

# Complex interactions of in-stream DOM and nutrient spiralling unravelled by Bayesian regression analysis

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**Abstract.** Uptake and release patterns of dissolved organic matter (DOM) compounds and co-transported nutrients are entangled, and the current literature does not provide a consistent picture of the interactions between the retention processes of DOM fractions. We performed plateau addition experiments with five different, complex DOM leachates in a small experimental stream, impacted by diffuse agricultural pollution. By including leachates of cow dung, pig dung, corn leaves, leaves from trees, and nettle plants, the study used a wide range of different DOM qualities. We measured changes in nutrient and DOC concentrations along the stream course and determined DOM fractions by fluorescence measurements and parallel factor (PARAFAC) decomposition. To assess influences of hydrological transport processes, we used a 1-D hydrodynamic model.

We developed a non-linear Bayesian approach based on the nutrient spiralling concept, which we named “Interactions in Nutrient Spirals using Bayesian Regression” (INSBIRE) approach. This approach can disentangle complex interactions of biotic and abiotic drivers of reactive solutes’ uptake in multi-component DOM sources, show their variability, and quantify their error distribution. Furthermore, previous knowledge on nutrient spiralling can be included in the model using prior probability distributions. We used INSBIRE to assess interactions of compound-specific DOM and nutrient spiralling metrics in our experiment.

Bulk DOC uptake varied among sources, showing decreasing uptake velocities in the order corn > pig dung > leaves > nettles > cow dung. We found no correlations of bulk DOC uptake with the amounts of protein-like compounds or co-leached SRP. The fastest uptake was observed for SRP and the tryptophan-like component, while the others more or less resembled the bulk DOC uptake. Almost all DOM components showed a negative relationship between uptake and concentration, known as efficiency loss. In addition, we observed a few negative and (weak) positive interactions between the uptake and the concentration of different components, such as a decreased uptake of protein-like compounds at high concentrations of a high-molecular humic-like compound. We also found an influence of the wetted width on the uptake of

soluble reactive phosphorus (SRP) and a microbially derived humic substance, which indicates the importance of the sediment-water interface for P and humic C cycling in the studied stream.

Overall, we show that bulk DOC is a weak predictor of DOC uptake behaviour for complex DOM leachates and that individual DOM compound uptake, including co-leached nutrients, is controlled by different internal (quality-related) and external (environmental) factors within the same aquatic ecosystem. We conclude that cycling of different C fractions and their mutual interaction with N and P uptake in streams is a complex, non-linear problem, which can only be assessed with advanced non-linear approaches, such as the presented INSBIRE approach.

## 1 Introduction

Dissolved organic matter (DOM) from terrestrial sources plays a key role in the metabolism and the ecological state of streams and rivers by controlling the activity and the composition of microbial communities (e.g. Freixa et al., 2016) and influencing a variety of aquatic biogeochemical processes (Tank et al., 2010). The quantity and the quality of DOM affect the aquatic bacterial respiration (e.g. Besemer et al., 2009; Niño-García et al., 2016), change the ratio between autotrophy and heterotrophy (Lutz et al., 2012; Martínez et al., 2017), alter the toxicity of pesticides (Bejarano et al., 2005), and influence the microbial uptake of dissolved inorganic nitrogen (DIN; e.g. Bernhardt and Likens, 2002; Taylor and Townsend, 2010; Wymore et al., 2016) and soluble reactive phosphorus (SRP; Gibson and O'Reilly, 2012; Stutter et al., 2020; Weigelhofer et al., 2020), amongst others.

The influence of DOM on nutrients is mutual (e.g. Mineau et al., 2013; Stutter et al., 2020; Weigelhofer et al., 2020) due to the demand of microbes for carbon (C), nitrogen (N), and phosphorus (P) approximating their molar C:N:P ratio (Small et al., 2009; Stutter et al., 2018; Welti et al., 2017; Godwin and Cotner, 2018). The importance of the stoichiometric control of organic carbon and nutrient uptake in streams and rivers has long been known (Cross et al., 2005; Dodds et al., 2004), but is increasingly gaining attention with the discovery of anthropogenic impacts on pristine C:N:P ratios and DOM compositions (Stutter et al., 2018; Xenopoulos et al., 2021). Intensive land use has changed the origin, amount, and transport of terrestrial DOM to streams, thereby possibly altering the DOM in-stream processing (Weigelhofer et al., 2020).

According to ecological stoichiometry, in-stream DOM uptake and retention is largely related to the availability of inorganic nutrients, whether they already exist in the freshwater ecosystem (environmental control of DOM uptake) or are provided by the DOM source itself (intrinsic control through the DOM quality; e.g. Bernhardt and McDowell, 2008; Graeber et al., 2015; Gücker et al., 2016; Wickland et al., 2012). Field and laboratory studies show that DOC uptake can be positively affected by N and P concentrations in the water column (Catalán et al., 2018; Mineau et al., 2013) and is also high in N- and P-rich DOM sources such as, e.g., leaves from fertilized trees or agricultural areas (Mineau et al., 2013; Mutschlecner et al., 2018; Weigelhofer et al., 2020).

In addition to nutrient interactions, DOM uptake depends on the structure and the bioavailability of the individual DOM compounds (Guillemette and Giorgio, 2012; Mineau et al., 2016). High uptake rates have been observed for protein-rich,

low-molecular DOM sources such as leachates of fresh leaf litter, macrophytes, and periphyton, for example (Berggren et al., 2010; Koehler et al., 2012). In contrast, the biodegradability of soil leachates has been described as generally low (e.g. Fellman et al., 2009b; Hansen et al., 2016). Finally, the in-stream uptake of DOM may be influenced by environmental factors other than nutrient concentrations, such as the hydrology and morphology of the respective reach or the composition of the biofilms (Casas-Ruiz et al., 2017; Romani et al., 2004; Weigelhofer et al., 2020).

Thus, unravelling the underlying mechanisms of in-stream DOM uptake is complex. While a considerable part of the reactive N and P exists as small and simple molecules, dissolved organic carbon (DOC) is bound in a mixture of differently structured organic molecules, with retention times varying by several orders of magnitude (Cory and Kaplan, 2012; Mineau et al., 2016). The production of new compounds during DOM decomposition may further complicate an accurate assessment of the DOM uptake (Stevenson and He, 1990; Tsutsuki and Kuwatsuka, 1979). While mass balances approaches or calculations of first-order decay curves from addition experiments have already been successfully used in numerous studies to estimate in-stream uptake of DIN, SRP, and even DOC (e.g. Bernhardt and McDowell, 2008; Catalán et al., 2018; Covino, 2012; Ensign and Doyle, 2005; Mineau et al., 2013; Schiller et al., 2011; Stream Solute Workshop, 1990; Weigelhofer et al., 2018b), these methods are often limited in quantifying the uptake of individual DOM components. This limitation restricts and complicates the analyses of interactions among different DOM components and their role in the overall DOC uptake (Mineau et al., 2013; Stream Solute Workshop, 1990; Weigelhofer, 2017; Weigelhofer et al., 2018b).

Our study aimed to investigate the effects of DOM quality on the in-stream DOM uptake, to provide an approach to quantify complex interactions between individual DOM compounds and co-leached N and P, and to elucidate their combined role in the overall DOM retention. For this purpose, we performed several short-term plateau additions with different DOM sources in an agriculturally influenced headwater stream according to the nutrient spiralling concept (Stream Solute Workshop, 1990). We used leachates from natural and human sources (e.g. leaves, manure) to see how in-stream DOM processing may be altered due to anthropic land use changes. Because of the diverse composition of DOM, we decided to extend the equations from the nutrient spiralling concept (Stream Solute Workshop, 1990) and use a Bayesian approach to analyse interactions between and influences of different DOM components and nutrients, including uncertainty propagation. Bayesian statistics is a suitable tool for ecological and biogeochemical questions, allowing us to assess natural variability and assign degrees of belief in hypotheses based on measured data (Arhonditsis et al., 2008; Berger and Berry, 1988; Cox, 1946; Ellison, 2004; Jaynes, 2003; McCarthy, 2007). We incorporated non-linear nutrient uptake models observed in previous studies, such as the Michaelis-Menten or the nutrient efficiency loss model (Dodds et al., 2002; O'Brien et al., 2007), in our approach to describe relationships between concentration and uptake velocity mathematically. Our approach enabled us to (1) analyse how uptake processes of different components influence each other, (2) test our mathematically pre-formulated assumptions with the measured data, including the remaining error, (3) consider the natural variability of each parameter, and (4) include knowledge on nutrient and DOC uptake kinetics from previous studies in our models. We called our approach "Interactions in Nutrient Spirals using Bayesian Regression" (INSBIRE). With INSBIRE, we addressed the following questions:

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1. What are the differences in bulk DOC uptake velocity of different leachates?
  2. How do selected DOM components behave in comparison to the bulk DOC uptake velocity?
  3. Which factors and interactions influence the uptake velocity of the bulk DOC as well as the uptake of the individual DOM components and the co-transported nutrients N and P?

We expected nutrient- and protein-rich leachates to show higher uptake velocities than the others, whereby low-molecular, protein-like compounds show a faster and high-molecular, aromatic compounds show a slower uptake than the bulk DOC. We also expected to find positive influences of co-transported nutrients on the bulk DOC uptake and negative influences of low-molecular protein-like compounds on high-molecular, aromatic compounds.

