



Decoupling of dissolved organic matter patterns between stream and riparian groundwater in a headwater forested catchment

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10 **Abstract.** Streams are important sources of carbon to the atmosphere, though whether they merely outgas terrestrially derived carbon dioxide or mineralize terrestrial inputs of dissolved organic matter (DOM) is still a big challenge in ecology. The objective of this study was to investigate the influence of riparian groundwater (GW) and in-stream processes on the temporal pattern of stream DOM concentrations and quality in a forested headwater stream, and whether this influence differed between the leaf litter fall period (LLF) and the remaining part of the year (no-LLF). The spectroscopic indexes (fluorescence index, biological index, humification index, and PARAFAC components) indicated that DOM had an eminently protein-like character and was most likely originated from microbial sources and recent biological activity in both stream water and riparian GW. However, paired samples of stream water and riparian GW showed that dissolved organic carbon (DOC) and nitrogen (DON) concentrations as well as the spectroscopic character of DOM differed between the two compartments throughout the year. A simple mass balance approach indicated that in-stream processes along the reach contributed to reduce DOC and DON fluxes by 50% and 30%, respectively. Further, in-stream DOC and DON uptake were unrelated to each other, suggesting that these two compounds underwent different biogeochemical pathways. During the LLF period, stream DOC and DOC:DON ratios were higher than during the no-LLF period, and spectroscopic indexes suggested a major influence of terrestrial vegetation on stream DOM. Our study highlights that stream DOM is not merely a reflex of riparian GW entering the stream and that headwater streams have the capacity to internally produce, transform, and consume DOM.

25 1 Introduction

The transport of dissolved organic matter (DOM) through fluvial networks is of major importance for understanding the links between continental and coastal biogeochemical cycles (Seitzinger and Sanders, 1997; Battin et al., 2008). Stream DOM is a combination of terrestrially derived and in-stream produced DOM. The former originates mostly from terrestrial systems (i.e. soils, vegetation and microbes) and it is transported to streams via surface and groundwater flow paths, while the latter derives from in-stream metabolic activity and leachates of litter falling into the stream especially during the leaf litter period (Qualls



and Haines, 1991, 1992). The bioavailability of DOM can differ substantially between terrestrial and in-stream sources, and thus, a good assessment of the origin and quality of stream DOM is of great importance to understand the capacity of aquatic ecosystems to store and transform carbon (C) and nitrogen (N) (Cole et al., 2007; Battin et al., 2008; Tranvik et al., 2009). Yet, our knowledge of the contribution of terrestrial vs in-stream sources to total stream DOM and its variability over time and space is far from complete.

The strong correlation found between dissolved organic carbon (DOC) and nitrogen (DON) in temperate and boreal streams have suggested that the soil organic pool is a major factor controlling the fate and form of stream DOM (Perakis and Hedin, 2002; Hedin et al., 1995; Brookshire et al., 2007; Sponseller et al., 2014). These previous observations are the cornerstone of the passive carbon vehicle hypothesis, which states that soil DOM is stoichiometrically static and behaves almost conservatively when travelling throughout the catchment and stream ecosystems (Brookshire et al., 2007). However, there is an increasing body of studies reporting differences in DOC:DON ratios between terrestrial sources and stream water. For instance, stream DOC:DON ratios can change as a consequence of in-stream heterotrophic DOM production during periods of high ecosystem respiration (Caraco and Cole, 2003; Kaushal and Lewis, 2005; Johnson et al., 2013). Moreover, stream biota can show a strong capacity to process DOM (McDowell, 1985; Bernhardt and McDowell, 2008), with whole-reach DOM uptake rates being even higher than for essential nutrients such as nitrate (Brookshire et al., 2005). The processing of DOM within the stream can lead to a decoupling between stream DOC and DON concentrations because stream DOC is mostly used as an energy source, while DON can alternatively be used as a nutrient source (Kaushal and Lewis, 2005; Lutz et al., 2011; Wymore et al., 2015). Therefore, an important fraction of stream DOM could be transformed within the stream, either degraded or eventually mineralized, or produced from in-stream activity.

Despite the potential role of in-stream biota on processing DOM, its ability to modify DOM concentrations and regulate terrestrial DOM fluxes remains elusive. First, the high variety of molecules used during in situ DOM additions (from monomeric carbohydrates to complex leachate molecules) limits the possibility to compare whole-reach DOM uptake rates among sites and to link manipulative experiments with actual DOM processing under natural conditions (Newbold et al., 2006; Bernhardt and McDowell, 2008). Second, the intrinsic complexity of up-scaling reach scale measurements constrains our understanding of the potential of in-stream processes to modify DOM export at catchment scale (Wollheim et al., 2015). Recent synoptic studies suggest that changes in stream DOC concentrations can be mostly explained by hydrological mixing of different water sources, thus suggesting minimal removal of DOC within streams (Tiwari et al., 2014; Wollheim et al., 2015). Yet, these studies are mostly performed during particular periods (usually summer low flow conditions) and in catchments with large wetland and peatland areas that provide large quantities of terrestrial DOM to aquatic ecosystems (Wollheim et al., 2015). Studies with a network perspective are still scarce and usually deal with a high amount of uncertainty because the quantity and quality of DOM in groundwater entering the stream is poorly characterized (Tiwari et al., 2014; Casas-Ruiz et al., 2017).



The objective of this study was to investigate the influence of DOM inputs from riparian groundwater (GW) and in-stream processes on the temporal pattern of stream DOC and DON concentrations and quality (DOC:DON stoichiometry and spectroscopic descriptors) in a Mediterranean forested headwater stream. To do so, we assessed the temporal variation of DOM quantity and quality in stream water and riparian GW along 1.5 years. We expected that differences between riparian GW and stream DOM would be small if terrestrial sources dominate the temporal pattern of DOM inputs and DOM is transported passively along the stream as stated by the carbon vehicle hypothesis. Alternatively, differences between riparian GW and stream water would indicate DOM generation and/or processing of terrestrial DOM within the stream. Specifically, we expected large differences between riparian GW and stream DOM associated with the leaf litter fall period because leachates from fresh material stored in the streambed may increase DOM concentration and fuel heterotrophic stream metabolism.

2 Study Site

The study was conducted from October 2010 to December 2011 in the Font del Regàs catchment (14.2 km²), located in the Montseny Natural Park, NE Spain (41° 50' N, 2° 30' E, 300-1200 m a.s.l.). The climate is sub-humid Mediterranean, with mild winters and dry summers. Mean annual precipitation (975 mm) and temperature (12.9 °C) during the study period fall within the long-term annual average for this region (Catalan Meteorologic Service: <http://www.meteo.cat/servmet/index.html>).

