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The Use of Fluorescent Organic Matter as a Natural Transit Time Tracer in the Unsaturated Zone of the Fontaine De Vaucluse Karst System

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Abstract: The fluorescence index called the Transit Time index (TTi) is based on the fluorescence of natural organic matter in order to qualitatively assess the transit time of karst groundwater, using springs affected by human activities. This study aims to further evaluate the potential of fluorescent compounds as a natural tracer of transit time when applied to unsaturated zone flows with natural catchments, in contrast to the first study. For this purpose, a bi-monthly sampling of one year of monitoring for organic matter fluorescence, TOC, major elements and water-stable isotopes was performed. A conceptual model of the sources and fates of fluorescent compounds is built, emphasizing the allochthonous origin of humic-like C compounds, and the autochthonous production of humic-like M and protein-like compounds within the unsaturated zone. Fluorescent compound intensity interpretation according to this model reveals consistent relative transit times with flow behavior and also provides complementary information. The results also show the TTi's ability to summarize fluorescent compounds, its consistency with relative transit time, and its higher sensitivity as compared to other natural tracers. However, prior to its use, a thorough assessment of soil organic matter, microbial activity, and potential anthropogenic contamination is required, encouraging interdisciplinary collaboration between hydrogeologists, microbiologists and soil scientists.

Keywords: geochemical tracers; transit time; hydrogeology; karst; fluorescence of organic matter; unsaturated zone; fluorescence index

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1. Introduction

Karst aquifers contribute to 13% of the total global withdrawal of groundwater and provide 9.2% of the world's drinking water [1]. Karsts are therefore essential to water supply but they are also very complex and fast reacting systems due to their hierarchized network of conduits [2] that heavily impacts water availability and quality. The characterization of karst fast flows is a challenging task that may benefit from the characterization of organic matter, as organic compounds are supplied from the soil and rapidly transformed during their circulation from the soil to the aquifer outlet [3].

Fresh organic matter (litter, dead organisms and roots) provided by the land cover is incorporated into the soil. Soil organic matter is transformed by decomposer organisms in response to environmental conditions (e.g., nutrients, redox conditions) and availability of organic matter (e.g., adsorption to mineral surfaces, or aggregate formations [4]). During rain events, the infiltration of rainwater increases the saturation of water in soils, resulting in changes in environmental conditions for soil organic matter decomposers. The water that infiltrates into the aquifer is rainwater enriched with organic matter mobilized from the soil. Hydrology has been identified by [5–7] as the main factor in the amount of organic matter transported within hydrosystems.

From its incorporation into soils to its transport within aquifers towards an outlet, organic matter is continuously transformed. The degradation model developed for soil can be applied to the fate of organic matter in water, from large biopolymers, small biopolymers, monomers, to carbon dioxide release (CO_2 [4]). Small biopolymers can be reused to produce large biopolymers, monomers to produce small biopolymers, and at each transformation stage, organic matter can adsorb and desorb from mineral surfaces and form/break aggregates [4].

The molecular complexity and size of fluorescent compounds can be assessed by the emission wavelength of organic matter, which increases with the number of conjugated bonds and aromatic nuclei [8,9]. Fluorescence can thus be used to distinguish families and types of compounds in some aquatic organic matter [10].

Organic matter is usually divided into two groups with different molecular structures and weights: humic-like and protein-like compounds. Protein-like compounds correspond to aromatic amino acid groups such as tryptophan. They are used as qualitative indicators of short transit time, because they are usually associated with fecal contamination from wastewater, which indicates rapid infiltration within the karst network (e.g., karst conduits, fractures, sinkholes [11,12]).

To characterize groundwater transit time using organic matter fluorescence, [13] proposed the use of the Humification IndeX (HIX). This index was first developed to assess the maturity of soil organic matter [9,14] according to an old model of organic matter decomposition [15], which suggests that protein-like compounds are decomposed to produce humic-like compounds, a process called humification, which has been reconsidered by [4]. [13] developed a formula between transit time and HIX by applying a fluorescence index developed for soil to karst groundwater of the Fontaine de Vaucluse. This study suffered from several methodological biases exposed such as the transferability of fluorescence indices from one environment to another (here from soil to groundwater) which are now being reconsidered [16]. The limitations identified by [16] lead to the proposal of a new index named the Transit Time index (TTi), which uses excitation–emission matrices (EEM) instead of emission spectra at the 254 nm excitation wavelength used for the HIX, in order to adapt the calculation of the index to each environmental context, and thus overcome the methodological biases identified. The TTi demonstrated its ability to qualitatively evaluate transit time, to compare the overall behavior of different springs from the Fontaine de Vaucluse karst observatory (southeastern France), and to identify pre-existing water (piston effect) and freshwater inflow (rapid infiltration). However, these promising results assumed no production of fluorescent compounds within the aquifer because of the use of the old model of organic matter decomposition, which suggests that protein-like compounds are decomposed faster than humic-like compounds.

The present study aims to deepen the use of fluorescent compounds as a natural tracer of transit time by (i) reconsidering the assumptions of no autochthonous production of fluorescent compounds within the Fontaine de Vaucluse karst system, as groundwater from this aquifer was used in previous studies [13], (ii) proposing a conceptual model of the source and fate of fluorescent compounds, (iii) and checking the consistency of fluorescent compound information and the TTi with the expected transit time of unsaturated zone flows.

2. Materials and Methods

2.1. The Study Site

The main outlet of the Vaucluse karst system (southeastern France), the Fontaine de Vaucluse spring, has one of the highest average discharge rates in Europe at 23.3 $m^3 \cdot s^{-1}$. This region of France has a Mediterranean climate with an average annual rainfall of approximately 800 mm. Several minor springs are also located in the recharge area of the Vaucluse karst system, the most important being the Millet, Saint Trinit and Nesque springs (Figure 1, Table 1). This karst system is also characterized by a particularly thick unsaturated zone (~800 m). The artificial galleries of the LSBB (https://lsbb.cnrs.fr) allow the observation of three to more than fifty unsaturated zone flows, depending on the hydrodynamic conditions, at depths ranging from ~30 m to almost 500 m below the ground surface. In this study, we consider 3 perennial flow points (A, B, D) and 2 ephemeral flows (AJ, C). In previous studies, perennial flows were considered "slow flows" due to their low TOC and high magnesium content, while temporary flows were considered "fast flows" due to their high TOC and low magnesium content [17]. The discharge of LSBB flows varies from less than 1 to more than 100 $mL \cdot s^{-1}$, with perennial flows having lower and less variable discharge than ephemeral flows. Soils are poorly developed in the Fontaine de Vaucluse system. Their thickness is approximately 10 cm above LSBB flows [18], and reaches a few decimeters in spring catchments.

Table 1. Main characteristics of monitored flow points in [16] from [18–22] and field observations.