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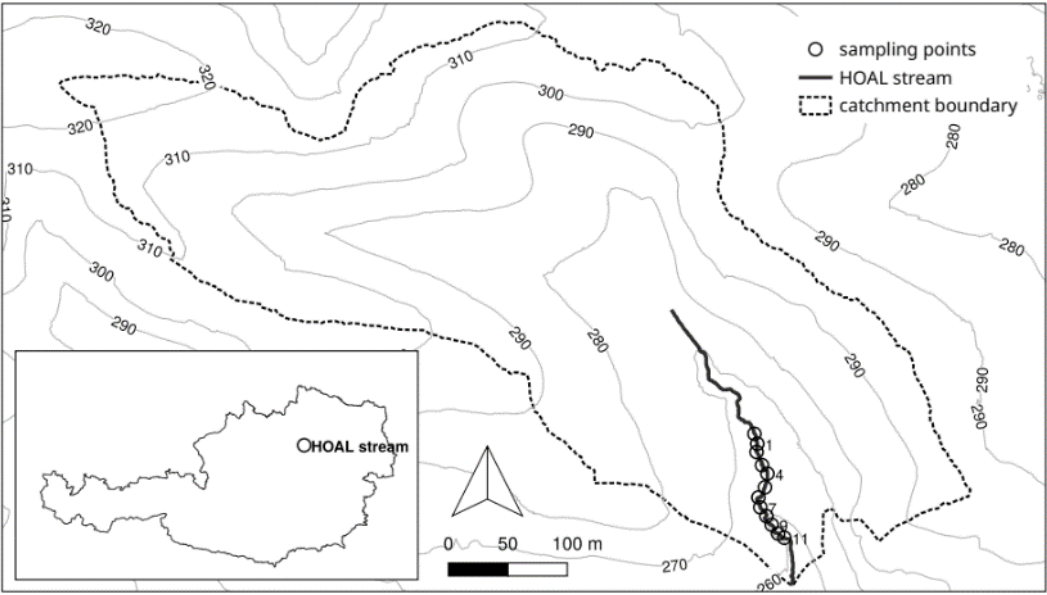
## 2 Methods

### 2.1 Site description

110 The experiment was carried out in the Hydrological Open Air Laboratory (HOAL: <https://hoal.hydrology.at/the-hoal>; Figure 1) in Petzenkirchen, Austria (Blöschl et al., 2016). The HOAL is a small catchment, transformed into a hydrologic observatory to foster scientific research. It features several permanently installed sensors measuring discharge, different water parameters, and the weather. In the past, many studies on surface and subsurface flow paths, evaporation, soil erosion, sediment transport, and nutrient dynamics have been performed (Blöschl et al., 2016). The 1<sup>st</sup> order stream has several inflows, two natural springs, six drainage pipes, and one site with groundwater infiltration from a small wetland. The stream is characterized in sections by (dense) grass growth on the banks, with deciduous forest dominating at the beginning and end of the study reach. All inflows as well as the stream discharge are continuously monitored regarding water quantity and quality. Sediments are dominated by clay washed in from the adjacent fields during storm events. Table 1 shows the extent and basic environmental characteristics of the stream.

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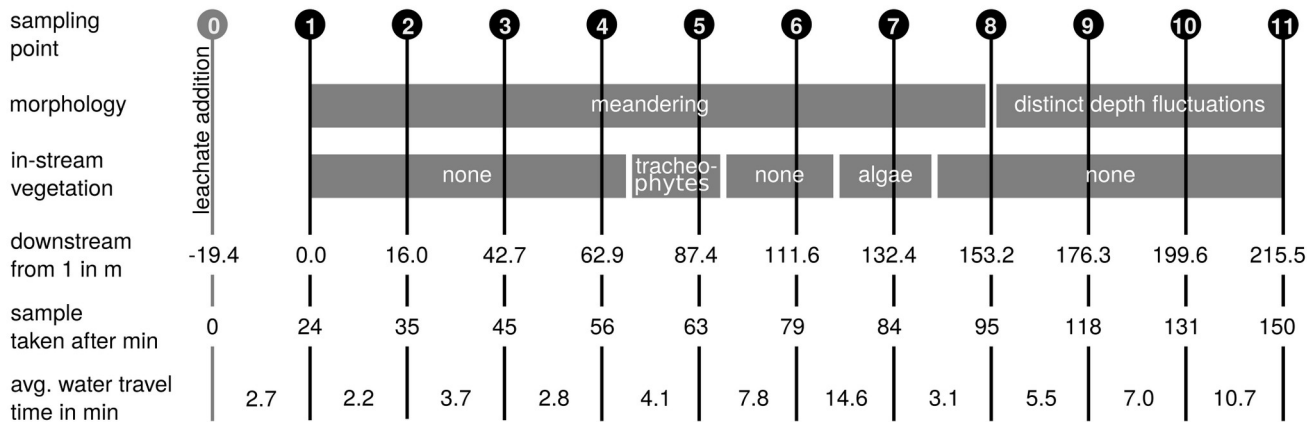
**Figure 1: Hydrologic open-air lab HOAL: catchment, stream, sampling points and location within Austria**

**Table 1: Extent and environmental characteristics of the HOAL**

Characteristic	Value	Unit
Length	620	m
Catchment size	0.66	km <sup>2</sup>
Arable land coverage	90	%
Mean annual discharge	0.004	m <sup>3</sup> s <sup>-1</sup>
Peak discharge	2	m <sup>3</sup> s <sup>-1</sup>
Mean annual temperature	9.5	°C
Mean annual precipitation	820	mm yr <sup>-1</sup>

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To avoid any lateral inflow, we chose a reach of 215 m situated between two lateral inflows for the experiments. We divided the study site into subsections of 16 to 26 m, depending on accessibility. The stream is characterized by a meandering course but is stretched with frequent pools (up to 24 cm in depth) at the end of the study reach. Between point 4 and point 5, *Equisetum palustre* and *Juncus sp.* grow in this open section's water (Figure 2). At point 7, the patchy canopy cover facilitates the growth of algae on the stream bed. During the experiment, the median temperature was 16.7 °C (IQR = 2.4) and the median conductivity was 633  $\mu\text{S cm}^{-1}$  (IQR = 23).



**Figure 2: Sampling scheme and general parameters of the stream.**

### 2.2 Experimental design

The experiment was performed during six consecutive weeks in July and August 2018. No major rain event occurred in the study area and the average discharge was between 0.38 and 0.93 L s<sup>-1</sup>. Ten additions with DOM leachates from five different sources were injected into the study reach using short-term plateau additions according to the Stream Solute workshop protocol (Stream Solute Workshop, 1990; Weigelhofer et al., 2012). The respective leachate plus a NaCl solution as conservative tracer were pumped into the stream at point 0 over 2 to 2.5 hours via a peristaltic pump (Fig. 2). The first sampling point (point 1) was chosen to ensure full mixing with the stream water based on equal and stable conductivity values at several points across the stream transect during a pre-experiment with NaCl. We used a mobile conductivity meter to identify plateau conditions in the stream at each sampling point. Approximately 10 min after reaching stable plateau conditions, water samples were taken at each sampling point for the analysis of nutrient concentrations, organic carbon concentrations, and DOM composition. Thus, the sampling time exceeded the average water travel time by far, ensuring proper mixing and a stable state during sampling (see numbers in Fig. 2). After shutting the addition off, the change in conductivity was recorded until salt concentrations had returned to ambient levels. Additions were limited to a maximum of two times per week with at least 48 h between two consecutive samplings, allowing the system time to recover. Each leachate was added twice to the stream with an interval of five to seven days and the added material created concentration peaks equal to or below local rain events to reduce adaption of the microbial community and interferences among leachates. Each Monday, we sampled ambient concentrations to interpolate background conditions for the days with addition experiments. All samples were taken between 10:00 and 14:00 to ensure comparability. As the environment changes naturally over time (e.g. discharge, temperature), different additions cannot be compared if the interval between them is too long. However, extremely short intervals and/or long addition times may lead to adaptations of the microbial community. Thus, the above-mentioned sampling schedule represents a compromise based on our experiences in nutrient additions

experiments (Weigelhofer, 2017; Weigelhofer et al., 2012, 2018b) and the long-term weather and discharge data of the stream. During our experiments, environmental changes were negligible due to extremely stable weather conditions and no human activities in the experimental area. We also observed no systematic changes of the DOM, N-NO<sub>3</sub>, and SRP uptake over time, indicating that any potential adaptations or responses of the microbial community to these rather short and low pulses did not affect the results of the study significantly.

### 2.3 Preparation of the leachates

The leachates were prepared from 50 g L<sup>-1</sup> dry matter of cow and pig dung, foliage from local trees (*Acer platanoides*, *Acer pseudoplatanus*, *Lonicera xylosteum*, *Pteridium aquilinum*, *Sambucus nigra*), nettles (*Urtica dioica*), and corn plant (*Zea mays*) leaves. We leached with nutrient-poor water from a local well under aerated conditions in a barrel over 24 h. The leachates were filtered in steps of 2 mm and 0.5 mm using stainless steel sieves and 50 µm using a 25 cm spun filter cartridge (PureOne PS-10). The end volume was between 40 and 60 L. To avoid post-leaching changes in DOM, the leachates were prepared freshly for each addition.

