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Seasonal and longitudinal dynamics of DOM within headwater streams of a tropical evergreen forest

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ABSTRACT

Dissolved organic matter (DOM) contributes to the energy flow in tropical rivers that dominate the global budget of riverine runoff to the ocean. Yet, the processes that control downstream DOM concentration and composition in the tropics are much less understood compared to higher latitudes. Here, we investigate seasonal and downstream changes in DOM concentration and composition in headwater streams draining the seasonally dry evergreen forest of the Río Tempisquito watershed in Costa Rica. We used dissolved organic carbon (DOC) analysis as a measure of DOM concentrations and UV–Vis, fluorescence spectroscopy and FT-ICR-MS to measure DOM composition. Seasonal differences in litterfall and day-to-day changes in rainfall controlled the DOC concentration. Elevated DOC concentrations were associated with inputs of terrestrial, humic-like DOM into the stream, and light rain falling on fresh leaf litter in the dry season led to nearly equivalent DOC concentrations as heavier rain falling on degraded litter in the wet season. The average nominal oxidation state of carbon (NOSC) in DOM molecular formulae differed between wet and dry seasons (seasonally) and with distance downstream (longitudinally). A higher NOSC of stream DOM in the dry versus wet season may be due to a peak in fresh litterfall in the dry season followed by the leaching of degraded litter during precipitation in the wet season. The shift in DOM toward a higher NOSC downstream in both seasons may be due to the selective metabolism of a more reduced, labile pool within DOM during downstream transport. We propose that the downstream changes in DOM composition in the dry tropical headwater streams of this study are driven by the preferential microbial metabolism of labile, reduced DOM compounds. These findings extend the River Continuum Concept by showing that DOM oxidation state can be a sensitive indicator of microbial metabolism in the absence of measurable decreases in downstream DOM concentrations.

1. Introduction

Dissolved organic matter (DOM) exported from terrestrial environments to streams and rivers in a watershed fuels heterotrophic metabolism (Meyer, 1994; Tank et al., 2010) and the subsequent outgassing of metabolically derived greenhouse gases to the atmosphere (Richey et al., 2002; Raymond et al., 2013). DOM fluxes in rivers as carbon metabolized to greenhouse gases or as carbon exported downstream are on the same order of magnitude as the net transfer of carbon between land and the atmosphere and are thus important in the global carbon cycle (Battin et al., 2009; Moody et al., 2013; Aho et al., 2021). The fluxes of DOM metabolized can be inferred from outgassing corrected for groundwater inputs and DOM exported downstream. However, knowledge of the processes that control DOM concentration and composition and determine how it is parsed between metabolism versus export in streams and along river networks remains poorly constrained (Hosen et al., 2021).

Rates of ecosystem metabolism suggest high rates of microbial metabolism of DOM to carbon dioxide (CO_2) in streams and rivers (Cole & Caraco, 2001; Cory & Kaplan, 2012; Battin et al., 2023) and thus concomitant decreases in DOM concentration with distance downstream are expected. However, few studies report downstream reductions in

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DOM concentrations, measured as carbon (DOC), as would occur if more DOM were metabolized in a stream reach to CO_2 than delivered to the reach from allochthonous inputs from plants, soils, and groundwater plus autochthonous inputs associated with the excretions of aquatic plants, animals, and microorganisms (Kaplan & Cory, 2016). Most studies report no change or an increase in DOC concentrations with distance downstream (Lush & Hynes, 1978; Mulholland & Kuenzler, 1979; Sabater et al., 1993; Kim et al., 2006), suggesting that the influx of DOM equals or exceeds the removal of DOM. This observation complicates efforts to estimate the DOM metabolism taking place during transport from downstream changes in DOC concentration.

Given the challenges of making direct measurements of reach-scale DOM uptake (Kaplan et al., 2008; Lutz et al., 2012; Mineau et al., 2016), the consumption or production of DOM during downstream transport has been inferred from the longitudinal succession of DOM composition in river networks. This approach was first presented in the River Continuum Concept (RCC) with predictions that a large interface with the terrestrial environment in headwaters would lead to a peak in diversity of organic compounds followed by a sharp decline in DOM diversity caused by the selective metabolism of labile organic compounds (Vannote et al., 1980). Mosher et al. (2015) confirmed the RCC prediction of peak DOM chemodiversity at 1st order streams using ultra-high-resolution mass spectrometry. However, Mosher et al. (2015) also showed high DOM chemodiversity throughout 2nd and 3rd order streams sampled in tropical and temperate biomes, in contrast to the RCC prediction of dramatically decreased DOM diversity in higher order streams. Consistent with this finding, other studies have observed little or no decrease in DOM diversity with distance downstream in higher order streams. For example, Danczak et al. (2023) found an increase in functional diversity of DOM with increasing drainage area in the Yakima River basin and Peter et al. (2020) found no distinct pattern in the number of DOM molecular formulae along downstream gradients in the Ybbs River network.

Creed et al. (2015) extended the RCC by suggesting a downstream shift in system dominance from hydrologic drivers that link terrestrial DOM sources with streams in the headwaters to dominance of in-stream biogeochemical processes trending towards chemostasis in rivers. Using specific ultraviolet absorbance, a coarse resolution optical proxy for aromaticity, Creed et al. (2015) analyzed data from nearly 10,000 measurements covering 200 sites within the continental U.S. and proposed that the downstream shift in system drivers could explain preferential losses of aromatic DOM and preferential gains in aliphatic DOM. Downstream shifts from aromatic to aliphatic DOM also have been observed using UV–Vis and fluorescence spectroscopy in a study of 60 sites from 14 river basins in the continental U.S. (Hosen et al., 2021). Hosen et al. (2021) attributed the change in DOM composition downstream to a shift from allochthonous to autochthonous DOM sources and proposed that the balance of the two sources was dependent on the flow-weighted travel time as a metric of the continuum of DOM composition from headwaters to rivers. While these findings by Creed et al. (2015) and Hosen et al. (2021) demonstrate that downstream changes in DOM composition can be influenced by the hysteresis and travel time in streams and rivers, the combination of a broad range of land uses, landcovers, climates, typographies, hydrologic conditions, and underlying geologic settings across the study sites plus the lack of chemical sensitivity of the DOM characterizations confound the ability to reveal the specific processes controlling changes in DOM composition. For instance, while not mutually exclusive, two processes – the degradation of up-stream aromatic DOM to aliphatic compounds versus a reduction in aromatic DOM inputs coupled with autochthonous production of aliphatic DOM – could generate the same downstream DOM patterns as observed in these studies.

