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The Dynamics of Macroinvertebrate Assemblages in Response to Environmental Change in Four Basins of the Etueffont Landfill Leachate (Belfort, France)

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Abstract We investigated the relationships between the composition and structure of macroinvertebrate communities and some environmental variables over a year in four basins of the Etueffont landfill leachate (Belfort, France) using co-inertia analysis. Culicidae larvae were the dominant macroinvertebrate group in the studied basins, contributing to 87% of the total zoobenthos density, followed by Corixidae (8.8%), Chironomids (2.5%) and other larvae (each <1%). The lowest density of chironomid larvae was recorded in the first basin which is used as a discharge system for the leachate produced by the landfill. In basin 4, however, the Baetidae, Orthocladiinae (*Orthocladius* spp., *Chaetocladius* spp. and *Isocladius* spp.) and Tanypodinae (*Psectrotanypus* spp.) developed favoured by low levels in ammonia, COD, BOD, EC, metals and high oxygen concentrations. The co-inertia analysis illustrated both temporal and spatial variabilities in the basins and revealed a strong relationship between environmental conditions and benthic macroinvertebrates assemblages. This ordination technique showed that the chironomid community structure might be used successfully to differentiate between sites with different levels and types of pollution.

Keywords Landfill leachate · Chironomids · Pollution · Co-inertia analysis

1 Introduction

In aquatic ecosystems, the dynamics of communities are closely related to natural and human interactions e.g., trophic interaction network, flooding, eutrophication, pollution, etc. (Reynolds 1984; Alaoui et al. 1994; Verneaux and Aleya 1998a; Beman et al. 2005). These constraints may dramatically translate into a loss in biological diversity (Aleya 1991; Kimberling et al. 2001; Ribaudo et al. 2001; Harris 2002; Dyer 2005; McAbendroth et al. 2005). The classification and ordination of communities have been recently focussed on the water quality assessment. Benthic macroinvertebrate communities have been shown to be widely acceptable in indicating water quality and might effectively reveal the ecological status of aquatic environments (Hynes 1960; Hawkes 1979; Hellawell 1986; Verneaux and Aleya 1998b; Hart and Lovvorn 2005). While there is now abundant literature

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on the use of macroinvertebrate communities as ecological integrity indicators of aquatic ecosystems (Verneaux and Aleya 1998b; Kay et al. 1999; Kimberling et al. 2001; Spanhoff et al. 2004; Hynynen and Merilainen 2005; Brooks et al. 2005; Gandouin et al. 2006), little is known about the seasonal variation of macroinvertebrate assemblages in stabilization ponds constructed to treat landfill leachate generated from the biodegradation of municipal solid wastes. These stabilization ponds, which are generally designed to treat only an average quality of the leachate, are commonly put under extreme pressure due to enhanced overload of organic matter. Furthermore, while landfilling is the most attractive option for waste disposal, it may be a source of large quantities of organic and inorganic matters and heavy metals into groundwater/surface water (Kabata-Pendias and Pendias 2001; Schwarzbauer et al. 2002). In particular, heavy metals that accumulate in biomass with long biological half-lives (Radha et al. 1997) have been shown to cause several health hazards (Omura et al. 1991; Thacker et al. 1992). In addition, the leachate composition has been shown from laboratory experiments (Bookter and Ham 1982; Blaky 1992) and in situ (Kjeldsen et al. 2002) to be closely linked to climate, hydrology and waste-hiding techniques. From a strictly epidemiological point of view, it was recently shown that chironomid eggs collected from a waste-stabilization may serve as an intermediate 'host' reservoir for *Vibrio cholerae*, facilitating its survival and multiplication in freshwater bodies (Broza and Halpem 2001).

This study aimed thus at investigating, for the first time to our knowledge, the impacts of the leachate quality on the distribution of macroinvertebrates over a year and to determine the dominant taxa that can be used to predict the overall resilience of the macroinvertebrate community in four stabilization ponds of the Etueffont landfill (Belfort, France).

2 Materials and Methods

2.1 Study Site

The Etueffont landfill (Fig. 1) is situated on 2.2 ha, started operating in 1974, and remained in-use until 2000. In 1999, the disposal site was full holding 200,000 tons of refuse. The width, length and depth are respectively 110, 200 and 5 m. The landfill was placed on a ground schistose and operates in the open air without cover after grinding the waste before landfilling. It was covered when exploitation of the site ended (July 2000) by a layer of vegetal soil coming from the old crushed organic waste (papers, wood, shearings of lawns, straws, fabrics; Khattabi et al. 2006). The leachates were collected downstream by a draining system and treated by lagooning process (Fig. 1). The morphometric characteristics of the four basins are reported elsewhere (Khattabi et al. 2002).

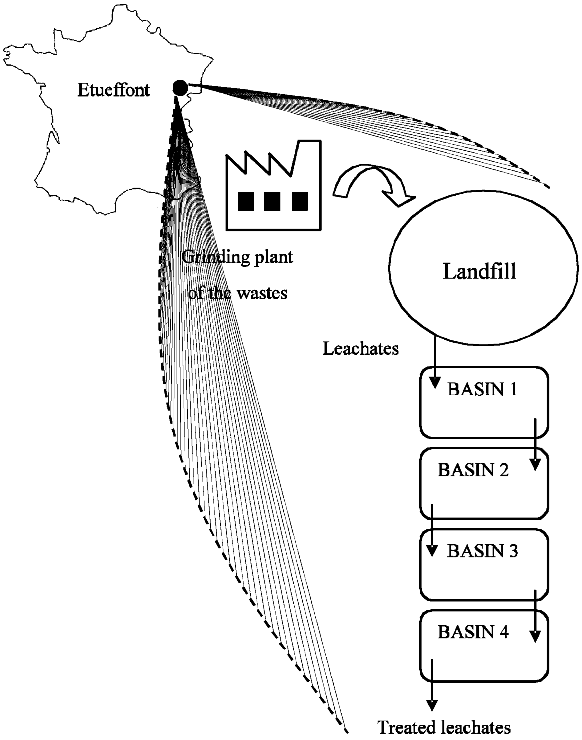

Fig. 1 Geographical location of the studied area, together with the four sampling sites and the morphometry of basins

2.2 Physico-chemical Analyses

Samples for the physico-chemical examination were collected monthly in the morning at each basin to minimize influence of the daily discharge fluctuations and correctly ensure data comparability. The samples destined for cation and metal analysis were acidified with nitric acid solution (65%). Sample preservation was accomplished by storing the bottles at 4°C immediately after sampling. During an annual cycle, samples were taken monthly (from May 1998 to May 1999). The temperature (T), pH, dissolved oxygen

(DO), and electrical conductivity (EC) were determined in situ with a portable multiparameter probe (WTW, Multiline P3 PH/LF-SET). Whatman GF/F glass fibre filters were used for total suspended matter (SM) measurements. Filters were previously precombusted at 450°C and weighed to the nearest 10^{-2} mg. Duplicated samples were filtered and briefly rinsed with distilled water. Filters were dried for 24 h at 60°C and then re-weighed. The carbonate content (HCO_3^-) of the leachate was determined using the volumetric method according to Rodier (1984). The concentrations of anions were measured using a ion-chromatography (Dionex DX-100, Ion Chromatograph). Concentrations of heavy metals and ammonia (NH_4^+) were determined by colorimetry with spectrophotometers (WTW Photolab Spectral and Rodier (1984), respectively). The biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) were measured by colorimetry using a spectrophotometer (WTW Photolab Spectral).