The catchment is dominated by biotitic granite and it has steep slopes (28%). Evergreen oak (*Quercus ilex*) and beech (*Fagus sylvatica*) forests cover 54% and 38% of the catchment area, respectively (Fig. 1). The upper part of the catchment (2%) is covered by heathlands and grasslands. Population density within the catchment is <1 person km⁻². Hillslope soils (pH ~ 6) are sandy and have a 3cm deep organic layer (O-horizon) followed by a 5 to 15cm deep mineral layer (A-horizon). The riparian zone is relatively flat (slope < 10 %), and it covers 6 % of the catchment area. Riparian soils (pH ~ 7) are sandy-loam and they have a 5cm deep O-horizon followed by a 30cm deep A-horizon. The width of the riparian zone increases from 6 to 32 m from the upper to the lower part of the catchment, whereas the total basal area of riparian trees increases by 12 fold (Bernal et al., 2015). *Alnus glutinosa*, *Robinia pseudoacacia*, *Platanus hybrida*, and *Fraxinus excelsior* are the most abundant riparian tree species followed by *Corylus avellana*, *Populus tremula*, *Populus nigra*, and *Sambucus nigra*. During base flow conditions the riparian GW table is well below the soil surface (~ 50 cm), though it can reach the superficial soil organic layers during storm events (Lupon et al., 2016a).

The catchment is drained by a perennial 3rd order stream. At the headwaters, the streambed is mainly composed of rocks and cobbles (70 %) with a small contribution of sand (~10 %). At the valley bottom, sands and gravels represent 44 % of the stream substrate and the presence of rocks is minor (14 %). Stream discharge at the valley bottom averages 70 L s⁻¹. The stream gains water in net terms along the reach but it can lose water towards the riparian zone during summer months (Bernal et al., 2015).



3 Material and Methods

3.1 Field sampling

We selected 15 sampling sites along a 3.7km reach that were located from 110 to 600 m apart from each other (Fig. 1). At each sampling site, we installed a 1m long PVC piezometer (3cm Ø) in the riparian zone (~1.5 m from the stream channel edge). We collected stream water (from the thalweg) and riparian GW from each sampling site every 2 months from October 2010 to December 2011. Groundwater samples were collected with a 100ml syringe connected to a silicone tube. Water samples were collected with pre-acid washed polyethylene bottles after triple-rinsing them with either stream water or groundwater. Field sampling was conducted during base flow conditions to capture the influence in-stream processes on DOM dynamics when they are expected to be the highest. Moreover, by avoiding storm flows, we ensured that riparian GW was the main catchment water source contributing to stream runoff. All field campaigns were performed at least nine days after storm events, except for October 2011. At each sampling site, we measured stream discharge (Q , in $L s^{-1}$) by adding 1 L of NaCl-enriched solution to the stream (Gordon et al., 2004). The empirical uncertainty associated with Q was calculated considering pairs of measurements conducted under equal water depth conditions as described in Bernal et al. (2015). On each sampling date, we also collected stream water and measured Q at the four permanent tributaries discharging to Font del Regàs stream, which drained 1.9, 3.2, 1.8, and 1.1 km^2 , respectively (Fig. 1). These data were used for mass balance calculations (see below).

3.2 Laboratory analysis and DOM quality indexes

Water samples were filtered through pre-ashed GF/F filters (Whatman®) and kept cold ($< 4 ^\circ C$) until laboratory analysis ($< 24h$ after collection). Chloride (Cl^-) was used as a conservative hydrological tracer and analyzed by ionic chromatography (Compact IC-761, Methrom). DOC and total dissolved nitrogen (TDN) concentrations were determined using a Shimadzu TOC-VCS coupled to a TN analyzer. DOC was determined by oxidative combustion infra-red analysis and TDN by oxidative combustion-chemiluminescence. DON concentration was calculated by subtracting nitrate (NO_3^-) and ammonium (NH_4^+) concentrations from TDN. Concentrations of NO_3^- and NH_4^+ were determined by standard colorimetric methods (details in Bernal et al., 2015).

We used different metrics to assess the quality of DOM and to infer its origin. First, the DOC:DON ratio was used as a general proxy of DOM quality, high values being indicative of plant organic matter sources (Bernal et al., 2005). Then, we assessed DOM properties by optical spectroscopy. Fluorescence excitation-emission spectra were recorded on a Shimadzu RF-5301 PC spectrofluorimeter over an emission range of 270-700 nm (1nm steps) and an excitation range of 230-430 nm (10nm steps). Measurements were done at room temperature (20-25 $^\circ C$) and corrected for instrument baseline offset. A Milli-Q blank was subtracted from each sample to eliminate Raman scattering, sampling blanks were included to assess for leaching of DOM during the sampling procedure. We followed the procedure in Kothawala et al. (2013) for inner filter correction. Briefly, UV-Vis absorbance spectra (200-800 nm) were obtained in a Shimadzu UV-1700 spectrophotometer, using 1cm quartz cuvette.



Due to fatal circumstances, absorbance spectra could not be recorded for some samples. In these cases, we used the modeled mean absorbance spectra for either riparian GW or surface stream water to apply the inner filter correction. All the corrections were applied using the FDOM correct toolbox for MATLAB (Mathworks, Natick, MA, USA) following Murphy et al. (2010). We calculated three spectroscopic descriptors: (i) the fluorescence index (*FI*) which typically ranges from ~1.2 to ~2 and is

5 linked to the DOM origin with low values being characteristic of terrestrial higher-plant DOM sources and high values of microbial DOM sources (Jaffé et al., 2008), (ii) the biological index (*BIX*), for which higher values indicate a higher contribution of recently produced DOM (i.e. biological activity or aquatic bacterial origin) (Parlanti et al., 2000; Huguet et al., 2009), and (iii) the humification index (*HIX*) as a proxy of the humification status of DOM (i.e. higher values indicating higher humification degree) (Ohno, 2002; Fellman et al., 2010).

10 Parallel Factor Analysis (PARAFAC) was used to identify the main fluorescence components of DOM (Stedmon et al., 2003). The analysis was performed using the DrEEM toolbox for MATLAB (Mathworks, Inc., Natick, MA) according to Murphy et al. (2013). Scatter peaks and outliers were removed and samples normalized to its total fluorescence prior to fitting the PARAFAC model. The appropriate number of components was determined by visual inspection of both the residual

15 fluorescence and the components behavior as organic fluorophores. The PARAFAC modeling of EEM spectra from the analyzed samples revealed four independent components (F1-F4; Fig. S1 in Supplementary Information). Components F2 and F3 corresponded to humic-like materials, while components F1 and F4 to protein-like fluorescence (Table S1 and S2). The four components model was validated by split-half analysis and random initialization with 10 iterations. Finally, the level of coincidence of the obtained model against other PARAFAC models published in the online repository OpenFluor data base (<http://www.openfluor.org>) was assessed applying a Tucker congruence coefficient of 95 % (Murphy et al., 2014).