Average DOC concentrations in the stream water were about 1.3 mg L<sup>-1</sup>. We aimed to achieve an increase by about 3 mg L<sup>-1</sup> DOC in the experiments. Some sources proved difficult to leach in sufficient amounts and parts of the leached DOC was degraded even during short storage. Thus, the DOC increase achieved during the experiments was between 0.2 and 2.3 mg L<sup>-1</sup>. Even within the same source, leached amounts varied in concentration and composition between different additions. We consider this unproblematic since we defined the leachates by their measured composition and not solely by their source. On the contrary, the fluctuations broaden the distributions of measured values and can provide more stable models as well as a more general picture of the uptake processes.

### 2.4 Analyses

Before the analyses in the lab, samples were filtered through combusted Whatman glass microfiber filters, Grade GF/F (0.7 µm) for syringes. We measured inorganic nitrogen as N-NO<sub>3</sub><sup>-</sup>, nitrite (N-NO<sub>2</sub><sup>-</sup>) and ammonium (N-NH<sub>4</sub><sup>+</sup>) as well as SRP with a Continuous Flow Analyzer (accuracy ± 0.1 µg L<sup>-1</sup>). Dissolved organic carbon (DOC) was measured with a Sievers\*900 portable TOC-Analyzer (accuracy ± 2%). We measured the DOM quality (Excitation-Emission-Matrices) via Fluorescence Spectroscopy with a Hitachi Fluorescence Spectro-photometer F-7000 and DOM absorbance with a Shimadzu UV-1700 spectrophotometer.

We analysed the data using R software version 3.5 (R Development Core Team, 2019) and tidyverse (Wickham et al., 2019). The DOM EEMs (11 sampling points, 16 sampling dates, 176 samples in total) were pre-processed using eemR (Massicotte, 2019), the PARAFAC analysis was done with staRdom (Pucher et al., 2019). The measured fluorescence EEMs were corrected for inner-filter effects, samples of ultra-pure water were subtracted, scatter bands were removed and interpolated and the samples were normalized to Raman units. Samples were screened visually and no unusual noise was found. After obtaining first models, three outliers were identified using the samples' leverages and excluded from the model. The



185 components' spectra were visually checked for plausibility. After that, a suitable model was validated using a split-half analysis. The final model did not express any problems related to those criteria. The outliers were included again to calculate loadings under the already fixed components. For calculating the PARAFAC models and the split-half validation, we used 256 random initializations, a tolerance of  $10^{-11}$  and staRdom's standard way to split the data (Pucher et al., 2019). We used Openfluor.org (Murphy et al., 2014) to compare and link the found components with other studies (Table 2).

190 **2.5 Hydrodynamic modelling**

A hydrodynamic 1D-model was used to calculate the necessary hydraulic parameters using the software package HEC-RAS. For the creation of the terrain model, a cross-sectional approach was applied, where 64 cross-sections were recorded at a distance of 0.8 m to 6.8 m depending on structural variations and accessibility. A total of 251 points were measured in the stream with a theodolite (Leica TC805) and then merged with a 1 x 1 m floodplain area model (based on the official laser scan data of the province of Lower Austria) using the software package Surface-water Modeling System (Aquaveo, LLC). The model was calibrated with the discharge data recorded at the HOAL site by comparing the measured water surface elevation with the modelled one. The calibrated 1D model was used to calculate the hydraulic parameters flow velocity, water depth, wetted width and water travel time at each sampling point for each sampling day.

200 **2.7 Calculating Interactions in Nutrient Spirals using Bayesian Regression (INSBIRE)**

The nutrient uptake was calculated using a Bayesian non-linear model and solved with a Markov chain Monte Carlo (MCMC) algorithm as provided in the R package brms (Bürkner, 2017) relying on stan (Carpenter et al., 2017). For hypothesis testing and model comparisons, we used the Bayes factor (BF, Goodman, 1999a, b), which is the ratio of the marginal likelihood of two competing hypotheses or models. A BF of 10 in favour of a particular hypothesis or model means that this model is 10 times more likely to explain the measured data. The interpretation of the BF was conducted according to (Kass and Raftery, 1995). In that way, a BF of more than 3.2 is considered as "substantial evidence", while values below are "barely noteworthy". A BF <1 corresponds to the inverse of the BF, but in favour of the other hypothesis. Model selection using the BF also allows to remove models prone to collinearity problems (Ghosh and Ghattas, 2015). The Bayes  $R^2$  (Gelman et al., 2019) for each model was calculated to demonstrate the accuracy of the analysis.

210 We used the equations of the nutrient spiralling concept provided by the Stream Solute Workshop (1990) to develop our solute spiralling model INSBIRE. All equations providing the base for the model from the Stream Solute Workshop (1990) as well as all equations derived, transformed, and developed from these basic equations for the model development are shown and explained in detail in Supplement section S1. For a straightforward solving scheme as INSBIRE, a single-step analysis is necessary to determine the posterior distributions of all interdependent parameters at once. Interactions, model weaknesses, collinearity (Ghosh and Ghattas, 2015), and the variation of parameters can then be assessed and interpreted in a consistent way.



Commonly, uptake length ( $s_w$ ), uptake velocity ( $v_f$ ) and areal uptake rate ( $U$ ) are used to describe nutrient uptake (Dodds et al., 2002; O'Brien et al., 2007; Trentman et al., 2015; Weigelhofer et al., 2018b). We fitted all three parameters to the equations. The uptake length  $s_w$  is known to change with different discharges, while  $v_f$  should compensate this problem (Dodds et al., 2002), and  $U$  incorporates the concentration of the solvent. While these values can be easily transformed into each other, we found  $v_f$  most suitable to address our research questions because the compensation of hydrologic conditions makes general uptake patterns better visible. In alignment with Bayesian statistics (e.g. McCarthy, 2007), we defined prior distributions (a priori distributions for each parameter based on past experience) based on knowledge from other studies (e.g. Mineau et al., 2016) to keep the parameters (e.g.  $v_f$ ) within realistic ranges and foster a stable fitting procedure. We provide an exemplary R script that demonstrates INSBIRE (Pucher, 2020). A detailed mathematical description of the INSBIRE approach can be found in section S1 in the supplementary material.

We used data from all experiments combined to perform the parameter estimation, thus increasing the number of points in our model. By that, we got a better insight into processes and interactions underlying uptake that can only be observed with different nutrient and DOM ratios. Unless in a fitting algorithm, that determines only the most suitable value, the result of a Bayesian fitting is a distribution of probable parameter values showing the variability in the stream and between experiments. Furthermore, we set a threshold for complete retention of the added solutes, at which the difference between plateau and ambient conditions was equal two times the accuracy of the lab analyses. Measured values below this threshold were removed from the analyses. Since the fluorescence of DOM increases linearly with concentration (Kothawala et al., 2013), we used  $F_{\max}$  of the PARAFAC components analogously to concentrations in these models.

We tested trends in the longitudinal nutrient concentrations by comparing the assumption of constant concentration with that of exponential decay as proposed in the nutrient spiralling concept (Stream Solute Workshop, 1990). The BF between those two models was calculated to show which one is more likely.

For the analysis of the uptake of bulk DOM and individual components (research questions 1 and 2), we used the equations from the nutrient spiralling concept (Stream Solute Workshop, 1990) to calculate  $v_f$  via a one-step fitting procedure (for details, see Supplement S1, Eq. S4).

By adding the leachate source to the uptake models as a random factor, we could determine general quality-related differences between the leachate sources. Previous studies showed a difference in DOM quality even when similar natural matter sources were used for the production (e.g. Ohno and Bro, 2006). Thus, we added the sampling date as a random effect to our models to see if there are differences between the two sampling dates of the same leachate source (e.g. Ohno and Bro, 2006). A systematic change with the sampling date for all or at least most nutrients and components can also reveal experimental or analytical problems in the execution process, but was not observed in this study.

The analyses of influencing factors on bulk DOM and component-specific uptake (research question 3), required the adaption of the original concept to include relationships among different DOM components and co-leached nutrients. This step was motivated by nutrient addition studies showing different uptake models such as linear functions, power functions (efficiency loss model), and Michaelis-Menton kinetics (Dodds et al., 2002; O'Brien et al., 2007; Trentman et al., 2015). We

additionally tested an exponential function and an asymptotic regression function. We decided to present only the results of the power function (Eq. 1) because these models showed the highest BF<sub>s</sub> (highest probability to explain the observed data) for most variables. A big advantage of the power function is that there is only one parameter to fit, which makes it less prone to over-fitting in complex models (e.g. discussed in McElreath, 2016, chapter 7). Also, in our experiment, concentrations did not reach uptake limits. In such cases, uptake rate curves often exhibit a power function, probably representing the lower part of a saturation model within a concentration range below saturation and thus often naturally met in stream systems. O'Brien et al. (2007) limited the exponent of the power function ( $m_i$  in Eq. 1) to negative values ( $m < 1$  with respect to  $U$ , but  $m < 0$  in case of  $v_f$ ) to describe the efficiency loss behaviour. However, positive  $m_i$  can also be used in the models to describe situations where a substance improves the uptake of another (e.g. Stutter et al., 2020). In our study, we were looking for both positive and negative interactions among components and thus did not restrict the sign of  $m_i$ . For positive exponents  $m_i$  in Eq. (1), the function would pass through zero, which means that the absence of a stimulating component automatically leads to a complete collapse of DOM or nutrient uptake. Sometimes, this is significant, so we incorporated an added value 1 in Eq. (1) as a degree of freedom, whose relevance was tested during the model selection process.