2.3 Identification and Enumeration of Invertebrates

Zoobenthos samples were collected in the basins from June 1998 to May 1999 using an Ekmann grab (225 cm^2). Samples were washed through a sieve bag with a mesh size of 250 μm, and the remaining material was preserved in formaldehyde (5%). Formaldehyde-preserved larvae were dropped into 10% KOH v/v left for 2–3 days until most of the internal issues had dissolved and the body appeared clear. The preserved materials were examined microscopically in order to sort out, prepare, and identify the genus of chironomids or, whenever possible, the species. Mounting and identification of chironomids larvae mainly followed the methods of Wiederholm (1983), but other techniques were also used for identification of particular species (Pankratova 1970; Saether 1975; Simpson et al. 1983; Coffman 1984; Schnell and Aagaard 1996).

Pupal skins left by emerging adults (exuviae) have been harvested with the help of a net dragged to the surface of the water of every basin. Pupal exuviae were left in 10% KOH overnight. They were then transferred to 70% ethanol and any remaining scales and debris removed as far as possible without damaging the cuticle and mounted on a slide. The key and illustrations of Wiederholm (1980, 1983) were used to identify the chironomids.

2.4 Statistical Analysis

Co-inertia analysis (CIA) was used to simultaneously examine the structure in the environmental and faunistic data, and to identify the corresponding variations (i.e. co-structure) of both structures (Dolédec and Chessel 1994). Co-inertia analysis is a two-table ordination method, as is canonical correspondence analysis (CCA). However, CIA enables the joint analysis of tables having similar (even low) as well as different numbers of environmental variables, species, and/or samples (Dolédec and Chessel 1994). In contrast, in CCA a small number of environmental variables is required to predict the faunistic structure, otherwise it would be reduced to a simple correspondence analysis of the faunistic table. Environmental data were normalized to ensure equal weights for all variables, whereas the faunistic data were $\log_{10}(x+1)$ transformed and centred to reduce strong inter-taxonomic differences in densities. The temporal and spatial components of variation in both data sets were examined using within-class CIA (Franquet and Chessel 1994; Franquet et al. 1995). All multivariate analyses were computed using the ADE-4 software (Chessel and Dolédec 1996; Thioulouse et al. 1997).

3 Results

3.1 Spatio-temporal Changes in Leachate Physicochemistry

The study of the temporal variation of the Etueffont leachate pointed out significant monthly fluctuations of most of the studied parameters (Khattabi et al. 2002). Overall, the concentrations of several pollutants in the leachate (Fig. 2, Table 1) were higher during the dry (summer) period, due to reduced percolation; selected COD values varied between 879 and 1,550 mg l^{-1} at the end of July (Fig. 2), while ammonia nitrogen concentrations reached up to 198 mg l^{-1} (Fig. 2). The other pollution parameters behaved similarly (Fig. 2). During the wet (winter/spring) season, the rain water percolating through refuse beds have extracted, dissolved and solubilized several constituents, producing a larger volume of diluted leachate, while during the summer dry months, the concentrations of several parameters (COD, Cl^-, EC, HCO_3^-) were high.