There is a need for an alternative indicator of DOM composition beyond measures of chemodiversity or an aromatic to aliphatic shift that could capture reach-scale DOM uptake. Ideally, that alternative measurement would have the potential to distinguish changes attributable to microbial metabolism from a combination of other processes including autotrophic production, photo-oxidation, adsorption/desorption reactions, and changing terrestrial DOM subsidies. We propose that quantifying the oxidation state of DOM may provide sufficient explanatory power to function as a marker for aerobic metabolism in forested stream ecosystems where shading limits photo-oxidation.

LaRowe and Van Cappellen (2011) put forward the nominal oxidation state of carbon (NOSC) as proxy for a thermodynamic limitation of microbial organic matter degradation particularly in anaerobic environments (e.g., Boye et al., 2017). In aerobic environments where oxidation of DOM is coupled to the reduction of O_2, no thermodynamic limitation is expected as a function of the NOSC of specific DOM substrates (LaRowe and Van Cappellen, 2011). Rather, it is thought that microbes may prefer to metabolize the most energy-rich substrates, i.e., relatively reduced substrates with a lower NOSC compared to more oxidized substrates (Sun et al., 1997). However, inconsistent results on a potential microbial preference for high or low NOSC DOM have been reported in aerobic environments. For example, NOSC was highest in the headwaters of the Yangtze River basin (Li et al., 2023) but lowest in the smallest watersheds within the Yakima River basin (Danczak et al., 2023). In the Ybbs River network, Peter et al. (2020) found positive correlations between NOSC and mass transfer coefficients, suggesting preferential uptake of relatively more oxidized DOM. Additionally, Kim et al. (2006) observed selective biological uptake of DOM with higher molar ratios of O/C and lower molar ratios of H/C which generally result in higher NOSC values, when water from temperate and tropical streams were fed through laboratory-based bioreactors. The inconsistent results observed across these studies may be confounded by processes other than microbial metabolism that can influence the NOSC of stream DOM, such as increasing autotrophic production, which supplies reduced, aliphatic-like DOM to streams (Kaplan and Bott, 1982) or changing terrestrial subsidies, which can supply reduced lignin-like DOM or tannin/oxidized lignin-like DOM to streams (Franklin et al., 2020; Hensgens et al., 2021). To address whether NOSC provides a measure of DOM composition that distinguishes among the processes impacting DOM, it would be useful to constrain an investigation to a stream ecosystem characterized by fewer processes that drive the fate of DOM.

Here, we studied DOM dynamics in highly oxygenated waters of headwater montane streams within a tropical evergreen forest, where microbial degradation is expected to dominate the processing of DOM because dense forest shade limits autotrophic production and light absorption by DOM. Given that light absorption by DOM is the first step in photo-oxidation, photochemical processing is unlikely to be a control on DOM degradation in these streams. Tropical stream ecosystems are relatively understudied systems despite their contributions to tropical rivers that dominate the global budget of riverine runoff (Russell and Miller, 1990) and the roles of tropical forests in regulating regional and global climate dynamics (Lewis et al., 2009). Dry tropical forests and streams that experience distinct seasonal patterns in precipitation (Newbold et al., 1995) and litterfall (Lawrence, 2005; de Queiroz et al., 2019) are also understudied compared to their wet forest counterparts (Siyum, 2020). Seasonal patterns should impact DOM concentration and composition as increasing discharge resulting from precipitation (Wen et al., 2020) and forest litter inputs (Meyer and Wallace, 1998) are both well established as regulators of DOM input. The presence of relatively young volcanic allophane soils which contain poorly crystalline clay minerals associated with many tropical dry forest ecosystems (Rivero-Villar et al., 2022) may also control the concentration and composition of DOM exported to these streams because DOM can be adsorbed onto clay minerals (Conte et al., 2011).

To study stream DOM dynamics in a seasonally dry tropical forest ecosystem, we measured DOM concentration and composition across seasons as the dominant temporal attribute of the ecosystem. Superimposed on the seasonal pattern, however, the sampling of streams connected longitudinally allowed us to assess changes in DOM during downstream transport. Our approach involved augmenting measures of

DOC concentration with measures of optical properties through UV–Vis and fluorescence spectroscopy (Bowen et al., 2020a) and molecular composition through ultra-high-resolution mass spectrometry (Kim et al., 2003). In doing so, we evaluated aromaticity, oxidation state, and chemodiversity as indicators of the seasonal and longitudinal shifts in the stream DOM composition in a seasonally dry tropical forest ecosystem. Further, we examined whether oxidation state of the DOM pool provides an extensive and inclusive measure of both the longitudinal and seasonal shifts in the DOM composition and whether it offers a sensitive metric for use within headwater streams and beyond.

2. Methods

2.1. Study sites

Headwater streams were sampled within the Río Tempisquito drainage near the Maritza Biological Station in the Guanacaste Conservation Area of northwestern Costa Rica (Fig. 1). Seasonality of this site in the dry tropics is characterized by a four-month dry season from January through April and an 8-month wet season from May to December (Newbold et al., 1995). Draining the western slope of the central Guanacaste Cordillera (Mata & Alvarado-Hernandez, 2016), the streams arise within an area that has experienced volcanism but minimal anthropogenic disturbance. Background water chemistry of streams in the Río Tempisquito drainage reflects their volcanic origin and has been characterized as cation and nitrate rich, with intermediate levels of total dissolved phosphorus, and low levels of DOC relative to other neotropical streams and rivers (Newbold et al., 1995; Brookshire et al., 2012).