From a modelling point of view, any available variable could be included and tested at that point. We decided to include the wetted width in the formula because we expected an influence of the available benthic surface on the uptake processes and the stream showed a promising fluctuation of wetted width by a factor of 2.8 between different dates and cross sections. This resulted in the following equation:

$$v_f = kw \left( 1 + \prod_i C_{i,x,t}^{m_i} \right) \quad (1)$$

$v_f$  ... nutrient uptake velocity

$k$  ... uptake rate factor (model parameter)

270  $w$  ... wetted width, constant 1 to represent no influence (calculated by Hec-RAS, then fixed)

$1$  ... additive value (model parameter)

$i$  ... index of DOM component or nutrient

$C_{i,x,t}$  ... concentration of compound  $i$  at point  $x$  and date  $t$  (measured variable)

$m_i$  ... exponent determining the strength of the relations (model parameter)

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The same fitting algorithm as for research questions 1 and 2 was used to derive the parameters  $k$ ,  $m_i$ , and  $1$  in Eq. (1). Additional informations are provided in the Supplement section S1, Eq. (S8). Relationships among components (including co-leached nutrients) were tested individually and in different combinations by adding factors of power functions, according to the single factors in Eq. (1). The different combinations were compared to the initial model, as used for research question 2, as well as the next simpler models and were rated according to their BF<sub>s</sub>. When models with specific variables did not improve the predictability of the observed data, they were rejected and are not presented in the results section below. By

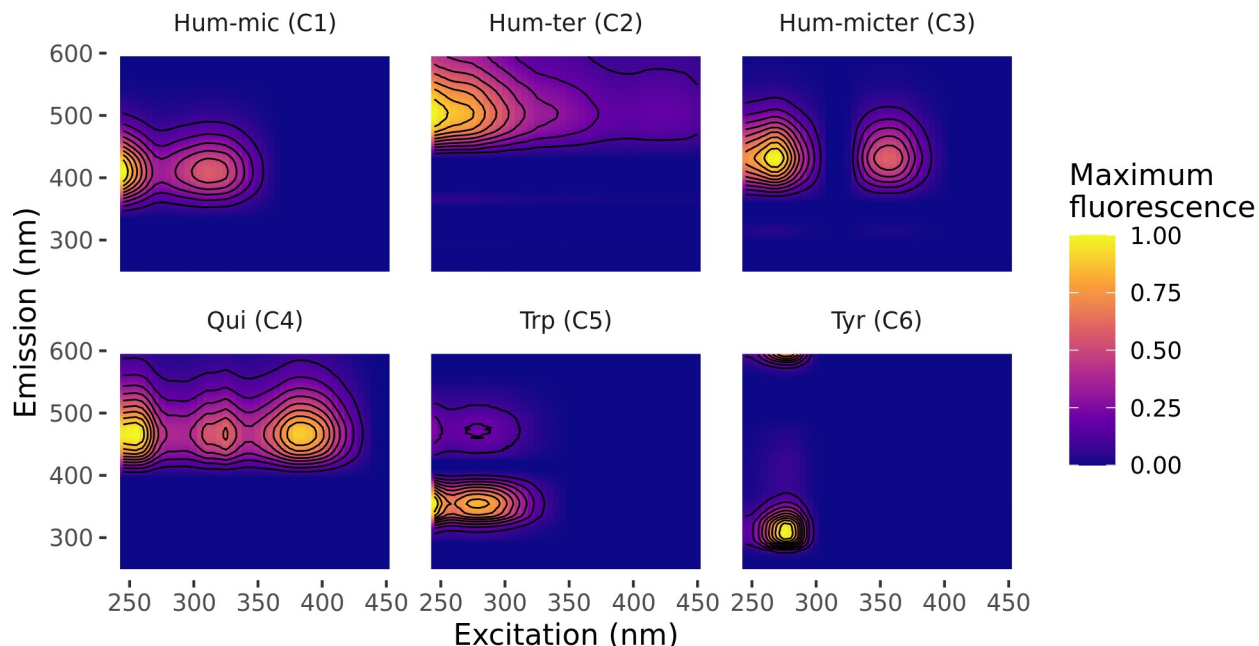
that, we determined models with meaningful component relationships and derived BF<sub>s</sub> for each variable included at a step, representing the strength of evidence to support this inclusion.

For the comparisons of  $v_f$  of all co-leached nutrients and DOM fractions, we transformed the equation from the nutrient spiralling concept (Stream Solute Workshop, 1990) to derive  $v_f$  for each nutrient and DOM component and between all pairs of sequent points directly. Uptake velocities between nutrients and DOM fractions were compared using a Bayesian test for linear correlation (Jeffreys, 1998; Ly et al., 2016) implemented in the R package BayesFactor (Morey et al., 2018) to be aware of and avoid effects of collinearity on the models calculated. Furthermore, the distributions of differences between  $v_f$ s of different DOM components and nutrients were calculated using a Monte-Carlo-Simulation. By that, we gain a probability distribution of differences, that can be used to measure the evidence in favour of a difference (motivated by the posterior distribution of difference in means, Kruschke, 2013).

### 3 Results

#### 3.1 PARAFAC components

We could successfully fit a six-component PARAFAC model (Figure 3, Table 2). Leachates of pig and cow dung characteristically exhibited high levels of tryptophan-like (Trp, C5) and tyrosine-like (Tyr, C6) compounds. Leaf leachate showed high peaks in microbially produced humic-like (Hum-mic, C1) fluorescence, which is assumed to represent low-molecular, aliphatic DOM originating from microbial degradation. Ambient water was characterized by humic-like material from terrestrial sources (Hum-ter, C2) and microbially processed terrestrial DOM associated with agriculture (Hum-micter, C3). Another humic-like fluorophore with some resemblance to pure quinone was identified in all sources (Qui, C4). The ambient DOM composition resembled the leachate from pig dung.



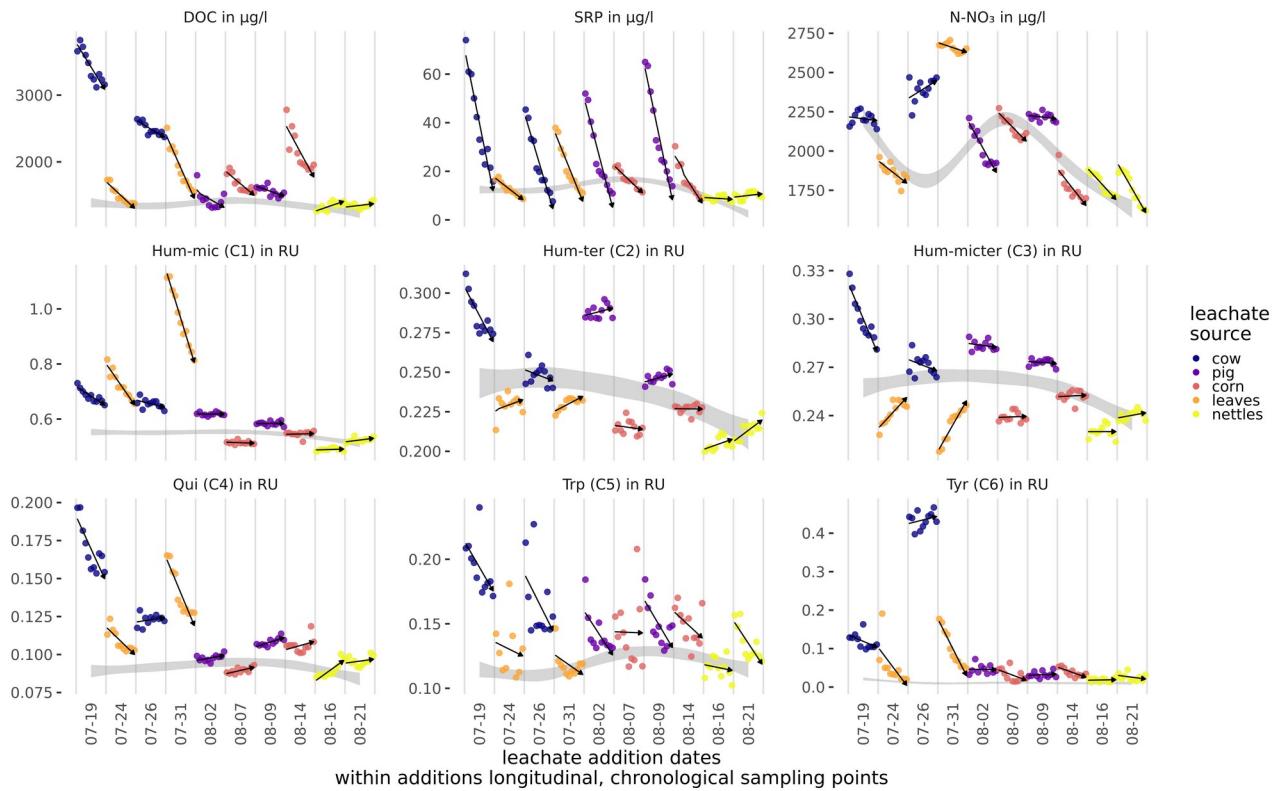
**Figure 3: Fluorescence spectra of the identified PARAFAC components.**

