

Assessment of the effects of wastewater treatment plant effluents on receiving streams using oligochaete communities of the porous matrix

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Abstract – Human activities can disturb the natural dynamics of exchanges between surface water and groundwater in rivers. Such exchanges contribute to the self-purification of the environment and an excess of infiltration can lead to contamination of groundwater. In addition, the porous matrix (coarse surface sediments and hyporheic zone), through which water exchanges occur, is a sink for pollutants. For environmental monitoring programs, it is therefore essential to take into account both the dynamics of vertical hydrological exchanges and the biological quality of this matrix. The functional trait (FTR) method, which is based on the study of oligochaete communities in coarse surface sediments and the hyporheic zone, was proposed as a tool to simultaneously assess the dynamics of vertical hydrological exchanges and the effects of pollutants present in the porous matrix. Here, we applied this method during two different periods (in March and September 2016), upstream and downstream of locations affected by discharges from wastewater treatment plants (WWTP) located in Switzerland. The biological quality of surface sediments and the hyporheic zone was shown to be better upstream of the WWTP in both campaigns. In addition, results suggested that the capacity for self-purification was lower downstream of the WWTP, and that groundwater at these locations was vulnerable to pollution by surface water. The FTR method proved valuable as a field method for detecting the effects of point source contamination on receiving streams. In the near future, this community-based approach will benefit from advances in the use of DNA barcodes for oligochaete species identification.

Keywords: annelids / bioindication / porous matrix / stream functioning / contamination

Résumé – **Évaluation des effets des rejets de stations d'épuration des eaux usées sur des cours d'eau récepteurs à l'aide de l'étude des communautés d'oligochètes du milieu poreux.** Les activités humaines peuvent perturber la dynamique naturelle des échanges entre les eaux de surface et les eaux souterraines dans les cours d'eau. De tels échanges contribuent à l'autoépuration du cours d'eau et un excès d'infiltrations peut conduire à la contamination des eaux souterraines. De plus, le milieu poreux (sédiments grossiers superficiels et zone hyporhéique), à travers lequel se produisent les échanges hydrologiques, a la propriété de stocker les polluants. Il est donc essentiel de prendre en compte la dynamique des échanges hydrologiques verticaux et la qualité biologique du milieu poreux dans le cadre des programmes de surveillance de la qualité des cours d'eau. La méthode des traits fonctionnels (TRF), basée sur l'étude des communautés d'oligochètes des sédiments grossiers superficiels et de la zone hyporhéique, a été proposée comme outil permettant d'évaluer simultanément la dynamique des échanges hydrologiques verticaux et les effets des polluants présents dans le milieu poreux. Dans la présente étude, cette méthode a été appliquée lors de deux périodes différentes (mars et septembre 2016) en amont et en aval de sites affectés par les rejets de stations d'épuration des eaux usées (STEP) situées en Suisse. Une meilleure qualité biologique a été observée en amont des STEP lors des deux campagnes. De plus, les résultats ont suggéré que la capacité d'autoépuration du milieu était inférieure au niveau des sites situés en aval des STEP et qu'au niveau de ces sites les eaux souterraines étaient vulnérables aux pollutions des eaux de surface. La méthode TRF s'est

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avérée appropriée pour détecter les effets globaux de sources ponctuelles de contamination sur des cours d'eau récepteurs. Dans un futur proche, cette méthode bénéficiera des avancées de la recherche en cours sur l'utilisation de codes-barres génétiques pour l'identification des espèces d'oligochètes.

Mots clés : annélides / bioindication / matrice poreuse / fonctionnement des cours d'eau / contamination

1 Introduction

It is well recognized that human activities negatively impact watercourse ecosystems in four different ways: reduction of the connectivity between surface water and groundwater, modification of hydrological exchange processes, contamination of surface water and groundwater and reduction of habitat diversity. Such impacts can affect the biodiversity as it is strongly influenced by the interactions between the dynamics of vertical hydrological exchanges, the geomorphic context and the inputs of chemical substances (*i.e.* nutrients and pollutants) (Lafont, 2001; 2011; Lafont *et al.*, 2010a; 2012; Schmitt *et al.*, 2011; 2016).