Sampling sites were arrayed along elevational gradients from 400 m to approximately 600 m, all within evergreen forests and the largest catchments extending upslope to elevations ranging from 1250 to 1540 m; high elevation areas were inaccessible due to steep slopes and dense forest growth. The sampling site closest to the Maritza Biological Station, Río Tempisquito [TEM], served as a primary monitoring station where effects of precipitation on stream DOM concentration, measured as DOC, and composition were assessed. Nine other streams were sampled along a transect at similar elevations as TEM to evaluate how DOM concentration and composition varied across streams of different catchment area. Together these ten streams were grouped into three size classes with larger streams (Río Tempisquito [TEM], Río Tempisquito Sur [TSM] and Quebrada Kathia [KAM]) draining catchments ranging from 3.01 to 10.3 km^2, intermediate-sized streams (Quebrada Marilin [MAM], Quebrada El Jobo [JOM], and Quebrada Zompopa [ZOM]) draining catchments ranging from 0.36 to 0.57 km^2, and a series of small spring seeps (Quebrada Cangrejo [CAN], Quebrada Rosa [ROS], Quebrada Origina [ORG], and Quebrada Leasa [LSA]) draining catchments of less than 0.1 km^2. The three largest streams [TEM, TSM, KAM] were also sampled at downstream locations [KAM2, TSM2, CTT] for an additional three sampling sites or a total of thirteen sampling sites arrayed across longitudinal gradients.

All the streams experience little annual temperature fluctuation and substantial seasonal rainfall differences with over 80 % of the annual rainfall occurring between May to December (Newbold et al., 1995). Total daily precipitation was measured at TEM using a Productive Alternatives Stratus rain gauge, stage height was continuously measured with a Solinst Levelogger II as well as instantaneous readings of the staff gauge when a daily water sample was taken for DOC analysis from this stream. Sampling at the remaining sites was performed during dry (20-Feb through 03-March 2016) and wet (03-July through 19-July 2016) seasons.

2.2. Streamwater collection and processing

A total of 38 stream water samples were collected across sampling sites. Stream waters typically were collected in multiple persulfate-cleaned (10 % w/v sodium persulfate) 2-L plastic bottles (Nalgene

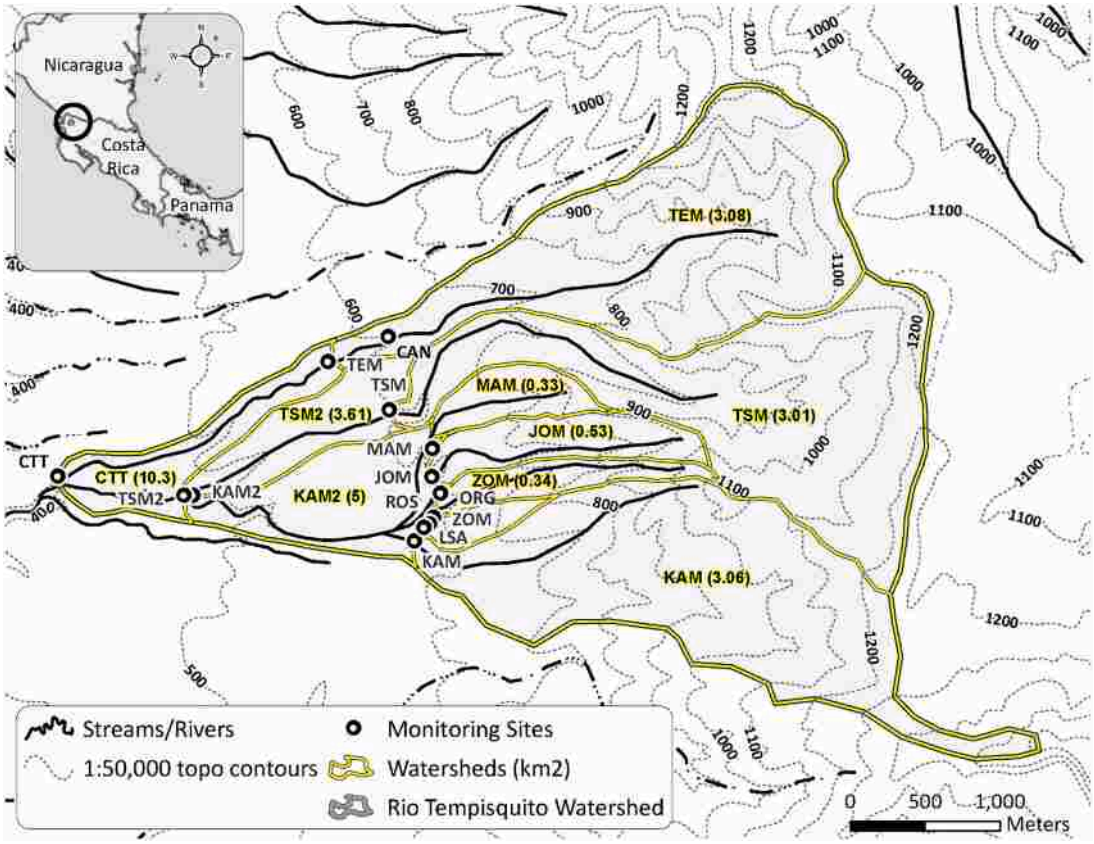

Fig. 1. Map of the streams monitored for DOM concentration and composition within the Río Tempisquito watershed.

PETE), returned to the laboratory, and filtered through pre-combusted 0.7 μm glass fiber filters (Whatman, GF/F) with a peristaltic pump and filter holder. Filtrates were mixed thoroughly before aliquots were taken for analyses that included DOC, UV–Vis and fluorescence spectroscopy, and FT-ICR-MS. Deviations from this protocol involved the stream water collected for the daily DOC analysis at TEM which was collected in the field using a syringe and filtered into pre-combusted 40-mL vials through a syringe-type filter holder containing a pre-combusted GF/F filter (Kaplan, 1994). Streamwater chemistry data collected as field measurements included temperature, dissolved oxygen, and pH (Thermo Scientific, Orion Star A329 meter).

2.3. DOC

Filtered stream water aliquots for DOC analysis were transferred to triplicate pre-combusted 40-mL borosilicate vials, covered in pre-combusted aluminum foil, sealed with Teflon-backed silica septa, and stored in the refrigerator at 4 °C until analysis. For analysis, the septa were removed, the vials were loaded into an autosampler, and DOC was measured with a Sievers-900 total organic carbon analyzer equipped with an inorganic carbon removal module. Samples were acidified during analysis with 6 M phosphoric acid to assist in inorganic carbon removal and the samples were oxidized with UV-promoted persulfate oxidation.

