne stream received urban inputs discharged through combined sewer overflows (CSOs). The water quality was not severely impaired, with pollution mainly of organic origin. Oligochaete assemblages were studied in the coarse surface sediments and the hyporheic zone and sampled at 4 sites on 7 occasions during 2000 and 2001. The seasonal distribution of oligochaete assemblages was analyzed by a PCA, the oligochaete species being assigned to functional traits (FTrs). Site 1, located upstream of the CSOs, was characterized by FTrs 1 and 2 (species indicating permeability and those intolerant to water pollution). Below the CSOs, high densities of oligochaetes occurred in the benthic layer of sites 3 and 4, with a predominance of FTr3 (species with tolerance to water pollution). At site 4, FTr4 species (indicative of sludge conditions) constantly predominated in the hyporheic system, but predominated in the benthos only during low stream discharges associated with peaks in CSOs. FTr3 was related to amounts of the oxidized forms of nitrogen, high stream discharges and probably to groundwater upwellings and the sludge tolerant species group (FTr4) was associated with high NH<sub>4</sub><sup>+</sup> contents. We are now testing the relevance and generalisation of this new approach.

#### 1. Introduction

The assessment of the ecological impacts of combined sewer overflows (CSOs) has became a priority in urban aquatic systems (Cyr et al. 1998, Paul & Meyer, 2001; Rogers et al. 2002). The alteration of urban watercourses complicates prediction of the effects of CSOs, which are strongly related to rainfall patterns. In addition, the pollution originating from storm-water runoff is generally accompanied by other pollution sources, including permanent inputs and polluted overflows from impervious urban surfaces. These multiple sources of pollution are a common feature in urban aquatic areas, and are generally poorly known (Faulkner et al. 2000). The recent EC Water Framework Directive (UE, 2000) emphasized the conservation or restoration of good ecological condition in all aquatic ecosystems, including those located in urbanized areas.

In this study, we tried to assess the ecological effects of a CSO on the Chaudanne stream, a suburban watercourse of Lyon's urban area (France). We focused on the surface coarse sediments and hyporheic system which largely predominates in the studied stream. Invertebrate assemblages were mainly composed of oligochaetes and crustaceans, and aquatic insect larvae were not abundant (Bernoud, 1998). This was related to the urbanization of the surrounding landscape which had altered the physical habitats. The preserved area located near the source of the stream was poor in aquatic insects, however it was naturally rich in oligochaetes and crustaceans (Bernoud, 1998). Consequently, we focused on benthic and hyporheic oligochaetes as i) they were the most abundant interstitial invertebrates in the study site and ii) the present study was supported by data sets considering the oligochaete assemblages in the surface coarse sediments and hyporheic system of polluted and unpolluted watercourses (Lafont, 1989; Lafont & Durbec 1990; Lafont et al. 1992, 1996; Lafont & Malard, 2001; Malard et al. 2001).

It was necessary, in this preliminary work, to assess the relevance of the information value of oligochaetes, in particular to see if gradients of alterations might be evident in a relatively unimpaired situation such as the Chaudanne stream. In addition, the choice of a suburban site was dictated 1) by the fact that catchment management is easier to control than in urban watercourses; 2) that the imperviousness of the urbanized surrounding landscape is not constant; and 3) that relatively

preserved areas can still be found. If alteration gradients could be illustrated by oligochaete worms in a situation of intermediate disturbance, the generalization to similar or more altered situations should be possible in the future.