Spring	Catchment Area	Karstification	UZ Thickness	Lithology	Land Cover
Millet	~2.5 km^2	Complex karstification-anastomoses	Thick ~ 70 m	Cretaceous/Barremian limestones (marine)	Forest, lavender cultivation
St Trinit	~2 km^2	High degree, large karst conduit	Thin ~ 10 to 20 m	Cretaceous/Aptian limestones (marine)	Anthropogenic activities, organic farming, town
La Nesque	~1 km^2	Low degree, conduits of centimetric scale at the outlet	Thin ~ 10 to 20 m	Marneous limestones, Oligocene (lacustrine)	Lavender cultivation
Fontaine de Vaucluse	~1160 km^2	Variable but high in average	Very thick ~ 800 m	Cretaceous limestones (marine)	Cultivations, cities, forests
LSBB	≤1 km^2	Variable	35 à 518 m	Cretaceous limestones (marine)	Forest and cultivation

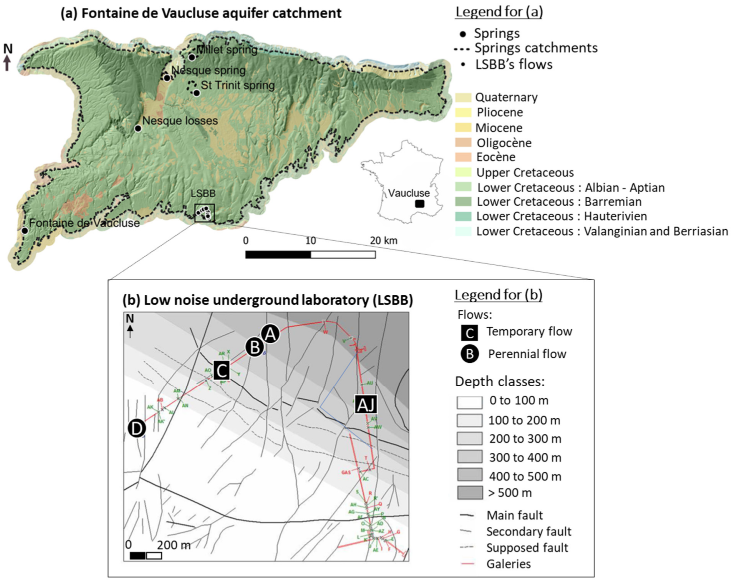

Figure 1. (**a**) Location of monitored flow points and spring catchment delineation on a background of a 1:50,000 geological map (BD-CHARM from BRGM). Catchment delineation for Millet, St Trinit and Nesque springs were based on mass balance and hydrogeological information; catchment delineation of Fontaine de Vaucluse was taken from [22]. (**b**) Enlarged view on LSBB flows on a background of a structural map (modified from [23]).

2.2. Sampling and Fluorescence Analysis Methods

Bi-monthly sampling of the springs of St Trinit, Millet and Nesque and of the unsaturated zone flows A, B, C, D, and AJ of the LSBB was carried out during more than one year of monitoring from June 2020 to October 2021. More frequent sampling is not possible for technical reasons, but continuous monitoring of temperature, electrical conductivity and discharge or water level ensures that the samples taken are representative; as does long-term point-in-time monitoring at the Fontaine de Vaucluse and LSBB flows (since 2002 for LSBB flows and 1981 for Fontaine de Vaucluse). Measurements of major elements, Total Organic Carbon (TOC) and water-stable isotopes were performed by UMR 1114 EMMAH, and those of the excitation–emission matrix (EEM) were performed at HydroSciences Montpellier (for details see: [16,24]).

2.3. Study Layout

To deepen the use of fluorescent compounds as a natural tracer of transit time, the Fontaine de Vaucluse karst system is used as it was the original field of development of the relationship between fluorescent compounds and transit time.

First, the assumption that there is no autochthonous production of fluorescent compounds within the Fontaine de Vaucluse karst system is reconsidered. For this purpose, fluorescent compounds are identified and quantified in all considered springs and flows using the excitation–emission matrix by performing a PARAFAC modeling (more details in: [16,24]). Then, a conceptual model of the source and fate of fluorescent compounds is elaborated, based on the bibliography and on the comparison of the presence of fluorescent organic matter compounds in groundwater and soil (data from a previous study).

Secondly, the consistency of transit time identified thanks to fluorescent compounds is checked with transit time known from previous studies and interpreted thanks to discharge and major element contents. For this purpose, the LSBB's flow variations in discharge and major element during the studied field campaign are analyzed in terms of the relative transit time and compared with previous studies using descriptive statistics and boxplots.

Then, the relative transit time of the LSBB's flows is evaluated using fluorescent organic matter compounds using the previously defined conceptual model of the source and fate of fluorescent compounds. This relative transit time obtained with fluorescent compounds is compared with the relative transit time evaluated using major elements and discharge in order to check the consistency of the use of fluorescent of organic matter to assess transit time, using time series, boxplots and descriptive statistics.

Finally, the Transit Time index (TTi) is calculated according to [16], corresponding to the ratio of humic-like compounds to the sum of humic and protein-like compounds; extracted from the PARAFAC analysis. The relative transit time of LSBB flows is estimated using the TTi and compared with the relative transit time using the fluorescent compounds separately in order to check that it summarizes the information well. Time series are also used to identify the added value of the TTi and the use of fluorescent compounds to qualitatively assess transit time.

3. Results and Discussion

3.1. Organic Matter Sources and Fate

3.1.1. Identification of Fluorescent Organic Matter Types in the Fontaine De Vaucluse System

Total Organic Carbon (TOC) is an approximate measure of the total amount of organic matter present in a water sample [25], including non-fluorescent compounds. Since organic matter is primarily derived from soil [10] and its decomposition is mainly related to degradation by microbial activity during its transfer in the aquifer, increases in TOC content may be associated with the arrival of water with short transit times [26]. Degradation of organic matter is estimated to be almost complete after 6 months, resulting in a low TOC content [19], but organic matter can persist for years to decades if protected from decomposing organisms [16].

Fluorescent compounds are a small fraction of the organic matter represented by TOC [10]. They result from microbial degradation of soil organic matter, either directly or indirectly [27]. For example, humic-like C compounds are derived directly from fresh soil organic matter, while humic-like M are derived from the complexation of microbial activity products [28] and protein-like compounds (also called aromatic amino acid-like) correspond to biological fluorophores [10]. Therefore, humic-like C are essentially produced in soils while humic-like M and protein-like (such as tyrosine and tryptophan) can be produced in both soils and aquatic environments. Anthropogenic pollution can also be a source of fluorescent compounds. For example, the influence of sewage effluents has been extensively documented as being a source of protein-like compounds [29,30]. The identification of fluorescent compounds and a good knowledge of their sources and fate is therefore a first step towards their use as natural tracers. The identification of these fluorescent compounds is based on the fact that organic matter families have different excitation and emission wavelengths, resulting in specific peaks in the excitation–emission matrix. For each of the aforementioned families, two peaks are often observed together: C and AC for humic-like C, M and AM for humic-like M, T and AT for tryptophan, B and AB for tyrosine ([10]; Figure 2).

In a previous study [16], we performed a four-component PARAFAC modeling of the excitation–emission matrix in order to identify and quantify the fluorescent compounds in karst waters. The first component was attributed to humic-like organic matter, component 2 to tryptophan, component 3 to a proteic compound called P1, and component 4 to tyrosine. In fact, components 1, 2 and 4 correspond to peaks AC and C (humic-like C), peaks AT and T (tryptophan), and peaks AB and B (tyrosine) of [31], respectively, confirming their identification as humic-like, tryptophan and tyrosine compounds (Figure 2). However, component 3, which was attributed by [16] based on [32] to the protein-like P1 compound, corresponds to the humic-like acid AM from [31] (Figure 2).

P1 was identified by [32] as a protein-like compound resulting from anthropogenic contamination because of its good correlation with indicators of fecal contamination, and also because its emission wavelength was below the 380 nm boundary between protein-like and humic-like compounds as defined in [3,33]. However, anthropogenic contamination that promotes microbial activity does not only produce protein-like compounds. In fact, humic-like M organic matter may also result from the complexation of microbial activity products. Furthermore, the 380 nm limit is reconsidered in [10], as the emission wavelength of humic-like M compounds is between 350 and 400 nm.