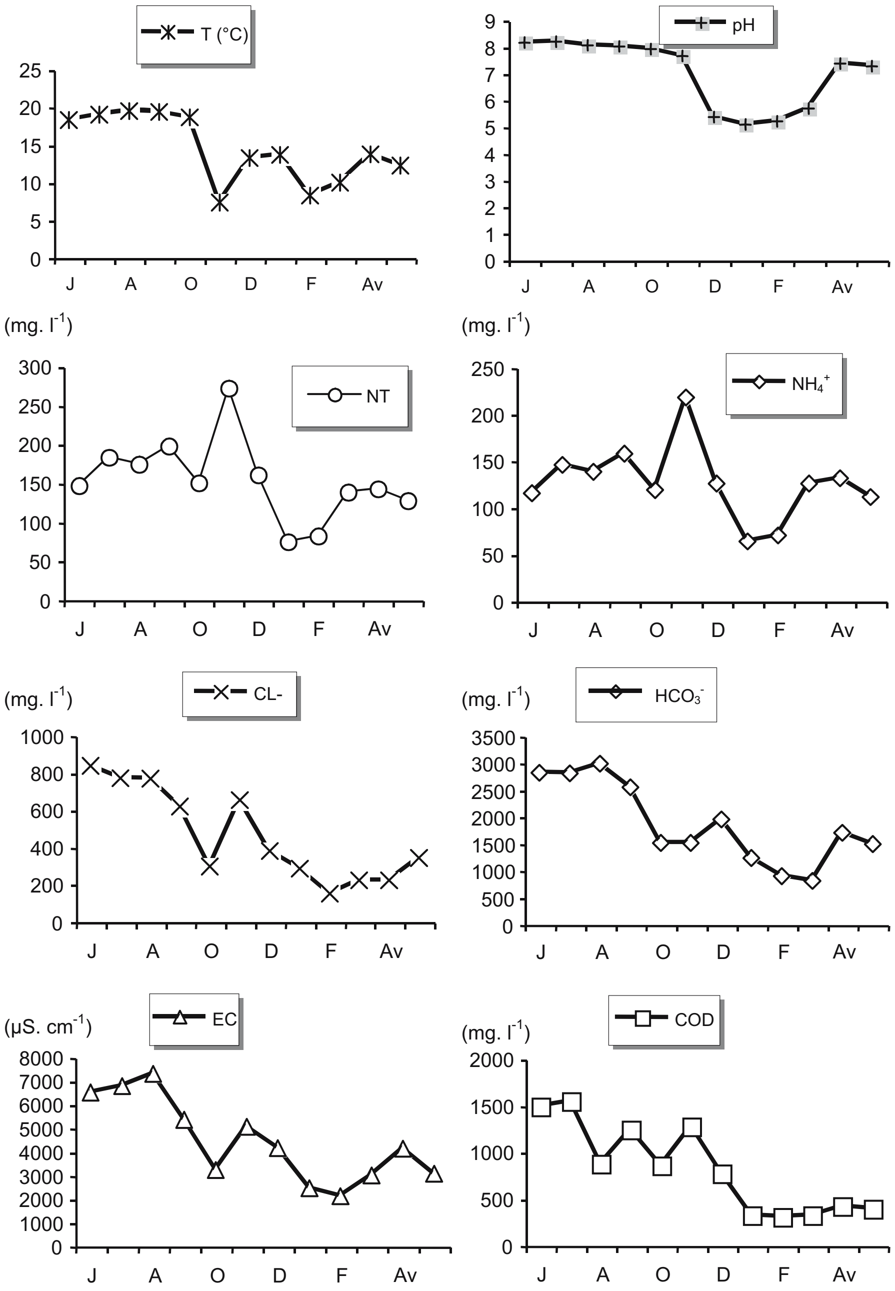

Fig. 2 Monthly variations in 1998–1999 of temperature (T) and the pH, NT and NH4 (mg l^{-1}), Cl^{-1} (mg l^{-1}) and HCO$_3^-$ (mg l^{-1}) and EC (μS cm^{-1}) and COD (mg l^{-1}) of Etueffont leachate

3.2 Macroinvertebrates

Total densities of macroinvertebrates in Etueffont basins showed both temporal and spatial variations. Culicidae larvae were the dominant macroinvertebrate group in the studied basins, contributing to 87% of the total zoobenthos density, followed by Corixidae (8.8%), Chironomids (2.5%) and other larvae (each <1%). The lowest density of chironomid larvae was recorded in the first basin (Table 2).The statistical analysis in presence of the Corrixidae and the Culicidae was chiefly explained by these two very abundant families. In order to highlight the spatio-temporal variation of the remaining invertebrate families, subsequent statistical analysis excluded the Corixidae and the Culicidae.

Table 1 Monthly environmental parameters of the basins

		CE	T	pH	DO	Eh	SM	BOD	COD	Na+	NH4+	HCO3−	F−	CL−	NO2−	NO3−	PO43−	SO42−	NK	PT	Cu	Ni	Mn	Fe	Zn	Cr	Es
B1	J	3,857	26	7.9	6.36	19	21	90	602	587	151	1,475	0.6	476	9.6	240	0	107	119	0.72	0.16	0.081	2.3	10.1	0.55	0.09	0.5
	Jl	3,467	21	8	12.5	94	76	35	735	437	153	1,797	0.7	533	0	1.2	0.9	79	120	0.73	0.18	0.103	1.6	10.9	0.88	0.14	1.17
	A	4,567	17	7.8	2.55	44	41	29	540	602	139	1,590	0	542	7	27	0	245	109	0.65	0.31	0.092	3.4	8.4	1.2	0.39	0.9
	S	4,197	19	7.5	0.47	−80	42	41	539	393	127	1,470	0	371	2.2	76.5	0	199	99	0.59	0.3	1.28	4.06	3.8	0.32	0.32	0.59
	O	2,473	13	7.3	0.17	−90	24	60	573	278	123	938	3.2	236	11.9	0.5	0.3	127	96	0.15	0.22	0.81	2.78	1.41	0.22	0.03	0
	N	2,783	5	7.3	1	16	24	69	419	270	140	1,229	2.3	264	0	2	0	96	110	0.65	0.54	6.68	2.39	4.61	0.34	0.21	0.4
	D	2,773	9	6.2	2.34	50	28	45	468	137	107	1,229	0.5	274	2.1	138.5	0.2	248	125	0.44	0.22	0.7	2.89	3.72	0.73	0.31	0.02
	Ja	1,940	4	5.4	9.86	81	14	18	180	150	46	1,335	0.3	208	0	63.3	0	131	57	0.39	0.28	8.5	0.47	2.29	2.21	0	0
	F	1,508	9	5.4	3.6	36	31	26	239	213	65	793	1.2	136	0	0.2	0	90	82	0.26	0	0.92	5.15	0.08	3.11	0	0
	Mr	2,150	9	4	1.5	−66	39	15	181	456	70	751	0.2	130	0	48.2	0	45	64	0.2	0.37	0.42	7.43	3.48	0.32	0.03	0.01
	Ap	2,649	15	7.1	1.76	−52	20	100	242	311	86	1,057	0.06	227	0	16.1	0	68	96	3.18	0.09	0.6	0.84	3.4	0.42	0.41	0
	M	2,202	24	7.4	1.4	19	25	44	307	150	91	1,159	0	159	159	52.9	0	63	103	0.5	0.42	1.44	4.39	5.8	0.23	0.05	0.02
B2	J	3,857	26	7.9	8.5	19	21	90	602	587	97	1,475	0.6	476	9.6	240	0	107	75	0.43	0.09	0.047	1.7	5.8	0.62	0.15	0.45
	Jl	2,603	21	8	3.65	27	98	10	356	428	58	942	0.6	356	0	0.4	0	77	44	0.23	0.07	0.063	1.7	5.2	0.78	0.28	0.65
	A	2,705	18	8.2	8.83	123	25	25	370	429	59	801	5	323	0	62.9	2.7	97	45	0.24	0.12	0.068	1.4	4	1.26	0.2	0.57
	S	2,797	18	7.8	1.75	66	37	22	443	389	50	1,139	0.03	319	0	4.7	0	211	38	0.19	0	1.12	3.82	2.6	0.55	0.2	0.53
	O	1,130	9	7.4	2.42	42	26	5	225	126	47	396	0.02	101	0	22.4	0	78	36	0.34	0.07	0.35	0.79	0.68	0.48	0.08	0
	N	2,375	3	5.4	0.25	26	22	54	354	213	113	926	0.9	182	1.3	13.6	0	77	88	0.48	0.24	1.32	1.45	2	0.24	0.14	0.17
	D	2,217	5	6.2	4.11	90	10	31	312	212	72	856	0.3	227	3.6	255.2	0.2	137	88	0.28	0.16	0.64	3.01	1.76	0.59	0.19	0
	Ja	1,903	2	5.9	8.5	99	6	14	277	271	72	751	0.5	233	0.1	58.5	0	125	89	0.64	0	8.2	1.76	3.4	2.42	0	0
	F	1,087	7	5.1	3.03	53	10	15	227	145	57	611	0.12	112	0.3	5	0	66	74	0.16	0	1.17	3.5	1.97	2.22	0	0.22
	Mr	1,590	8	4.6	4.33	77	35	13	187	238	76	625	0.7	149	0	39.8	0	62	70	0.2	1.27	0.37	4.72	1.95	1.2	10.03	0
	Ap	2,153	16	6.7	5.26	75	10	13	232	181	58	720	0	123	0	179.3	0	42	72	0.18	0.11	0.66	3.97	3.5	0.42	0.13	0
	M	2,216	23	7.8	13.6	113	9	16	220	146	54	708	0	111	111	65.2	0	58	62	0.4	0.23	0.79	1.18	3.4	0.58	0.03	0.01
B3	J	2,617	19	8.2	3.26	186	29	15	366	378	72	986	0.1	323	3	26.8	0	91	55	0.30	0.07	0.002	2.8	6	0.87	0.1	0.6
	Jl	2,240	20	8	4.35	71	85	2	430	367	62	758	0.5	266	0	11.1	0	61	47	0.25	0.06	0.053	2.8	5.3	1.06	0.18	0.83
	A	2,167	19	8.3	10.33	132	18	19	326	354	54	645	0.7	292	0	57.9	0	95	41	0.21	0.06	0.053	1.2	2.7	1.22	0.12	0.7
	S	2,357	16	7.9	2.45	144	84	24	368	337	52	912	0	251	0	24	0	82	39	0.20	0.24	0.78	3.18	2.1	0.4	0.2	0.14
	O	1,179	9	7.6	2.30	71	12	7	206	132	48	388	0	101	0	23.8	0	63	36	0.14	0.06	0.41	0.72	0.68	0.32	0.06	0
	N	2,070	1	5.1	1.5	110	22	34	226	173	74	659	1	147	0	3.8	0	119	57	0.24	0.08	0.61	0.65	0.55	0.07	0.07	0.02
	D	1,886	4	6.9	4.68	109	12	27	281	231	93	726	0.4	197	2.7	304.1	0.2	97	72	0.23	0.13	0.58	3.34	1.71	0.28	0.12	0.01
	Ja	1,739	2	5.7	8.23	78	5	17	246	231	59	715	0.2	170	0	48.6	0	97	70	0.45	0.17	8.21	0.36	2.54	2.1	0.02	0
	F	1,167	7	4.7	5.43	81	13	13	211	219	51	586	0.1	132	0.2	0.4	0	53	67	0.1	0	0.51	2.94	2.05	2	0.01	0.03
	Mr	1,443	8	4.7	4.56	67	33	22	166	262	73	635	0.5	126	0	23.3	0	35	66	0.31	0.09	0.37	4.44	2.88	0.29	0.04	0.07
	Ap	1,917	17	7.5	5.34	217	30	12	202	214	48	567	0	169	0	225.2	0	53	60	0.09	0.18	0.43	3.69	2.7	0.64	0.19	0.03
	M	2,144	23	8.1	17.11	70	29	23	199	209	37	653	0	113	113	77.2	0	60	52	0.36	0.03	0.44	3.48	2.6	0.69	0.02	0
B4	J	2,087	22	8.2	6.36	−141	8	4	282	308	59	824	0.2	250	0.8	70.7	0	80	45	0.31	0.04	0.04	2.7	5.1	1.12	0.05	0.39