20 3.3 Whole-reach net DOM uptake rates

We investigated the influence of in-stream biogeochemical processes on stream DOM fluxes by applying a mass balance approach for the whole reach. Briefly, we calculated the net flux resulting from in-stream gross uptake and release along the reach (*U*, in $\mu\text{g m}^{-2} \text{s}^{-1}$) by including all hydrological input and output solute fluxes (upstream-most site, tributaries, and riparian GW) in the mass balance. For each sampling date, *U* for either DOC or DON was approximated with:

$$25 \quad U = (Q_{top} \times C_{top} + \sum_{i=1}^4 Q_{tr,i} \times C_{tr,i} + \sum_{j=1}^{14} Q_{gw,j} \times C_{gw,j} - Q_{bot} \times C_{bot})/A, \quad (1)$$

where Q_{top} and Q_{bot} are the discharge at the top and at the bottom of the reach, Q_{tr} is the discharge from tributaries, and Q_{gw} is the net riparian GW inputs (all in L s^{-1}). Q_{gw} was estimated as the difference in Q between consecutive sampling sites and

30 could be either positive (net gaining) or negative (net losing) (Covino et al., 2010). Top and bottom fluxes were calculated by multiplying Q by stream water solute concentration at the top (C_{top}) and at the bottom (C_{bot}) of the segment, respectively. For each stream segment j , riparian GW fluxes were estimated by multiplying Q_{gw} by solute concentration (C_{gw}) as described in



Bernal et al. (2015). Briefly, C_{gw} averaged riparian GW concentration at the top and bottom of the segment for net gaining segments ($Q_{gw} > 0$), while it averaged stream water concentrations at the top and bottom of the segment for net losing segments ($Q_{gw} < 0$). For each tributary i , the input flux to the stream was calculated by multiplying Q_{tr} and solute concentrations (C_{tr}) at the outlet of the tributary. The total active streambed (A) was 8860 m² and it was estimated by multiplying the total length of the reach (3.7 km) by the mean wetted width (2.4 m) that varied $< 10\%$ across the different sampling dates. The values used to calculate U for each sampling date are detailed in Table S3. Finally, we calculated an upper and lower limit of U based on the empirical uncertainty associated with discharge measurements (Q and Q_{gw}) (Bernal et al., 2015).

The mass balance approach used in the present study was similar to that applied for Cl^- , NH_4^+ , and NO_3^- for the same study reach and period in Bernal et al. (2015). We considered Cl^- as a hydrological reference because this conservative tracer showed $U \sim 0$ for the whole study period (Bernal et al., 2015). For DOC and DON, $U > 0$ indicates that gross uptake prevails over release, $U < 0$ indicates the opposite, $U \sim 0$ indicates that gross uptake \sim release. Therefore, we expected $U \neq 0$ if DOM does not behave conservatively and in-stream gross uptake and release processes do not fully counterbalance each other. We assumed that U was indistinguishable from 0 when the range of upper and lower limits contained zero.

To assess the contribution of in-stream net uptake to stream DOM fluxes, we calculated the ratio between $U \times A$ (absolute value) and the total input flux (F_{in}) for each compound (i.e. DOC and DON) and sampling date. F_{in} was the sum of fluxes from upstream ($Q_{top} \times C_{top}$), tributaries ($Q_{tr} \times C_{tr}$), and riparian GW ($Q_{gw} \times C_{gw}$). The later was included in the calculation only when the main stream was gaining water in net terms (i.e. $Q_{gw} \times C_{gw} > 0$). We interpreted a high $|U \times A|/F_{in}$ ratio as a strong potential of in-stream processes to modify input fluxes (either as a consequence of gross uptake or release). The relative importance of in-stream DOM uptake and release was estimated with $U > 0/F_{in}$ and $|U| < 0/F_{in}$, respectively.

3.4 Statistical analysis

The data set was divided in two groups based on the temporal pattern of leaf litter fall because we expected large differences between riparian GW and stream DOM associated with the input of fresh leaf litter to the stream. During the two water years, leaf litter fall began in early October and peaked in early November. In 2010, the litter fall period finished in late November, while it lasted until late December in 2011. There were four sampling dates within the leaf litter fall period (hereafter, LLF) and six sampling dates during the remaining part of the year (hereafter, no-LLF). Median values for each sampling date were used for analyzing the seasonal pattern of stream DOM concentration and quality (DOC:DON ratio and spectroscopic descriptors). We used a Mann Whitney test to analyze differences in DOM concentrations and quality between the LLF and no-LLF periods for both stream water and riparian GW (Zar, 2010). Moreover, we used the linear regression models to investigate whether DOM stoichiometry (i.e. the relationship between DOC and DON concentration) was similar between riparian GW and stream water.

We explored the influence of riparian GW on the temporal pattern of stream DOM by analyzing the difference between DOM concentrations in these two water compartments with a Wilcoxon paired rank sum test. Tests were run separately for the LLF



and no-LLF periods. Moreover, we compared the temporal variation of longitudinal trends in DOM spectroscopic descriptors between stream water and riparian GW. Longitudinal trends were analyzed by applying linear regression and the standardized regression coefficient (r) was used as a measure of the strength of the longitudinal pattern along the reach. For a particular sampling date, we expected similar longitudinal trends between stream water and riparian GW (and thus similar r) if riparian

5 GW was a major source of DOM to the stream and in-stream processes had a small influence of DOM quality.

Finally, we explored differences in U between LLF and no-LLF periods with a Mann Whitney test. Moreover, we used Spearman's ρ correlations to test (i) whether U_{DOC} and U_{DON} followed the same temporal pattern, and (ii) whether they were behaving conservatively, and thus, similar to U_{Cl} .

We chose non-parametric tests for comparing groups of data because the residuals of variables were not always normally
10 distributed (Zar, 2010). All statistical tests were run with JMP v.5.0 statistical software (SAS Institute, Cary, NC).

4 Results

4.1 Temporal pattern of DOM in stream water

During the study period, median DOC concentration in the stream (i.e. main stream) was higher for the LLF (843 [643, 1243] [25th, 75th percentiles] $\mu\text{g C L}^{-1}$) than for the no-LLF period (406 [304, 580] $\mu\text{g C L}^{-1}$) (Mann Whitney test, $Z = 2.55$, $\text{df} = 1$, $p = 0.008$) (Fig. 2a). Median stream DON concentration was 58 [35, 78] $\mu\text{g N L}^{-1}$ and showed no seasonal pattern (Mann Whitney test, $Z = -0.85$, $\text{df} = 1$, $p > 0.05$) (Fig. 2b). The median stream DOC:DON ratio was higher during the LLF (DOC:DON = 22 [14, 43]) than during the no-LLF period (DOC:DON = 8 [5, 15]) (Mann Whitney test, $Z = 1.98$, $\text{df} = 1$, $p = 0.033$) (Fig. 2c). Median values of FI (> 2) were typical of microbial DOM sources, while low values of HIX (< 2) indicated that the humification of the samples was low (Fig. 2). Regarding the PARAFAC model, the components F1 and F4 (associated with protein-like
20 materials) were responsible for the major part of the total fluorescence of stream water samples (50 [46, 53] % and 25 [24, 28] %, respectively). The components F2 and F3 (associated with humic-like materials) accounted for 13 [11, 15] % and 11 [9, 13] % of the total fluorescence, respectively (Fig. 3).

There were differences in stream DOM quality between the LLF and no-LLF period, though most of the spectroscopic metrics (BIX , HIX , F1, F2, and F4) were similar between the two periods (in the five cases, Mann Whitney test, $p > 0.05$). In contrast,
25 values of FI and the humic-like component F3 were higher during the LLF than during the no-LLF period (in the two cases, Mann Whitney test, $Z < 2.24$, $\text{df} = 1$, $p < 0.05$). The relative contribution of F3 to the total fluorescence was higher during the LLF than during the no-LLF period (Mann Whitney test, $Z = 3.43$, $\text{df} = 1$, $p < 0.0006$), while the protein-like component F4 showed the opposite pattern (Mann Whitney test, $Z = -2.23$, $\text{df} = 1$, $p < 0.025$).