The co-occurrence of AM and M humic (humic-like M organic matter) was first observed in marine environments [31] and later in nonmarine environments [34]. No M peak is observed in our dataset. However, such a secondary peak could be merged with components 1 (peak C) and 2 (peak T). Indeed, the shape of component 2 is slightly distorted towards Coble's peak M (Figure 2), suggesting that peak M may have been integrated into peak T in the PARAFAC model. Peak M would therefore have a lower intensity than peak AM, which is common for humic-like substances [10], and which is also illustrated by component 1 having a higher intensity at peak AC than at peak C. Figure 3a,b show the raw excitation–emission matrix of the sample with the most intense component 3 (St Trinit spring, 1 February 2021).

The peak AM shadow expands towards longer excitation wavelengths up to the base of peak M window as defined by [10]. At least one other study using PARAFAC modeling encountered the same problem [35]. Integration of peaks M and T into the same PARAFAC component implies joint occurrence, and proportional intensities. The co-occurrence of peaks M and T is very likely, as both compounds may be directly or indirectly derived from microbial autochthonous activity [27]. Based on these elements, we assume that component 3 can be identified as a humic-like M compound.

Two types of protein-like compounds (tyrosine associated with peaks AB and B, and tryptophan associated with peaks AT and T) and two types of humic-like compounds (humic-like C associated with peaks AC and C, and humic-like M associated with peaks AM and M) are thus observed at the Fontaine de Vaucluse observatory.

In the absence of anthropogenic contamination, tyrosine, tryptophan, peaks AM and M are reported to be rarely found within hydrosystems [10]. However, tryptophan and humic-like M are found in unsaturated zone flows and springs of the Fontaine de Vaucluse observatory (Table 2). Humic-like M has the lowest intensity except on some water samples from St Trinit spring (which is most affected by anthropogenic activity) which reach high fluorescence intensities. This very large variability, with high values being reached at a spring with high vulnerability to anthropogenic activity, suggests a relation with anthropogenic contamination, for example through the spreading of manure practiced in this basin. This is consistent with the higher nitrate contents found at the St Trinit spring (from 10 to 15 $mg \cdot L^{-1}$) compared to Millet (from 0 to 3 $mg \cdot L^{-1}$) and Nesque springs (from 0 to 10 $mg \cdot L^{-1}$). However, humic-like M are detected in all monitored flow points including LSBB flow points. Since there is no anthropogenic input at the LSBB, it is therefore very likely that humic-like M result from microbial activity within the soil or the aquifer. The variability of humic-like M intensities within LSBB flows is likely due to the variability of transit time or that of microbial activity along flow paths [28]; whereas the high values reached at St Trinit spring are likely due to the input of degraded organic matter (amendments, sewage) and/or an increased microbial activity within the hydrosystem due to nutrient supply from anthropogenic activities. The increased microbial activity may be associated with higher nutrient contents.

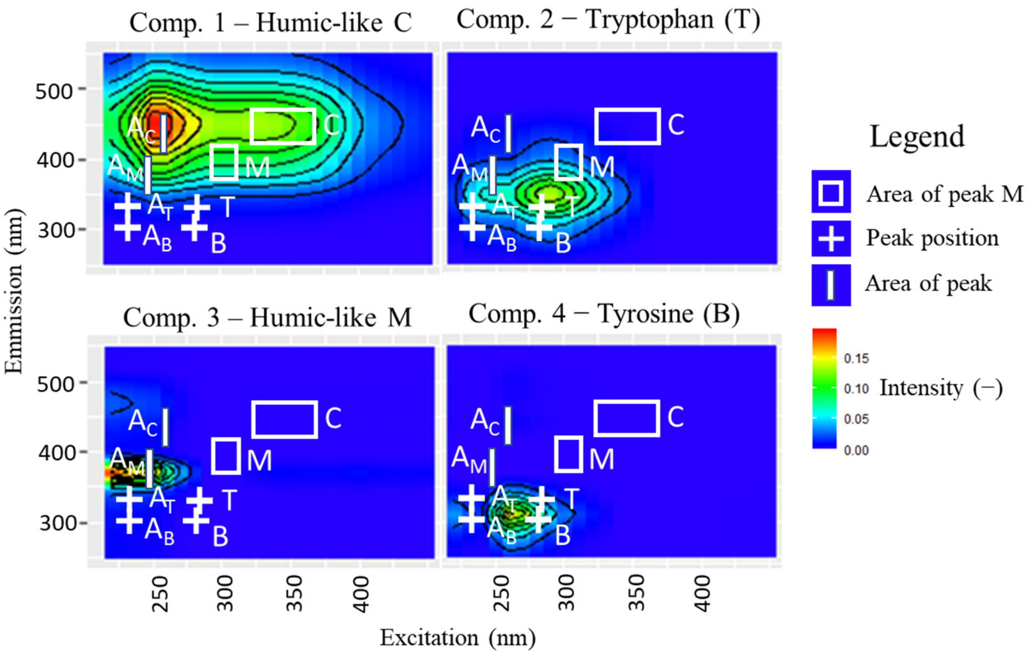

Figure 2. Comparison of organic matter components location in EEMs from PARAFAC modeling and literature.

