Behind the Scenes: Mechanisms Regulating Climatic Patterns of Dissolved Organic Carbon Uptake in Headwater Streams


Abstract Large variability in dissolved organic carbon (DOC) uptake rates has been reported for headwater streams, but the causes of this variability are still not well understood. Here we assessed acetate uptake rates across 11 European streams comprising different ecoregions by using whole-reach pulse acetate additions. We evaluated the main climatic and biogeochemical drivers of acetate uptake during two seasonal periods. Our results show a minor influence of sampling periods but a strong effect of climate and dissolved organic matter (DOM) composition on acetate uptake. In particular, mean annual precipitation explained half of the variability of the acetate uptake velocities ($V_f$acetate) across streams. Temperate streams presented the lowest $V_f$acetate together with humic-like DOM and the highest stream respiration rates. In contrast, higher $V_f$acetate were found in semiarid streams, with protein-like DOM, indicating a dominance of reactive, labile compounds. This, together with lower stream respiration rates and molar ratios of DOC to nitrate, suggests a strong C limitation in semiarid streams, likely due to reduced inputs from the catchment. Overall, this study highlights the interplay of climate and DOM composition and its relevance to understand the biogeochemical mechanisms controlling DOC uptake in streams.

Plain Language Summary Headwater streams receive and degrade organic carbon and nutrients from the surrounding catchments. That degradation can be assessed by measuring the uptake of simple compounds of carbon or nitrogen such as acetate or nitrate. Here we determine the variability in acetate and nitrate uptake rates across headwater streams and elucidate the mechanisms behind that variability. The balance between nutrients, the composition of the organic materials present in the streams, and the climatic background is at interplay.

1. Introduction Headwater streams receive large organic matter subsidies from their adjacent terrestrial ecosystems, subsidies that are essential resources for headwater streams’ food webs (Fisher & Likens, 1972; Vannote et al., 1980). Terrestrially derived dissolved organic carbon (DOC) is actively processed in streams, and only less than...
half of the inputs are exported to the ocean, due to the storage in sediments or emissions to the atmosphere (Cole et al., 2007; Tranvik et al., 2009). Although the processing of DOC along fluvial networks has received attention due to its relevance for the global C cycling, the magnitude of the DOC biodegradation processes and the main drivers controlling their variation remain unclear.

The study of DOC processing is intimately linked to the pool of dissolved organic compounds in the water. Indeed, stream dissolved organic matter (DOM) is a mixture of thousands of molecules with different origin and reactivity (Mosher et al., 2015; Sleigher et al., 2014) that shapes the structure and function of microbial communities inhabiting streams (Freixa et al., 2016; Ruiz-González et al., 2015; Wymore et al., 2016). The complexity of DOM composition challenges the methodological approach to study in-stream DOC processing. The most common techniques used to study in-stream DOM biological processing are as follows: laboratory bioassays (Catalán et al., 2016), whole-stream ecosystem metabolism (i.e., by monitoring and modeling the change in oxygen concentrations; Hall & Tank, 2003), and mass budget approaches (i.e., by quantifying DOC inputs and outputs in a given reach; Butturini et al., 2016; Casas-Ruiz et al., 2017; Fisher & Likens, 1972). However, lab bioassays and mass budget approaches do not really integrate the longitudinal flow of DOC (Fisher et al., 2004). Stream metabolism relies on uncertain respiratory quotients for the conversion of oxygenic into carbonic metabolic rates (Berggren et al., 2012), and most importantly, it is extremely hard to determine the fraction of ecosystem respiration corresponding to DOC uptake. Alternatively, metrics describing uptake of a specific solute at reach scale have been proven useful to assess the biological processing of nitrogen and phosphorous in streams (e.g., Peterson et al., 2001). For DOC, reach-scale uptake metrics are usually calculated based on the uptake of simple, highly bioavailable organic molecules such as acetate, sugars, or amino acids (see Mineau et al., 2016, for a review). The use of these simple compounds might result on a magnified response when compared with the uptake of ambient DOC but allows for standardization, comparison across systems, and studies and informs on how reactive a system is in response to the in-stream DOC availability (Bernhardt & Likens, 2002; Mineau et al., 2016; Newbold et al., 1982).