4.2 Temporal pattern of DOM in riparian GW

During the study period, median DOC concentration in riparian GW was higher for the LLF (1411 [1133, 2311] $\mu\text{g C L}^{-1}$) than for the no-LLF period (864 [626, 1414] $\mu\text{g C L}^{-1}$) (Mann Whitney test, $Z = 5.49$, $\text{df} = 1$, $p < 0.001$). In contrast, median DON concentrations in riparian GW were lower during the LLF (67 [45, 157] $\mu\text{g N L}^{-1}$) than during the no-LLF (113 [64, 195] $\mu\text{g N L}^{-1}$) (Mann Whitney test, $Z = -1.96$, $\text{df} = 1$, $p = 0.049$). Riparian GW showed higher DOC:DON ratios during the LLF (DOC:DON = 27 [14, 43]) than during the no-LLF period (DOC:DON = 10 [6, 14]) (Mann Whitney test, $Z = 4.98$, $\text{df} = 1$, $p < 0.001$).

Similar to stream samples, the PARAFAC components related to the protein-like fluorescence (F1 and F4) were responsible for the major part of the total fluorescence of riparian GW samples (44 [38, 49] % and 26 [23, 29] %, respectively). The fluorescence components associated with humic-like materials (F2 and F3) accounted for 16 [13, 21] % and 12 [9, 17] %, respectively.

Values of *FI*, *BIX*, and *HIX* in riparian GW showed no differences between the LLF and no-LLF period, with medians equaling to 2.49 [2.41, 2.61], 1.11 [0.85, 1.68], and 0.67 [0.61, 0.74], respectively (for the three indexes, Mann Whitney test, $\text{df} = 1$, $p > 0.05$). Regarding PARAFAC, three out of the four fluorescence components (F1, F3, and F4) showed higher values in riparian GW during the LLF than during the no-LLF period (for the three components: Mann Whitney test, $Z > 2.2$, $\text{df} = 1$, $p < 0.015$). However, the relative contribution of the four components to the total fluorescence did not change between the two periods (for the four components, Mann Whitney test, $\text{df} = 1$, $p > 0.05$).

4.3 Influence of riparian GW on stream DOM

The paired test comparing stream water and riparian GW samples collected simultaneously along the study reach showed that DOC and DON concentrations were higher in riparian GW than in stream water during both the LLF and the no-LLF period (Table 1). However, there were no differences in DOC:DON ratios between riparian GW and stream water in any of the two periods. During the LLF period, concentrations of DOC and DON were uncorrelated to each other, while stream water and riparian GW showed a positive relationship between DOC and DON concentrations during the no-LLF period (Fig. 4).

Spectroscopic descriptors also show differences between the two water bodies which were not consistent between the two study periods. During the LLF period, the *FI* was higher in stream water than in riparian GW, while the opposite trend was observed for indexes associated with both humic-like substances (*HIX* and F2) and protein-like compounds (*BIX* and F4) (Table 1). During the no-LLF period, *HIX*, F2, F3, and F4 were lower in stream water than in riparian GW, while no differences between the two water bodies were observed for *FI*, *BIX*, and F1 (Table 1).

The longitudinal trends in DOM quality differed between stream water and riparian GW. Values of *FI* in stream water increased along the reach in eight out of 10 sampling dates, while values of *HIX* did so in four out of 10 cases ($r > 0$ in Fig. 5). Longitudinal trends in stream DOM spectroscopic properties were observed during both the LLF and no-LLF period. In contrast, riparian GW showed no significant longitudinal patterns for either *FI*, *BIX* or *HIX* in any of the sampling dates. Regarding PARAFAC



components, both stream water and riparian GW showed significant changes along the reach in some particular sampling dates. The most consistent pattern was the longitudinal increase in humic-like components (F2+F3) which was observed in four out of 10 sampling dates (Figure S2).

4.4 Contribution of in-stream processes to stream DOM fluxes

5 Values of $U > 0$ were measured for both DOC and DON indicating that in-stream processes influenced stream DOM fluxes at Font del Regàs. During the study period, median values of U_{DOC} were $197.7 [58.3, 315] \mu\text{g C m}^{-2} \text{ h}^{-1}$, whereas values of U_{DON} were $22.3 [4.6, 44.3] \mu\text{g N m}^{-2} \text{ h}^{-1}$. Differences in the contribution of in-stream processes to stream DOM fluxes between the LLF and the no-LLF period were not statistically significant (for both U_{DOC} and U_{DON} , $Z > Z_{0.05}$, $\text{df} = 1$, $p > 0.05$). At reach scale, U contributed to modify stream fluxes ($|U \times A|/F_{in}$) by $32 [19, 46] \%$ for DOC and $40.5 [29, 52] \%$ for DON. These values
 10 were 10 fold higher than for Cl^- (the conservative tracer) for which U_{Cl} represented $3.6 [1.9, 9.4] \%$ of the input fluxes (Fig. 6a). The stream acted as a net sink of DOM ($U > 0$) in seven and eight out of 10 sampling dates for DOC and DON, respectively. In these cases, in-stream processes contributed to reduce stream fluxes by $44 [36, 54] \%$ and $37 [28, 40] \%$ for DOC and DON, respectively (Fig. 6b and c, bars).

There was no significant relationship between U for the different compounds considered in this study. No correlation was
 15 found between U_{Cl} and either U_{DOC} or U_{DON} (in the two cases, $\rho < 0.3$, $p > 0.05$), indicating that both DOC and DON behaved differently than expected from a conservative tracer. Moreover, U_{DOC} and U_{DON} were unrelated to each other (Fig. 7a).

5 Discussion

The capacity of stream ecosystems to mineralize terrestrial DOM, and thus their ability to contribute to C storage and emission at global scales, remains elusive mostly because available results are contradictory. Most of the uncertainties associated with
 20 the estimation of biogeochemical processing rates at large scales (reaches $> 100 \text{ m}$) rely on the fact that GW inputs are rarely measured (Tiwari et al., 2014; Casas-Ruiz et al., 2017). Our unique synoptic approach explicitly considers GW inputs, allowing for more reliable C and N budget calculations (Bernal et al., 2015). Moreover, we characterized the spectroscopic properties of DOM in both stream water and riparian GW which helped us to elucidate whether stream DOM was just a reflex of terrestrial sources or if in-stream processes were able to modify DOM quality.

25 Our study highlights that DOM in the Font del Regàs stream and riparian GW had an eminently protein-like character, most likely originated from microbial sources and recent biological activity. For instance, the fluorescence of the samples was dominated by F1 and F4 (up to 75% of the total fluorescence), two PARAFAC components that presented wavelengths typically attributed to tyrosine and tryptophan (Fellman et al., 2010) (Table S1). Moreover, the whole range of BIX values measured in water samples (from 0.4 to 1.63) depicted a strong influence of autochthonous DOM sources (Huguet et al., 2009),
 30 while all measured HIX values were < 6 , indicating low humification of the samples (Fellman et al., 2010). These values



contrast with those reported for stream water samples from boreal and temperate catchments with large peatlands and wetland areas, which usually have high DOC concentrations ($> 10 \text{ mg C L}^{-1}$) and high colored humic materials (e.g. Kothawala et al., 2016). However, similar values of both *BIX* and *HIX* to the ones presented here have been reported previously in systems with low DOC concentrations and not very colored DOM, such as ground caves and spring waters (Birdwell and Engel, 2010; Simon et al., 2010) as well as in soils (Traversa et al., 2014) and some rivers (Huang et al., 2015).