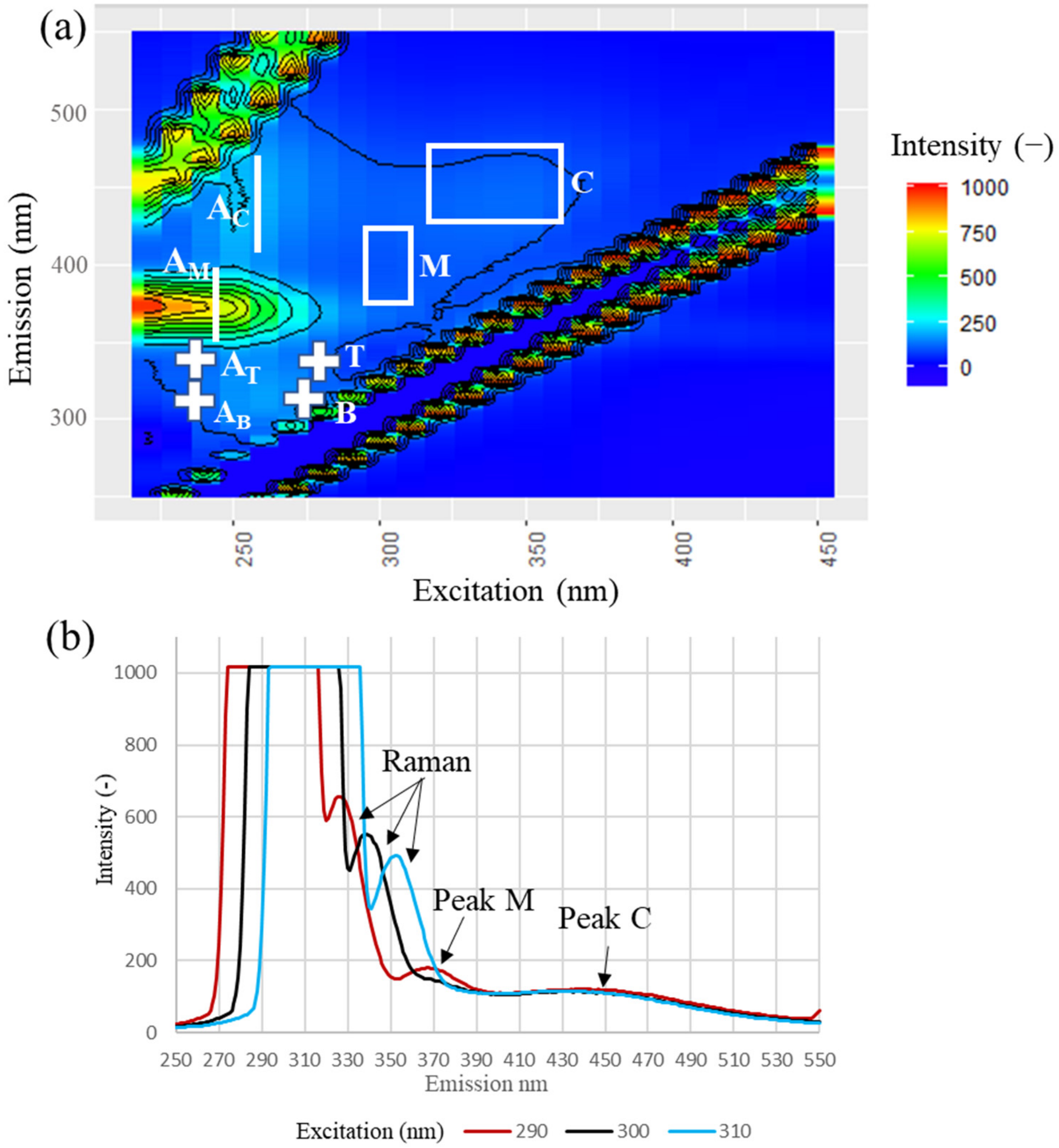

Figure 3. (**a**) The raw excitation-emission matrix of St Trinit spring sampled the 1st of February 2021. Intensity in arbitrary units. (**b**) raw 2D spectra for excitations 290, 300 and 310 nm extracted from (a). Intensity in arbitrary units and same peak positions from [10] than in Figure 2.

3.1.2. The Source and Fate of Fluorescent Organic Matter in the LSBB Hydrosystem

Fluorescent organic matter from soil leachates was characterized at LSBB by [18] using monthly monitoring from December 2005 to April 2008. This study shows both a short-term variability of fluorescence intensity and dissolved organic carbon content, and thus of the amount of organic matter provided by land cover, and a relative temporal stability of organic matter types, as humic-like C compounds are found (Figure 4). The presence of humic-like C was expected, as these compounds are commonly produced in soils [10], but the absence of protein-like and humic-like M compounds likely means that protein-like compounds identified in water samples from LSBB flow points result from microbial production within the hydrosystem. The temporal gap between soil and water monitoring is unlikely to be an issue, as soil monitoring covered a long period (more than 2 years) and no major change in vegetation cover occurred between the two studies.

Therefore, the production of these compounds in aquifers may not be insignificant, even in natural hydrosystems.

Table 2. Min, max, mean, standard deviation (SD) and SD/mean values of the organic compound fluorescence intensity normalized to the water raman: humic-like C, humic-like M, tryptophan (Trp), tyrosine (Tyr) for St Trinit (30 samples), Millet (29 samples), Nesque (30 samples) springs and LSBB flows A (33 samples), B (33 samples), C (17 samples), D (29 samples), and AJ (12 samples), from June 2020 to October 2021.

Parameter	Spring	Min	Max	Mean	SD	SD/Mean
Humic-like C (C and A_C)	St Trinit	0.33	2.31	0.78	0.54	0.70
	Millet	0.44	0.75	0.60	0.09	0.15
	Nesque	0.87	1.79	1.06	0.17	0.16
	A	0.35	0.60	0.43	0.06	0.14
	B	0.29	0.56	0.37	0.07	0.19
	C	0.72	1.81	0.92	0.25	0.27
	D	0.42	0.66	0.55	0.06	0.11
	AJ	0.49	0.84	0.69	0.09	0.13
Humic-like M (P1 or A_M)	St Trinit	0.03	12.6	1.13	2.71	2.40
	Millet	1.1×10^{-20}	1.8	0.23	0.50	2.14
	Nesque	1.3×10^{-20}	0.25	0.1	0.10	0.97
	A	0	0.43	0.08	0.11	1.35
	B	0	0.78	0.17	0.23	1.33
	C	0	0.30	0.08	0.14	1.71
	D	0	0.30	0.08	0.10	1.14
	AJ	0.01	0.09	0.05	0.06	1.21
Tryptophan (T and A_T)	St Trinit	0.07	1.99	0.64	0.42	0.65
	Millet	0.01	2.47	0.46	0.46	1
	Nesque	1.7×10^{-3}	1.58	0.46	0.30	0.66
	A	0.05	0.80	0.29	0.17	0.58
	B	0.04	1.05	0.33	0.24	0.72
	C	0.05	0.73	0.29	0.18	0.64
	D	0	0.64	0.30	0.17	0.56
	AJ	0.18	0.75	0.39	0.20	0.51
Tyrosine (B and A_B)	St Trinit	0.01	2.59	0.65	0.62	0.96
	Millet	0.04	2.16	0.77	0.63	0.82
	Nesque	0.09	2.57	0.90	0.79	0.88
	A	0	2.18	0.55	0.59	1.07
	B	0.02	2.96	0.58	0.70	1.21
	C	0	1.05	0.43	0.38	0.88
	D	0	1.76	0.61	0.58	0.94
	AJ	0	1.76	0.61	0.58	0.94

Soil organic matter is transported to the aquifer by rain infiltration. Recent work by [36] has highlighted the slower transport velocity of humic-like compounds as compared to protein-like compounds (tryptophan) when artificially injected into a shallow hole. [37] also highlighted the higher migration capacity of protein-like substances compared to humic-like substances. This difference may be due to the larger size of humic-like molecules, with numerous aromatic nuclei and carboxylic groups, which increases their potential to be adsorbed, especially in the presence of oxides, hydroxides and clays [38]. Therefore, the decrease in humic-like C content may be caused by the adsorption of molecules, just like TOC, since humic-like compounds constitute 30–50% of TOC in water [39].

Since protein-like and humic-like M compounds are not found in LSBB's soil leachates, we assume that these compounds result from microbial activity occurring in the LSBB hydrosystem. Their production may be influenced by the characteristics of both organic matter available to the microbial communities (e.g., amount and type) and the microbial communities themselves (e.g., types and numbers of microorganisms). Karst environments are oligotrophic and poorly colonized compared to soil, which means that decomposition of organic matter takes time because the likelihood of decomposer organisms encountering and decomposing organic matter is low. Because fluorescent compounds are degradable and have low susceptibility to adsorption, transit time may be a significant, but not unique, factor of variability for these compounds. For example, given similar microbial communities and soil supply, and assuming negligible autochthonous production, an increase transit time would likely result in a decrease in content. Conversely, assuming significant autochthonous production, an increased transit time would likely result in an increased content.