#### 2. Study site

The Chaudanne stream is a tributary of the Yzeron river (Bernoud, 1998). The Yzeron river enters the Rhône river at Oullins, a city located in the southwestern area of the Lyon's urban area. The length of the Chaudanne stream is 2743 m and it flows southeast, from 443 m elevation to its entry into the river Yzeron at 306 m (average gradient = 5 %). The basin area is 2.83 km<sup>2</sup>. Its head water is located in a rural area (2.22 km<sup>2</sup>). The length of the studied reach is about 2.5 km. Four sites were studied (Fig. 1). Site 1 is located near the spring, uninfluenced by agricultural, road or urban inflows. It is therefore considered as a reference site. Site 2 receives the first polluted input, from the drainage ditch of a factory. Site 3 is located 150 m below the main polluted input (Combined Sewer Overflow CSO of the "Pont de la Barge"). Site 4 is 500 m downstream of the CSO and receives a third input from the car park of a commercial zone (Nogueira, 2001, Fig. 1). The imperviousness of the surrounding urbanized landscape of sites 2, 3 and 4 does not exceed 15% (Bernoud, 1998). The stream bed is composed of stones, gravel and sand. The current velocity of surface waters is about 30 cm.s<sup>-1</sup> in the riffles where the biological and chemical samples were collected. The surface water velocity decreases to less than 10 cm.s<sup>-1</sup> during low stream discharges (August, October 2000). The temperature of surface waters varies from 6.5°C in winter (February 2000) to 18°C in summer (August 2000). The temperature of hyporheic waters (-20 to -30 cm depth) varies from 6.4°C in winter to 17°C in summer. The pH and conductivity are similar in surface and hyporheic waters and, respectively, vary from 6.5 to 8.3 and from 170 μS.cm<sup>-1</sup> (site 1) to 532 μS.cm<sup>-1</sup> (site 4) (Namour, unpublished data). Daily stream discharge measurements were available from a gauge located at site 2.

#### 2. Material and methods

Benthic and hyporheic oligochaetes were sampled at the four sites on seven occasions (April, June, August, October and December 2000, February and April 2001). Benthic sediments were collected using a Surber-type net (400 cm<sup>2</sup> aperture; net mesh-size: 0.160 mm). In the hyporheic system, 10 litres of material (sediments and interstitial water) were pumped from a depth of 20 to 30 cm using a Bou-Rouch pump (Bou & Rouch, 1967). At each site, three replicates were collected (a sample each 10 m). The three benthic replicates were pooled at each occasion. The three hyporheic replicates were kept separate. Samples were preserved in the field with 4% formaldehyde. In the laboratory, the mineral particles (stones, gravel, sand) of benthic and hyporheic samples were separated from the organic fractions (organic fragments and invertebrates) by decanting, and the organic fractions were washed through a 0.160 mm sieve. The residue from the sieving of each sample was poured into a gridded sub-sampling box. 100 oligochaete specimens were sorted by hand under a binocular microscope, if necessary from sub-samples taken randomly with a pipette from the squares of the sub-sampling box. Worm specimens were mounted on slides in a mixture of lactic acid and glycerin (50% glycerin, 50% lactic acid), covered by a cover-glass, and identified to species when possible under a microscope. Stream discharge was very low during August 2000, allowing benthic sampling of sites 2 and 3 only, the others being dry. The hyporheic sediments of the control site (Site 1) were sometimes clogged by silt, and the hyporheic system was dry during August 2000 at Site 4. Hyporheic samples with total oligochaete densities in the 3 replicates less than 10 specimens were not retained, as the relative abundances of oligochaete species were mainly considered in the statistical analyses. To allow a comparison with pooled benthic collections, the data of the hyporheic replicates were pooled together, each pooled sample being characterized by the mean values (calculated from the 3 replicates) of the biological variables. Consequently, 18 hyporheic and 26 benthic samples were retained for analyses. The surface replicates were pooled in the field to allow a comparison with previous data sets. Conversely, the hyporheic replicates were kept separately and pooled only in the multivariate analysis. The data of each hyporheic replicate remain available and will be considered in other papers.

#### Data analysis

The seasonal distribution patterns of oligochaete assemblages at the 4 sites were analysed by a standardized Principal Component Analysis (PCA, software: ADE-4, Thioulouse et al. 1997). The benthic system (26 samples) was separated from the hyporheic system (18 samples). The oligochaetes were gathered into 4 ecological groups (Table 1), called "functional traits" (FTrs, Lafont et al. 2000). The FTrs were derived from the ecological knowledge of indicator oligochaete species that reveals the ecological functioning of the functional units "coarse surface sediments" and "hyporheic system" (FU3 & 4, Lafont 2001). Two other variables were considered: the total number of oligochaete species and the densities per sample,  $Log_{10}(n + 1)$  transformed for normality (NOSP and EFBO, respectively). These two last variables enabled incorporation of the species whose ecological preferences are not known or not considered as ecologically distinct.