Table 1 (continued)

	CE	T	pH	DO	Eh	SM	BOD	COD	Na+	NH4+	HCO3−	F−	CL−	NO2−	NO3−	PO43−	SO42−	NK	PT	Cu	Ni	Mn	Fe	Zn	Cr	Es
Jl	2,000	21	8.7	16	102	20	2	362	340	61	605	0.5	268	0	22	0	70	46	0.24	0.06	0.046	1.5	4.1	1.26	0.1	0.47
A	1,955	20	8.8	16.17	141	22	26	379	315	59	660	2.4	261	1.9	3.4	1.9	17	45	0.24	0.03	0.044	0.3	1.9	2.12	0.09	0.3
S	1,923	16	8.2	10.2	136	98	31	328	309	57	756	0	226	0.3	36	0	203	43	0.22	0.04	0.72	2.8	2	0.45	0.24	0.15
O	1,375	8	7.7	2.27	78	2	6	230	143	56	489	0	45	0	8.1	0	27	42	0.28	0.06	0.48	1.88	1.06	0.36	0.06	0.01
N	1,530	0	6	1.55	93	22	49	317	181	91	774	0	145	0	6.1	0	80	70	0.33	0.27	0.97	0.96	1.14	0.66	0.08	0.09
D	1,685	4	6.7	3.6	146	18	28	258	223	56	692	0.4	181	2.5	269.7	0.2	99	68	0.25	0.12	5.11	1.71	0.83	0.54	0.12	0
Ja	1,678	2	5.5	6.73	90	4	17	211	210	50	622	0.1	167	1.9	56.5	0	94	57	0.43	0.96	7.54	2.07	2.32	1.93	0	0
F	1,259	6	4.5	5.83	92	21	14	194	177	42	545	0.2	117	0	67.6	0	83	57	0.35	0	0.5	3.07	1.78	2.11	0	0.04
Mr	1,263	8	5	4.83	63	34	11	153	264	68	681	0	129	0	158.8	0	49	65	0.17	0.12	0.27	3.95	2.02	0.86	0.02	0.04
Ap	1,754	17	7.8	8.62	183	10	17	182	218	41	486	0	153	0	243.4	0	48	52	0.09	0.1	0.59	3.33	2.9	0.19	0.225	0.04
M	1,899	24	8	10.07	103	22	26	179	201	37	619	0	103	103	70.2	0	50	47	0.33	0.12	0.58	2.72	1.8	0.89	0.03	0

Table 2 Macroinvertebrate densities in the basins

	Taxa	Basin 1	Basin 2	Basin 3	Basin 4
Oligochaeta	-	0	1	0	0
Plecoptera	-	0	3	0	2
Tricoptera	Brachycentridae	39	25	4	7
	Sericostomatidae	1	0	0	0
	Glossomatidae	0	0	0	1
Coleoptera	Dysticidae	1	47	41	8
	Dryopidae	5	5	1	10
Heteroptera	Corixidae	272	1,792	841	658
Odonates	Gomphidae	1	0	0	1
Ephemeroptera	Baetidae	0	3	32	79
Odonates	Zygoptera	0	1	0	1
Diptera	Stratiomydae	4	29	1	2
	Culicidae	32,542	1,564	1,026	3
	Tabanidae	0	0	0	1
	Chaoboridae	0	0	0	8
	Chironomidae	198	381	212	239

3.3 Species Richness and Diversity of Chironomids

Overall, 1,030 Chironomids larvae were identified and enumerated from the samples and seven chironomid taxa were recorded in the Etueffont basins. A relative abundance of the Chironomid taxa found in the studied basins is presented in Table 3. The lowest number of taxa was found in the first basin, the observed richness being two, six, seven and five taxa in B1, B2, B3 and B4, respectively (Table 3).