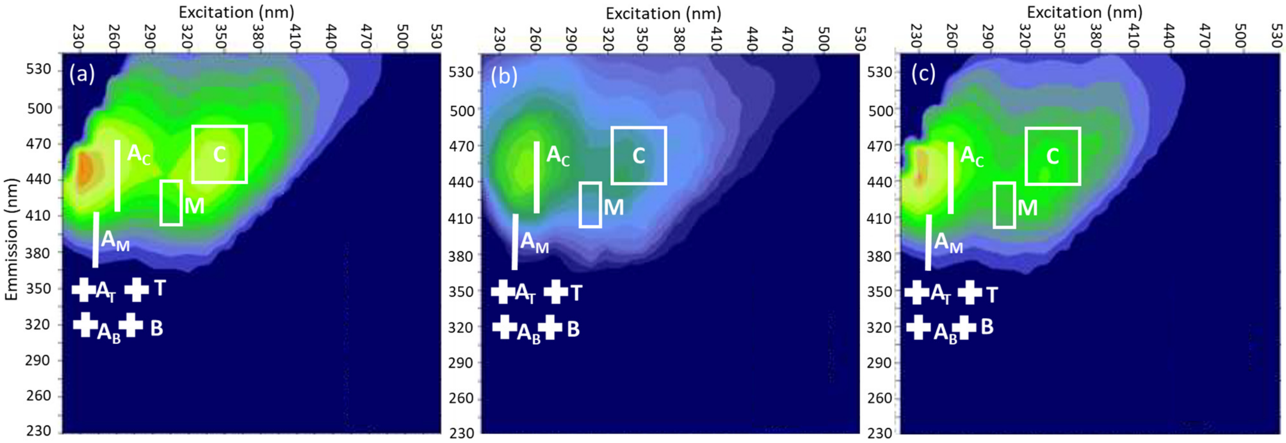

Figure 4. The representative excitation–emission matrix of the monthly monitoring of LSBB soil leachate from December 2005 to April 2008 (modified from [18]). (**a**) Rendosol found upon the plateau; (**b**) lithosol from the slopes below grass; (**c**) shrub and grass. Samples were collected using lysimetric plates (area of 850 cm^2) and artificial rain. Peaks B, T, M, C and AB, AT, AM and AC are the same as in Figure 2; only peaks C and AC are observed.

The recharge area of LSBB flow points covers an area over which the vegetation can be considered as homogeneous. Assuming homogeneous soil input and similar microbial activity at subsurface flow paths, a conceptual model of organic matter source and fate in the LSBB hydrosystem can be proposed (see illustration in Figure 5):

(1) Soil organic matter is supplied by the surface biocenosis. Some of it may be solubilized by rainwater infiltration and incorporated into soil water [40].

(2) Within the soil, organic matter is decomposed, resulting in the release of humic-like C substances derived from lignin, which can also be solubilized in soil water [10,40].

(3) Infiltration of rainwater improves the connection between water-bearing soil pores, and can also contribute to the solubilization of soil organic matter, including humic-like C substances. Infiltration of dissolved organic matter results from a mixture of rainwater and soil water [5].

(4) During transport within the unsaturated zone, organic matter can be adsorbed or delayed (especially humic-like C compounds [36]) and degraded, resulting in the production of other fluorescent compounds [27]. Both molecule breakage during degradation and the production of protein-like compounds can lead to the release of inorganic compounds such as free ions (mineralization process [10]).

(5) According to the continuum of soil organic matter decomposition proposed by [5], degradation results in the release of simpler molecules down to inorganic carbon. Therefore, protein-like compounds can be degraded to inorganic matter. However, the degradation model of [4] also suggests that, to a lesser extent, simpler molecules can be reused to synthesize larger molecules. Humic-like M substances are large molecules derived from microbial products (simpler molecules [10]). Since protein-like substances are simple molecules derived from microbial activity, it seems very likely that protein-like compounds could be reused to produce humic-like M compounds if the continuum of soil organic matter decomposition is applied to aquatic organic matter. Since this degradation process is less direct, it seems logical that humic-

like M substances would be present in lower concentrations than other fluorescent compounds. This is what is observed in LSBB waters (see Section 3.1.1 and Table 2). For the same reason, the presence and relative increase in humic-like M compounds may be associated with longer transit times. This assumption is consistent with [41] which observed a higher proportion of microbial humic-like compounds in deep groundwater during non-monsoon seasons.

(6) As humic-like C, humic-like M compounds can either be adsorbed or may be degraded again.

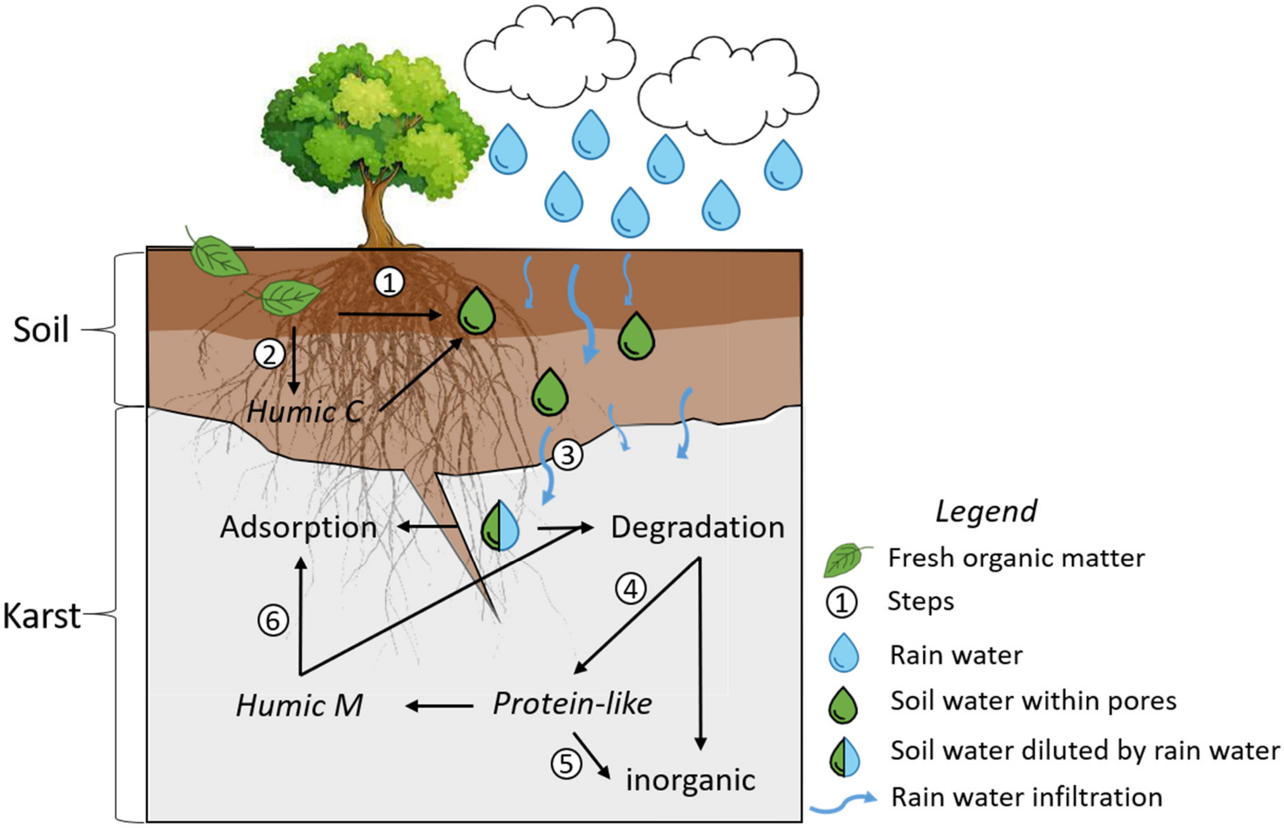

Figure 5. Conceptual model about the fate of the fluorescent organic compounds from LSBB's flows considering a soil only providing humic-like C organic matter (natural catchment without anthropogenic activities).

In this particular case of the LSBB (absence of anthropogenic activity, thin soil providing only humic-like C compounds), the conceptual model of organic matter sources and fates (Figure 5) suggests that the relative content of fluorescent compounds may be related to transit time. Under these conditions: (i) the presence of only humic-like C is associated with short transit times; (ii) intermediate transit times are characterized by the additional presence of protein-like compounds; and iii) at long transit times, humic-like M compounds may be detected.