The PCA reduces the number of input variables, and was here expected to describe meaningful ecological gradients and spatio-temporal patterns of benthic and hyporheic assemblages. Because relationships among variables could be slightly different between the hyporheic and the benthic systems and as the sampling devices were different, we performed a PCA for each system.

## 4. Results

A total of 46 taxa was found in the surface sediments and hyporheic system. The immature Tubificidae (with and without hair setae) and Lumbriculidae were not counted as individual taxa because adults of them were present. The unidentified Enchytraeidae accounted for 10 taxa. A notable record was *Rhyacodrilus ardierae*, which was first discovered near the spring of the river Ardières, in a headwater reach similar in size to the Chaudanne stream. It is considered a pollution-intolerant species (Lafont & Juget, 1993).

## Distribution patterns of oligochaete assemblages

For the benthic samples (Fig. 2A), the axis F1 (53.6 % of the total variance) of a PCA of oligochaete assemblages separated species-rich samples, dominated by the species considered as indicators of hydrological exchanges between surface and ground water (AED species) and by pollution-intolerant ones (NSPO, FTr1, FTr2), that typically reflected the assemblage features of site 1, from assemblages characteristic of sites 2, 3 and 4 (Fig. 2B). These were characterized by high densities of oligochaetes (EFBO) and a predominance of pollution-tolerant species (FTr3). The second axis F2 (26.8 %) discriminated the samples rich in Tubificidae (FTr4) (site 2 October 2000, site 3, August and October 2000, site 4 June and October 2000). In the surface sediments, the factor F1 of the PCA was considered as characterizing organically polluted waters. The factor F2 characterized another type of pollution, with a predominance of Tubificidae worms indicating the presence of sludge in the sediment interstices. The reference site (1) was clearly separated from the remaining sites by an increasing dominance of FTr3+4 species, indicating a downstream pollution gradient, although the site located just below the combined sewer overflow (3) was apparently no more impaired than the site immediately above the outflow (2).

In the hyporheic zone (Fig. 3A), axis F1 accounted for 44.4 % of the total variance. It illustrated the difference between the global predominance of the Tubificidae (FTr4) at site 4 (Fig. 3B), from an association of AED and pollution-intolerant species (FTr1 & 2), particularly at sites 1 and 3. Axis F2 (24.5 % of the total variance) was mainly correlated with the absolute densities of oligochaetes (EFBO), and characterized the sites 3 (December 2000) and 4 (June and October 2000). The F1-F2 plane discriminated site 1 from all the others. This site was frequently clogged by mineral particles and no oligochaetes were collected. But when silt was absent or reduced, the assemblages of the hyporheic zone were dominated by AED (FTr1) and pollution-intolerant species (FTr2).

#### Correlations with chemical features.

In the benthic system, the distribution pattern of oligochaetes on the F1-F2 plane was associated with an increasing gradient of nitrates (Fig. 2B), particularly from below the CSO (sites 3

and 4). Site 1 had good chemical quality compared to the 3 other ones, except for phosphorus. But the high standard deviation of phosphorus concentrations at the other sites reflected the phosphorus (and ammonium) peaks, characteristic of CSOs in urbanized environments (Paul & Meyer, 2001; Walsh et al. 2001). In the hyporheic system (Fig. 3B), site 1 exhibited relatively high values of NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup>, probably related to mineral clogging at this site. The chemical alteration was marked at site 4 when considering ammonium salt contents (1.44 mg.L<sup>-1</sup>, Fig. 3B), but not at the first site below the CSO (site 3). The nitrate contents also declined at site 4, implying reducing conditions, in contrast to sites 2 and 3, where high nitrate contents indicated oxidized situations.