**Table 2: PARAFAC components and their comparison to other studies. The used abbreviations and symbols stand for: a: ambient, m: corn, c: cow dung, l: leaves, n: nettles, p: pig dung, ▲: high, —: intermediate, ▼: low.**

component	similar components in other studies	interpretation	relative share in leachates					
			a	m	c	l	n	p
Hum-mic (C1)	G2 (Murphy et al., 2011), C2 (Lambert et al., 2016b), D2 (Shutova et al., 2014)	microbial humic-like, DOM produced during the microbial degradation of terrestrial DOM within freshwaters	—	▼	▼	▲	▼	—
Hum-ter (C2)	C2 (Lambert et al., 2016a), F3 (Heibati et al., 2017)	terrestrial humic-like, high molecular weight and aromatic compounds of terrestrial origin.	▲	▼	▲	▼	▼	▲
Hum-micter (C3)	C5 (Lambert et al., 2017), C4 (Williams et al., 2010), C5 (Williams et al., 2013)	microbial humic-like, positively correlated with bacterial activity and croplands in the catchment, associated with microbial transformation of terrestrial organic matter.	▲	▼	▼	▼	▼	▲
Qui (C4)	C2 (Yamashita et al., 2011),	humic-like, A and C peaks, terrestrial	—	▲	▲	—	▲	—



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**Figure 4: Measured concentrations of DOC, SRP, and N-NO<sub>3</sub>, and Raman units (RU) of DOM fractions along the stream course for the different samplings. The values are corrected for dilution effects. (see also Table S1). Arrows show the general trend of the concentrations/RUs from up- to downstream. The ambient concentrations were interpolated from measurements taken in-between leachate additions and are visualized as grey ribbons (see Table S1 for ambient conditions and additional amounts from leachate additions at the upstream station).**

**Table 3: Linear correlation of nutrient concentrations and DOM fraction fluorescence; BFs in brackets; only shown, if BF > 1.**

	Hum-mic (C1)	Hum-ter (C2)	Hum-micter (C3)	Qui (C4)	Trp (C5)	Tyr (C6)	DOC
Hum-micter (C3)	0.87 (5.47)	0.62 (2.34)					
Qui (C4)		0.86 (3.25)	0.59 (1.46)				
Trp (C5)			0.73 (2.45)	0.87 (8.22)			
Tyr (C6)			0.58 (1.03)				
DOC			0.56 (1.38)	0.80 (12.62)	0.91 (8.83)		
SRP			0.47 (1.18)		0.69 (4.74)	0.37	0.41 (1.99)

	(1.35)
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325 **3.3 Results from the INSBIRE approach**

During the experiment, discharge varied (0.41 to 0.93 L s<sup>-1</sup>) and we could clearly see more stable fitting behaviour using  $v_f$  than  $s_w$ . Out of all tested functions, we found the power function the most suitable one for the concentration-uptake velocity relations.

**3.3.1 Uptake velocities in dependence of the leachate source**

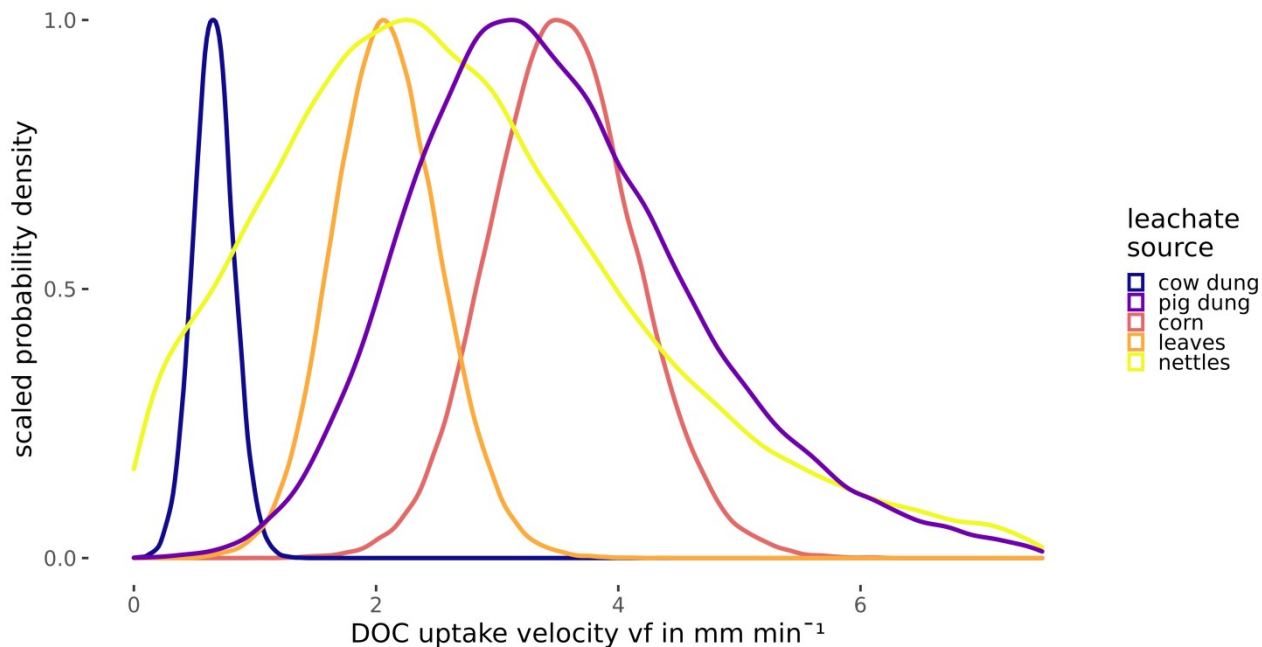
330 The probability density of DOC (Figure 5) from corn leachate, leave leachate, and cow dung leachate was narrow, allowing for a clear distinction of  $v_f$  between these three (Table 4). Here, corn leachate was taken up fastest followed by leave and cow dung leachate. The probability density of the uptake velocities of nettle and pig dung leachates was much broader than those of the other leachates, making  $v_f$  distinction more difficult. During nettles and pig dung leachate additions, the DOC peaks were lower and therefore measurement errors have a higher influence. This demonstrates how a low number of

335 observations or erroneous data influences results in Bayesian statistics. However, although we cannot make reliable statements in all relation to other leachates, we get the probable range of uptake velocities. In specific, we can assume that the uptake velocities of nettles and cow dung leachates do not exceed 6 mm min<sup>-1</sup> and are faster than the  $v_f$  of cow dung leachate.

340 **Table 4: Comparison of  $v_f$  of DOC depending on leachate source by the BF of one  $v_f$  being lower than the other one. Additionally, the table presents median values of the  $v_f$  distributions in mm min<sup>-1</sup>.**

	$v_f$ median	BF for $v_f$ (row) < $v_f$ (column)			
	in mm min <sup>-1</sup>	leaves	nettles	pig dung	corn
cow dung	0.66	981	7.04	650	> 1000
leaves	2.08		1.40	7.33	38.7
nettles	2.42			2.24	2.74
pig	3.37				1.19
corn	3.54				





**Figure 5: Posterior density distribution curves of uptake velocity  $v_f$  of DOC depending on the leachate source.**

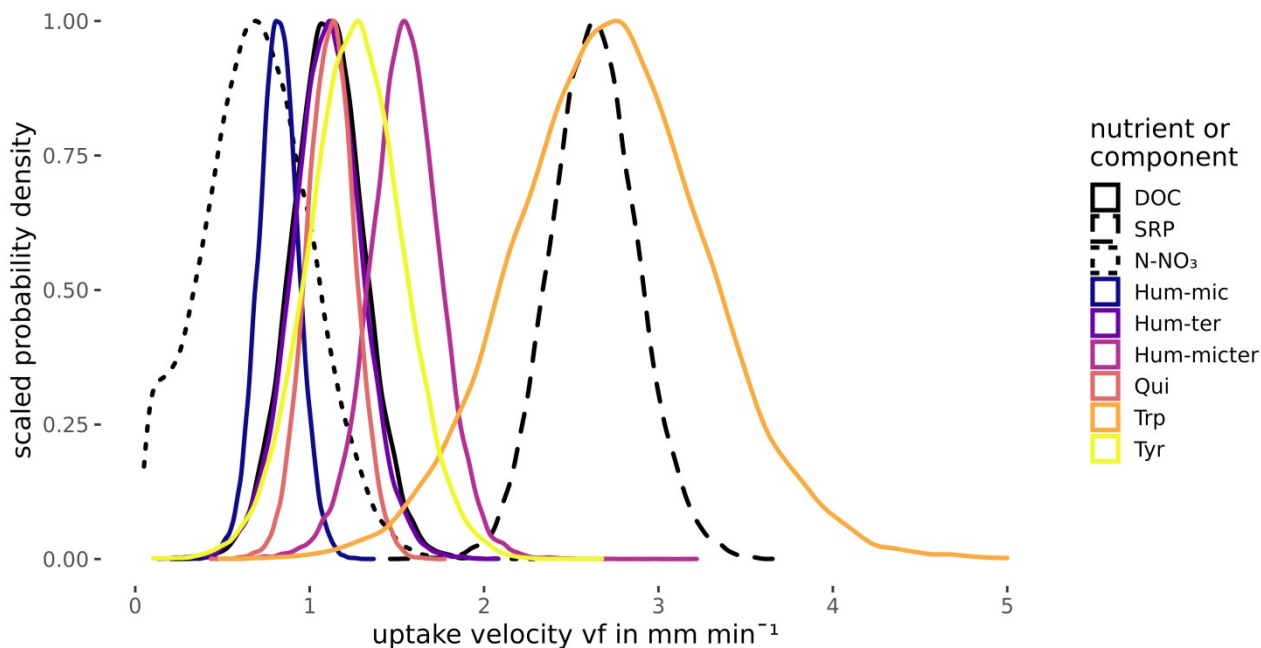
### 3.3.2 Uptake velocities of different DOM fractions and nutrients

Modelling  $v_f$  of the different components and nutrients without considering interactions or influencing factors showed that the uptake of the bulk DOC reflected the average uptake of the different DOM components. N-NO<sub>3</sub> and Hum-mic (C1) were taken up slower, SRP, Hum-micter (C3) and Trp (C5) were taken up faster than the bulk DOC and all other components (Figure 6, Table 5).