Mechanisms affecting DOC processing at different temporal and spatial scales also hamper our quest to appraise controls on DOC processing (Lisboa et al., 2016; Mineau et al., 2016). For instance, seasonality is likely to influence stream DOC uptake, as seasonal variability is tightly related to discharge. Discharge variability has strong effects on stream water chemistry, DOM concentration, and sources (Bernal et al., 2005; Hood et al., 2006) and modifies the water residence time of drainage networks, which is a key factor controlling DOC reactivity and processing (Casas-Ruiz et al., 2017; Catalán et al., 2016). Local environmental conditions, such as nutrient availability or DOM composition, are also likely to influence DOC processing. The few studies examining the influence of nitrogen availability on DOC uptake metrics have reached contrasting results (Bechtold et al., 2012; Johnson et al., 2009; Marti & Sabater, 2009; Mutschlechner et al., 2017). Furthermore, research assessing the effects of ambient DOM composition on DOC uptake is even scarcer (Mineau et al., 2016; but see Lutz et al., 2012). Similarly, the relationship between stream metabolism and DOC uptake at the reach scale is still poorly explored. To our knowledge, only one study has found a link between stream DOC uptake velocity and ecosystem metabolic rates (Newbold et al., 2006), showing a positive relationship between uptake and respiration.

At large spatial scales, the effect of climatic factors on DOC processing might encompass both terrestrial inputs into the stream and their posterior processing. For example, mean annual precipitation (MAP) has been shown to determine the organic carbon content of terrestrial soils (Craine et al., 2015) and litter fall inputs to streams (Benfield, 1997) at the global scale. MAP is also a main factor explaining differences in stream metabolism across biomes (Dodds et al., 2015; Pastor et al., 2017). In a comprehensive review, Mineau et al. (2016) identified climatic factors, particularly MAP, as a significant predictor of DOC uptake velocities across the published studies. Still, the underlying mechanisms driving this relationship are not yet fully understood.

In this study, we aimed to fill some of these knowledge gaps by examining uptake rates of DOC (as acetate) across streams located at distinct ecoregions (sensu Olson et al., 2001), as well as other functional metrics that can control or explain DOC uptake rates in streams (i.e., metabolism and nitrogen uptake). We assessed molar ratios of DOC: NO₂ and between their uptake rates to evaluate energy and nutrients limitations and characterized ambient DOM composition to bridge the gap between DOM composition and expected bioavailability and in-stream processes. We used slug additions of acetate, a simple highly bioavailable organic compound, to obtain a standardized functional metric on DOC uptake and compare the metrics among
sampling sites and previous studies. We assessed the variability in acetate uptake rates across 11 European headwater streams spanning five ecoregions using pulse acetate additions by means of a coordinated distributed experiment (Fraser et al., 2013). The streams were sampled during two periods of expected contrasting flow conditions (i.e., summer and fall). We predicted that acetate uptake would vary among climatic regions and sampling periods, linked to the ambient DOM composition. We expected that acetate uptake will be negatively linked to discharge and positively coupled to nitrate uptake rates and ecosystem respiration at the reach scale.

2. Materials and Methods

2.1. Study Sites

We sampled 11 headwater streams located across a wide geographical area in Europe (Figure 1), during summer and fall 2014. Stream discharge ranged from 2.9 to 31.5 L/s (Table 1) and the ecological status of the riparian zones was acceptable to very good according to the riparian forest quality index ([QBR] = 55–95; Munné et al., 2003). We selected representative reaches that ranged from 40- to 90-m length and received no lateral hydrological inputs. Streams comprised five ecoregions (Olson et al., 2001; Figure 1). Two streams corresponded to Cantabrian mixed forest (CMF), two to Iberian conifer forest (ICF), Mediterranean forest (MF), two to European broadleaf forest (EBF), and one to Pyrenean conifer and mixed forest (PCMF). MF and ICF are characterized by hot and dry summers and mild winters. CMF and PCMF are both situated between the Euro-Siberian and Mediterranean regions of Europe. While CMF sites present warm Atlantic conditions, with mild temperatures and high precipitation, PCMF is characterized by a colder and drier weather. Finally, a temperate humid climate predominates at EBF all yearlong. The stream selection was the result of a collaborative project, the first coordinated distributed experiment exclusively leaded and conducted by early-career researchers. Stream reach selection was performed by each site group under the supervision of the project coordinators. The requested characteristics of the streams, detailed in the protocol, were the following: headwater streams, low discharge (<100 L/s, which allows for accuracy in the slug addition), and channel not affected by morphological alterations. Stream were aimed to be sampled at basal discharge conditions (<3 days after a high-flow event). The protocol used to coordinate all the sampling is available

Figure 1. Location of study streams (see Table 1) and their corresponding ecoregions across Europe. Acronyms in the legend correspond to Pyrenean conifer and mixed forested (PCMF), Cantabrian mixed forests (CMFs), Iberian conifer forest (ICF), Mediterranean forest (MF), and European broadleaf forests (EBF).
We characterized the catchment and climatological attributes of the sampled streams using both the virtual watershed approach by means of the NestStream software (National Geographic Institute; Álvarez-Cabria et al., 2016; Benda et al., 2007) and data from different public databases (SIMPA and Occupation Information system of Soil for Spain; Corine Land Cover 2006 and Deutscher Wetterdienst for Germany; Swiss land use data and SwissMetNet for Switzerland). MAP and mean annual catchment temperature were also obtained from public databases (AEMET, Deutscher Wetterdienst, and SwissMetNet). Further details can be found in Pastor et al. (2017).