## Relations between stream discharge and assemblage features

The sludge effect, characterized by pollution-tolerant Tubificidae and *Lumbricillus* spp., was observed in the benthic system at all sites (Fig. 4) when the stream bed discharges were very low with high CSO peaks (August and October 2000), but was least pronounced at the reference site (site 1). The sludge effect was also strongly pronounced at site 4 during June, when stream discharges were falling. These results suggested that rainfall led to overflows from the surrounding environment and brought sediments into the stream, even at the reference site, and that the purification capacity of the stream decreased (low discharges). The sludge effect (FTr4) remained most pronounced at the sites below the sources of pollution (2, 3 and 4). When stream discharges increased (> 14 L.s<sup>-1</sup>), the sludge effect was much less marked in the benthic layer, but it remained strong in the hyporheic layer of site 4 (Fig. 4).

#### 5. Discusssion

The species richness of the Chaudanne stream was high (46 taxa), probably because we considered both the surface and subsurface layers. The reference site (site 1) was predominated by pollution-intolerant species, which also are AED species, together with a greater species richness. We found similar ecological traits in a glacial unpolluted river (river Roseg, Lafont & Malard, 2001; Malard et al. 2001), where the surface and hyporheic habitats were colonized by pollution-intolerant AED species, particularly where upwellings of groundwater occurred. Site 3, located just below the

main CSO, exhibited a distribution of epigean oligochaete assemblages similar to that of the site 2 above the CSO. In the hyporheic layer, the distribution of oligochaetes typified a rehabilitated situation compared to site 2 (Fig. 3B), with a predominance of pollution-intolerant AED species. This suggests that the effect of the CSO is not detrimental just below its inputs, and that benthic and hyporheic systems at site 3 were preserved by ground water upwellings. The high stream discharges had also a beneficial effect, probably by taking away the polluted sludge of the CSOs. Site 4 was the most vulnerable to a sludge effect, particularly in the hyporheic system, that might be attributed to an attenuation of the slope of the stream and the predominance of downwellings of polluted surface waters. These last observations emphasize the fact that the hyporheic system can accumulate nutrients and polluted inputs (Hynes, 1983; Jones & Mulholland, 2000) and retain residues of past and present pollution (Danielopol, 1989; Giere, 1993; Lafont et al. 1996).

The alteration of chemical quality is seen below the CSO, but with relatively low concentrations of solutes. The mean ammonia salt contents reached 1.44 mg.L<sup>-1</sup> at site 4. These contents are considered indicative of a "fair" quality in urban ecosystems (Faulkner et al. 2000). An increase in phosphorus and nitrogen loads was observed between site 2 (just above the CSO) and site 3 just below the CSO, but did not reach alarming values. The hyporheic zone of site 1 exhibited relatively high values of ammonia salts and phosphorus contents, potentially related to the clogging of interstices by mineral particles. This conflicts with the good biological quality of the site. We assume that the comparison between solute concentrations and oligochaetes will be better understood by considering the fluxes of solutes, rather than their instantaneous concentrations. Probably, nitrogen and phosphorus exhibited relatively high contents in the hyporheic zone at site 1 because they were not intensely assimilated by living organisms, which were scarce or absent.

The temperatures of surface and hyporheic waters are important factors, particularly for deriving patterns of downwelling surface water and upwelling ground water (Evans & Petts, 1997). However, temperature was not considered an important factor because temperature ranges did not reach extreme values in Chaudanne stream. Temperature, as well as other important factors like oxygen and organic matter (Strayer et al. 1997), will be considered in future studies,

The FTrs illustrated clear-cut gradients of oligochaete distribution in a situation not still severely polluted and where organic pollution predominated. The FTrs were empirically defined from literature data and our own experience and appear an attempt that by-passes regional and spatial heterogeneities and defines the functionalities of the studied habitats (Lafont et al. 2000). We are conscious that this approach is debatable. FTtr1 (permeability) seems relevant and supported by literature. FTr4 (sludge effect) is also relevant and supported by field experience on polluted coarsegrained habitats in running waters. The FTr2 (intolerance to water pollution) and FTr3 (tolerance to water pollution) are questionable. Moreover, the tolerance/intolerance to pollution of a given species is also strongly related to the habitat where it has been collected (Lafont, 1989).