**Table 5: Comparison of  $v_f$  of DOM components and nutrients by the BF of one  $v_f$  being lower than the other one. Additionally, the table presents median values of the  $v_f$  distributions in mm min<sup>-1</sup>.**

	$v_f$ median		BF for $v_f$ (row) < $v_f$ (column)						
	in mm min <sup>-1</sup>	Hum-mic (C1)	Hum-ter (C2)	DOC	Qui (C4)	Tyr (C6)	Hum-micter (C3)	SRP	Trp (C5)
N-NO <sub>3</sub>	0.73	1.60	2.98	3.21	3.52	3.97	6.66	133	38.4
Hum-mic (C1)	0.82		4.73	4.91	5.61	7.68	20.8	> 1000	42.9
Hum-ter (C2)	1.10			1.10	1.18	2.66	6.28	255	25.0
DOC	1.11				1.16	2.51	6.06	235	24.6
Qui (C4)	1.12					2.65	7.56	613	24.2

Tyr (C6)	1.27	3.88	42.9	17.1
Hum-micter (C3)	1.56		10.8	7.44
SRP	2.63			1.58
Trp (C5)	2.76			



**Figure 6: Posterior density distribution curves of uptake velocity  $v_f$  for different compounds and nutrients.**

### 3.3.3 Influencing factors and component interactions

Differences between samplings using the same source can be caused by day-dependent characteristics such as discharge and weather or by differences in the leachate despite the same source. Hence, we tested whether the date of the different experiments and/or the source significantly affected the uptake of the bulk DOC, the DOM components, and the leached nutrients. Hum-mic (C1) retention was substantially (BF 4.6) and Trp (C5) retention was decisively (BF 134.2) influenced by the addition date. Bulk DOC and Tyr (C6) retention was influenced by both the DOM source and the date. However, for bulk DOC retention, the source had a stronger effect (BF 1563) than the date (BF 146), while the reverse was true for Tyr (BF 10.7 and  $10^8$  for source and date, respectively). Hum-ter (C2), Hum-micter (C3), and Qui (C4) as well as SRP and  $\text{NO}_3$  showed conservative uptake behaviour independent of the source or addition date (BF < 1, see also supplement Table S3).

To further disentangle the interaction effects between nutrient and DOM component uptake velocities as proposed in Eq. (1), component concentrations or fluorescence and wetted width, representing the influence of hydrology, were included and evaluated in the models (Eq. 1, for details, see Suppl. section S1, Eq. S8).

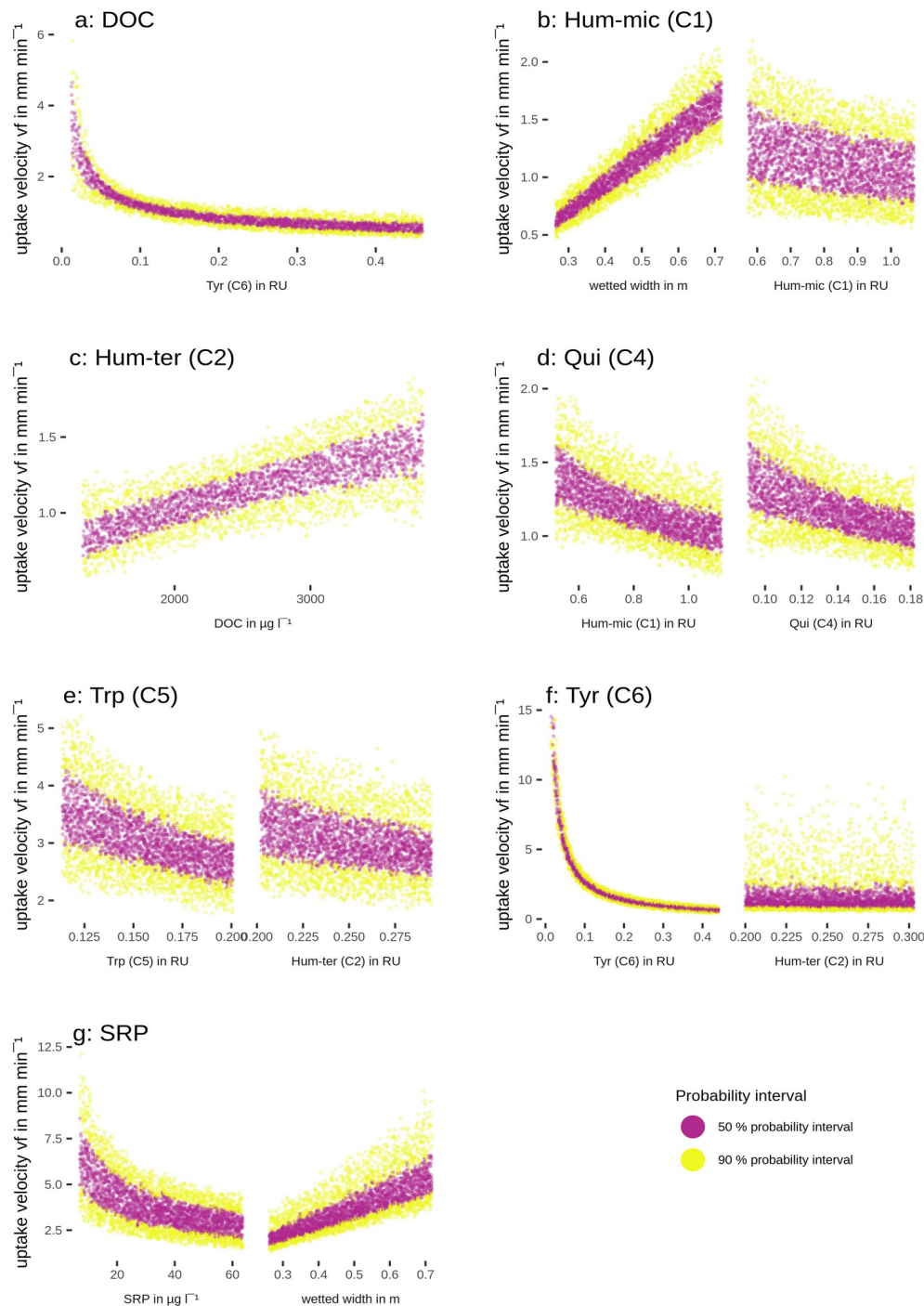
Efficiency loss effects (i.e. decreasing uptake velocities of a component with increasing concentrations or fluorescence of the same component) were observed for SRP, Hum-mic (C1), Qui (C4), Trp (C5), and Tyr (C6) (Table 6, Fig. 7). The uptake velocities of both SRP and Hum-mic (C1) also increased with wetted width. Including wetted width and concentration improved the Hum-mic (C1) model even more than including the addition date (Table 6).

Furthermore, we found several attenuating or stimulating effects among different components. Bulk DOC uptake velocity was lower at higher concentrations of Tyr (C6), although there is strong evidence that the leachate source variable offers a better explanation. Hum-ter (C2) retention was stimulated by higher DOC concentrations and Qui (C4) retention decreased with increasing Hum-mic (C1). The Trp (C5) retention decreased with Hum-ter (C2), but the inclusion of this interaction could not outperform the model with the sampling date included. Tyr (C6) was retained slower with higher fluorescence of Hum-ter (C2). Although the model improved decisively in comparison to the one without interactions, it could not exceed the model with the sampling date in probability. Other than in the Hum-mic (C1) model, the sampling date variable still contained more important information than the interactions found for Trp (C5) and Tyr (C6) uptake velocities. For Hum-mictic (C3) and N-NO<sub>3</sub>, no additional information could be gained from the available data. We found no effects of variable collinearity within the models (Table 6, additional information in Tables S2 and S4).

We analysed correlations between uptake velocities of nutrients and different DOM components to check for concurrent retention, which might indicate interrelations among or dependencies of different microbial metabolic processes, such as, e.g., the combined need of these substances in the microbial metabolism (Table S2). We found substantial evidence that  $v_f$  of Qui (C4) correlated with  $v_f$  of Tyr (C6) and DOC, indicating that the retention of Qui (C4) concurred with Tyr (C6) and DOC.