3.2. Consistency of Relative Transit Times Derived from Fluorescent Compounds Contents—An Assessment at LSBB

3.2.1. Characterization of LSBB Flows Based on Discharge and Major Elements Variations

A study on the hydrochemical variability of LSBB flows based on physico-chemical parameters, major elements and TOC was carried out by [42] on 12 LSBB flows over a period of 10-year (2002–2012). This study highlighted the relationship between several parameters and hydrochemical processes. At LSBB, high relative magnesium content is attributed to long relative transit times due to dissolution of limestones dissolution in the absence of Mg-rich carbonates [26,43]. Sulfate content is inversely related to magnesium, suggesting a decrease with long transit times. The process related to this decrease could not be accounted for with certainty by [42]. This study also showed that at LSBB, electrical conductivity is mainly influenced by the calco-carbonic equilibrium and seasonal variations in soil pCO_2, resulting in variable mineralization of infiltrating water. For all LSBB flows, the low temporal variability of electrical conductivity is therefore assumed to be related to an important buffering effect corresponding to slow transit. In the following, flow velocity will be characterized as fast or slow, and transit time as short or long. The transit time depends on both the flow velocity and the flow depth.

This 10-year chronicle study found a crescent-shaped variability in electrical conductivity from flow D, A and B, to C, therefore suggesting a decreasing buffering effect and faster flow velocity. Low magnesium and TOC contents found at flows D suggested a transit time long enough to allow a TOC to decrease, but too short to allow magnesium to increase. Higher magnesium and lower TOC contents at flows A and B are consistent with slow flows and relatively longer transit times, possibly with similar pathways due to their spatial proximity [42]. The shortest transit times were attributed to flow C, which has the lowest magnesium.

Our dataset confirms the main results of [42], showing the similarity of magnesium and TOC contents of flows A and B, lower magnesium and higher TOC at flow C, and the same distribution of the electrical conductivity variation in the flows (Figure 6 and sd/mean ratio in Table 3).

Table 3. Variability of electrical conductivity, the TTi, magnesium and discharge for flows A (33 samples), B (33 samples), C (17 samples), D (29 samples), and AJ (12 samples) from June 2020 to October 2021.

	A	**B**	**C**	**D**	**AJ**
CE sd/mean	0.06	0.05	0.09	0.03	0.22
TTi sd/mean	0.47	0.49	0.31	0.47	0.27
Magnesium sd/mean	0.11	0.08	0.11	0.11	0.12
Discharge sd/mean	0.26	0.68	0.94	0.22	2.33

Flow AJ is not included in the 10-year chronicle study, but [42] assumptions suggest that its very high variability in electrical conductivity (Table 3) and discharge (Figure 7) indicates very rapid circulation. Figure 6 shows close TOC and magnesium contents of flows C and AJ. This result suggests that flow AJ has a transit time close to that of flow C, despite of its location being 100 m deeper, which is consistent with the fast circulation assumed by the electrical conductivity variations. Therefore, we conclude that the major elements and discharge indicate a crescent transit time from AJ, C, D, to A and B.

3.2.2. Contribution of Fluorescent Organic Compounds to Relative Transit Time Assessment at LSBB

In what follows, we assume that the organic matter supply to the unsaturated zone is similar in quality (i.e., ratio of fluorescent compounds) and quantity for all LSBB flows. This assumption is supported by the geographic proximity of these flow points and the similarity of soils and vegetation across the slope under which LSBB stands. In addition, local changes in land cover and slope, which may be associated with a local variability of organic matter supply, are likely to be averaged within the flow catchment (≤ 1 km^2).

Similar organic matter supply thus allows a direct comparison of TOC and fluorescent organic matter intensity at LSBB flow points.

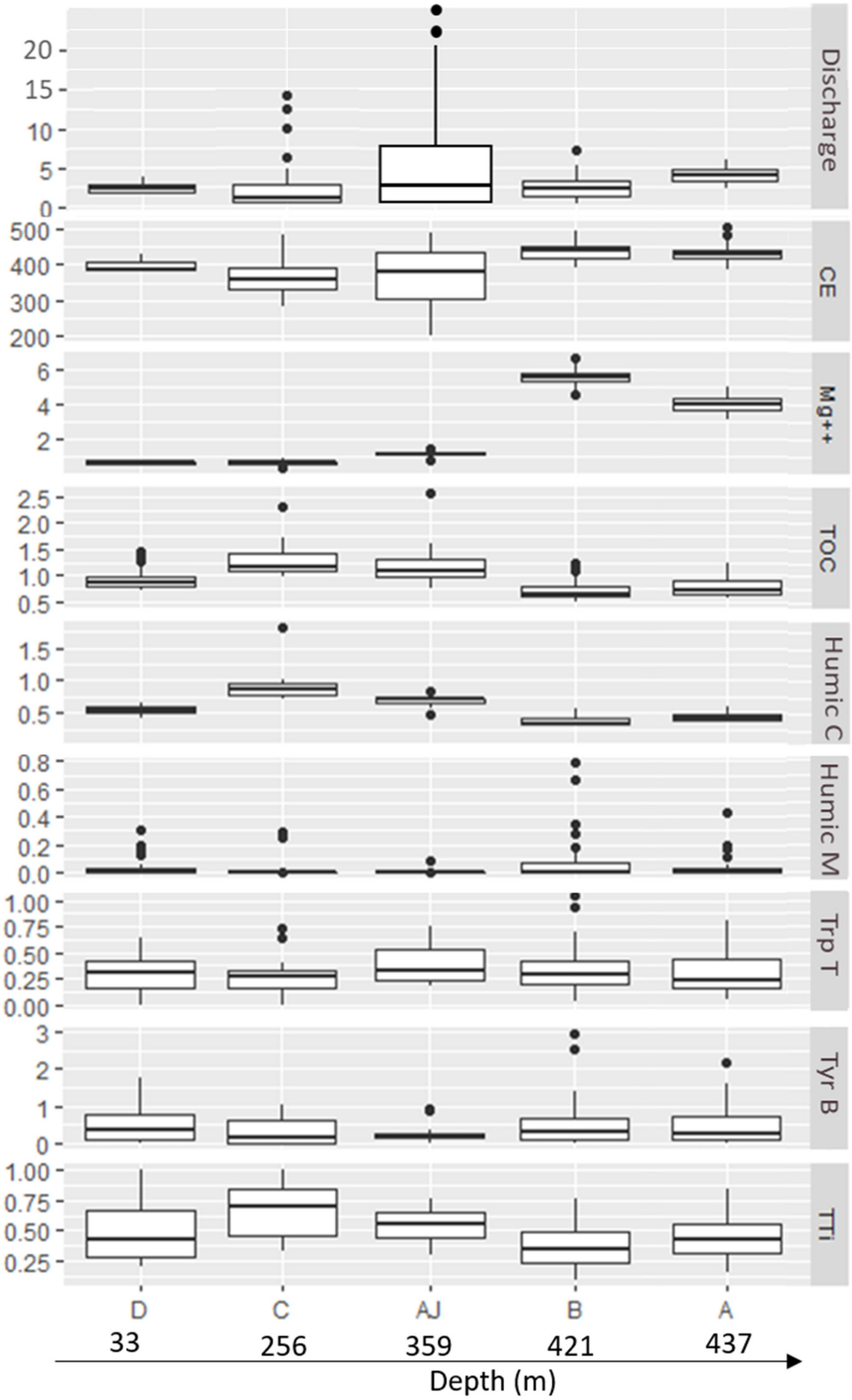

Figure 6. Boxplots of discharge (mL.s^{-1}), electrical conductivity (µS.cm^{-1}), magnesium (mg.L^{-1}), TOC (mg.L^{-1}), humic-like C, humic-like M, tryptophan (Trp T), tyrosine (Tyr B), and the Transit Time index (TTi) for LSBB's flows from different depths within the unsaturated zone: A (33 samples), B (33 samples), C (17 samples), D (29 samples), and AJ (12 samples), from June 2020 to October 2021.