The great majority of pollution studies regarding oligochaete communities considered fine sediments (see Chapman, 2001) and not coarse sediments or hyporheic systems. *Nais communis* is more tolerant of organic pollution in coarse than in fine sediments (Lafont, 1989). The pollution-tolerant species of fine sediment-dwelling Tubificidae are collected in coarse sediments only when polluted sludge is trapped within the interstices (Brinkhurst, 1965, Datry et al. 2003). Without polluted sludge, these species are not collected, even if the interstitial water is polluted. The ecological preferences of oligochaete species are also dependant upon the salinity of waters (Leland & Fend, 1998), habitat heterogeneity (Carter & Fend, 2001) and time (Verdonschot, 1999). But the hyporheic system is less sensitive to physical disturbances like floods than the benthic system, and its consideration in the ecological studies of watercourses has been recommended (Boulton, 2000; Boulton et al. 2003; Hynes, 1983; Lafont, 2001; Petts, 2000). The evaluation of the tolerance/intolerance to pollution of oligochaete species is probably more reliable in the hyporheic layer. Indeed, in the hyporheic layer, the species stay in more direct and constant exposition to pollutants than in the superficial zone, where flow regimes are prevailing factors (Bunn & Arthington, 2002) and reduce the probability of species exposure to pollutants.

Another issue is that several species occupy 2 FTrs while others occupy only one (e. g. *C. atrata* is characteristic of FTrs 1 & 2, the Tubificidae of FTr4). This means that the value of *C. atrata* is 0 to FTr4, and that the value of Tubificidae is 0 to FTr1 & 2, etc. This is an empiric simplification that needs several improvements. For example, *Chaetogaster diastrophus* is a common and abundant

species in the Chaudanne stream, but it is above all a species which is tolerant to organic enrichment, but intolerant to severe organic pollution or toxic substances (Lafont, 1989). It did not fit any of the 4 FTrs considered here, but other FTrs will be investigated, and we are conscious that the functioning of coarse and hyporheic sediments cannot be described by only 4 FTrs.

This first general survey was a feasibility study. We now plan to conduct multidisciplinary research on the Chaudanne stream, including studies of crustaceans and hydraulics modeling. This research is expected to improve our present knowledge about the value of oligochaete species and present or future FTrs as functional descriptors (Lafont, 2001; Ruysschaert & Breil, 2004).

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Table 1. Functional traits (FTrs) of the surface and hyporheic sediments.

abundances of oligochaete assemblages per sample.

Functional traits FTrs	Oligochaete species characterizing each FTr				
FTr1: Percentages of oligochaete species	Trichodrilus strandi, Stylodrilus heringianus,				
which indicate active hydrologic exchanges	S. parvus, Rhyacodrilus ardierae, R. coccineus,				
between surface and ground water (AED species) (Gaschignard, 1984; Juget, 1984; Juget & Dumnicka, 1986; Juget & Lafont, 1994; Lafont, 1989; Lafont & Durbec, 1990; Lafont et al. 1992, 1996)	R. falciformis, R. subterraneus, Haber speciosa Pristina aequiseta, P. jenkinae, P. osbora Cernosvitoviella atrata, Achaeta vesicular Marionina argentea, Haplotaxis gordioides				
FTr2: Percentages of oligochaete species which are intolerant to water pollution (Lafont 1989, Lafont & Juget 1993; Lafont et al. 1996)	R. ardierae, R. falciformis, R. subterraneus, C. atrata, A. vesiculata, M. argentea, Eiseniella tetraedra				
FTr3: Percentages of oligochaete species which are tolerant to water pollution in coarse sediments (op. cited; Giani, 1984)	Nais elinguis, P jenkinae, Dero digitata,  Marionina riparia				
FTr4: Percentages of taxa which indicate the presence of polluted sludge within coarse sediment interstices (Brinkhurst 1965; Datry et al. 2003; Lafont, 1989; Lafont et al. 1996,).	Immatures of Tubificidae with and without hair setae, <i>Tubifex ignotus, T. tubifex, Limnodrilus hoffmeisteri, Bothrioneurum</i> sp, <i>Lumbricillus</i> spp. (in bacterial sewage-treatment beds, Solbé, 1975)				
•	e assemblages in the Chaudanne stream, except represented by the species richness and the absolute				

## Figure captions

## Figure 1

Sampling site locations in the stream Chaudanne.