**Table 6: Interactions between uptake velocity and concentrations of other nutrients or DOM components using the INSBIRE approach.  $v_f$ : uptake velocity, k: uptake rate factor, w: wetted width, C<sub>i</sub>: fluorescence of PARAFAC components, m<sub>i</sub>: exponent of relation, l: additive parameter**

fraction/ nutrient	most probable model (Eq. 1)	Bayes R <sup>2</sup>	BF vs. v <sub>f</sub>	estimates of parameter values
Hum-mic (C1)	$v_f = k w C1^{mc1}$	0.60	16.74	$k = 2.11$ $mc1 = -0.38$
Hum-ter (C2)	$v_f = k (1 + DOC^{mc})$	0.34	7.69	$K = 0.11$ $l = 3.16$ $mc = 0.32$
Hum-micter (C3)	$v_f = v_f$	-	-	-
Qui (C4)	$v_f = k C1^{mc1} C4^{mc4}$	0.44	3.13	$K = 0.71$ $mc1 = -0.25$ $mc4 = -0.35$
Trp (C5)	$v_f = k C2^{mc2} C5^{mc5}$	0.30	3.87	$k = 0.85$ $mc2 = -0.44$ $mc5 = -0.55$
Tyr (C6)	$v_f = k C2^{mc2} C6^{mc6}$	0.45	1.51e7	$k = 0.27$ $mc2 = -0.23$ $mc6 = -0.96$
DOC	$v_f = k C6^{mc6}$	0.28	10.50	$k = 0.30$ $mc6 = -0.62$
NO <sub>3</sub>	$v_f = v_f$	-	-	-
SRP	$v_f = k w SRP^{mp}$	0.63	1.45e4	$k = 26.18$ $mp = -0.31$



**Figure 7: Simulated change of uptake velocity  $v_f$  with variation of one variable using the fitted models from Table 6. The colours show the 50 % (violet) and the 90 % (yellow) percentile intervals.**

### 3.3.4 Brief propagation of uncertainty

390 The simulated probability density of the residuals (Figure S1) was compared to the expected accuracy of the instruments for DOC and SRP. The models depended on three measured values ( $C_{x,t}$ ,  $C_{amb,t}$ ,  $C_{x-1,t}$ ), so we multiplied the instrument errors by 3 to get the effect, their uncertainty would have on the model. For DOC measurements with an accuracy of 2%, three of our concentrations at around  $2000 \mu\text{g L}^{-1}$  would have an approximate effect on the model uncertainty of  $120 \mu\text{g L}^{-1}$ . The 95% probability interval of residuals of the DOC model (mixed model including leachate source) was between  $-172$  and  $131 \mu\text{g L}^{-1}$  and is already close to the error assumed from the measurements. Hence, we do not expect a more sophisticated model to reveal any more details. In contrast, the instrument accuracy for SRP multiplied by 3 was  $0.3 \mu\text{g L}^{-1}$ , and the 95% probability interval of the residuals was between  $-4.74$  and  $4.85 \mu\text{g L}^{-1}$  for the model with wetted width and SRP concentration included in the exponent (Table 6). The higher error of the model compared to the assumed effect of the measurements on the accuracy shows that the model for SRP has still potential for improvement by, e.g., adding meaningful variables not measured in this study or by increasing the number of observations. A similar analysis of the PARAFAC components is not as simple because there is no conventional way of calculating the accuracy of a PARAFAC model's sample loadings.

## 4 Discussion

### 4.1 Uptake of bulk DOC from different sources

The uptake velocity of bulk DOC varied between leachate sources (Figure 5), as was also observed in previous studies (e.g. Bernhardt and McDowell, 2008; Mineau et al., 2016; Mutschlecner et al., 2018). However, in contrast to our original hypothesis, neither a high SRP content nor an increased amount of low-molecular, protein-like compounds could be linked to higher  $v_f$  of bulk DOC (Table 2). Cow and pig dung leachates, for example, had both high peaks of the tryptophan-like component C5, which showed the fastest uptake of all DOM fractions indicating a high bioavailability. Nevertheless, cow dung leachate was taken up slowest, while pig dung leachate was among the leachates with the fastest uptake. Corn leachate showed the highest uptake of all sources, while the uptake velocity of leaf leachate was intermediate. Interestingly, we observed the same sequence of increasing uptake velocities from cow dung leachate to leaf leachate and corn leachate in a laboratory flume experiment using the same organic matter sources as this field study, but different sediments (Weigelhofer et al., 2020). There, however, DOC uptake was positively influenced by the SRP concentrations in the leachates. Comparisons with the literature are difficult, as other field and laboratory studies have used a variety of different organic matter sources. Among those, leaf leachates have been used most frequently so far. Leaf leachates show a wide range of biodegradability, depending on the respective species, the region, the pre-treatment, and the decomposition or leaching stage (e.g. Wickland et al., 2007). Mutschlecner et al. (2018), for example, could demonstrate the stimulating effect of long-term fertilization of trees with phosphorus on the leaf leachate uptake. Reported uptake velocities for leaf leachates range between  $0.002$  to  $7.8 \text{ mm min}^{-1}$ , showing a high variability among leaf sources and aquatic systems (review by Mineau et al., 2016;

420 Graeber et al., 2019). The median  $v_f$  of our leaf leachate was  $2.08 \text{ mm min}^{-1}$  and thus lies within this range. Regarding the other sources used in our study, we only found one addition study using cow manure (Kuserk et al., 1984; uptake velocity calculated in Mineau et al., 2016), showing a median uptake velocity of  $0.31 \text{ mm min}^{-1}$  that was slightly lower than the one observed in our study ( $0.66 \text{ mm min}^{-1}$ ).

## 4.2 Uptake of DOM fractions and nutrients

425 The various DOM fluorophores were retained with different uptake velocities, whereby the velocity density curves partly overlap (Fig. 6, Table 5). Hum-mic (C1), described as product of microbial degradation of terrestrial organic matter, was taken up slowest. Hum-ter (C2; high-molecular, aromatic), Qui (C4; aromatic), and Tyr (C6; tyrosine-like) showed large overlaps and exhibited uptake velocities comparable to the bulk DOC, followed by slightly higher uptake velocities for Hum-micter (C3). As expected, the fastest uptake was observed for the tryptophan-like component C5 (Trp), concordant  
430 with previous studies of different amino acid-like fractions (Findlay and Sinsabaugh, 2003). Several studies report about a high biodegradability of protein-like components, while humic-like, aromatic components proved to be much more refractory (e.g. Fellman et al., 2009a, b; Casas-Ruiz et al., 2017). However, in contrast to C5, the uptake velocity of the tyrosine-like component C6 (Tyr) was only intermediate in our study. This may have been caused by the release of Tyr (C6) as degradation product of humic substances during the experiment (Stevenson and He, 1990; Tsutsuki and Kuwatsuka,  
435 1979). Other studies also report the generation of protein-like components during passage through the system, due to either the release of algal exudates or the decomposition of humic substances (Casas-Ruiz et al., 2017; Weigelhofer et al., 2020).

The uptake of  $\text{N-NO}_3$  was the lowest of all components due to its high background concentrations in the water column exceeding even those of the ambient DOC (Fig. 4). In contrast, the co-leached SRP showed the highest uptake velocity (together with Trp, C5). An equally fast uptake was observed in the flume experiments, especially in the presence of algae  
440 (Weigelhofer et al. 2020). Despite the low to moderate background concentrations of P in the stream water (Fig. 4), background molar C:P ratios in the water column of our study stream were usually below 80:1, displaying an ideal ratio for a huge number of different bacterial strains (Cross et al., 2005; Godwin and Cotner, 2018). The C:P ratios were even decreased by the additions to  $< 30:1$  at point 1, followed by an increase to background ratios in the downstream sections. While stoichiometry has been shown to be a key factor for C, N, and P uptake (e.g. Cross et al., 2005; Gibson and O'Reilly,  
445 2012; Stutter et al., 2020), we do not believe that stoichiometric control played a large role in the P uptake in our study stream. Rather, we assume that the co-leached P was taken up faster than the DOC due to the demand of both bacteria and algae (Oviedo-Vargas et al., 2013; Weigelhofer et al., 2020). In general, the bioavailability of a fraction is not only depending on the chemical composition, but also on the ecosystem and the involved microbial community (Kamjunke et al., 2015), the overall availability of different fractions and nutrients (Berggren and Giorgio, 2015; Bernhardt and McDowell,  
450 2008; Mutschlecner et al., 2018) and transport characteristics (Ejarque et al., 2017). We performed the experiments in a small homogeneous stretch of a stream and already found considerable variability in DOM fluorophore-specific uptake between sampling dates. Thus, we propose that the bioavailability of DOM fractions and/or different DOM sources should



be determined under in-situ conditions in different stream reaches, seasons, and under different environmental conditions in addition to laboratory incubations to determine the effective biodegradability range of the respective components.