2.4. UV–Vis and fluorescence spectroscopy

Filtered stream water aliquots for analysis of the chromophoric and fluorescent fractions of DOM (CDOM and FDOM, respectively) were transferred to triplicate pre-combusted 40-mL borosilicate vials sealed with persulfate-cleaned, Teflon-backed, silica septa, and then the water samples were made biologically stable using a modified Tyndallization procedure (Tyndall, 1877). Briefly, vials were placed in a water bath and heated three times by keeping the water bath at 60 °C for 5 min followed by a 30-minute interval at 25 °C (Bowen et al., 2020a). After this procedure, samples were kept at room temperature until they were shipped to the University of Michigan and analyzed.

CDOM was analyzed from 240 to 600 nm using one of two UV–Vis spectrophotometers (Cary Varian 300 or Aqualog, Horiba) depending on the pathlength (10 or 1 cm, respectively) against laboratory-grade deionized water blanks. CDOM absorption coefficients ($a_{CDOM,\lambda}$; m^{-1}) were calculated as the absorbance at wavelength λ multiplied by 2.303 and divided by the pathlength (m). The spectral slope ratio (S_R; a proxy for the average molecular weight of DOM) was calculated from the CDOM absorbance spectrum as previously described in Helms et al., (2008). The specific ultraviolet absorbance at 254 nm ($SUVA_{254}$, L $mg^{-1}C$ m^{-1}; a proxy for the aromatic content of DOM) was calculated as the absorbance at 254 nm divided by the pathlength and the concentration of DOC (Weishaar et al., 2003).

FDOM was analyzed using a fluorometer (Aqualog, Horiba) with a 1-cm pathlength cuvette alongside laboratory-grade deionized water blanks following Bowen et al. (2020a). Briefly, excitation emission matrices (EEMs) were collected for excitation wavelengths 240–450 nm with 5-nm increments and emission wavelengths 320–550 nm with 2.33-nm increments. Integration times varied from 3 to 6 s. EEMs were corrected for inner-filter effects, instrument-specific excitation and emission corrections, and for the laboratory-grade deionized water blanks in MATLAB (version 8.5). Fluorescence intensities were smoothed over the emission wavelengths collected at 2.33-nm increment to obtain 5-nm increments and the resulting intensities were then converted to Raman units (RU; Stedmon and Bro, 2008). Parallel factor analysis (PARAFAC) was used to separate the dataset of EEMs into mathematically and chemically independent components and five FDOM components were validated (Table S1; see the Supplementary Text S1 for details). The spectrum of each component was compared to those previously published in the OpenFluor database using a 95 % similarity criteria (Murphy et al., 2014) and the components were identified as: humic-like C1, humic-like C2 and C3 corresponding to allochthonous peaks A and C, respectively (Coble, 1996), and the amino acid-like C4 and C5 that overlap with the fluorescence of the tryptophan and tyrosine, respectively (Cory and McKnight, 2005; Cory and Kaplan, 2012; Stedmon and Markager, 2005). Of the eight studies of headwater streams with spectral matches for C1, two have classified it as a pool of refractory compounds (Williams et al., 2010; Lambert et al., 2017), four have attributed it to microbial degradation products of humic-like DOM (Yamashita et al., 2010; Yamashita et al., 2011; Osburn et al., 2018; Attermeyer et al., 2022), and two have found it associated with plant organic matter (Derrien et al., 2018; Graeber et al., 2021); Table S2). Each FDOM component is reported as the relative abundance within an EEM (F_{max} value in RU; Cory & Kaplan, 2012) or as the percent abundance of all FDOM components. The fluorescence index (FI; a proxy for the aromatic content of DOM; McKnight et al., 2001) was calculated as the fluorescence intensity at excitation / emission wavelength of 370 nm / 470 nm divided by the intensity at excitation / emission wavelength of 370 nm / 520 nm (Cory et al., 2010).

2.5. Ultrahigh resolution mass spectrometric analysis (FT-ICR-MS)

Samples from 8 of the 13 stream sites (ROS, MAM, KAM, KAM2, TEM, TSM, TSM2, and CTT) were used for molecular-level analyses by FT-ICR-MS by dispensing aliquots of the filtered stream waters into pre-combusted 40-mL borosilicate vials. Because one or more water samples were collected from each stream site, (see Table S3) a total of 26 samples were analyzed by FT-ICR-MS from the dry and wet seasons. Briefly, DOM from each sample was concentrated using PPL cartridges (Agilent Technologies, Bond Elut PPL, 100 mg styrene divinyl copolymer) following Dittmar et al (2008). The PPL resin cartridges were stored in the freezer, transported to Old Dominion University frozen, and thawed for an hour followed by flushing with nitrogen gas to remove any condensation on the resin prior to extraction and analysis. Methanol (Optima LC-MS grade MeOH, Fisher Scientific) was used to elute the extracted DOM from the cartridges following Dittmar et al. (2008)and the effluent was then diluted to obtain a 1:1 MeOH:H_2O mixture.

A Bruker Daltonics 12 Tesla Apex Qe FT-ICR-MS with an Apollo II negative mode electrospray ionization source was used for analysis of the extracted DOM as described previously (Sleighter and Hatcher, 2008). Briefly, 100 μL of each 1:1 MeOH:H_2O mixture was injected into the instrument. External calibration was performed daily using a standard of polyethylene glycol over the mass range of 200–1000 Da. Each spectrum was internally calibrated to compounds that are naturally present in DOM, such as fatty acids, dicarboxylic acids, and other compounds that are part of a CH_2 homologous series.

The FT-ICR-MS spectra were processed by an in-house Matlab program TEnvR to correct for salt peaks and process blanks, remove ^{13}C isotopologues, align the spectra among all samples in the dataset (Mantini et al., 2007; Thompson et al., 2021), and assign molecular formulae (Goranov et al., 2023). Molecular formulae were assigned to peaks using C, H, O, N, S, and P elements in the range of $^{12}C_{5\text{-}\infty}$, $^{1}H_{5\text{–}100}$, $^{16}O_{1\text{–}30}$, $^{14}N_{0\text{–}5}$, $^{32}S_{0\text{–}4}$, and $^{31}P_{0\text{–}2}$. For each FT-ICR-MS spectra, the intensity of each peak with a molecular formula assignment was normalized to the sum of all peak intensities to remove any bias from tuning or ionization efficiency. Molar H/C and O/C ratios were calculated for each unique molecular formula and plotted on van Krevelen diagrams as described previously (Kim et al., 2003). Compound classes were assigned to each molecular formula following Hertkorn et al. (2006) and Kim et al. (2003): lignin-like (H:C = 0.7–1.5; O:C = 0.1–0.67), tannin/oxidized lignin-like (H:C = 0.5–1.5; O:C = 0.67–1.2), and lipid-like (H:C = 1.5–2.5; O:C = 0–0.3). Aliphatic-like molecular formulae were defined as those with a modified aromaticity index (AI_{mod}) < 0.5 (Dittmar et al., 2008) and condensed aromatic-like formulae were defined as those with $AI_{mod} \geq 0.67$. Carboxylic-rich alicyclic-like molecules (CRAM)-like formulae were identified using double bond