2.2. Field and Laboratory Work

Sampling campaigns were performed simultaneously and using a uniﬁed protocol. We quantiﬁed whole-reach nitrogen and acetate uptake using pulse releases (Martí & Sabater, 2009) of potassium nitrate (KNO3) and sodium acetate (NaCH3COO). Acetate (CH3COO−/CO2) is a ubiquitous low-molecular weight compound naturally found in streams and is readily used by stream microorganisms (Berggren et al., 2010; Johnson et al., 2009; Mineau et al., 2016). For each pulse release, we prepared a solution of NaCH3COO, KNO3, and salt (NaCl; used as conservative tracer), adjusting the concentration of each solute to the discharge of each stream and sampling period. Discharge was determined through a NaCl addition the day prior to the experimental DOC and N addition (Gordon et al., 2004). The solution was released in a single pulse at the upper end of the reach where turbulence ensured adequate mixing with the stream water. At the downstream station, conductivity changes were recorded and water samples collected at different time intervals according to the conductivity breakthrough curve measured a priori, summing up 30 ± 2 samples per experiment. All water samples were filtered in the ﬁeld through preashed glass ﬁber ﬁlters (Whatman GF/F, nominal pore of 0.7 μm) and kept frozen until laboratory analyses. Two sites (GER and MAU; Table 1) could not be visited during fall, and samples for the addition of acetate for REI in summer and of NO3−/CO2 for MAU in summer and for REI in fall were lost.

At each sampling, stream water temperature (T, in degrees Celsius), pH, and conductivity (EC, in microsiemens per centimeter) were measured in situ with hand probes. Dissolved oxygen concentrations (milligrams per litter) were recorded over at least one daily cycle to obtain metabolic parameters (i.e., gross primary production and ecosystem respiration, see Pastor et al., 2017, for details). We calculated speciﬁc discharge both as discharge by average reach width (Q/w) and as discharge by catchment area (Q/A).

Water samples were analyzed for acetate and NO3− using ion chromatography (Metrohm IC system 883 Basic IC Plus ﬁtted with a Metrosep A Supp 4/5 guard column and a Metrosep A Supp 5 analytical column).
Concentrations of $\text{NH}_4^+$ were determined manually by the salicylate-nitropruside method (Baethgen & Alley, 1989) using a PharmaSpec UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Dissolved inorganic nitrogen (DIN) was determined as the sum of $\text{NO}_3^-$ and $\text{NH}_4^+$. DOC concentrations were analyzed by oxidative combustion infrared analysis on a Shimadzu TOC-VS (Shimadzu Corporation, Kyoto, Japan) on preacidified samples (HCl, final sample pH = 2–3).

### 2.3. Spectroscopic Analyses for DOM Composition and PARAFAC Modeling

DOM composition in streams was characterized using spectroscopic methods. Absorbance spectra (200 to 800 nm) were measured at 1-nm intervals with a Lambda 40 spectrophotometer (Perkin-Elmer, Waltham, USA). Samples were measured in a 1-cm quartz cuvette, and Milli-Q water was used as blank. Absorption coefficients ($\alpha_\lambda$) were determined using the following equation:

$$\alpha_\lambda = \frac{2.303 \times A_\lambda}{l}$$

where $A_\lambda$ is the absorbance measurement and $l$ the path length in meters. The slopes ($S$) of the spectra at different wavelength ranges were obtained by nonlinear fitting of the exponential curve (Stedmon et al., 2000). The slope ratio $S_{275-295}/S_{350-400}$ (Helms et al., 2008) inversely related with molecular weight was calculated. The specific ultraviolet absorbance (SUVA) was calculated as the absorbance at 254 nm ($A_{254}$) normalized by DOC concentration (L·mg C$^{-1}$·m$^{-1}$; Weishaar et al., 2003). Colored DOM ($a_{440}$) was quantified as the absorption coefficient at 440 nm (Kirk, 1994). Fluorescence excitation-emission matrices (EEMs) were obtained using a spectrofluorometer (SPEX Fluoromax-4, Horiba Jobin Yvon). Excitation wavelengths ranged from 250 to 445 nm at intervals of 5 nm and emission wavelengths from 300 to 600 nm at increments of 4 nm. A Milli-Q blank was run the same day and subtracted from each sample to eliminate Raman scattering. The area underneath the water Raman scan was used to normalize sample intensities to Raman units. Correction factors supplied by the manufacturer were used to correct for instrument-specific biases and the absorbance-based approach was used to correct for the inner filter effect (Kothawala et al., 2013; McKnight et al., 2001). Corrections were applied using the FDOMcorr toolbox for MATLAB (Mathworks, Natick, MA, USA) according to Murphy et al. (2010). The fluorescence index (FI), a proxy for DOM origin with higher values related to labile, algal-, or microbial-like sources (Jaffé et al., 2008), the biological index, positively related with biological activity (BIX; Huguet et al., 2009), and the humification index, indicator of the DOM humification status (HIX; Ohno, 2002) were also calculated.