## Figure 2

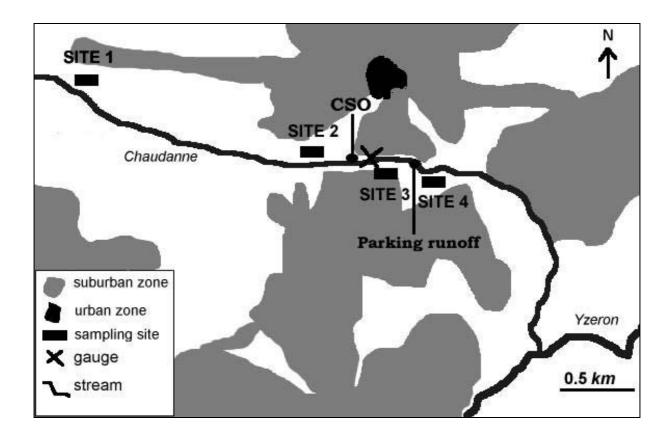
Standardized PCA on benthic oligochaete assemblages (26 rows, 6 columns); **A**: correlation circle (F1-F2 plane); **B**: plots and dispersion polygons of biological samples from the 4 sites on the first plane; NSPO: number of oligochaete species; EFBO: densities per 0.1 m<sup>2</sup> of oligochaete assemblages; FTr1, FTr2: functional traits permeability and intolerance to water pollution; FTr3, FTr4: functional traits tolerance to water pollution and sludge effect; the chemical variables are expressed as mg.L<sup>-1</sup>; the standard deviations are put in brackets; NH4: ammonium salts; NO3: nitrates; PO4: orthophosphates.

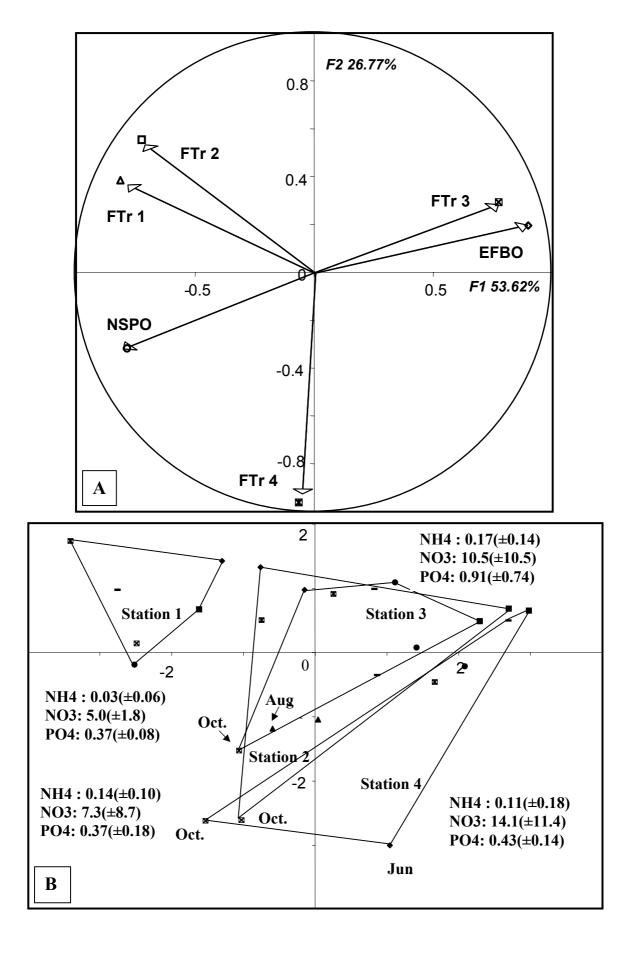
## Figure 3

Standardized PCA on hyporheic oligochaete assemblages (18 rows, 6 columns); **A**: correlation circle (F1-F2 plane); **B**: plots and dispersion polygons of biological samples from the 4 sites on the first plane; NSPO: number of oligochaete species; EFBO: densities per 10 l of oligochaete assemblages; FTr1, FTr2: functional traits permeability and intolerance to water pollution; FTr3, FTr4: functional traits tolerance to water pollution and sludge effect; the chemical variables are expressed as mg.L<sup>-1</sup>; the standard deviations are put in brackets; NH4: ammonium salts; NO3: nitrates; PO4: orthophosphates.