Larval exuviae examination showed that four species belonged to Chironomidae family (*Chironomus dorsalis, Chironomus nuditarsis, Chironomus melanotus, Chironomus sp.)*, one species to each of the Orthocladiinae (*Orthocladuis barbadipes*) and Tanypodinae (*Psectrotanypus varius*) families (Table 4). Of these, the dominant groups were *Chironomus nuditarsis* (36%) and Psectrocladius barbatipes Kieffer 1923 (26%). One specie was present only in B3 and B4. The total exuviae larvae increased from the first to last basin (42 at B1, 118 at B2, 195 at B3 and 477 at B4).

3.4 Density of Chironomids

Total density of chironomid larvae was overall higher in basins 2, 3 and 4 than B1 most likely due to increasing distance from the landfill (Table 3). Chironomid larvae were absent in spring in the four

Table 3 Relative abundance of the Chironomid taxa found in the studied basins

		J	Jl	A	S	O	N	D	Ja	F	Mr	Ap	M
Basin 1	*Chironomus*	15	0	11	0	17	0	0	0	0	0	0	0
	Glyptotendipes	3	0	28	0	0	0	124	0	0	0	0	0
Basin 2	*Chironomus*	5	7	0	0	0	0	74	0	0	0	0	13
	Glyptotendipes	5	46	0	30	0	0	140	0	0	0	0	3
	Isocladius	9	1	0	0	0	0	0	0	0	0	0	5
	Einfeldia	0	2	0	0	0	0	20	0	0	0	0	0
	Polyatilum	0	0	0	0	0	0	1	0	0	0	0	0
	Psectrotanypus	2	0	0	0	0	0	16	0	0	0	0	2
Basin 3	*Chironomus*	33	2	0	0	0	0	6	0	0	0	0	0
	Glyptotendipes	13	23	1	4	0	0	9	7	0	0	0	0
	Orthocladius	11	17	17	10	0	0	0	2	0	0	0	0
	Isocladius	4	10	10	6	0	0	0	1	0	0	0	0
	Einfeldia	0	2	2	3	0	0	0	0	0	0	0	0
	Psectrotanypus	7	5	5	1	0	0	0	0	0	0	0	0
	Chaetocladius	0	0	0	0	0	0	0	1	0	0	0	0
Basin 4	*Chironomus*	1	0	0	0	3	2	0	2	0	0	0	1
	Glyptotendipes	6	23	1	45	38	94	7	4	0	0	0	1
	Isocladius	0	1	1	0	2	0	0	2	0	0	0	2
	Einfeldia	0	0	1	0	1	0	0	0	0	0	0	0
	Psectrotanypus	0	0	0	0	1	0	0	0	0	0	0	0

basins. This absence translated in a more pronounced absence of pupal exuviae from October 98 to February 99 (Table 4).

3.5 Fauna – environmental Factors Relationships

The temporal stability of macroinvertebrate assemblages at each basin assessed using a within-basins CIA had a significant co-structure ($P=0$). The first two axes explained 89% of total inertia ($F1=78\%$, $F2=11\%$). The $F1\times F2$ factorial plane showed a distinctive seasonal shift for all sampling basins that became even more distinct with increasing distance from the landfill (Fig. 3c). Positions of June an July samples on the $F1\times F2$ factorial map (Fig. 3c) were related to high concentrations of all studied environmental parameters (Fig. 3a) that were imported from the landfill. At a faunistic level, Brachycentridae, Dysticidae, *Isocladius* spp. and *Chironomus* spp. contributed highly to the total community that developed in June in the basins (Fig. 3b, c).

Lastly, the spatial typology was investigated by means of a within-dates CIA. The existence of a significant co-structure was confirmed (permutation test, $P=0$). The first two CIA axes explained 71 and 13% of total inertia. EC, HCO_3^-, NH_4^+, BOD, COD, Cl^-, Fe^{++} and SM (suspended matter) were negatively related to Factor 1 of CIA, while Eh showed a positive relationship (Fig. 4a). Factor 2 was related to SO_4^-, Mn^{++}, Cu^{++} and SM in the positive region. Axis $F1$ of the corresponding faunistic structure (Fig. 4b) was best explained by *Orthocladius* spp., *Psectrotanypus* spp., *Isocladius* spp., *Glyptotendipes* spp., *Einfeldia* spp. and Baetidae, whereas axis $F2$ was described by *Chironomus* spp. and *Glyptotendipes* spp. Although longitudinal patterns differed among dates, two groups of sites were observed (Fig. 4c). Although the first basin overall contrasted with basins 2, 3 and 4 (Fig. 4c), the grouping of basins showed more important winter correlations between environmental and faunistic parameters. Thereafter, in summer the basins statistically separated as the aforementioned correlations became less significant.

4 Discussion

Our findings indicate a clear spatial distribution in both abundance and richness of the benthos from the first to the last basin. The number of species of Beatidae was lower in the polluted basins (B1 and B2) (Table 2), whereas the density and relative

Table 4 Monthly densities in the four basins of four species belonging to the Chironomidae family and one species to each of the Orthocladiinae and Tanypodinae families

		Orthocladius barbadipes	*Chironomus nuditarsis*	*Chironomus melanotus*	*Chironomus dorsalis*	*Chironomus paginus*	*Psectrotanypus varius*
J	B1	1	1	12	13	0	0
	B2	38	4	28	7	0	0
	B3	0	0	0	0	0	0
	B4	18	0	3	0	0	2
Jl	B1	8	0	3	3	0	1
	B2	5	1	5	2	0	0
	B3	1	0	2	0	0	0
	B4	11	0	0	1	0	0
A	B1	0	0	0	0	0	0
	B2	5	0	0	0	0	0
	B3	46	9	43	4	0	0
	B4	3	2	11	0	0	3
S	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	4	0	2	0
	B4	1	2	24	0	0	0
O	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
N	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
D	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
Ja	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
F	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
Mr	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	43	0	7	0	0
	B4	0	225	10	0	0	0
Ap	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	B3	0	0	0	0	0	0
	B4	0	0	0	0	0	0
M	B1	0	0	0	0	0	0
	B2	14	0	7	1	0	0
	B3	24	1	5	0	0	0
	B4	48	16	14	0	79	0

abundance of Culicidae was highest in B1 and of Chironomidae Corixidae was highest in B2. Our hypothesis is corroborated by various works made in aquatic polluted ecosystems that pointed out a strong resistance of the Chironomids and Culicidae to the mineral and organic pollutions (Fabela et al. 2001; Bouallam and Nejmeddine 2001; Bettinetti et al. 2005).