The porous matrix, comprising the coarse surface sediments and hyporheic zone, constitutes an important habitat, and acts as a biological, chemical and physical filter of the water that travels through it. The filtering function is stimulated by the dynamics of water exchanges between surface water and groundwater (Hynes, 1983; Brunke and Gonser, 1997; Boulton, 2000; Jones and Mulholland, 2000; Hancock, 2002; Hancock and Boulton, 2005; Boulton and Hancock, 2006; Malard *et al.*, 2006; Boulton, 2007; Breil *et al.*, 2007). Indeed, active hydrological exchanges allow to supply the porous matrix with oxygen and therefore to stimulate degradation of organic matter by aerobic microbial and faunal activity (Hancock, 2002). In addition, exfiltration of good quality groundwater can lead to an improvement of the porous matrix and surface water quality. The self-purification potential of a watercourse is therefore controlled by these processes. When the infiltration of surface water into groundwater dominates, contaminants present in surface water can pollute the hyporheos and groundwater, which may lead to impairment of ecosystem function. Similarly, polluted groundwater exfiltration may lead to a degradation of surface water quality (Lafont *et al.*, 2006). The dynamics of infiltration and exfiltration may be disturbed by anthropogenic activities (Lafont and Vivier, 2006; Breil *et al.*, 2007; Lafont *et al.*, 2010a). Infiltration of surface water into groundwater can be caused, for example, by a lowering of the groundwater table by excessive pumping, high discharges from combined sewer overflows, sudden water releases from reservoirs, or flash floods associated with an increase in impervious surfaces in the catchment area of a stream. The importance of taking into account surface water/groundwater exchanges for achieving a complete and robust evaluation of the ecological integrity of a watercourse has been emphasized for decades (Hynes, 1983).

The dynamics of the vertical hydrological exchanges and the specific quality of the porous matrix are generally not assessed in current monitoring programs of environmental water quality. While biotic indices based on invertebrate, oligochaete (in fine/sandy sediments), macrophyte and diatom communities (AFNOR, 2004; 2014; 2016a; b; OFEV, 2007; 2010) are widely accepted and applied, they only provide

information on surface habitats. Nonetheless, restoration strategies aiming to improve the ecological status of streams need to consider and understand the functioning of streams, *i.e.* the interactions between the dynamics of vertical hydrological exchanges, the geomorphic context and the input of chemicals, as well as integrate knowledge of the biological quality of all compartments (Lafont, 2001).

Because oligochaetes are dominant in the interstices of the porous matrix and comprise species with a wide range of pollution tolerance, as well as species characteristic of surface sediments or groundwater, community assessments of this organism group provides important information on the functioning of a watercourse (Lafont *et al.*, 2006). The oligochaete functional traits (FTR) method, which is focused on characterizing oligochaete communities of the porous matrix, can simultaneously assess the biological quality of the porous matrix and the dynamics of the hydrological exchanges between surface water and groundwater (Vivier, 2006; Lafont *et al.*, 2006; 2010a). Several studies undertaken in France and Switzerland showed that this approach was suitable to distinguish between preserved and anthropogenically altered sites (Lafont *et al.*, 2010a; Vivien *et al.*, 2015).

In this study, we applied the FTR method for detecting the effects of WWTP effluents on the biological quality and the functioning of the receiving streams. We used this method during two periods of the year (in March and September 2016) upstream and downstream of two WWTP whose discharges significantly increased concentrations of pollutants at the downstream locations. The applicability of this approach is discussed and the prospects for future development are presented.