equivalence (DBE) where DBE/C = 0.30–0.68, DBE/H = 0.2–0.95, and DBE/O = 0.77–1.75 (Hertkorn et al., 2006). NOSC for each molecular formula was calculated following LaRowe and Van Cappellen (2011) as:

$$NOSC = 4 - \left(\frac{(4 * Carbon) + (Hydrogen) - (3 * Nitrogen) - (2 * Oxygen) - (2 * Sulfur) + (5 * Phosphorus)}{Carbon}\right.$$

The average percentage of molecular formulae making up each compound class and the average NOSC value of DOM formulae are reported for each stream sample collected in Table S3.

Four separate analyses were carried out using the molecular formulae assigned in the mass spectra to evaluate seasonal and longitudinal patterns for DOM. First, the molecular formulae present in the dry season versus the wet season were compared by identifying the molecular formulae common across all stream water samples within each season. Second, shifts in the molecular formulae present within DOM with rainfall in each season were evaluated by identifying the molecular formulae unique to stream waters collected on days with low rainfall versus those collected on days with high rainfall relative to the average rainfall of each season. For instance, in the dry season, days with low rainfall were defined as those with < 0.5 mm of precipitation and days with high rainfall were defined as those with > 0.5 mm of precipitation. In the wet season, days with low rainfall were defined as those with ⟨ 10 mm of precipitation and days with high rainfall were defined as those with ⟩ 10 mm of precipitation. Third, shifts in the molecular formulae present within DOM along longitudinal gradients were evaluated by identifying the molecular formulae unique at an upstream site relative to a downstream site across both seasons. For the dry versus wet season comparison and the upstream versus downstream comparison, an inter-sample ranking was performed following Herzsprung et al. (2012) (see the Supplementary Text S2 for details). The last step involved carrying out three different principal component analyses (PCA) to analyze the effect of rainfall on DOM composition (see the Supplementary Text S3 for details).

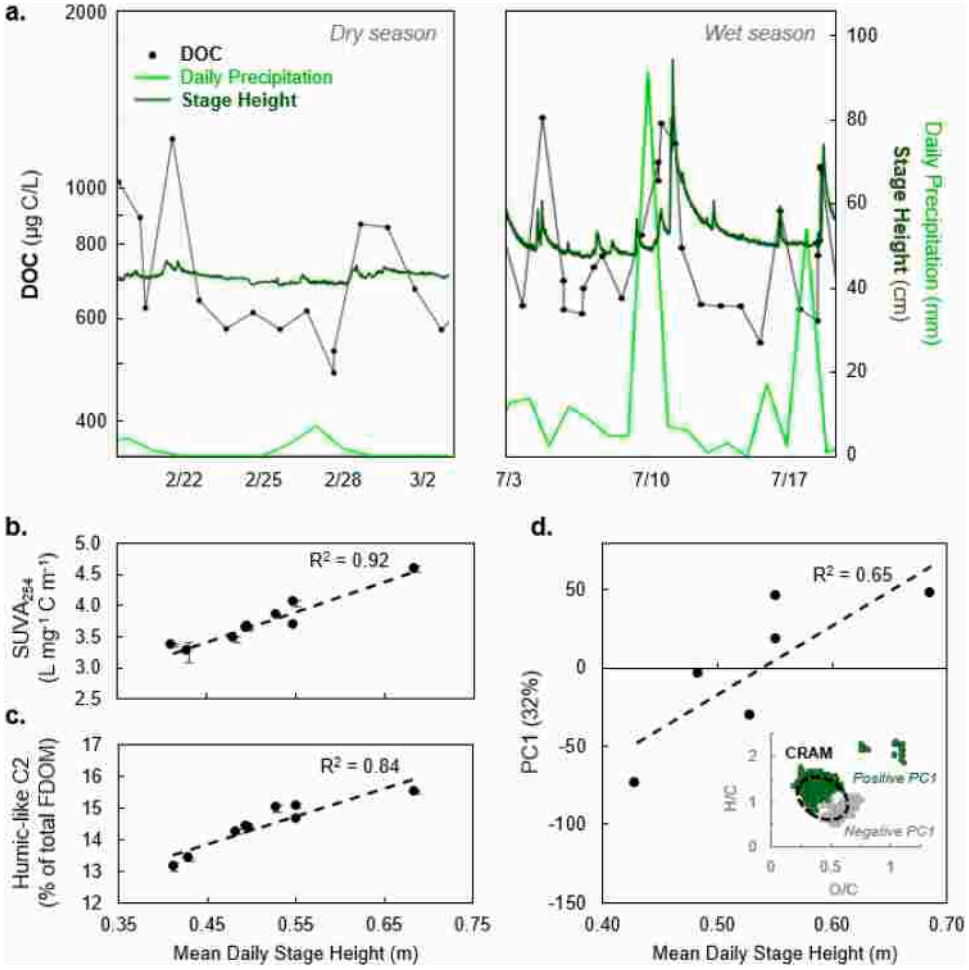

Fig. 2. Effects of rainfall on DOM concentration and composition in the Río Tempisquito stream (TEM). (a) DOC concentrations, daily precipitation, and stage height at the TEM monitoring site during the dry and wet season sampling periods. Correlations of stage height at TEM with (b-c) DOM composition and (d) the first principle component (PC1) from PCA of DOM formulae analyzed by FT-ICR-MS ($n = 6$). Data in (b-d) were fit with a least-squares linear regression where $p < 0.05$. All values on the y-axis in (b-c) are shown as the average ± 1 SE of triplicate measurements. The inset in (d) shows a van Krevelen diagram of the positive and negative loadings for PC1 (Fig. S1).