Pollution sensitive taxa such as Baetidae and Ephemeroptera were found only at basins 3 and 4, whereas pollution tolerant taxa (Culicidae, Corixidae) dominated in the remaining basins. These trends have been well described in temperate and cold water regions of the world (Gaufin and Tarzwell 1954; Karr 1991; Battegazzore and Renoldi 1995; Gandouin et al. 2006). Among all zoobenthos, Oligochaeta were absent, except in the second basin where they were found in December 1998 with only one species. This absence of Oligochaeta is difficult to explain. Both anoxia and high ammonia concentrations in the leachate might have adversely affected the development of Oligochaeta (Casellato and Caneva 1994).

The species richness of Chironomids was generally low in the first basin, most likely due to the polluting compounds originating from Etueffont leachates. A similar pattern has been reported by Winner et al. (1980) who observed a decrease in species richness from 39 at the least polluted site to 15 at the most polluted site. A trend of increasing species richness and diversity with decreasing water pollution has also been documented within the chironomid communities (Sheehan and Winner 1984; Sheehan and Knight 1985; Rehfeldt and Sächtig 1991). Therefore, chironomid species richness seemed to be a reliable indicator of leachate pollution (Seire and Pall 2000). The contribution of the tribe Chironomini to species richness (100% in the B1, 90% in B2, 45% in B3 and 96% in B4) was higher than that recorded in lakes and rivers (Serra-Tosio and Gay 1978; Vernaux and Aleya 1998a).

Total density of the Chironomid larvae was higher in basins 2, 3 and 4 than in basin 1, indicating that chironomid larvae numbers increased with increasing distance from the landfill (i e., sources of pollution). The density of chironomid larvae which was chiefly represented by *Chironomus* spp. and *Glyptendipes* spp., was high in December in the first basins. This may be ascribed to a low mineral charge load (low ammonia, chiefly) imported by the landfill during this month and the increase of NK which is shown by the increase of organic nitrogen concentrations associated with enhanced organic matter levels. However, the chironomid larvae communities in basins 3 and 4

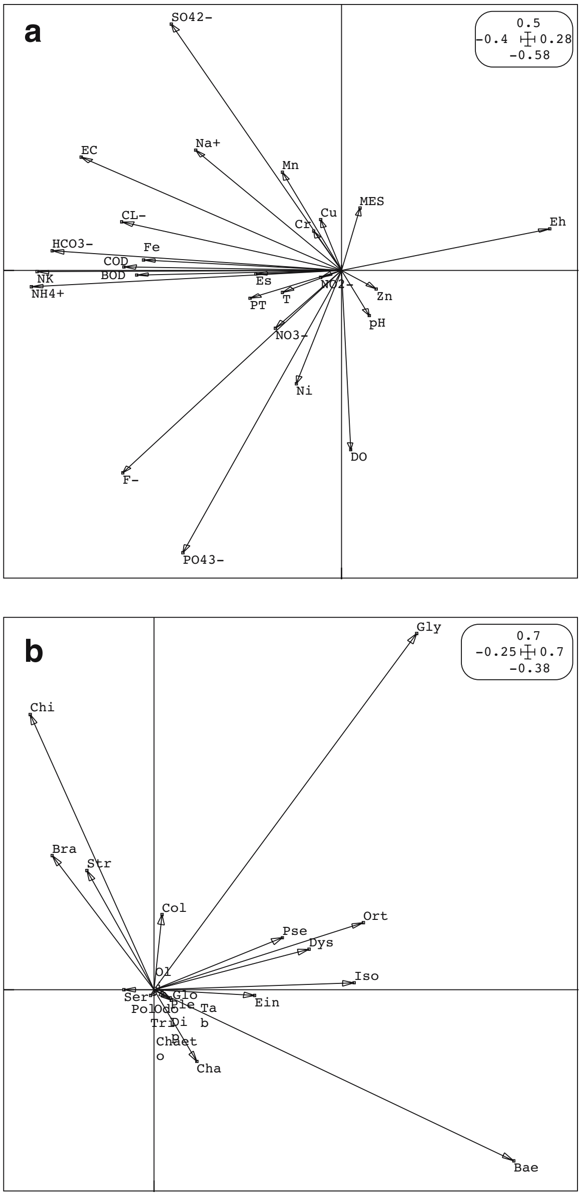

Fig. 3 Within-date co-inertia analysis (CIA) (spatial typology). **a** Co-inertia scores of the environmental variables, and **b** of the taxa on the $F1 \times F2$ factorial plane. **c** Standardized co-inertia scores of sampling sites of the environmental and faunistic data sets onto the $F1 \times F2$ factorial maps for each sampling date. In (**a**) the longer an arrow and the closer to an axis the stronger the relationship to this factor; in (**c**), in contrast, arrows measure the strength of the co-structure between the fauna and the environment, so the shorter the arrow the better the agreement between the two structures