Parallel Factor Analysis (PARAFAC) was used over 59 samples to identify the main components of the EEMs (Stedmon et al., 2003). The analysis was performed in MATLAB using the DrEEM toolbox (Murphy et al., 2013). Accordingly, scatter peaks and outliers were removed, and each sample normalized to its total fluorescence prior to fitting a PARAFAC model. The appropriate number of components was determined by visual inspection of the residual fluorescence and of the components behavior as organic fluorophores (Lakowicz, 2006; Murphy et al., 2013). The model was then validated by split-half analysis and random initialization with 10 iterations. Three PARAFAC components (C1, C2, and C3; Figure S1) were found to provide a robust description of DOM fluorescence within the data set. We report PARAFAC components both in Raman units and their relative abundance (e.g., $\%$ C1 = $C1/(C1 + C2 + C3)$). The results of the PARAFAC model were queried (Tucker's...
congruence coefficient = 95%) in the OpenFluor database (http://www.openfluor.org), in order to search for quantitative matches with previously published and validated PARAFAC models (Murphy et al., 2014). Components C1 and C2 correspond to humic-like materials and C3 to protein-like fluorescence (Tables S1 and S2 and Figure S1 in the supporting information).

2.4. Calculations of OC and N Uptake Metrics

We estimated the uptake rate coefficient \( K_t \) (in per second) using a mass balance between the N (N-KNO\(_3\)) or C-acetate (C-CH\(_3\)CO\(_2\)^-) mass released into the upstream station \( (M_i \) in milligrams of C) and the mass exported at the downstream station of the study reach \( (M_d \) in milligrams of C):

\[
K_t = \frac{\ln \left( \frac{M_i}{M_d} \right)}{t}
\]

where \( t \) is the time taken for the concentration to arrive at the downstream end of the reach. The masses were obtained from the integration of the concentration curves (Álvarez et al., 2010). From \( K_t \), we then calculated the uptake length \( (S_w, \) in meters), the uptake velocity \( (V_f, \) in millimeters per minute), and the areal uptake rate \( (U, \) in milligrams per square meter per second) of acetate and N-NO\(_3^-\) as:

\[
S_w = \frac{v}{K_t}
\]

\[
V_f = \frac{h \cdot v}{S_w}
\]

\[
U = C_b V_f
\]

where \( v \) is the water velocity (m/s), \( h \) is mean depth (m), and \( C_b \) is the natural background concentration of the added solute (mg/L).

2.5. Statistical Analysis

Statistical analysis were performed according to these points: (a) to test for differences between periods or ecoregions and (b) to determine the descriptors associated with uptake metrics and within those and particularly the DOM composition.

In order to evaluate the temporal variability across the set of streams, differences on uptake metrics between periods were assessed through paired tests. As variables presented a nonnormal distribution, we applied both a Wilcoxon signed rank test using function \texttt{wilcox.test} and paired \( t \) tests on log-transformed metrics (i.e., for confirmation of the results obtained with the first) both from R package \texttt{stats}. Because we did not observe statistical differences between the two sampling periods with any of the methods, we grouped data from both periods to run subsequent analyses. The collaborative nature of this work led to an uneven distribution of the sites within ecoregions, with \( n = 2 \) in two regions (PCMF and EBF), preventing us from applying statistical tests for region as factor.

To initially examine (i) the correlation between biogeochemical, geomorphological (i.e., stream and catchment descriptors) and climatic variables and (ii) the association between metabolic rates, Spearman’s correlation tests were computed (\texttt{cor} function from R package \texttt{stats}) and plotted (\texttt{corrplot} function from R package \texttt{corrplot}). Correlations were considered statistically significant when \( p < 0.05 \). Based on these results, ordinary least squares regression analysis was performed to illustrate the relationships between the metrics of organic carbon (OC) uptake and the most influential climatic (mean annual catchment temperature and MAP), functional (NO\(_3^-\) uptake, metabolism metrics and molar ratio DOC: NO\(_3^-\)) and reach-scale (nutrients concentrations and specific discharge) descriptors. Variables were log transformed when necessary to avoid skewed distributions, and the assumptions for general linear models were checked by inspection of diagnostic plots and tests and met in all the cases. The differences between the linear models relating MAP and \( V_f_{Acetate} \) for this study and the reviewed data in Mineau et al. (2016) were tested by bootstrapping the differences between the parameters of the two models (Davison & Hinkley, 1997) using package \texttt{boot} for R.
In order to assess DOM composition control on acetate uptake, a redundancy analysis (RDA) was performed using the `rda` function in R package `vegan` (Oksanen et al., 2017). RDA seeks a series of linear combinations of the explanatory variables (i.e., the DOM spectroscopic descriptors: SUVA, \(S_{350-600}\), BIX, FL, \(S_R\), HIX, \(a_{440}\), C1\%, C2\%, and C3\%, and DOC concentration) that best explain the variation in the response matrix (i.e., acetate uptake metrics; Borcard et al., 2011). Multicollinearity of variables was inspected by computing the variance inflation factor (VIF) and variables with VIF > 10 were excluded (i.e., fluorescence components C1 and C2). A forward selection of the explanatory variables was performed based on an initial RDA model in order to obtain a most parsimonious model (function `ordistep` in R package `vegan`; Borcard et al., 2011). Prior to this analysis, \(V_{\text{acetate}}\), \(U_{\text{acetate}}\), \(K_{\text{acetate}}\), and \(S_{\text{acetate}}\) data were log transformed in order to fit the model assumptions and the explanatory variables scaled and centered. All statistical tests were performed in R 3.3.2 (R Core Team, 2017).