5.1 Empirical evidence of in-stream DOM processing

We found that stream DOM did not exhibited a conservative behavior because the stream showed a large capacity to change DOM fluxes (by 30-40%) compared to Cl^- fluxes (by 3%). The predominant protein-like character of stream DOM at Font del Regàs could explain, at least partially, why U_{DOC} and U_{DON} differed from zero during most of the study period. This result indicates that in-stream DOM uptake and release processes were not counterbalancing each other (otherwise U would approach to zero). For both DOC and DON, we found that in-stream uptake usually predominated over release (i.e. $U > 0$), suggesting higher DOM consumption than production. Our mass balance calculations indicated that in-stream processes were capable to decrease reach scale fluxes up to 80 % and 50 % for DOC and DON, respectively. These findings imply that biogeochemical processes occurring within the stream were able to modify DOC and DON concentrations and fluxes to downstream ecosystems, contrasting with results reported in previous studies (Temnerud et al., 2007; Tiwari et al., 2014; Wollheim et al., 2015). Yet, our results are representative of base flow conditions which represent ca. 60 % of the annual DOC and DON flux in the study catchment (unpublished data). Further studies including storm flow conditions would be needed to gain a more complete picture of the role of in-stream processes on DOM dynamics and whether the headwater stream shifts from acting as a reactor to a pipe with increasing discharge (Casas-Ruiz et al., 2017; Raymond et al., 2016).

Noteworthy, median values of in-stream net uptake ($U_{\text{DOC}} = 198 \text{ } \mu\text{g C m}^{-2} \text{ h}^{-1}$ and $U_{\text{DON}} = 22.3 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$) were from 10-1000 fold lower than rates of in-stream gross uptake and DOM production reported for DOM addition experiments in other headwater streams (Lush and Hynes, 1978; McDowell, 1985; Maranger et al., 2005; Bernhardt and McDowell, 2008; Johnson et al., 2013). These discrepancies could be partially explained by the fact that some of these manipulative experiments used monomeric carbohydrates that are easily bioavailable. Moreover, and as previously reported for nutrients, differences between estimates of in-stream gross and net uptake suggest that DOM consumption and production likely occur simultaneously within the stream, and that the former is counterbalanced to some extent by the latter (von Schiller et al., 2015). Supporting this idea, median values of U_{DOC} were >100 fold lower than DOC consumption inferred from measurements of ecosystem respiration calculated from diel cycles of dissolved oxygen concentrations in the same study stream (Lupon et al., 2016b).

The observed differences in the spectroscopic properties of DOM between the stream and riparian GW further support the existence of an autochthonous source of labile DOM in the Font del Regàs stream. For instance, riparian GW presented higher humic-like fluorescence (i.e. higher values of *HIX*, F2 and F3) than stream water, which is in agreement with a recent study comparing stream and groundwater DOM (Huang et al., 2015). Moreover, the contribution of the protein-like component F1



to the total fluorescence was higher in stream water (50.6 %) than in riparian GW (43.9 %), while the contribution of F2, a ubiquitous humic component related with fulvic acids and re-processed humics, was higher in riparian GW (17.8 %) than in stream water samples (13.1 %). Finally, the lack of longitudinal trends in DOM quality in riparian GW contrasted with the consistent increase in *FI* observed for stream water along the reach (in eight out of 10 sampling dates). This finding suggests that stream DOM shifted towards a more microbial origin as moved downstream, and that this change was more related to in-stream processes than to changes in the spectroscopic character of riparian GW. Altogether, our results highlight that in-stream processes have the potential to change not only the quantity but also the quality of DOM, what reinforces their potential role as bioreactors rather than as merely carbon chimneys transforming dissolved inorganic carbon from terrestrial groundwater to CO₂ (Hotchkiss et al., 2015).

5.2 Decoupling between in-stream DOC and DON dynamics

We found small differences in DOC:DON ratios between stream water and riparian GW throughout the year. Moreover, water samples showed a positive and moderate relationship between DOC and DON concentrations, especially during the no-LLF period. Similar DOM stoichiometry between terrestrial and aquatic ecosystems has been typically understood as an indication of the recalcitrant and terrestrial nature of organic matter in stream waters (Perakis and Hedin, 2002; Rastetter et al., 2005). Therefore, these results could a priori suggest that terrestrial DOM inputs mostly dominated DOM in stream water. Yet, the spectroscopic analysis clearly indicated that the quality of DOM differed between these two compartments, and that stream DOM was likely highly available to biota given the high content of protein-like material, which was higher than in riparian GW entering the stream.

In concordance with the idea that stream DOM was not recalcitrant, we found (i) that U differed from zero for both DOC and DON, and (ii) that U_{DOC} and U_{DON} were unrelated to each other. This finding supports the hypothesis that these two compounds undergo different metabolic and biogeochemical pathways (Kaushal and Lewis, 2005; Lutz et al., 2011): DOC is mostly used as an energy source, while evidence is growing that DON can also be used as a nutrient (Wymore et al., 2015). The dual behavior of DON could partially explain why U_{DON} was unrelated to U_{DOC} , which contrasts with the strong relationship exhibited by in-stream net uptake rates for the two inorganic forms of N, U_{NO3} and U_{NH4} , which are both essential nutrients for biota (Fig. 7b). For DOC, a major fraction of what is taken up (~ 70%) follows catabolic pathways (respiration) and is removed to the atmosphere, while the remaining part (~ 30%) may be used for microbial growth (del Giorgio and Cole, 1998). Thus, considering that in-stream DOM uptake contributed to reduce terrestrial DOC fluxes by 36-54 % (25th and 75th percentiles), approximately one quarter (21-32 %) of the DOC entering or produced within the stream could be released as CO₂ to the atmosphere.



5.3 Influence of leaf litter fall on stream DOM dynamics and spectroscopic properties

Previous studies have reported large increases in stream DOC concentration and ecosystem respiration associated with large inputs of fresh leaf litter in autumn (e.g. Acuña et al., 2004). Thus, we expected large differences in stream DOM concentrations and quality between the LLF and no-LLF period, as well as between riparian GW and stream DOM during the LLF period.

5 Concordantly, the highest stream DOC concentrations and DOC:DON ratios were measured during the LLF period. Yet, the same pattern was observed for riparian GW, where concentrations of DOC during the LLF period were even higher than in the stream. In this case, higher DOC concentrations could be explained by increases in the groundwater table after autumn rains, which then flow through more superficial organic soil layers (Guarch-Ribot and Butturini, 2016). This idea is supported by the fact that riparian GW showed higher fluorescence during the LLF than the no-LLF period, but no changes in the relative

10 contribution of the four fluorescence components to the total fluorescence. In contrast, the relative contribution of F3 (humic-like component) and F4 (protein-like component) increased and decreased, respectively, in stream water during the LLF period. This result together with the higher values of *FI*, bears the idea that leaf litter inputs were a source of humic-like material but that, at the same time, were fueling microbial activity within the stream. The fact that DOM uptake predominated over release (U_{DOC} and $U_{DON} > 0$) even during some sampling dates within the LLF period supports the hypothesis that fresh particulate

15 organic matter was processed in route and that stream biota was consuming DOM (Battin et al., 2008; Fasching et al., 2014).