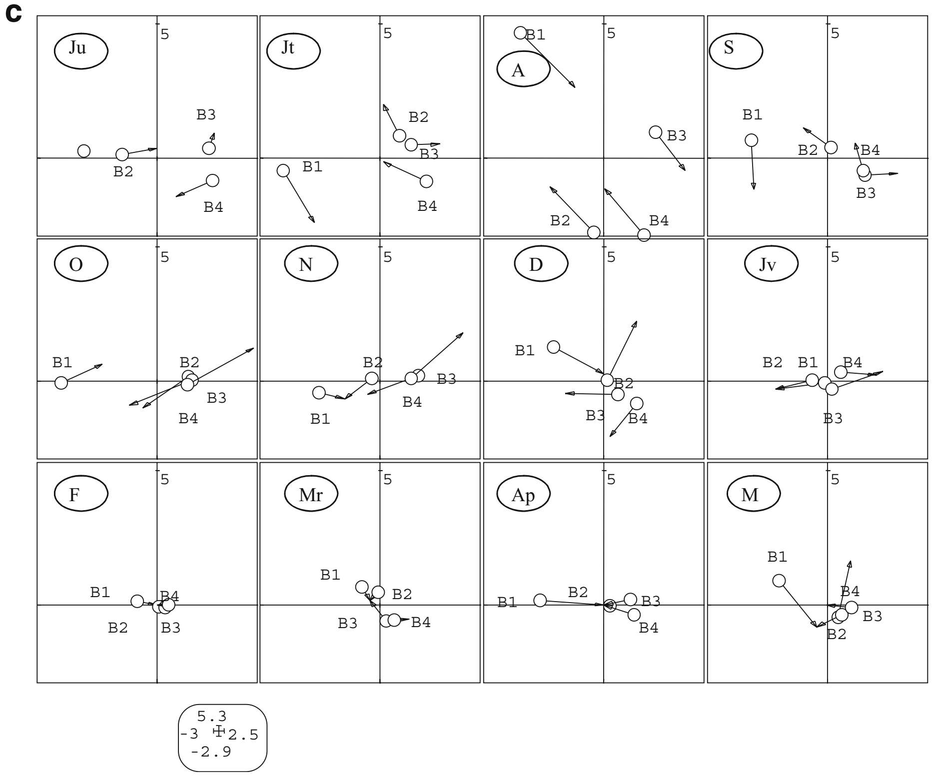

Fig. 3 (continued)

seemed to escape the influence of the leachate as they were located more downstream from the discharge. Increased ammonia concentrations in aquatic ecosystems has been reported to induce a decrease in the overall benthic invertebrate abundance, including Chironomidae (USEPA 1984; Versteeg et al. 1999). Overall, the absence or presence of a certain species in a contaminated habitat is not only influenced by its sensitivity or ability to adapt to the leachate quality, but also to its flexibility to climatic conditions.

In the first basin, the seasonal distribution of chironomid larvae was governed by the leachate quality. However, the absence of larvae in winter was due to low temperature and high metals concentrations chiefly Nickel concentrations (Table 1) (Winner et al. 1980; Wiederholm 1980; Rehfeldt and Sächtig 1991; Johnson et al. 1992; Richardson and Kiffney 2000). In summer, and especially in July and September 1998, the absence of chironomid larvae was most likely due to high ammonia concentrations (Table 1) (USEPA 1984, 1998; Versteeg et al. 1999) and low dissolved oxygen (Table 1; USEPA 1986) concentrations. The subsequent emergence in September 1998, in the first basin of adults may be ascribed to the decrease in chironomid larvae. In the second basin, we recorded a similar trend in the seasonal distribution of chironomid larvae with an absence of larvae during several months. In particular, basin 2 was larvae-free in August 1998, most likely resulting from high concentrations in metals (Cu and Zn) and oxygen (Table 1). The most extended temporal distribution of chironomid larvae was observed more downstream (last basins). This distribution was however marked by the absence of chironomid larvae from October to November 1998 and from February to May 1999 in basin 3 and only in February, March and April 1999 in basin 4, due to increasing water temperature and the emergence of adults. In addition, the abundance of macrophytes in these basins might have influenced the distribution of aquatic invertebrates by affecting predation (Mittelbach 1988; Schriver et al. 1995; Ferenc et al. 2000), food availability (Campeau et al. 1994), and because plants were useful in providing adequate attachment sites and materials that favoured building of protective retreats (Soszka 1975; Lodge 1985; Dudley 1988).

The distribution of benthic chironomids showed the overall dominance of the Chironomini which represented 100% of chironomids in the first basin, 96% in B2, 84% in B3 and 99% in B4 (Fig. 4). Besides, basin 1 was both Tanypodinae and Orthocladinae-free. The strong presence of the Chironomini in the first basin clearly reflected the highly polluted status of this basin. This is in accordance with the work of Bazzanti (2000) who showed from a polluted river that the Chironomini were the most dominant tribe in the most polluted stations studied. In the last basin, the Chironomini again became dominant. This trend may be explained by the enhanced phytoplankton biomass in basin 4 (Khattabi et al. 2006), which most likely stimulated the growth of larvae by the extracellular organic carbon released by algae via photosynthesis and that originated from lysing algae and detritus (Fogg 1983; Jones et al. 1983; Cole et al. 1988; Aleya et al. 1988; Boyd and Osburn 2004).

The Orthocladiinae were poorly present in basin 1 compared to basins 2, 3 and 4 (15 in B1, 58 in B3 and 8 in B4, Table 2). Winberg (1978) proposed a pollution index based on the fact that clean waters are dominated by larvae of the subfamily Orthocladiinae and polluted water by larvae of the subfamily Tanypodinae. Winner et al. (1980) did not validate this index when relationships were made with heavy metal-inducing pollution. They found, indeed a dominance of Orthocladiinae but not of Tanypodinae in heavy metals – polluted streams. Orthocladiinae dominated in the most polluted basins of Etuffont landfill which is overall in agreement with other reported observations (Clements et al. 1992; Richardson and Kiffney 2000). Our findings of low Orthocladiinae species richness in the most polluted basins diverged partially from the patterns observed in other studies, where high numbers of species was found at contaminated sites (Winner et al. 1980; Yasuno et al. 1985; Clements 1991; Wilson 1994; Poulton et al. 1995). Nonetheless, Clements et al. (1992) recommended caution when using their community sensitivity estimates in studies of other ecoregions.

The co-inertia analysis greatly helped us in determining the kind of relationships that established in Etuffont landfill between the benthic community structure and environmental conditions. Furthermore, it revealed the existence of a strong spatio-temporal overlapping the effects of which have been overcome through considering a within-individual classes examination of data. This statistical approch enabled a correct examination of the spatio-temporal changes in the