2.6. Statistical analyses

Statistical analyses were conducted using parametric *t*-tests and the non-parametric Mann-Whitney U test to assess the significance of the seasonality and longitudinal factors. For the DOC, CDOM, and FDOM data, independent two-sample *t*-tests were performed to compare the seasonal mean values. For NOSC values, Mann-Whitney U tests were conducted to compare the ranks of the sites and seasonal data. A significance level of $p < 0.05$ was considered statistically significant for both tests.

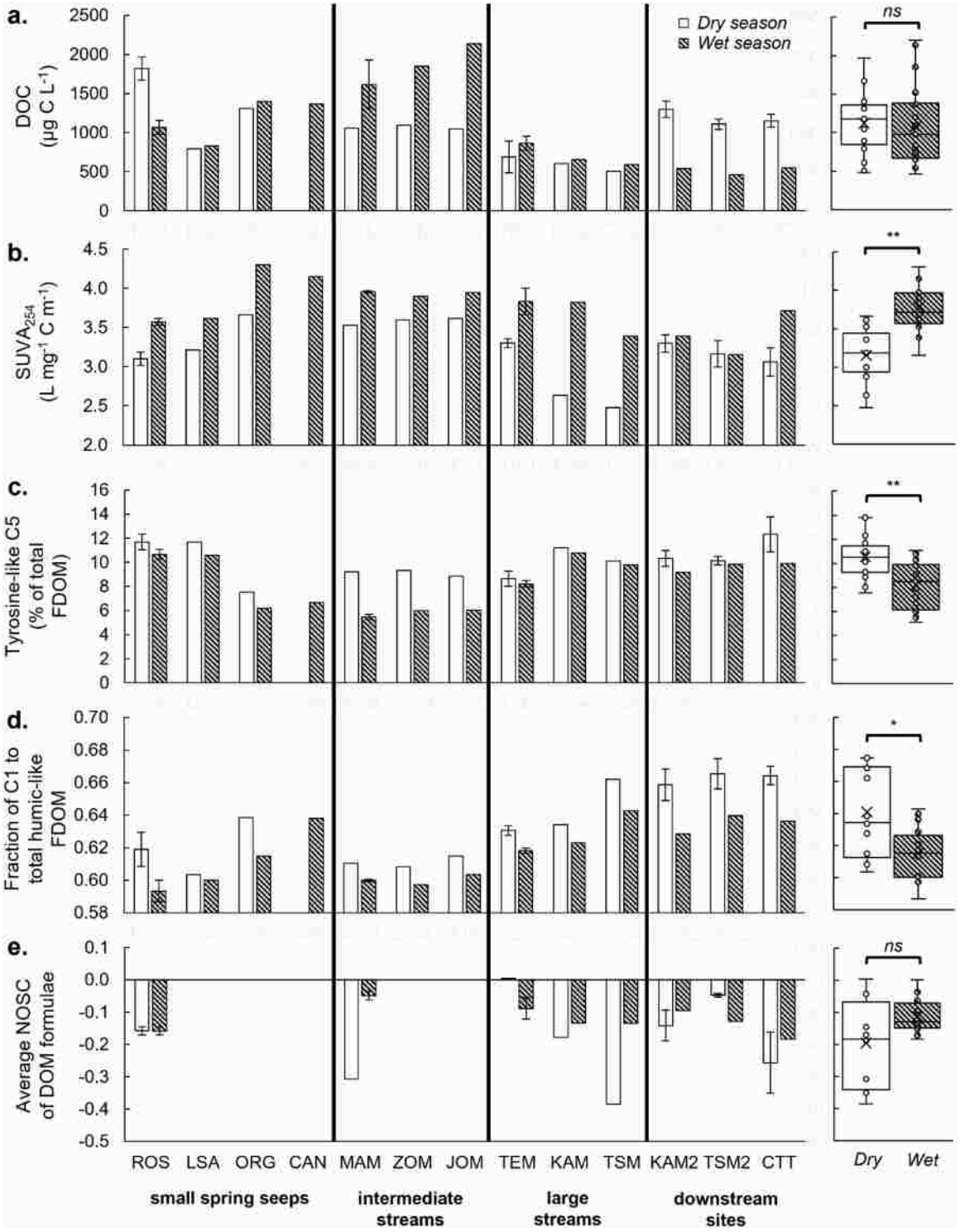

Fig. 3. Seasonal average DOM concentrations and compositions across all sampling sites. Bar graphs show the average ± 1 SE for water samples collected on separate days during the same season ($n = 2$, except MAM, where $n = 3$ in (a-d), and TEM, where $n = 6$ and 4 in (a-d) and (e), respectively). Box plots show the mean, median, interquartile range, and upper and lower limits for DOM concentrations and composition across sites within the dry season versus the wet season, where the upper limit was calculated as the third quartile plus the interquartile range multiplied by 1.5 and the lower limit was calculated as the first quartile minus the interquartile range multiplied by 1.5. ** and * indicate statistically significant differences with *p*-values < 0.001 and 0.05, respectively, whereas ns = not significant.

3. Results

3.1. Precipitation and discharge

Rainfall during the wet season led to consistently higher stage heights in the Río Tempisquito stream [TEM] than during the dry season, though discharge increased in response to precipitation during the dry season as well. The dry season sampling period from 20-February through 03-March 2016 was marked by infrequent, small amounts of rain, with measurable accumulations of 1.1 to 7 mm on five of the days (Fig. 2a). While no measurable precipitation occurred on the other eight days, brief morning mists occurred almost daily. During the wet season sampling period from 03-July through 19-July 2016 it rained on all but one of the days with amounts ranging from 1.1 to 92 mm. Rainfall amounts were variable, with a total of 31 mm between 06-July and the stream sampling on 10-July (< 10 mm of daily rainfall) and a total of 184.3 mm following the 10-July stream sampling through 18-July (> 10 mm of daily rainfall).

3.2. Impacts of precipitation and season on DOM concentrations and composition

3.2.1. DOC

Precipitation during both seasons caused spikes in DOC and the daily monitoring of DOC at TEM revealed concentration shifts within each season. Daily DOC concentrations at the TEM site showed similar concentration ranges across seasons of 485 to 1218 μg C/L and 549 to 1329 μg C/L for the dry season and wet season, respectively (Fig. 2a). Small amounts of precipitation during the dry season generated spikes in DOC concentrations, such as on 21 and 28-February (Fig. 2a). DOC concentrations also varied from day-to-day by over 10 % when no measurable rainfall was recorded at the site for over 72 hours (Fig. 2a). In comparison to the dry season, the shifts in DOC concentration with rainfall in the wet season were less pronounced. For example, 92 mm of precipitation on 10-July during the wet season caused an increase in DOC by 600 μg C/L, which was similar to the 400 μg C/L DOC increase observed on 27-February with 7 mm of precipitation.