## 3. Results

### 3.1. Stream Characteristics and Variability in DOC and N Uptake Metrics

Discharge varied 1 order of magnitude (2.9–31.5 L/s) across streams but did not change consistently between summer and fall, whereas water temperature tended to decrease during fall (Table 1). DIN concentrations spanned a wide range among streams, from the lowest concentration at the high-altitude mountain stream (PAU: 178 μg N/L) to the highest concentration at a Swiss stream (MAU: 9480 μg N/L; Table 1). The DOC concentrations were relatively low across streams (from 0.8 to 2.9 mg C/L).

Uptake metrics presented similar patterns between \(V_{\text{acetate}}\), \(U_{\text{acetate}}\), and \(K_{\text{acetate}}\) or the opposite in the case of \(S_{\text{acetate}}\), as is to be expected (Table S3 and Figure S2). To report the results, we have focused on \(V_{\text{acetate}}\) as it also corrects for differences in discharge and normalizes for solutes concentrations, facilitating cross-site comparisons. The other metrics are highlighted when providing additional insight. \(V_{\text{acetate}}\) spanned from 0.31 mm/min in the alpine stream (PAU) in fall to 7.9 mm/min in the most meridional stream in summer (BLA; Figure 2 and Table S3). The drier ecoregions (i.e., ICF and MF) presented higher \(V_{\text{acetate}}\) rates than humid regions (i.e., PCMF, CMF, and EBF; Figure 2a). \(V_{\text{acetate}}\) did not significantly differ between sampling periods (Wilcoxon-\(W = 23\), \(p = 0.55\); t test: \(t (7) = 0.687\), \(p = 0.51\); Figure 2b).

\(V_{\text{NO}_3}\) varied widely among streams from 0.4 to 21.1 mm/min (Table S4), and it did not present significant differences between sampling periods (\(W = 15\), \(p = 0.74\)). \(V_{\text{NO}_3}\) was not related to DIN or NO\(_3^--\) concentrations (Figure S2, \(p = 0.83\)).
3.2. Factors Controlling the Variability of Acetate Uptake Across Streams

MAP explained most of the variability of $V_{f_{\text{Acetate}}}$ across sites ($r^2 = 0.52$, $p < 0.01$; Figure 3a), and no differences were found between the intercepts or the slopes of this model and the one derived from Mineau et al. (2016) data (Table S5).

Acetate uptake did not vary uniformly with instantaneous discharge (Q) but specific discharge by catchment area (Q/A) was inversely related to $V_{f_{\text{Acetate}}}$ ($r^2 = 0.39$, $p < 0.01$, Figure 3b).

$V_{f_{\text{Acetate}}}$ was not correlated to DOC concentrations (Figure S2; $\rho = 0.24$, $p > 0.05$) but was positively related to DIN (Figure S3; $r^2 = 0.44$, $p = 0.003$) and negatively related to the stoichiometric ratio between DOC and NO$_3^-$ (DOC:NO$_3^-$; $r^2 = 0.45$, $p < 0.01$, Figure 4b).

$V_{f_{\text{Acetate}}}$ was lower than $V_{f_{\text{NO3}}}$ when pooling the data across sampling times (Figure 4a), although we did not find a significant relationship between both uptake velocities ($r^2 = 0.035$, $p > 0.05$). Mass ratios of acetate and N uptake rates ($K_{\text{Acetate}}:K_{\text{NO3}}$) ranged between 0.12 and 6.7, with only streams from ICF and MF regions presenting median $K_{\text{Acetate}}:K_{\text{NO3}} > 1$ (Figure 4c). Regarding metabolism, ecosystem respiration was negatively related to $V_{f_{\text{Acetate}}}$ (Figure 5), but no relationship was found between any of the acetate uptake metrics and gross primary production (Figure S2).