2 Material and methods

2.1 Sites

Wastewater treatment plant located in Hochdorf and in Buttisholz (Canton of Lucerne, Switzerland) were selected as study sites based on previous studies conducted in 2013 and 2014, which showed that the effluents of these WWTP substantially increased the load and concentrations of micro-pollutants at downstream locations (Munz *et al.*, 2017; Tlili *et al.*, 2017). In these two studies, a large number of pollutants was measured, as well as water quality parameters such as dissolved organic carbon, dissolved nitrogen and orthophosphates. The sampling locations upstream and downstream of the WWTP were the same as in Tlili *et al.* (2017), and a map showing the locations of these sites is provided by Tlili *et al.* (2017). The coordinates of the upstream and downstream locations of the WWTP of Hochdorf (Ron river) are 47.17462°N 8.27986°E (downstream) and 47.173540°N 8.280462°E (upstream); the coordinates of the sites upstream and downstream of the WWTP of Buttisholz (Tannebach river) are 47.115241°N 8.071179°E (downstream) and 47.114089°N 8.074437°E (upstream). The downstream sampling locations

Table 1. Overview and definition of functional traits (FTR1 to FTR4, FTRi) in coarse surface sediments and the hyporheic zone, with examples of characteristic oligochaete taxa.

Functional traits FTRs	Examples of characteristic taxa
FTR1: Includes active exchange describer (AED) species, <i>i.e.</i> species indicating active hydrologic exchanges between surface waters and groundwater	All Lumbriculidae (<i>Trichodrilus</i> spp., <i>Stylodrilus</i> spp., etc.) except <i>Lumbriculus variegatus</i> , <i>Haber</i> spp., <i>Pristina</i> spp., <i>Cernosvitoviella</i> spp., <i>Achaeta</i> spp., <i>Marionina argentea</i> , <i>Haplotaxis gordioides</i> , <i>Propappus volki</i> , <i>Chaetogaster parvus</i>
FTR2: Includes oligochaete species which are intolerant to chemical pollution	<i>Cernosvitoviella</i> spp., <i>Marionina argentea</i> , <i>Eiseniella tetraedra</i> , <i>Nais alpina</i> , <i>Vejdovskyella comata</i> , <i>Stylodrilus heringianus</i> , <i>Rhyacodrilus falciformis</i> , <i>Propappus volki</i> , <i>Haplotaxis gordioides</i>
FTR3: Includes oligochaete species which are tolerant to chemical pollution	<i>Nais elinguis</i> , <i>Pristina jenkiniae</i> , <i>Dero digitata</i> , <i>Globulidrilus riparius</i> , <i>Lumbriculus variegatus</i>
FTR4: Includes oligochaete species which indicate the presence of polluted sludge within sediment interstices (“sludge effect”)	Tubificinae with or without hair setae, <i>Lophochaeta ignota</i> , <i>Tubifex tubifex</i> , <i>Limnodrilus hoffmeisteri</i> , <i>Bothrioneurum</i> sp., <i>Lumbricillus</i> spp
FTRi: Includes oligochaete species which are moderately tolerant to chemical pollutions	<i>Chaetogaster diastrophus</i> , <i>C. diaphanus</i> , <i>Nais communis</i> , <i>N. christinae</i> , <i>N. barbata</i> , <i>N. pardalis</i> , <i>Slavina appendiculata</i>

had been chosen so that discharged effluents were completely mixed with stream water across the stream channel during low flow conditions (Tili *et al.*, 2017). Sampling was performed on March 15 and 29, 2016 and on September 12 and 26, 2016. These WWTP had not been upgraded between the time when chemical measurements of water samples were performed (2013–2014) and when we collected samples for this study (2016). Both rivers flow through agricultural, industrial and urban areas upstream of the sampling sites (Burdon *et al.*, 2016), which means that micropollutants were expected to be present even upstream of the WWTP. The distances between the sampling locations and the WWTP effluent input are: for Hochdorf 0.24 km (upstream) and 0.18 km (downstream); for Buttisholz 0.05 km (upstream) and 0.15 km (downstream).

2.2 Oligochaete community analysis

2.2.1 Sampling and laboratory procedures

Coarse surface sediments and the hyporheic zone were sampled separately. At each site, 3 subsamples (10–20 m apart) of coarse sediments and hyporheic zone were collected and subsequently combined.