6 Conclusions

Global studies highlight that streams and rivers are important sources of C to the atmosphere (Cole et al., 2007; Raymond et al., 2013). Yet, the potential role of streams to mineralize terrestrial DOC and its consequences at the catchment scale is still largely unknown (Hotchkiss et al., 2015). Our study sheds new light into this issue by showing that headwater streams have a

20 strong capacity to internally produce, transform, and consume DOM. The mass balance calculations revealed that in-stream processing substantially modify stream DOC and DON fluxes during base flow conditions. Moreover, we found that DOM concentration and spectroscopic character differed between stream water and riparian GW, which provides evidence that stream DOM is not merely a reflex of riparian DOM entering the stream. On the contrary, our findings suggest that both riparian leaf litter inputs and in-stream DOM cycling are essential controls of DOM dynamics in forested headwater streams.

25 Data availability

The data sets used in this paper can be obtained from the authors upon request.



Authors contribution

Susana Bernal designed the experiment. Susana Bernal and Anna Lupon carried it out. Sara Castelar, Anna Lupon and Núria Catalan performed all laboratory analysis. Susana Bernal, Anna Lupon, and Núria Catalan analyzed the data set. Susana Bernal prepared the manuscript with contributions from Anna Lupon, Núria Catalan, Sara Castelar and Eugènia Martí.

5 **Competing interests:** the authors declare that they have no conflict of interest.

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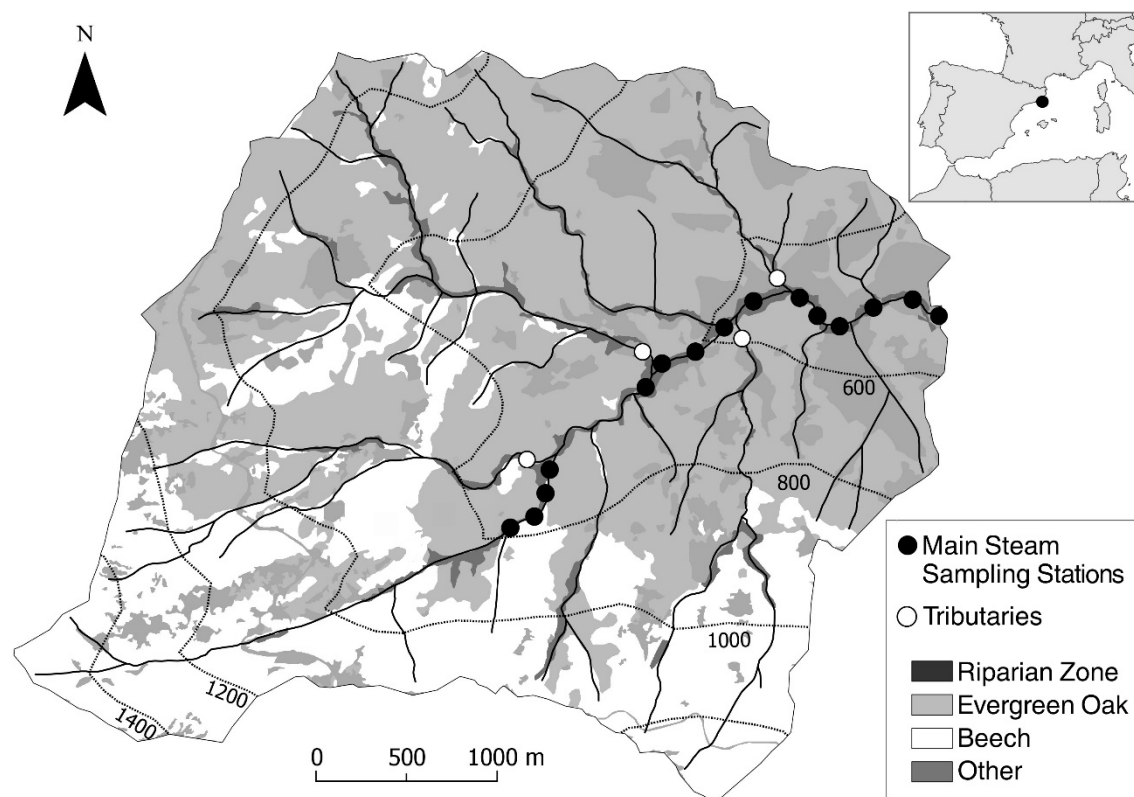
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Figures



5 **Figure 1. Map of the Font del Regàs catchment within the Montseny Natural Park (NE, Spain). The vegetation cover and the main stem sampling stations along the 3.7km reach are indicated. Four permanent tributaries discharged to the main stream from the upstream- to the downstream-most site (white circles). The remaining tributaries were dry during the study period.**

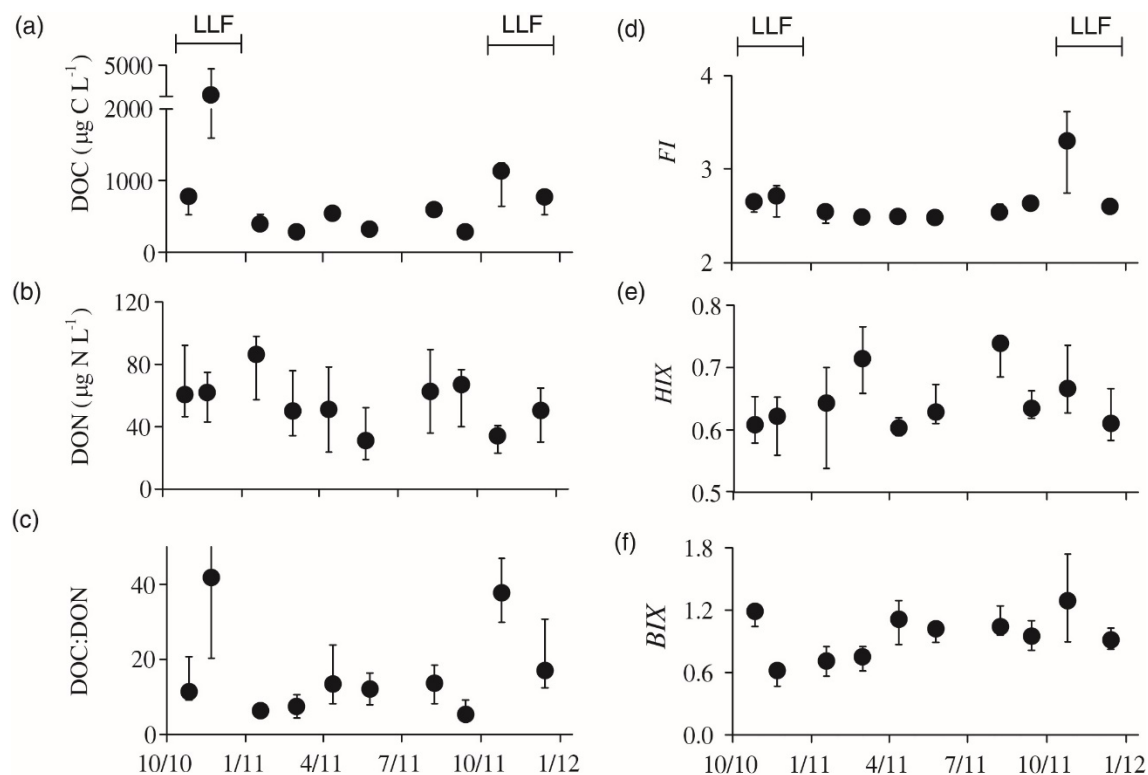


Figure 2. Temporal pattern of (a) dissolved organic carbon (DOC), (b) dissolved organic nitrogen (DON), (c) DOC:DON molar ratio, (d) fluorescence index (*FI*), (e) humification index (*HIX*), and (f) biological index (*BIX*) in stream water. *FI*, *HIX*, and *BIX* were calculated from fluorescence spectroscopy. Symbols are medians and whiskers are 25th and 75th percentiles for samples collected along the main steam. The leaf litter fall period (LLF) is indicated.