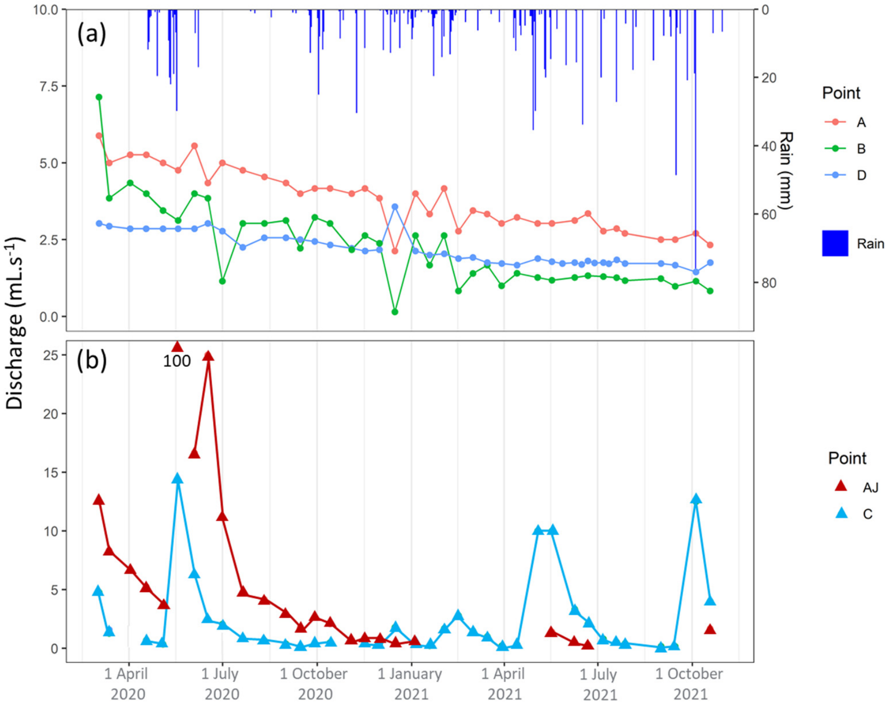

Figure 7. Discharge time series of the five LSBB flows considered compared with rain from the study site. (**a**) Flows A, B and D. (**b**) Flows AJ and C.

Figure 6 shows the distribution of the monitored parameters at the LSBB flow points. Humic-like C and TOC contents show a decreasing trend with depth, consistent with an increase in adsorption potential associated with longer flow paths, and an increase in degradation affecting TOC. Flow D is an exception to this general trend, as it is the shallowest and yet has low TOC and humic-like C contents. This flow point also has a very stable discharge related to a specific lithological context where flow occurs in a low permeability matrix [44]. This specific context is likely to favor trapping, confirming that this process may be the main factor of variability in humic-like C content during flow through the aquifer.

Flows A and D have very close humic-like M contents (the highest values being reached at flow A) and also comparable protein-like contents (Figure 6), suggesting close transit times, with transit to flow A being slightly longer than towards flow D, in accordance with the magnesium, TOC and electrical conductivity behavior (Section 3.2.1 and [42]).

Point B has a higher maximum intensity of humic-like M and protein-like peaks than flow A, suggesting a longer transit time. In our dataset, flow B also has a higher magnesium content and its mean TOC is slightly lower than that of flow A, suggesting that flow B has a longer mean transit time over the period considered (Figure 6). Fluorescent compounds thus provide the first distinction between flows A and B, suggesting that this tracer is more sensitive than major elements, allowing it to reach a different level of information.

AJ has higher tryptophan, but lower tyrosine and lower humic-like M peaks than flow C, which cannot be explained by our model and assumptions. This may be related to the high temporal variability of the hydrochemistry of flow AJ, which is already offset by electrical conductivity and discharge. However, on average, autochthonous compounds (sum of humic-like M and protein-like) are more abundant in flow C (0.89) than in flow AJ (0.69), which may mean that during the monitored period, flow AJ is characterized by faster flow and shorter transit time on average than flow C, even though it is deeper.

This analysis shows that fluorescent organic compounds provide transit time estimates consistent with the major elements and hydrodynamics (Section 3.2.1), which supports the conceptual model of the source and fate of fluorescent organic compounds developed in Section 3.1.2 (Figure 5). It confirms that the relative abundance of fluorescent organic compounds can provide insight into the relative transit time and that their sensitivity can improve the transit time estimation.

3.3. Limits of the Use of Fluorescent Compound Intensity as a Transit Time Tracer and Transit Time Index (TTi) Added Value

Previous sections have highlighted the interest of fluorescent compound intensities for a qualitative assessment of LSBB flow transit times. This comparison is made possible by the similar context of LSBB flows in terms of organic matter supply and biological activity. On a larger spatial scale, the spatial variability of these two parameters would prevent a direct comparison of fluorescent intensities and thus the relative transit times of different flow points. To allow the comparison of waters from different study sites, ratios of intensities such as the TTi must be considered instead of absolute values. The TTi was defined by [16] as the ratio of the intensities of humic-like C to all fluorescent compounds. Based on the conceptual model of the source and fate of fluorescent organic compounds elaborated in Section 3.1.2, a high TTi may be related to a strong soil fingerprint (high humic-like C content) and relatively short transit time, while a low TTi is related to the production of autochthonous fluorescent compounds (humic-like M and protein-like) indicating relatively long transit time. A strong assumption underlying this interpretation is the absence of anthropogenic contamination. Note that this interpretation is opposite to that developed by [16], who assumed no autochthonous production in the studied springs and a significant anthropogenic input due to manure spreading. These contrasting interpretations highlight the difference between the interpretation of the relative contents of fluorescent compounds that can be made in natural or anthropogenic catchments and the need for a careful assessment of organic matter sources and fates prior to any interpretation of fluorescence indices in terms of relative transit times.

At the LSBB, the median TTi is higher from flow C, AJ, D, A to B which is consistent with transit time assumptions from Section 3.2 (Figure 6). Therefore, the TTi seems to be an interesting tool to summarize fluorescence information related to transit time. At the Millet spring, the temporal variation in the TTi was consistent with the expected variation in transit time [16]. At the LSBB, the temporal variability of the TTi is high as compared to that of other tracers (Figure 8 and Table 3), which prevents effective comparison of transit time interpretations. This high temporal variability also implies that the 15-day sampling time step is too high to allow the assessment of TTi variability and needs to be shortened. The higher complexity of the TTi signal at the LSBB as compared to that at the Millet spring may mean that organic matter is a more sensitive transit time tracer than common natural tracers, and that its variations correspond to knowledge that is not accessible using other tracers. It may be related to (i) a faster reactivity of the unsaturated zone flows, which may specifically affect the variation in fluorescent compounds because of their higher sensitivity, (ii) a higher number of possible pathways of water from the surface to the outlet in the unsaturated zone of the LSBB, and (iii) a variable connection between soil/roots and aquifer or different sources of protein-like compounds and humic-like M compounds involving a more variable supply at the LSBB than at Millet (autochthonous at the LSBB, assumed to be allochthonous at Millet spring). Note that hypothesis (iii) highlights the need to improve the knowledge of microbial activity in this system in order to fully understand the production and the degradation of fluorescent compounds.