Coarse sediment (5–10 cm depth) was collected using a shovel, in a 5 L clean plastic bucket. The grain size of the coarse sediment samples was not measured, but was approximately similar between sites (mostly gravel, with sand and pebbles). Samples from the hyporheic zone (containing water, fine and sandy sediments) were collected in a 5 L clean plastic bucket by means of a probe inserted into coarse sediment to a depth of 20–30 cm and a Bou-Rouch pump (Bou & Rouch, 1967) fitted to the probe (Uwitec, Austria). The supernatant water was sieved in the field using a 0.16 or 0.20 mm mesh size sieve. The material retained in the sieve was transferred into the bucket. A volume of formaldehyde

37% (Thermodisher, Switzerland) was then added to the buckets containing the samples for a final concentration of 4% formaldehyde.

At the laboratory, coarse sediment and hyporheic samples were sieved on a column of stainless steel sieves of 5 and 0.2 mm mesh sizes (Fisherbrand, Fisher Scientific, Switzerland). The material retained on the 0.2 mm mesh sieve was transferred into a subsampling square box (5 × 5 cells). The content of randomly selected cells was transferred into a petri dish and examined under a stereo microscope (Olympus, Model SZ51, Switzerland). Successive cells were examined until 100 identifiable oligochaetes were obtained. Oligochaete specimens were mounted on slides. Oligochaete specimens were mounted on slides in a coating solution composed of lactic acid, glycerol and polyvinyl alcohol (mowiol 4-88) and identified to the lowest practical level (species if possible or genus or family) using a compound microscope (Olympus, Model BX43, Switzerland).

Generally, the number of specimens identified per site should be 100. However, the FTR method can also be applied when the number of specimens obtained is smaller than 100 per site, which is common in hyporheic zone samples. If the number of specimens is < 20, results of the community analysis should, however, be interpreted with caution.

2.2.2 Functional traits

Based on observations in the field (study of sites from pristine to highly altered, comparison of oligochaete community structure with physicochemical and hydrogeological data) and a literature study, Vivier (2006), Lafont and Vivier (2006) and Lafont *et al.* (2010a) classified oligochaete taxa of the porous matrix into 5 FTR categories (Tab. 1). FTR1 describes interactions between physical porosity of the coarse surface sediments and hyporheic zone and the hydraulic potentials.