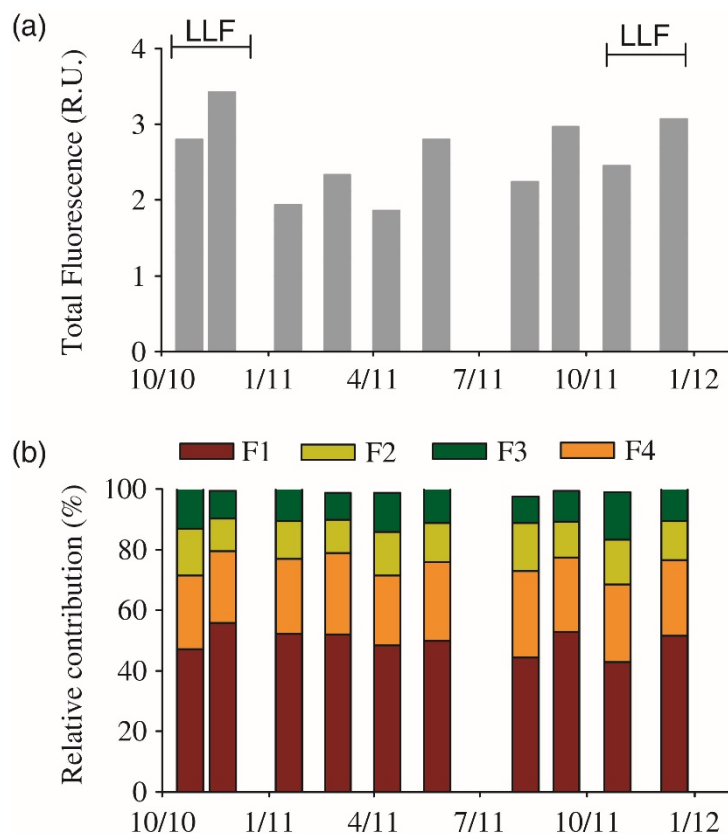


Figure 3. Temporal pattern of (a) total fluorescence of the four PARAFAC components and (b) their relative contribution to total fluorescence in the main steam of the Font del Regàs stream. The fluorescence components F1 and F4 corresponded to protein-like materials, while F2 and F3 corresponded to humic-like materials. Bars are median values for each sampling date. The leaf litter fall period (LLF) is indicated. R.U. are raman units. See more details on the obtained PARAFAC model in Table S1, S2, and Figure S4 (Supplementary Information).

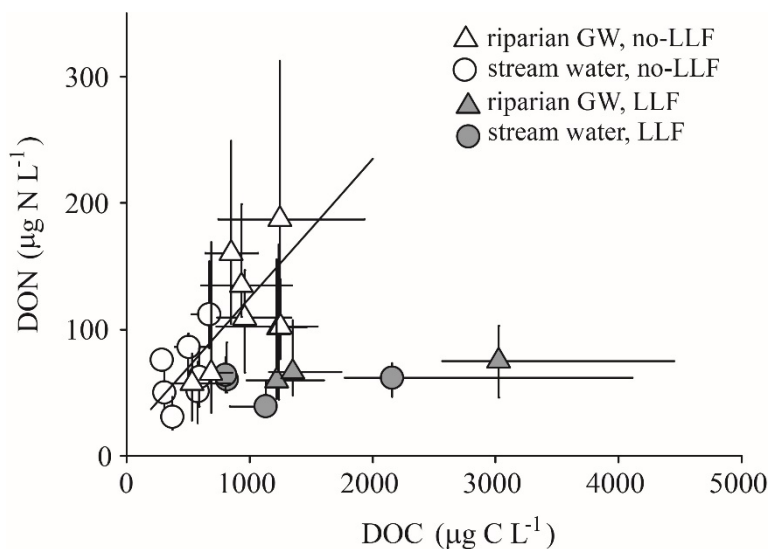


Figure 4. Relationship between dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations in stream water and riparian groundwater (GW). Symbols are median values and whiskers are 25th and 75th percentiles for each sampling date. The black line shows the DOC vs DON linear relationship for stream water and riparian GW samples pooled together for the no-LLF period (ANOVA, $F = 16.6$, $df = 13$, $p = 0.0015$). The relationship was not significant for the LLF period.

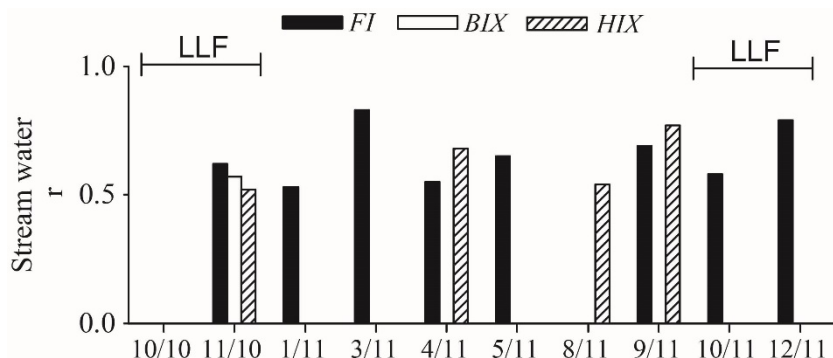


Figure 5. Temporal pattern of the standardized regression coefficient (r) obtained by fitting linear regression models to values of spectroscopic indexes measured along the 4km study reach. The r is shown for the fluorescence index (FI), biological index (BIX), and humification index (HIX) in stream water. For each sampling date, $r > 0$ indicates that values for a particular spectroscopic index increased significantly in stream water along the study reach. Bars are shown only when the model was significant ($p < 0.05$). The leaf litter fall (LLF) period is indicated. Note that none of the three spectroscopic indexes showed significant longitudinal patterns for riparian groundwater in any of the sampling dates.