The RDA identified a direct link between ambient DOM composition and acetate uptake metrics (Figure 6). The first two RDA axes explained together 66.9% of the variance (RDA1 = 63.6%; RDA2 = 3.3%), with an $R^2_{\text{adj}} = 44%$. The percentages of accumulated constrained eigenvalues of the first and second axes explained 40.7% and 2.1%, respectively. A permutational test of these results by axis (function anova.cca) showed that RDA1 was statistically significant ($F = 28.7$, $df = 1$, $p = 0.034$) but not RDA2 ($F = 1.49$, $df = 1$, $p = 0.94$). The forward selection of variables indicated that FI was the DOM descriptor explaining most of the variance in the uptake metrics ($R^2_{\text{adj}} = 27%$), but the full model is shown here to have a better overview on the links between DOM composition and uptake metrics. Accordingly, RDA1 separated on its positive side $V_{f_{\text{Acetate}}}$, $K_{\text{Acetate}}$, and $U_{\text{Acetate}}$ explained by FI, BIX, and $S_B$ and related with sites from the ICF and MF ecoregions. On its negative side, $S_{W_{\text{Acetate}}}$ appears related to high DOC concentration, $a_{440}$, and HIX and to the CMF, EBF, and PCMF sites.

4. Discussion

The results of our study reveal different mechanisms controlling DOC uptake (as acetate) across the studied ecoregions. The link between uptake and MAP sets the context to discuss stoichiometric and DOM composition factors that ultimately drive uptake DOC rates.
4.1. Acetate Uptake Across Seasons and Ecoregions

Selected stream locations within this study covered five European ecoregions. Consistently with this broad geographical gradient, we found a wide range of variability on acetate uptake metrics, which corresponded to 25% of the variability found for worldwide streams in a recent review on stream DOC uptake (Figure 3a; Mineau et al., 2016).

We hypothesized that acetate uptake would be influenced by the seasonal regime due to changes in DOC and nutrients inputs and discharge patterns. Although we did not find differences between summer and fall for $V_f$Acetate, we postulate that the link of acetate uptake with discharge comes modulated by the climatic pattern and as such, is not conspicuous. Specific discharge by catchment area ($Q/A$) is considered an indirect descriptor of climatic variability, as streams in arid regions typically have larger catchment areas than streams in humid regions for the same discharge (Álvarez-Cobelas et al., 2005). Here $Q/A$ presented a negative relationship with acetate uptake (Figure 3b) and streams in semiarid regions, with higher C demand, presented also lower $Q/A$. Thus, the link between hydrology and acetate uptake might be explained by long-term or climatic hydrological descriptors. Accordingly, we did not find a direct relationship between uptake of DOC (as acetate) and instantaneous discharge, as initially hypothesized and found elsewhere (Lisboa et al., 2016). Thus, indicating that acetate uptake did not vary uniformly with discharge across sites.

The atypical precipitation pattern of the hydrological year 2014, with high accumulated precipitation during summer and very late rains in fall (for most of the studied ecoregions, precipitation is usually low in summer), might be obscuring seasonal differences in acetate uptake across streams. In any case, discharge seems to be an influential variable on acetate uptake only when considering its climatic variability. The Stream Biome Gradient Concept (Dodds et al., 2015) proposes that stream ecosystem functions vary predictably along with climate, which directly influences hydrology and geomorphology. Accordingly, the present study showed differences in $V_f$Acetate across climates and found MAP to be the main descriptor explaining the variability of $V_f$Acetate (Figure 3a). Interestingly, in addition to explain half of the variability, our model for $V_f$Acetate explained by MAP did not statistically differ from the model previously reported in Mineau et al. (2016; Table S5), suggesting that MAP is a robust descriptor of the climatic variability driving acetate uptake in streams. The highest values of $V_f$Acetate correspond to the ecoregions with lower MAP (i.e., MF and ICF) and the lower uptake to the humid most regions.

Furthermore, the present collaborative distributed experiment allowed using a standardized methodology and a simultaneous sampling approach, which improved the confidence intervals of the $V_f$Acetate-MAP model parameters (Table S5), signaling the virtue of testing ecological questions through coordinated distributed experiments.