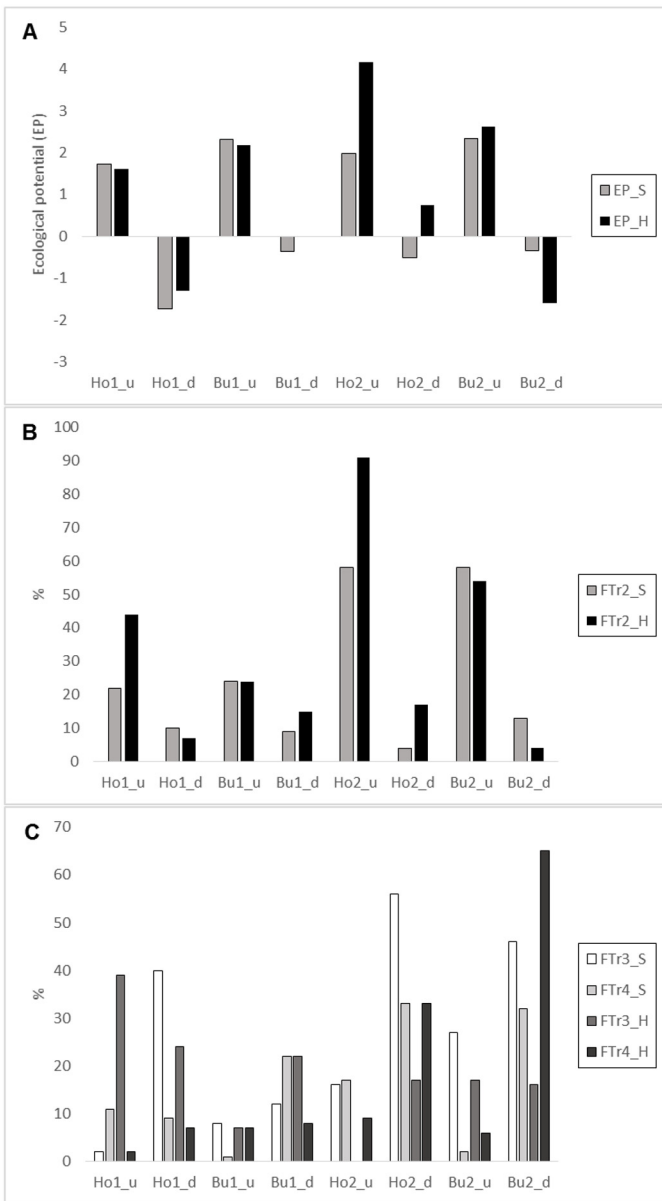


Fig. 1. Values of the ecological potential (EP) (A) and of the percentages of the functional traits 2, 3 and 4 of the sites situated upstream and downstream of the WWTP of Hochdorf and Buttisholz (B and C). EP_S=ecological potential in surface sediments; EP_H=ecological potential in the hyporheic zone; FTR2_S, FTR3_S, FTR4_S=functional traits 2, 3 and 4 in surface sediments; FTR2_H, FTR3_H, FTR4_H=functional traits 2, 3 and 4 in the hyporheic zone; Ho1_u=Hochdorf upstream in March; Ho1_d=Hochdorf downstream in March; Ho2_u=Hochdorf upstream in September; Ho2_d=Hochdorf downstream in September Bu1_u=Buttisholz upstream in March; Bu1_d=Buttisholz downstream in March; Bu2_u=Buttisholz upstream in September; Bu2_d=Buttisholz downstream in September.

FTR2, FTRi and FTR3 comprise, respectively, intolerant, moderately tolerant and fully tolerant taxa to chemical pollution. FTR4 includes taxa, which indicate the presence

of polluted sludge within the interstices of the porous matrix. These taxa are, in general, highly tolerant to pollution. FTR4 describes a highly altered functioning of the porous matrix. The percentages of these 5 FTRs are used to assess, in the porous matrix, the effects of interactions between physical factors like the geomorphic context, the water fluxes (mainly the dynamics of water exchanges between surface water and groundwater), and the inputs of substances, such as nutrients and pollutants.

FTRs provide information on the direction of water fluxes between surface water and groundwater, particularly when the coarse surface sediments and hyporheic zone are sampled at the same location. The predominance of active exchange descriptor (AED) species (FTR1) in surface sediments indicates the exfiltration of groundwater. The intensity of water exchanges and exfiltration may be roughly assessed as follows: FTR1 < 15%: low intensity; 16–30%: moderate intensity; 31–50%: high intensity; > 50%: very high intensity. Conversely, the predominance in the hyporheic zone of species/taxa belonging to another FTR than FTR1 indicates the infiltration of surface waters. Surface water infiltration is low when the percentage of these species/taxa in the hyporheic zone is < 15%, moderate (16–30%), high (31–50%), and very high (> 50%). AED species/taxa (FTR1) may belong to the FTR2 (intolerant species/taxa to pollution) or to the FTR3 (tolerant species/taxa to pollution). For example, *Marionina argentea* belongs to FTRs 1 and 2 and *Pristina jenkiniae* to FTRs 1 and 3 (Tab. 1). It is thus possible to assess the state of pollution of groundwater by analyzing the composition of the AED species present in the surface sediments and hyporheic zone.

2.2.3 Ecological potential

The ecological potential (EP) describes the state of the functioning of each compartment of the porous matrix based on the percentages of FTRs (Lafont *et al.*, 2010a). The EP is calculated using the following equation:

$$EP = \text{Log}_2[(FTR1 + FTR2) + 1]/[(FTR3 + FTR4) + 1].$$

The EP corresponds to the ratio between FTRs considered as representative of a preserved functioning and that representative of an impaired functioning due to anthropogenic or non-anthropogenic causes. The EP allows to distinguish between various states of functioning, from preserved to highly altered. As the relation is converted into \log_2 , negative values of the EP can be obtained. EP values ≥ 7 characterize a well-preserved functioning of the system. Values between 2 and 7 correspond to a slightly to moderately altered functioning. In running waters of urbanized environments, values ≥ 7 are rarely obtained, and the value of 2 becomes an objective of ecological potential corresponding to the lowest observed urbanization effects (Lafont *et al.*, 2008). EP values below 2 depict states of major alterations. The causes of these alterations may be diverse and not only due to anthropogenic pressures. But when values are close to zero or negative, the main source of alteration is clearly associated with anthropogenic pressures. In addition, a low EP in both

Table 2. Percentages of the functional traits (FTR1-4; FTRi) and ecological potentials (EP) obtained during the campaign of March and September (hypor= hyporheic zone; surf= coarse surface sediments).