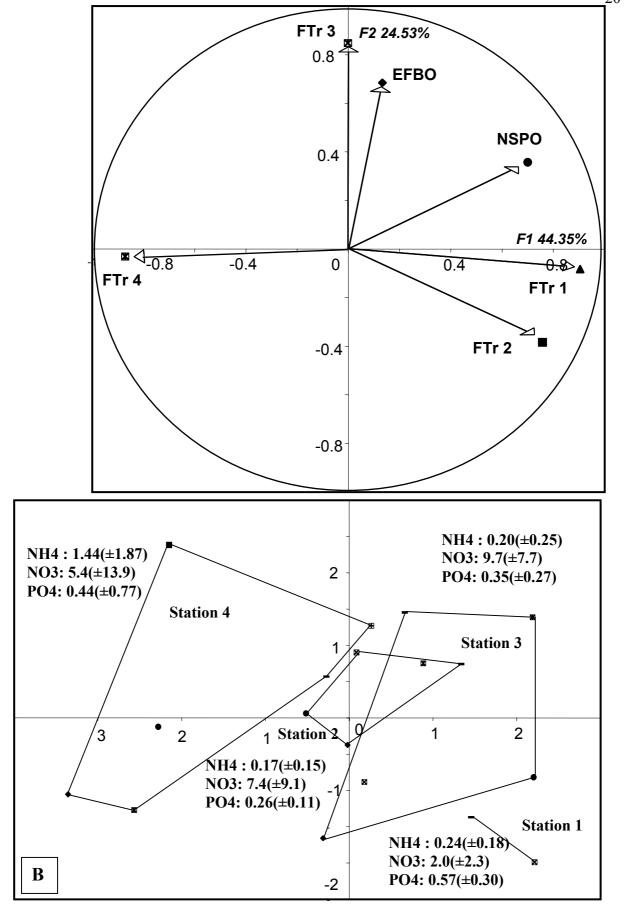
## Figure 4

Comparison between the stream discharge (unbroken lines), the CSOs' peaks and the sludge effect (FTr4), at the 4 stations of the stream Chaudanne; vertical arrows: dates of biological samples; stream discharge and CSOs' peaks are expressed as m<sup>3</sup>.s <sup>-1</sup>; ?: undetermined data.

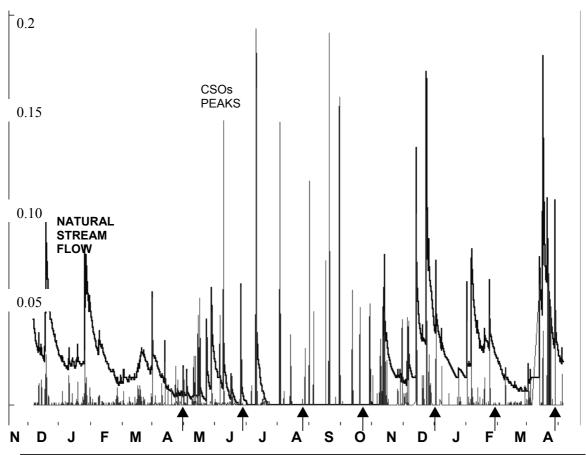








Lafont et al. Fig. 3



Surface sediments: percentages of the species indicating FTr4 (sludge effect)									
Sites	1	1%	0%	?	11%	0%	1%	7%	
	2	1%	2%	24%	54%	5%	14%	7%	
	3	0%	0%	30%	33%	0%	1%	0%	
	4	1%	71%	?	50%	22%	3%	16%	
Hyporheic zone: percentages of the species indicating FTr4 (sludge effect)									
Sites	1	0%	?	?	?	?	0%	?	
	2	?	24%	?	17%	7%	2%	9%	
	3	?	7%	?	8%	4%	7%	2%	
	4	40%	87%	?	30%	63%	31%	32%	