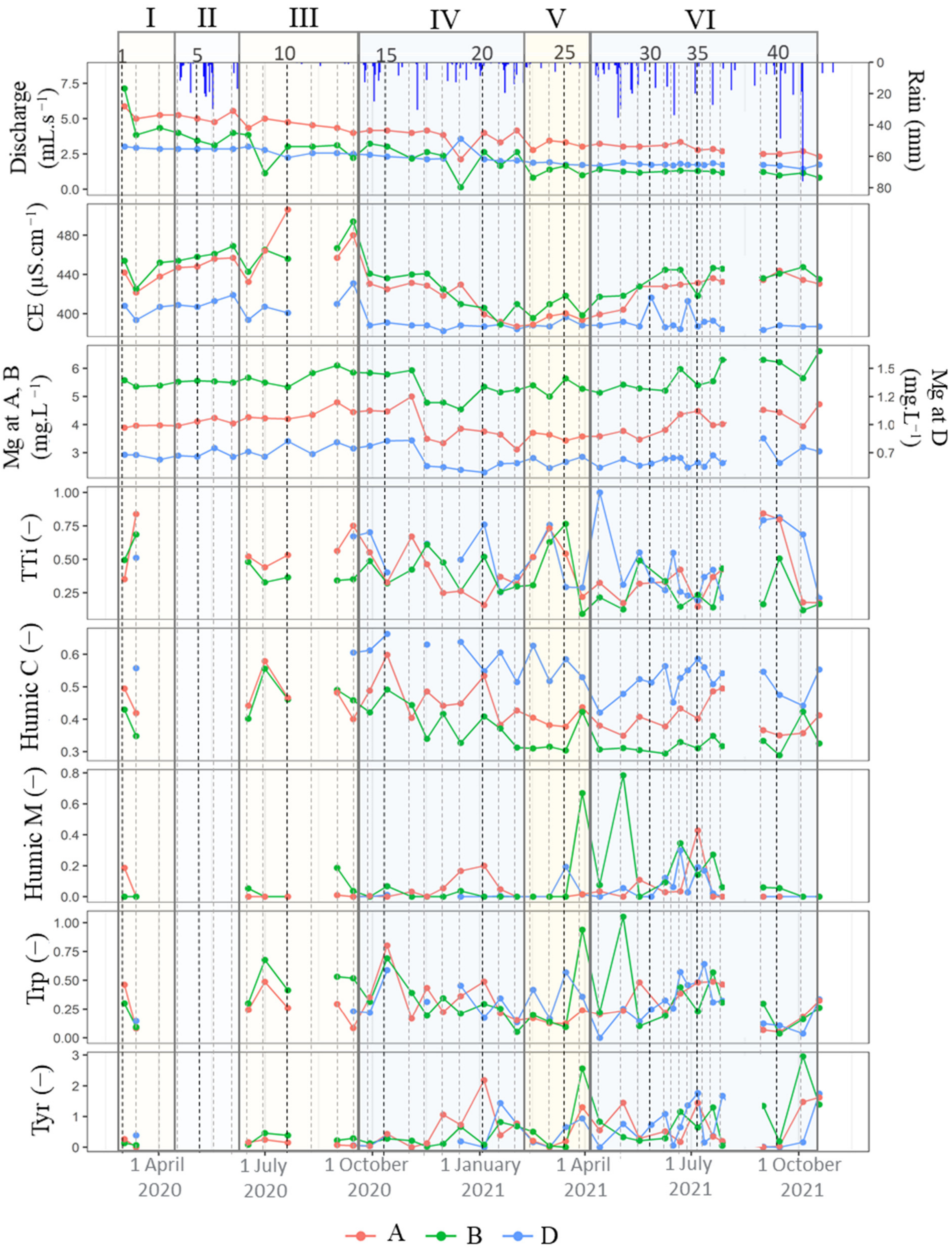

Figure 8. Time series of electrical conductivity (CE), discharge, magnesium, the TTi, humic-like C and M, tryptophan and tyrosine contents (normalized fluorescence intensities) for flows A, B, and D from the LSBB. Vertical lines correspond to the sample number. Periods were separated according to rain frequency and intensity. Dry periods are periods I (cumulative rainfall = 4mm, < 0.1 mm.d^{-1}), III (cumulative rainfall = 6.4 mm, <0.09 mm.d^{-1}) and V (cumulative rainfall = 33 mm, <0.5 mm.d^{-1}); wet periods are periods II (cumulative rainfall = 178 mm, 4.1 mm.d^{-1}), IV (cumulative rainfall = 277 mm, 2.0 mm.d^{-1}) and VI (cumulative rainfall = 511 mm, 2.9 mm.d^{-1}).

4. Conclusions

Reference [16] highlighted the potential of fluorescent organic matter to qualitatively assess transit time through the Transit Time index (TTi) in karst environments. The interpretation of this index was based on the assumption of anthropogenic contamination and no autochthonous production within the aquifers considered. When applied to the unsaturated zone flows of the Fontaine de Vaucluse observatory through the local scale of the LSBB galleries characterized by a natural environment without any anthropogenic activity, TTi interpretation appeared to be reversed. In fact, the excitation–emission matrix from monthly monitoring of LSBB soil leachates from December 2005 to April 2008 [18] shows only humic-like C compounds, whereas protein-like and humic-like M compounds were found in LSBB waters. The present work highlights that these types of compounds can be detected in natural environments unaffected by anthropogenic activity, whereas it is generally accepted that these compounds are almost exclusively of anthropogenic origin and very rarely present without contaminant input to the hydrosystem [10]. It also highlights that in natural catchments, the autochthonous production of fluorescent compounds is unlikely to be negligible. Furthermore, it confirms the interest of the LSBB site to study natural variations without anthropogenic contribution.

The careful analysis of the source and possible fate of the fluorescent compounds leads to a first conceptual model of their behavior from soil to karst, which allows the estimation of transit time. In the LSBB hydrosystem, we hypothesize that the presence of only humic-like C, produced in soil, is associated with a short transit time; intermediate transit times are characterized by the additional presence of protein-like compounds resulting from autochthonous production from the unsaturated zone; and long transit times are associated with the detection of humic-like M compounds.

At the five selected LSBB flows, the use of fluorescent compounds for relative transit time assessment is consistent with flows characterization from a 10-year chronicle study [42], validating the conceptual model of fluorescent compounds source and fate and also providing a more sensitive transit time information than major elements. A median TTi provides consistent results with previous flows characterization, demonstrating its ability to summarize fluorescent compounds information. The TTi time series show a high temporal variability compared to that of other natural tracers, emphasizing the sensitivity of TTi and the need for a short sampling time step to exploit its full potential.

The Transit Time index (TTi) therefore appears to be a powerful tool to qualitatively assess transit time. However, its application requires prior knowledge of organic matter sources and fate at the study site scale. Furthermore, fluorescent compounds and the TTi assessment are inexpensive and can be adapted to continuous monitoring to answer the fundamental question of transit times in karst systems. This study strongly encourages exchange between scientific communities (hydrogeology, microbiology, pedology, etc.) to advance in the understanding of the source and fate of fluorescent compounds, as they are essential to develop tools that allow a better understanding of karst systems, such as the TTi.

Author Contributions: M.B. (Matthieu Blanc) and L.S. took water samples that were analyzed for major elements, TOC, and water-stable isotopes by M.B. (Milanka Babic), J.D. and R.S. and for fluorescence of organic matter by L.S. C.B.-G., C.E., N.M. and L.S. provided critical feedback and helped to shape the research and the analysis. L.S. prepared this manuscript with contributions from all co-authors. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw excitation–emission matrices of organic matter fluorescence are available at: https://data.oreme.org/doi/view/8d6104e1-ae78-4b4e-8e50-198ccc5b19c9#2024 [45].

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Conflicts of Interest: The authors declare no conflicts of interest.

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