	Campaign of March								Campaign of September							
	Hochdorf				Buttisholz				Hochdorf				Buttisholz			
	Upstream		Downstream		Upstream		Downstream		Upstream		Downstream		Upstream		Downstream	
	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf
FTR1	84	23	5	4	43	25	15	17	88	76	67	58	94	94	22	48
FTR2	44	22	7	10	24	24	15	9	91	58	17	4	54	58	4	13
FTR3	39	2	24	40	7	8	22	12	0	16	17	56	17	27	16	46
FTR4	2	11	7	9	7	1	8	22	9	17	33	33	6	2	65	32
FTRi	13	63	58	42	40	55	52	50	0	5	0	3	0	2	6	5
EP	1.62	1.72	-1.3	-1.74	2.18	2.32	0	-0.37	4.17	1.99	0.74	-0.51	2.63	2.35	-1.6	-0.35

the surface sediments and the hyporheic zone indicates pollution of surface water with surface water infiltration, or strong pollution of groundwater with groundwater exfiltration.

3 Results

A total of 36 oligochaete taxa belonging to the families Naididae (8 taxa of Tubificinae, 14 taxa of Naidinae, 3 species of Pristininae and one taxon of Rhyacodrilinae), Enchytraeidae (5 taxa) Lumbriculidae (3 taxa) and Lumbricidae (2 taxa) were found. All the functional traits were well represented (Tabs. 2 and 3).

At both sites (Hochdorf and Buttisholz), oligochaete communities indicated a better environmental quality upstream than downstream of the WWTP in both surface sediments and hyporheic zone. This was observed in both sampling campaigns. Percentages of sensitive taxa (FTR2) were higher and those of resistant taxa (FTR3 and FTR4) lower at upstream than at downstream locations (Fig. 1, Tabs. 2 and 3). The differences between upstream and downstream were more pronounced in September than in March. In September, a strong sludge-like effect (*i.e.* high percentages of FTR4) was observed in surface sediment and the hyporheic zone at the downstream sites. At the downstream location of Buttisholz and Hochdorf in September, pollution-resistant AED taxa dominated communities in surface sediments, which suggested that contaminated groundwater entered the stream. As expected, the structure of oligochaete communities indicated moderate chemical pollution at upstream sites, likely due to non-point source contamination of the streams.

EP values were close to 2 or between 2 and 5 at the upstream locations, and negative, equal to 0 or between 0 and 1 at the downstream locations. Low EP values downstream were explained by both the strong dominance of pollution-resistant taxa and of taxa indicating infiltration of surface water into the hyporheic zone. The indication of surface water infiltration was more pronounced at downstream than at upstream sites, which can partially be explained by discharges of WWTP to the river. Results suggest that the capacity of self-purification was lower downstream than upstream of WWTP, and that the

groundwater was vulnerable to pollution by surface water at the downstream sites.

4 Discussion

This study showed that the FTR method was able to detect the effects of pollutant inputs from the WWTP on environmental quality of the receiving streams. In addition, it provided valuable information on the capacity of the stream environment to self-purify and on the vulnerability of adjacent groundwater resources to pollution by surface water. Our results suggest that the FTR method allows the categorization of sites according to the degree of pollution, and thus can be used to detect local disturbances in impacted areas.