Our estimates of DOC decay rates ($K_t$) using acetate additions were more than 1 magnitude higher than the ones using bioassays reported in previous studies, using either bulk riverine DOM, exclusively the smaller molecular weight DOM fraction, or simple substrates (Table S6). Whereas bioassays typically consider DOC consumption only in the water column, whole-reach approaches include DOC processing rates from the benthic and hyporheic stream compartments. These compartments are well known to increase stream transient storage and are hot spots of biogeochemical processing in streams (Battin et al., 2003; Mendoza-Lera &
Mutz, 2013). While decay rates estimated throughout bioassays are the net result of both DOC uptake, transformations, and mineralization, short-pulse addition methods measure gross uptake fluxes (Mineau et al., 2016). In addition, simple carbon compounds such as acetate cannot capture the complexity of the DOM pool degradation, which is represented by thousands of decay rates each corresponding to an individual compound (Mostovaya et al., 2017). The magnitude of the DOC processing rates derived from in situ additions has to be considered as an indicator of the potential response of the communities rather than a quantitative estimate of net DOC processing in stream ecosystems. Finally, uptake experiments represent a compromise between the accuracy on the addition measurements and the range of discharge selected, limiting them to headwater streams. A better comprehension of the results obtained through both approaches will strongly improve our understanding of C uptake processes in big rivers.

4.2. Mechanisms Controlling DOC Uptake in Streams: The Relevance of Ambient DOM

Acetate uptake appears strongly modulated by ambient DOM and elemental stoichiometry or resources availability. The negative relationship between $V_{\text{acetate}}$ and the molar DOC:NO$_3^-$ ratio in stream water reflects the interplay between energy and nutrient limitation. When the ratio is high, the energy demand decreases (i.e., lower $V_{\text{acetate}}$). The molar ratio DOC:NO$_3^-$ has been previously found to be a good predictor of NO$_3^-$ uptake velocities (Rodríguez-Cardona et al., 2016; Wymore et al., 2016). Indeed, DOC seems to strongly favor NO$_3^-$ uptake (Bernhardt & McDowell, 2008), and the opposite pattern is found here; high nutrients and low DOC availability relate with higher acetate uptake (Figure 4b). Acetate uptake also presents a positive relationship with DIN signaling that possible nutrient control on DOC uptake (Figure S3). Thus, low DOC demand in streams with high DOC:NO$_3^-$ could be showing a strong N limitation, in agreement with previous studies linking OC dynamics to N availability in streams (Bernhardt & Likens, 2002; Brookshire et al., 2005) but in contrast with several other studies that did not find a link between DOC uptake and N (Johnson et al., 2009; Mineau et al., 2016). $V_{\text{acetate}}$

![Figure 5.](image1.png)

**Figure 5.** Relationship between $V_{\text{acetate}}$ (mm/min) and ecosystem respiration (ER, g O$_2$ m$^{-2}$ day$^{-1}$) ($y = -0.691x + 0.409$, $r^2 = 0.41$, $p = 0.008$); axes are in logarithmic scale. The color coding in the legend stands for the different regions with acronyms as in Figure 1.

![Figure 6.](image2.png)

**Figure 6.** Redundancy analysis triplot of the acetate uptake metrics constrained by the DOM spectroscopic descriptors. The bottom and left-hand scales correspond to cases and response variables ($V_{\text{acetate}}$ = uptake velocity, $U_{\text{acetate}}$ = areal uptake rate, $Sw_{\text{acetate}}$ = uptake length, and $K_{\text{tacete}}$ = uptake rate coefficient). The top and right – hand scales are for the explanatory variables, corresponding to optical descriptors of DOM (FI = fluorescence index; Sr = ratio of spectral slopes; BIX = biological index; $S_{350_600}$ = spectral slope between 350 and 600 nm; SUVA = specific ultraviolet absorbance at 254 nm; DOC = dissolved organic carbon concentration; $a_{440}$ = coefficient at 440 nm; HIX = humification index; DOM = dissolved organic matter). The color coding in the legend stands for the different regions as in Figure 1.
and DOC did not appear to be related in the present study indicating that the balance between resources reflected by DOC:NO\textsubscript{3}\textsuperscript{−} is a better predictor of \( V_{\text{Acetate}} \) as well. Alternatively, other mechanisms could be at interplay, such as concomitant P limitation (Marti et al., 2009) or variation in DOM sources and thus on heterotrophic activity. Thus, although we cannot conclude whether energy or nutrients limitations are driving the variability on acetate uptake, we describe the control of DOM composition on DOC uptake below.

This variation in limiting resources presents a spatial trend across the studied ecoregions. Indeed, despite the absence of a general significant relationship between NO\textsubscript{3}\textsuperscript{−} uptake and acetate uptake (Figure 4a), mass ratios of acetate and NO\textsubscript{3}\textsuperscript{−} uptake rates (\( K_{\text{Acetate}}/K_{\text{NO3}} \)) indicate a noteworthy pattern across ecoregions. Streams more efficiently processing N than DOC (\( K_{\text{Acetate}}/K_{\text{NO3}} < 1 \); Figure 4c) corresponded to humid ecoregions with lower acetate uptake (i.e., PCMF, CMF, and EBF), while arid and semiarid sites (i.e., ICF and MF) process DOC more efficiently than N. A similar pattern is found for \( V_{\text{Acetate}} \) versus \( V_{\text{NO3}} \) (Figure 4a), and semiarid streams are located above the 1:1 line, while humid ecoregions are located below. DOC appears to be a limiting resource in arid streams that receive lower terrestrial inputs from soils and surrounding vegetation. At the global scale, soil DOC declines with decreasing MAP (Craine et al., 2015) and so do litterfall inputs to streams (Benfield, 1997), reflecting the link between catchment-specific net productivity and the aquatic C flux (Webb et al., 2018). Thus, soil DOC inputs from the watershed, typically enriched in humic compounds, are also likely to diminish in arid regions. Consequently, we propose regional patterns in mass ratios to be further explored in future studies as a tool to predict DOC to N retention efficiency across ecoregions.