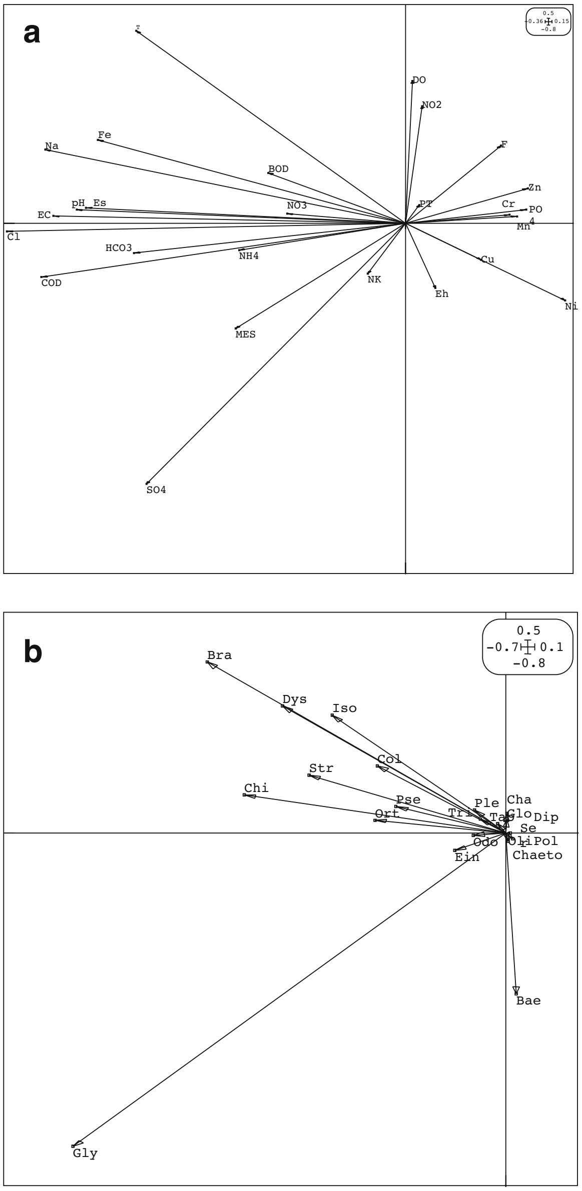

Fig. 4 Within-site co-inertia analysis (CIA) (temporal typology). **a** Co-inertia scores of the environmental variables, and **b** of the taxa on the $F1 \times F2$ factorial plane. **c** Standardized co-inertia scores of sampling dates of the environmental and faunistic data sets onto the $F1 \times F2$ factorial maps for each sampling site. See Fig. 3 caption for other explanations

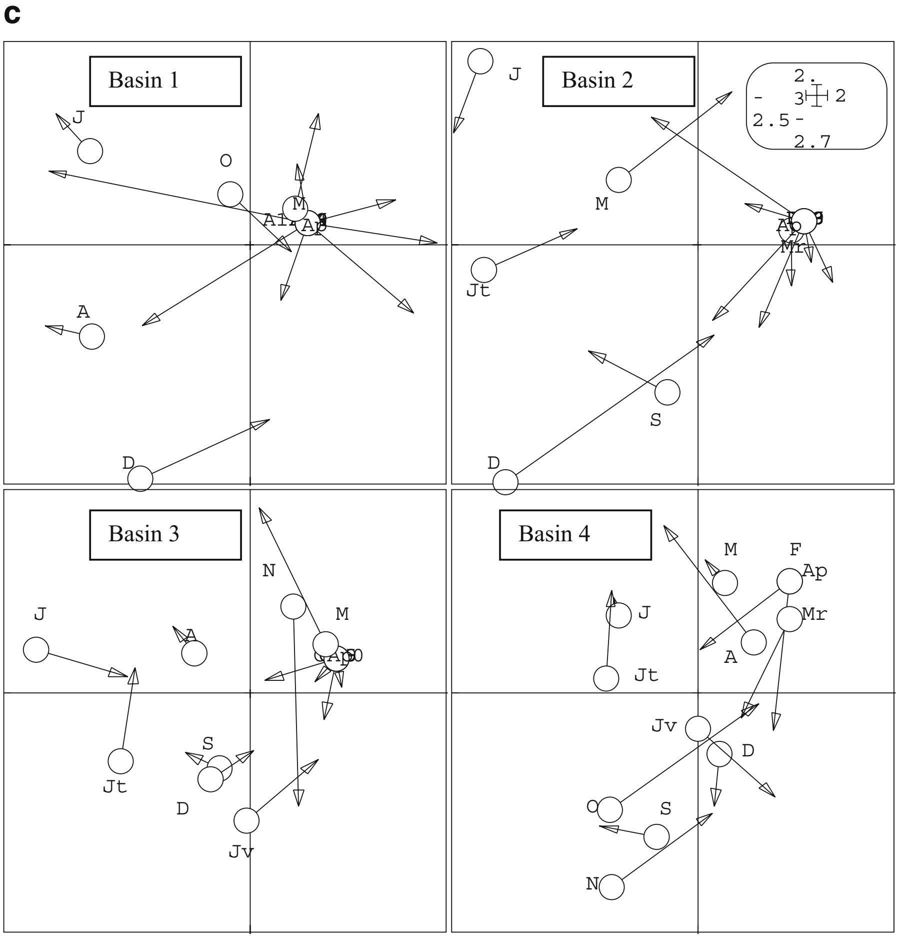

Fig. 4 (continued)

invertebrate communities. In addition, the elimination of the basin effect acheived by a within-dates statistical examination showed that species like *Chironomus* and *Glyptotendipes* which have the strongest absolute contribution to the first axis, were associated with valuable levels in preserving parameters such as chloride and temperature. The results demonstrated that the seasonal effect was markedly elevated at least for species with advantageous abilities to resist high polluting loads. The samples collected in the four basins from June to July 1998 resulted in the clearest pattern we ever observed when it came to illustrate the impacts of NH_4, BOD, COD and EC contamination on the chironomid communities. Our observations went along with the most frequently reported profundal zoobenthos sampling periods i.e. late summer or autumn (e.g. Johnson and Wiederholm 1989; Fjellheim et al. 2000; Servia et al. 2004; Lencioni and Rossaro 2005). The seasonal differences that are markedly higher in basins B1 and B2 than B3 and B4 may be ascribed to the effects generated by the inputs from the Etuffont landfill. Our results went along with the statements that separating the natural variation in community structure from the variation due to anthropogenic disturbances is one of the greatest challenges in biomonitoring studies using benthic macroinvertebrates (Johnson et al. 1992; Clements 1994; Poulton et al. 1995).

The spatial typology investigated through a within-basins CIA pointed out that the 4 basins seemed more polluted in summer. In summary, high levels in metals, NH_4^+, BOD and COD, influencing directly or indirectly other parameters such as dissolved oxygen concentrations and climatic conditions appeared to have marked impacts on the invertebrate communities. The within-basins CIA which eliminates the weight of seasonality on interpretation showed that species like Baetidae which have the strongest absolute contribution to the first axis were associated with high levels of COD, BOD and chiefly ammonia. Qualitative physico-chemical and faunistic profiles improved in the last basins most likely due to organism's abilities to induce a self-based epuration process.

Finally, by using ordination techniques like within-basins and within-dates CIA, the chironomid community structure might be used successfully to differentiate between sites with different degrees of pollution.

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