The FTR method is a valuable tool for biomonitoring programs as it can reveal alterations undetected by other biological indices. Burdon *et al.* (2016) studied macroinvertebrate communities upstream and downstream of 12 WWTP in Switzerland, among them Hochdorf and Buttisholz, the two WWTP studied here. These authors were unable to detect any impacts of the WWTP effluent using the IBCH macroinvertebrate index (OFEV, 2010), which is commonly used in monitoring programs throughout Switzerland. Similarly, the FTR method detected impacts of highway runoff on the receiving environment, while the French biomonitoring indices using diatoms, invertebrates and macrophytes were unable to detect such effects on the stream ecosystem (Lafont *et al.*, 2010b).

Oligochaete abundance is generally considered to be an indicator of organic matter concentrations (Masson *et al.*, 2010; Vivien *et al.*, 2014). At the sampling locations of the study by Burdon *et al.* (2016), oligochaete abundances were significantly higher at sites located downstream of WWTP than at upstream sites. Quantifying oligochaete abundance alone, however, does not provide comprehensive information on the effects of WWTP effluents. An increase in oligochaete abundance is not necessarily associated with a degradation of biological quality, or vice versa. As in other community indices, it is important to consider if sensitive or tolerant taxa are present. An assessment of the impacts of WWTP effluent is only possible based on thorough knowledge of community

Table 3. Oligochaete communities per sample during the campaigns of March and September. The values correspond to the numbers of specimens per taxon. The functional trait(s) FTR(s) of each taxon is (are) indicated in brackets (hypor= hyporheic zone; surf= coarse surface sediments).

	Campaign of March								Campaign of September							
	Hochdorf				Buttisholz				Hochdorf				Buttisholz			
	Upstream		Downstream		Upstream		Downstream		Upstream		Downstream		Upstream		Downstream	
	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf	Hypor	Surf
Tubificinae (Naididae)																
Tubificinae without hair setae (FTR 4)	1		1	1	2	1	5	5	2	11	1	17	1	2	23	13
<i>Limnodrilus hoffmeisteri</i> (FTR 4)				2	1			3								1
<i>Limnodrilus udekemianus</i> (FTR 4)																1
Tubificinae with hair setae (FTR 4)				3			2	11		2	1	3	1		30	17
<i>Potamothrix barbatus</i> (FTR 4)			2	2					1	1		8				
<i>Aulodrilus plurisetus</i> (FTR 4)																1
<i>Lophochaeta ignota</i> (FTR 4)	1	11	1	1								3				
<i>Tubifex tubifex</i> (FTR4)						1	3		3							
Naidinae (Naididae)																
<i>Amphichaeta leydigi</i> (FTRi)	2															
<i>Chaetogaster diastrophus</i> (FTRi)	7	38	20	30	4	31	43	26		3						2
<i>Chaetogaster langi</i> (FTRi)	3	23	1	8	2	5	3	16								
<i>Chaetogaster parvus</i> (FTR1 and 2)									4		2		7			2
<i>Chaetogaster limnaei</i> (FTRi)								2								
<i>Chaetogaster diaphanus</i> (FTRi)												1				
<i>Nais alpina</i> (FTR2)		2	3	6	4	4	6	2	1	2		1		2		6
<i>Nais elinguis</i> (FTR3)	1		13	41		2	18	8				3			3	9
<i>Nais communis</i> (FTRi)			6				2	2		1					4	1
<i>Nais breitscheri</i> (FTRi)	1	1	1	1	6	17	3	2					2			
<i>Nais pardalis</i> (FTRi)					4	3	2	1								
<i>Nais variabilis</i> (FTRi)			1	4				2		1		1				
<i>Nais christinae</i>												1			1	2
<i>Nais</i> sp (FTRi)			1													
Pristininae (Naididae)																
<i>Pristina aequiseta</i> (FTR1 and 3)	20	2			1	1	4	4		15		33	5	12	7	37
<i>Pristina jenkinae</i> (FTR 1 and 3)	18				2	5				1	1	15	1	14	1	1
<i>Pristina longiseta</i> (FTR1)	1															
Rhyacodrilinae (Naididae)																
<i>Rhyacodrilinae</i> sp (FTR1 and 2)				1												
Enchytraeidae																
<i>Achaeta</i> sp (FTR1 and 2)	1															
<i>Marionina argentea</i> (FTR1 and 2)	2							1	6	5			1	3	1	
<i>Globulidrilus riparius</i> (FTR 3)												1			2	
<i>Enchytraeus buchholzi</i> (FTRi)					1											
<i>Cernosvitoviella</i> sp. (FTR1 and 2)	41	20	1	1	6	20	8	5	21	47	1	1	18	44	2	5
Lumbriculidae																
<i>Lumbriculus variegatus</i> (FTR i)			1													
<i>Styiodrilus heringianus</i> (FTR 1)		1		1	5	5	1	2		1			2	2		1
Lumbriculidae g. sp (FTR1)	2		2	1	4	8	2	5	1	3	2	3	8	9	5	3
Lumbricidae																
Lumbricidae sp (FTR2)								1								
<i>Eiseniella tetraedra</i> (FTR 2)								1								