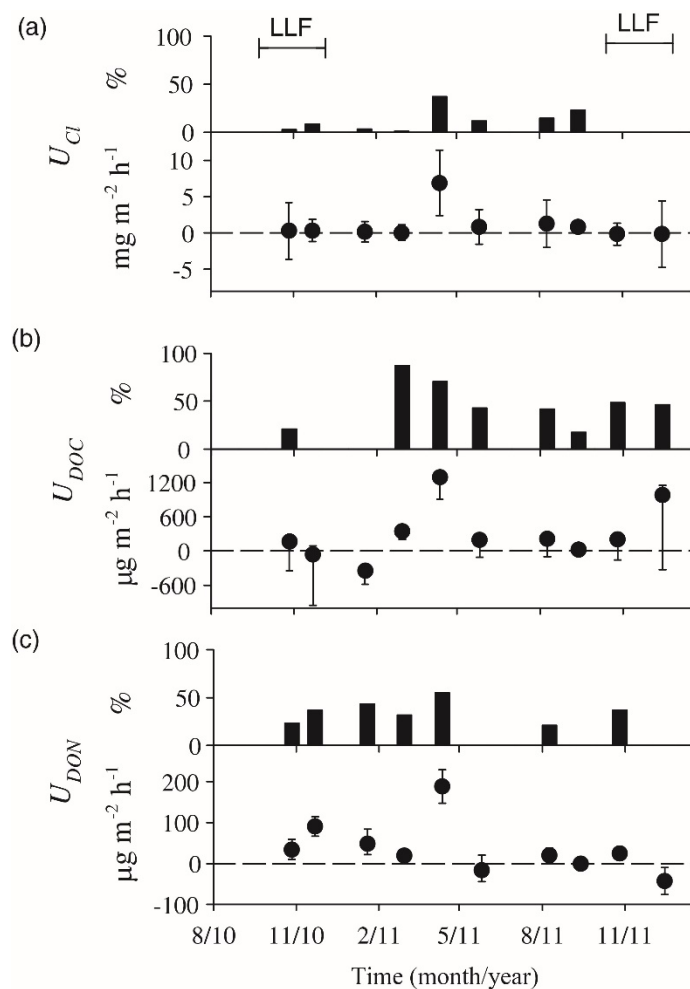


Figure 6. Temporal pattern of in-stream net uptake (U , either in μg or $\text{mg m}^{-2} \text{h}^{-1}$) for (a) chloride, (b) dissolved organic carbon (DOC), and (c) dissolved organic nitrogen (DON) at the whole reach scale. Whiskers are the uncertainty associated with the estimation of stream discharge from NaCl slug additions as in Bernal et al. (2015). Values of $U > 0$ indicate that gross uptake prevails over release, while $U < 0$ indicates the opposite. For cases with $U > 0$, the contribution of in-stream net uptake to decrease stream solute fluxes (i.e. $U \times A / F_{in}$, in %) is shown (black bars). The leaf litter fall period (LLF) is indicated.

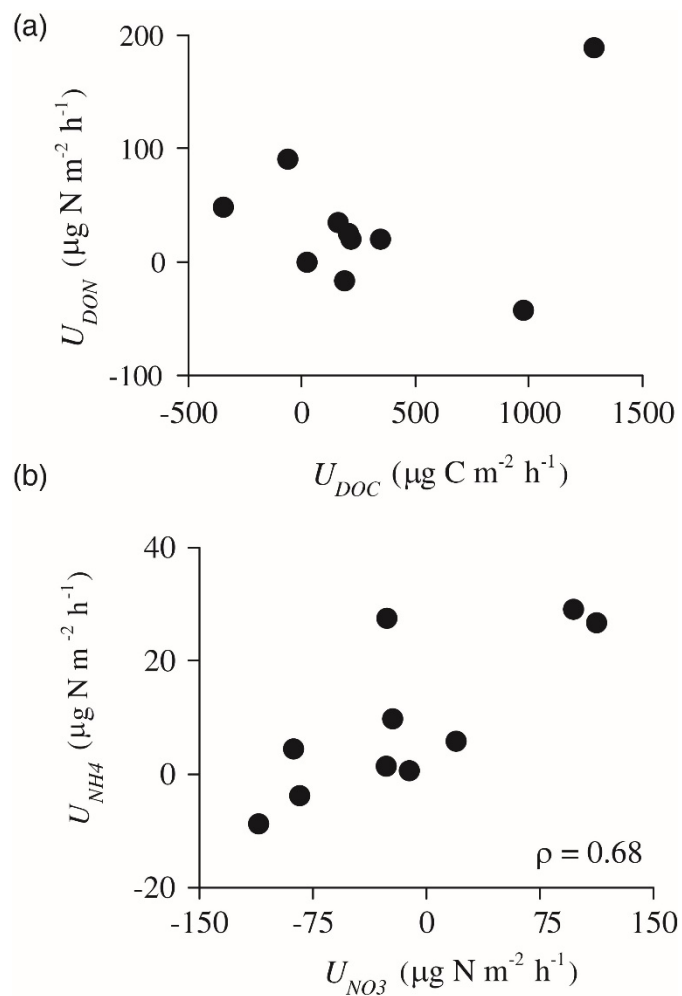


Figure 7. Relationship between in-stream net uptake along the study reach for (a) U_{DOC} and U_{DON} , and (b) U_{NO3} and U_{NH4} . The Spearman coefficient (ρ) is shown only when significant ($p < 0.05$).



Tables

Table 1. Characterization of dissolved organic matter (DOM) concentrations and quality in the main stem (stream) and in riparian groundwater (riparian GW) for the leaf litter fall period (LLF) and the no leaf litter fall period (no-LLF) at Font del Regàs. Values are medians and interquartile ranges [25th, 75th percentiles] for dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) concentrations, DOC:DON molar ratio, fluorescence index (FI), humification index (HIX), biological index (BIX), and the four PARAFAC components (F1, F2, F3, and F4). The number of cases is shown in parenthesis.

	LLF			no-LLF		
	Stream	Riparian GW	p-value	Stream	Riparian GW	p-value
DOC (µgC/L)	843 [643, 1243] (59)	1411 [1133, 2311] (56)	<0.0001	406 [304, 580] (102)	864 [626, 1414] (93)	<0.0001
DON (µgN/L)	48 [34, 67] (47)	67 [45, 157] (38)	0,012	63 [36, 87] (97)	113 [64, 195] (82)	<0.0001
DOC:DON	22 [14, 43] (47)	27 [14, 43] (38)	0,8	8 [5, 15] (93)	10 [6, 14] (82)	0,3
<i>Spectroscopic indexes</i>						
FI	2.79 [2.56, 2.83] (55)	2.59 [2.44, 2.62] (54)	0.0001	2.54 [2.47, 2.59] (84)	2.53 [2.41, 2.60] (79)	0,211
BIX	0.60 [0.60, 0.67] (55)	0.70 [0.63, 0.75] (54)	0,0072	0.67 [0.61, 0.71] (84)	0.67 [0.60, 0.73] (79)	0,646
HIX	1.03 [0.66, 1.24] (55)	1.51 [0.84, 1.82] (54)	0,0066	0.94 [0.75, 1.09] (84)	1.36 [0.86, 1.63] (79)	<0.0001
<i>PARAFAC components</i>						
F1	1.78 [1.19, 1.87] (55)	1.70 [1.14, 1.90] (54)	0,831	1.24 [0.99, 1.41] (84)	1.31 [1.03, 1.54] (79)	0,373
F2	0.45 [0.32, 0.50] (55)	0.80 [0.40, 0.94] (54)	<0.0001	0.31 [0.27, 0.36] (84)	0.58 [0.36, 0.67] (79)	<0.0001
F3	0.44 [0.28, 0.61] (55)	0.68 [0.28, 0.79] (54)	0,115	0.25 [0.20, 0.29] (84)	0.42 [0.24, 0.47] (79)	<0.0001
F4	0.89 [0.64, 1.02] (55)	1.02 [0.66, 1.16] (54)	0,021	0.65 [0.51, 0.77] (84)	0.83 [0.61, 0.93] (79)	<0.0001

*The *p* value of the Wilcoxon paired rank sum test is shown in each case.