#### 455 4.3 Relationships between uptake and concentrations of other compounds

In contrast to our assumptions, we found no influence of the co-leached SRP on the bulk DOC uptake, although there is evidence in other studies that DOC uptake can be stimulated by P especially in P-limited systems (Mutschlecner et al., 2018; Stutter et al., 2020). However, as the molar ratios of C:P were low in our stream, showing no P limitation, and we also did not raise the SRP concentrations in our stream additionally to the P content of the leachates, SRP-related effects on DOC  
460 retention might have stayed uncovered. Although the source-independent model showed a relationship between the bulk DOC uptake velocity and the Tyr (C6) fluorescence, the mixed effects model including the leachate source performed much better. This indicated that, apart from the fluorescence of Tyr (C6), other, probably non-fluorescent, components influenced the bulk DOC uptake, which we could not detect with our methods. Almost all DOM fractions showed a negative relationship between uptake and concentration of the same component. Lower uptake velocities with increasing  
465 concentrations have been previously described for nitrogen (Dodds et al., 2002; O'Brien et al., 2007). This efficiency loss can be explained by the processing capacity of the stream ecosystem, which is influenced by adaptations of the microbial community to usually occurring concentrations (Fasching et al., 2020; Tihomirova et al., 2012) and potential transport limitations between the source and the reactive sites (Weigelhofer et al., 2018a, b; Teissier et al., 2007; Ribot et al., 2013). Hum-mic (C2), Hum-micter (C3), and DOC retention showed no evidence of efficiency loss (BF was around 1) at the  
470 measured concentrations, indicating that the microbial community would have been able to retain more of these substances without a decline in uptake velocity. So far, we have not found any other studies presenting efficiency loss for DOM fractions.

Additionally, we observed both positive and negative interactions among different DOM fractions, with higher/lower uptake velocities of one component at increased concentrations of another component. These interactions can have different reasons  
475 and are, therefore, difficult to interpret. Positive effects on  $v_f$  can arise, e.g., from the stimulation of the uptake of one substance by the presence of another through priming (but see critical discussion in Bengtsson et al., 2018). Direct negative effects can be caused by the preferential uptake of one fraction over another (Brailsford et al., 2019) or inhibitory effects between different substances (Freeman et al., 1990). Furthermore, the degradation of DOM can cause one molecule to break down into others, causing an increase of the degradation product, while the degraded component decreases (Kamjunke et al.,  
480 2017).

In our study, the retention of Qui (C4) was lower at higher Hum-mic (C1) fluorescence. The molecular structures found in the literature (Stevenson and He, 1990; Tsutsuki and Kuwatsuka, 1979) suggest that Qui (C4) is a product of the Hum-mic (C1) degradation, resulting in a reduced net retention due to simultaneous production and degradation processes. Similarly, Trp (C5) and Tyr (C6) might have been degradation products of Hum-ter (C2). In contrast, Hum-ter (C2) degradation was  
485 stimulated by high DOC concentrations, probably due to the supply of energy in the form of carbohydrates or other essential

components needed for degradation (Bengtsson et al., 2018; Bianchi et al., 2015). We also saw a weak probability that the uptake velocity of Hum-ter (C2) was stimulated by Qui (C4, BF = 1.9) and Tyr (C6, BF = 1.8).

We found substantial evidence that Qui (C4) was degraded simultaneously with Tyr (C6) and bulk DOC. Good degradation conditions, such as low transport limitation (Weigelhofer et al., 2018b), ideal stoichiometric ratios for microbial metabolism (Cross et al., 2005; Godwin and Cotner, 2018; Stutter et al., 2018), or stretch-wise more productive microbial communities, can foster simultaneous turnover (Guillemette and Giorgio, 2012). We consider concurrent degradation as well as negative and positive interactions to be essential characteristics of the complex DOM degradation processes. Using the INSBIRE approach in future experiments may help to elucidate, which of the proposed mechanisms is responsible under certain environmental conditions.

Our models also revealed some hydromorphological effects on DOM fluorophore and nutrient uptake. The wetted width could partly explain the uptake of Hum-mic (C1), and SRP, probably due to sorption playing a role in the retention of these compounds. The adsorption of humic substances to clay is generally strong when the ionic strength is high (Theng, 2012). The conductivity around  $630 \mu\text{S cm}^{-1}$ , which was measured during the experiment, as well as the clay-dominated sediments offered good conditions for adsorption (Theng, 2012). The role of the sediment surface in the uptake of solutes is not surprising as such and has been observed elsewhere (Romani et al., 2004; Sabater et al., 2002; Battin et al., 2016). However, the component-specific influence of wetted width suggests that different DOM components are preferably taken up in different stream compartments. Unlike the common assumption that uptake processes are dominated by the benthic community (Battin et al., 2016; Wiegner et al., 2005), Graeber et al. (2018) and Kamjunke et al. (2015) proposed a potentially important impact of planktonic bacteria on in-stream DOM uptake processes. In our study stream, such planktonic uptake might be dominating for the uptake of most DOM fractions except Hum-ter (C1), where the substantial influence of wetted width indicates the importance of the benthic community.

#### 4.4 Potential and limitations of the INSBIRE approach

The INSBIRE approach was developed after the data from the experiment was acquired due to limitations in other data analysis methods developed for inorganic nutrient uptake (Stream Solute Workshop, 1990), such as the lack of a strategy to handle interactions among DOM components. Thus, our study represents a case study for the application of INSBIRE in the analysis of DOM uptake, but does not claim to be a systematic check of the developed approach. Using INSBIRE for our experimental data could reveal positive and negative interactions among different DOM fractions, that has not been done in such detail so far. We could also get some insights into the method, especially information about the potential, but also the limitations. Nevertheless, an application under controlled laboratory conditions is still open to thoroughly test the INSBIRE approach.

The underlying concepts, such as nutrient spiralling (Stream Solute Workshop, 1990) and Bayesian statistics, have been investigated and developed for at least some decades. With this available knowledge, it was possible to develop the approach on a solid theoretical basis and with already existing concepts and algorithms. INSBIRE can be adapted by changing the

underlying equations, using different solving schemes, and using different kinds of data. We used fluorescence measurements to determine the DOM quality, but INSBIRE is capable of incorporating any other data of different solvents (e.g. toxins or pesticides) and methods (e.g. mass spectroscopy, liquid chromatography). The power function has proven useful in our study, but the approach facilitates the use of other equations if suited better for the respective case. Due to the formal description of the uptake processes, extrapolations to different ambient or event-related concentrations can be done (Payn et al., 2005).

The presented plots of the  $v_f$  posterior density curves are intuitive to interpret and can help in our understanding and perception of the retention processes, in particular their variability. The presentation in form of probability distributions rather than single values corresponds to the experience that ecosystems are inhomogeneous while still assessable (McCarthy, 2007). For further studies, these posterior density curves can be directly used as prior information for similar models.

The Bayesian nature of the analysis allows us to evaluate even weak relations, that can be tested in further experiments. Also, we could show the limitation of the bulk DOC retention model due to the accuracy of the measurements and the heterogeneity of the measured molecules.

When a small number of observations is available, but the general knowledge about a topic is profound, it is possible to include data from previous studies as well as expert knowledge by means of non-conservative prior densities of the parameters. Then, results can be more precise and decisions can be based on both measured data and other available knowledge (Kuhnert et al., 2010; Lemoine, 2019). Even a low number of observations may show certain trends in DOM uptake (Fig. 5), which might be especially useful for monitoring or management decisions.

## 5 Conclusion

Human impacts, such as agricultural land use or wastewater discharges, have changed the quantity and composition of terrestrially derived DOM in stream ecosystems. Our study demonstrates that in-stream DOM uptake is source-depended and, thus, influenced by DOM quality, although we did not observe any significant correlations between bulk DOC uptake and those of DOM components, such as co-leached nutrients or specific fluorophores. One reason for this lack of correlation could be that DOM uptake comprises a variety of simultaneously or sequentially occurring microbial degradation and production processes. The presented INSBIRE approach provided evidence for positive and negative effects among the uptake of different DOM components, which indicate transformations of one substance into another during processing. Besides, identification of different DOM components via spectroscopic characterization may be too imprecise to reveal the influence of DOM components on DOM uptake, either because different molecules show similar fluorescent peaks or because of non-fluorescent components influencing bulk uptake. Thus, further studies on DOM processing under controlled conditions are required which identify important molecular groups, such as, amino acids, sugars, or humic acids, more accurately.

550 Our study also shows that the uptake of bulk DOC, but also that of specific DOM components may be subject to efficiency loss, so far only known from nutrient uptake. This means that the uptake efficiency declines with increasing concentration of the respective component. However, individual DOM components were not equally affected by efficiency loss or interactions with other components, indicating that the component-specific uptake capacity of benthic biofilms may depend on the respective microbial processes involved. Further studies need to look more closely into the underlying mechanisms of both efficiency loss and influences between components during DOM processing in aquatic ecosystems. Our study also demonstrates that the cycling of different C fractions and their mutual interaction with N and P uptake in streams is a complex, non-linear problem, which can only be assessed adequately with advanced non-linear approaches. Here, the developed INSBIRE approach may help to find concurrent retention and interactions of DOM components, thus providing an efficient tool for the analysis and the management of organic carbon cycling in aquatic systems affected by human impacts.

### **Code availability**

The codes necessary for applying the INSBIRE approach can be downloaded from <https://doi.org/10.5281/zenodo.4071851> (Pucher, 2020).

### **565 Author contribution**

Conceptualization: MP and GW; formal analysis: MP; funding acquisition: GW; investigation: MP, PF; methodology, MP, GW, KF, PF; project administration: GW; software: MP; supervision: TH, KF; validation: DG; writing – original draft and preparation: MP, GW; writing – review and editing: all co-authors.

### **Competing interests**

570 The authors declare that they have no conflict of interest.

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