Ambient DOC concentration in the stream water did not explain \( V_{\text{Acetate}} \) suggesting that it is not indicative per se of the ecosystem functioning in terms of metabolism or DOC uptake. Also, the studied streams presented overall low DOC concentrations (0.7 to 3 mg/L), which could lead to strong C limitation across all studied streams (Wymore et al., 2016). In contrast, several evidences point toward stoichiometry, as discussed above, and ambient DOM composition to exert a stronger role. Acetate uptake was higher in those streams where DOC might be limiting compared with N (Figures 4b and 4c). These DOC or energy limited sites correspond to streams in semiarid ecoregions, with DOM linked to higher FI, BIX, and S\textsubscript{4} values that indicate compounds most likely produced from in situ algal or microbial sources (Figures 6 and S3; Fellman et al., 2010). These indices have been linked to persistent or fast-cycling DOM constituents (Kellerman et al., 2015) which, together with the negative relationship with HIX, \( a_{440} \) or SUVA (Figure 6), indicate a dominance of these protein-like DOM compounds over humic constituents and thus low subsidies from terrestrial sources. Hence, the microbial community in semiarid streams will not be limited by nutrients but by DOM inputs from the catchment (e.g., soil organic matter or litter), which are expected to be lower in these ecoregions (Benfield, 1997; Craine et al., 2015). On the contrary, the non-C limited sites, presenting lower DOC demand, correspond to humid ecoregions’ streams. DOM in these streams was humic and colored (i.e., related to HIX, SUVA, and \( a_{440} \) values) most likely DOM derived from soils and vegetation (Fellman et al., 2010), pointing toward higher inputs from the terrestrial environment (Figure 6). Other ecoregion specific factors such as contrasting benthic communities or water chemistry covarying with DOM composition might influence DOC uptake response (Mineau et al., 2016), but this study does not allow a direct test on those. Overall, our results highlight the relevance of DOM composition on controlling DOC uptake. We stress out here the need to further explore these links and to routinely include ambient DOM characterization in DOC uptake experiments.

Finally, we found a significant relationship between metabolism and acetate uptake that relates to DOM composition variability across regions. In contrast with our hypothesis based on previous literature (Newbold et al., 2006), the negative relationship between acetate uptake and ecosystem respiration indicates that streams with higher ecosystem respiration present lower uptake of DOC (as acetate; Figure 5). This result most likely derives from the relevance of DOM composition in modulating DOC uptake, a factor not systematically explored before (as stated in Mineau et al., 2016). Streams with higher ecosystem respiration, as those from humid ecoregions, show humic DOM, no C limitation, and deciduous vegetation (Pastor et al., 2017), which suggests that these systems are strongly subsidized by the catchment. On the contrary, streams with lower ecosystem respiration, as those from semiarid ecoregions, depict higher acetate uptake, DOM from in situ sources (Figure 6), and C limitation, and thus, catchment C subsidies might be minor. DOM composition is thus indicative of the DOC processing capacity of headwater streams, with those regularly fed by humic compounds presenting a slower uptake response. Our results show that DOC uptake and metabolism in headwater streams can be negatively related across diverse ecoregions, with southernmost (driest) sites
showing higher DOC uptake, lower ecosystem respiration rates, and more labile ambient DOM. This grants the need to further explore this relationship across a wider climatic gradient that includes other ecoregions in arctic, boreal, and tropical realms.

5. Conclusions and Implications

The link between DOM composition and acetate uptake metrics presented here is a key finding that can help to further understand DOC processing in streams. Such finding might imply a different functionality of streams in terms of DOC processing depending on climate region but also on DOM inputs variability. Moreover, and in tropical streams, typically characterized by a higher proportion of protein-like compounds in their DOM, are underrepresented on the DOM spiraling literature (Lisboa et al., 2016). Linking ambient DOM composition to both metabolism and spiraling metrics offers a more inclusive picture that will help to provide a more complete understanding on the responses of the communities to different DOM sources, which are predicted to change linked to climatic drivers (Catalán et al., 2016; Kellerman et al., 2014). In order to get a comprehensive picture of DOC processing in river systems, C balance studies, based either on emissions or mass balances, should recover the DOC spiraling metrics as well as integrate DOM composition as modulator.

References