composition and the traits of individual taxa, and identification of oligochaetes to the species or genus level is therefore necessary.

The implementation of the FTR method into biomonitoring programs requires solid expertise in oligochaete taxonomy, which may dissuade environment managers and consulting

firms from using this methodology. It is generally preferred to use simpler and cheaper methods, even if they give incomplete information on the quality and the functioning of the ecosystem (Lafont, 2011; Lafont *et al.*, 2012). However, we would like to emphasize that the identification of oligochaetes is no more difficult than the identification, for example, of

diatoms, which are used routinely to monitor the water quality of streams. In addition, recent developments have shown that in the near future it will be possible, as part of biomonitoring programs, to genetically identify oligochaetes using high throughput sequencing and tagged primers, according to a methodology described in [Shokralla *et al.* \(2014\)](#).

Limiting the analysis of oligochaete communities to surface sediment only would significantly reduce the effort and cost per site. However, the inclusion of hyporheos samples allows a more reliable and complete assessment of the ecosystem functioning ([Lafont and Vivier, 2006](#)). The hyporheos tends to accumulate higher levels of pollutants than surface sediments; therefore, a focus on surface sediments alone would lead to an overestimation of environmental quality. Indeed, high flow episodes favor the decrease of pollution in surface sediments and the storage of pollutants in the hyporheic zone (due to infiltration of surface water), and surface sediments can be removed/transported during floods. In addition, the hyporheos is permanently saturated with water while surface sediments can periodically be dry (during low flow episodes). The FTR method can also be used specifically for the biomonitoring of hyporheos, as no other invertebrate monitoring methodology is yet available for that environmental compartment.

The FTR method is validated and ready to be applied in monitoring programs. A technical guideline for the methodology was provided by [Vivier \(2006\)](#), and the method is currently used by environmental consulting firms and research institutions in France and Switzerland. Nevertheless, the FTR method can and should be continuously improved as new data becomes available, for example, by adding information on functional traits, and by adapting the attribution of functional traits to each oligochaete species. For example, the FTRi was created specifically for species of the genus *Chaetogaster* and several species of the genus *Nais*, because these species did not fit any of the original FTR1-4. The ecological status of some taxa (for example *Nais christinae* and some species within the subfamily Rhyacodrilinae) remains uncertain, and more data are required to assign with certainty a functional trait(s) to these taxa.

The current prospects are to gain more experience and continue collecting data using the FTR method at sites with various degrees of chemical pollution and different dynamics of vertical hydrological exchanges, and up- and downstream of point sources of pollution. It is, for example, well suited for the study of Highly Modified Water Bodies (HMWB, *sensu* [EU, 2000](#)). The HMWB group encompasses all waterbodies whose physical context is altered by human activities to such a degree that a “good ecological status” cannot be reached, but “good ecological potential” exists (urban streams constitute a large part of HMWB). In the future, the application of the FTR method in such monitoring programs will be greatly facilitated by advances in the use of DNA barcodes for oligochaete species identification ([Vivien *et al.*, 2016; 2017; Lefrançois *et al.*, 2018](#)).

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Cite this article as: Vivien R, Lafont M, Werner I, Laluc M, Ferrari BJD. 2019. Assessment of the effects of wastewater treatment plant effluents on receiving streams using oligochaete communities of the porous matrix. *Knowl. Manag. Aquat. Ecosyst.*, 420, 18.