At the other streams, DOC concentration ranges were also similar across seasons with 505 to 1969 μg C/L during the dry season and 464 to 2198 μg C/L during the wet season (Fig. 3a). There were no consistent seasonal DOC concentration trends that extended across all streams. Among all streams, DOC concentrations were consistently higher during the wet season only for the intermediate-sized streams (MAM, JOM, ZOM) and consistently lower during the wet season only for the largest streams (KAM2, TSM2, CTT) (Fig. 3a).

3.2.2. CDOM and FDOM

DOM composition shifted with precipitation and differed between seasons. For example, $SUVA_{254}$ and the percentage of humic-like C2 increased in TEM as a function of mean daily stage height ($p < 0.05$; Fig. 2b-c). Across all streams, $SUVA_{254}$ and the percentage of humic-like C2 were significantly higher in the wet season compared to the dry season ($p < 0.001$; Table S3; Fig. 3b). FI and the percentage of tyrosine-like C5 were significantly lower in the wet season compared to the dry season ($p < 0.001$; Table S3; Fig. 3c). The fraction of humic-like C1 relative to the total humic-like FDOM was also lower at each site in the wet season ($p < 0.05$; Fig. 3d). For the intermediate-sized streams (MAM, JOM, ZOM), S_R was lower in the wet season compared to the dry season (Table S3).

3.2.3. Molecular composition of DOM

FT-ICR-MS analysis revealed that precipitation during both seasons led to similar proportions of molecular composition of stream DOM. A PCA analysis of the DOM molecular composition at TEM separated the composition of stream DOM on days with higher mean stage height (plotting within the positive region of PC1) and days with lower mean stage height (plotting within the negative region of PC1; Fig. S1a). The loading of PC1 increased with the mean daily stage height at TEM ($p < 0.05$; Fig. 2d). The molecular formulae associated with the PC1 loading in van Krevelen space were characterized by lignin and CRAM-like formulae in the positive region and more tannin/oxidized lignin-like formulae in the negative region (inset in Fig. 2d; Fig. S1).

A similar convergence towards CRAM-like formulae was also observed following precipitation at the other stream sites within the Río Tempisquito watershed (Fig. S2). For example, in the dry season, stream DOM shifted from carbohydrate-like and lignin-like formulae to more CRAM-like formulae on days with higher precipitation (increase in CRAM-like formulae from 50 to 62 %; Fig. S2a). Additionally, in the wet season, stream DOM shifted from tannin/oxidized lignin-like formulae to more CRAM-like formulae on days with higher precipitation (increase in CRAM-like formulae from 23 to 37 %; Fig. S2a). These shifts in DOM molecular composition with rainfall during the dry season and the wet season were also observed following PCA analysis (Fig. S2b-c).

A comparison of the average nominal oxidation state of carbon (NOSC) among DOM molecular formulae showed wider variation in NOSC among streams than between the two seasons. For example, the average NOSC of DOM formulae calculated for each stream during the wet season was generally higher than the average NOSC of DOM during the dry season, although this was not the case for ROS, TEM, and TSM2 (Fig. 3e). Across streams, the average NOSC value of DOM formulae varied from -0.386 to 0.004 (Fig. 3e). As a result of this variability in NOSC, there were no significant differences in the average NOSC of DOM across all streams in the dry season versus the wet season (Fig. 3e). Consistent with this result, there were no significant differences in the percentage of DOM formulae comprising each compound class between all streams in the dry season and all streams the wet season: lignin-like formulae (71 % and 71 %), tannin/oxidized lignin-like formulae (9 % and 9 %), lipid-like formulae (11 % and 7 %), and condensed aromatic-like formulae (4 % and 5 %), respectively (Table S3).

When constraining the comparison to the formulae that were found in all streams in the dry season to the formulae that were found in all streams in the wet season, the compound classes and NOSC values making up DOM differed between the two seasons. Many formulae were found in common across the streams studied: 668 out of 7714 formulae were consistently found in the dry season and 775 out of 8360 formulae were found in the wet season (Fig. S3). While lignin was the

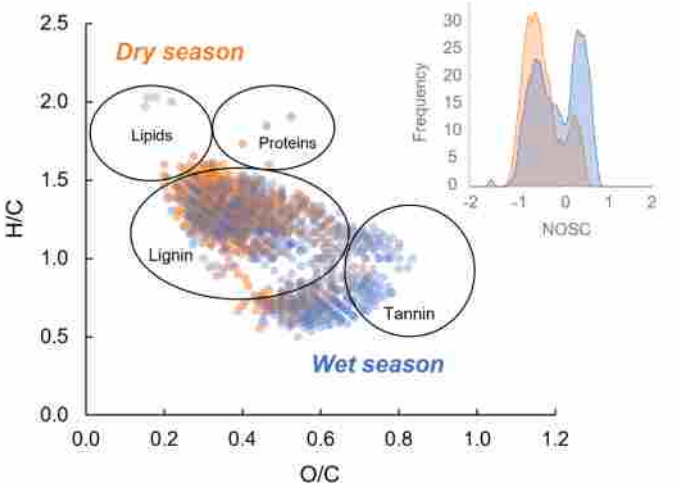

Fig. 4. Seasonal differences in DOM molecular composition. Van Krevelen diagram of the DOM formulae commonly found at all sampling sites within the dry season (orange) versus those commonly found within the wet season (blue). The inset shows the frequency distribution of the nominal oxidation state of carbon (NOSC) for the DOM formulae commonly found within each season ($U = 4.5\times10^7$, $p < 0.01$ following a Mann-Whitney U test). Colors are transparent to demonstrate where overlap occurred between the two seasons.

predominant compound class making up the common formulae present within DOM during each season, there was generally more oxygenated formulae during the wet season compared to the dry season (Fig. 4). The more oxygenated formulae present across streams in the wet season were tannin/oxidized lignin-like (making up 17 % of the formulae consistently found), whereas the more reduced formulae present in the dry season were non-CRAM aliphatic-like (making up 22 % of the formulae consistently found; Fig. 4). A plot of the distribution of NOSC values for formulae common to the dry season versus the wet season demonstrated a significant shift toward more oxidized formulae in the wet season (mean NOSC of -0.154) relative to the dry season (mean NOSC of -0.185; $p < 0.01$, inset in Fig. 4). Similar shifts in DOM composition between the dry season and wet season were observed across all five rankings from the inter-sample ranking analysis (Fig. S4).

3.3. Longitudinal trends in DOM concentration and composition across seasons

With stream sizes that drain catchments ranging from $< 1km^2$ to $>10km^2$, the sequence of five streams directly connected as tributary inputs (ROS, KAM, KAM2, TSM2, CTT; Fig. 1) offers the most direct assessment of longitudinal trends in DOM. The only measure of DOM composition or concentration that consistently changed across all five streams in the longitudinal sequence was the fraction of C1 relative to total humic-like FDOM, which increased from upstream to downstream during both seasons (Fig. 5). No clear pattern of DOM molecular diversity, expressed as richness and calculated from the total number of molecular formulae, was observed across the longitudinal sequence during either season, although molecular diversity was highest in the smaller spring seep sampled upstream, ROS, and lowest in the largest downstream site, CTT (Table S4). Constraining longitudinal comparisons to upstream/downstream sites on the same stream, KAM/KAM2 and TSM/TSM2, DOC and $SUVA_{254}$ increased in the dry season and decreased in the wet season. The percentage of tyrosine-like C5 decreased slightly in the wet season for KAM/KAM2 (Fig. S5).

The upstream/downstream shifts in DOM molecular composition were larger in magnitude for TSM/TSM2 than KAM/KAM2 (Fig. 6a-d). DOM in the upstream KAM and TSM sites was dominated by lignin-like and aliphatic-like formulae and had an average NOSC of -0.193 and -0.481, respectively (yellow and green in Fig. 6a-d). Moving downstream, DOM at KAM2 and TSM2 had a higher percentage of tannin/oxidized lignin-like formulae within the van Krevelen space (Fig. 6a,c). The downstream shift in DOM composition was more pronounced at TSM2 compared to KAM2 as shown by the higher frequency of NOSC values > 0 for the DOM formulae unique to TSM2 relative to upstream TSM (Fig. 6b,d). These patterns held for each upstream/downstream pair of streams during each season (as shown for TSM/TSM2 in Fig. S6). Despite there being a weaker shift in DOM composition for KAM/KAM2 compared to TSM/TSM2, similar shifts in DOM composition were observed between KAM and KAM2 across all five rankings from the inter-sample ranking analysis (Fig. S7). Of the 1253 formulae found at TSM, 445 were unique to that site and 808 were also found downstream at TSM2 (Table S5). Of the 1586 formulae found at KAM, 436 were unique to that site and 1150 were also found downstream at KAM2 (Table S5).

Unlike the shifts in DOM molecular composition observed at TSM/TSM2, longitudinal comparisons further downstream between TSM2 and CTT showed that DOM shifted from tannin/oxidized lignin-like to aliphatic-like formulae with lower NOSC values downstream (Fig. 6e-f, Table S3). Nearly all the molecular formulae unique to CTT relative to all upstream sites along the longitudinal sequence were aliphatic-like (>80 %) and only ~25 % were lignin-like (Fig. S8). From upstream to downstream, DOC and $SUVA_{254}$ increased in the wet season while the percentage of tyrosine-like C5 increased in the dry season (Fig. S5).

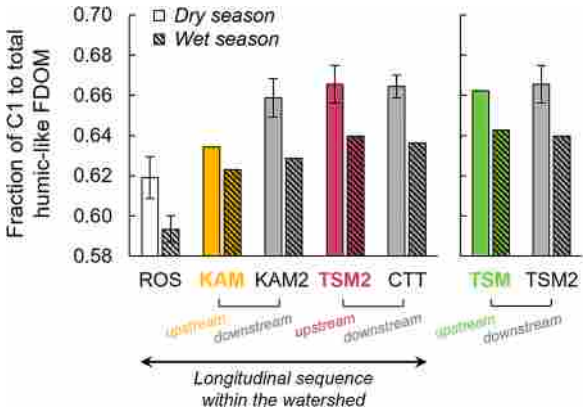

Fig. 5. Shifts in DOM composition along different longitudinal gradients within the watershed. Bar graphs show the average ± 1 SE for water samples collected on separate days during the same season ($n = 2$). The graph is organized to show three longitudinal comparisons of upstream/downstream sites (KAM/KAM2, TSM2/CTT, and TSM/TSM2), and the sequence of five streams directly connected as tributary inputs (ROS, KAM, KAM2, TSM2, CTT).

4. Discussion

Here, we show that daily rainfall controlled the concentration of DOC and the input of humic and CRAM-like DOM to streams draining the Río Tempisquito watershed. Peak litterfall during the dry season, discussed below, enhanced the export of biologically labile DOM to streams, whereas DOM shifted to a higher oxidation state as dry season litterfall underwent degradation and leaching in the wet season. During both seasons, the make-up of humic-like FDOM shifted with distance downstream and DOM shifted to a higher oxidation state downstream, which we propose are attributed to microbial metabolism of the most biologically labile compounds within DOM.

4.1. Daily rainfall controls the input of humic and CRAM-like DOM to streams

Baseflow DOC concentrations within the Río Tempisquito watershed of 500 μg C/L or less are some of the lowest concentrations reported globally for streams (Meybeck, 1982), including streams within the tropics (Brinson, 1976) and alpine streams above tree line (Fasching et al., 2016). Streams within the densely forested Río Tempisquito watershed have DOC concentrations similar to barren watersheds where glaciers have retreated (Boix Canadell et al., 2019) or where the forests have been clearcut (Meyer & Tate, 1983). One possible explanation for the low baseflow DOC concentrations in the Río Tempisquito watershed may be the presence of young, volcanic allophane soils (Morera, 1983; Mata & Alvarado-Hernandez, 2016) that can sequester organic carbon (Basile-Doelsch et al., 2007; Parfitt et al., 1999). These allophane soils may restrict carbon movement from the forest floor by binding DOM as water moves down through the vadose zone soils to the groundwater and to streams within the watershed. Consistent with this explanation, DOC concentrations below 500 μg C/L have been observed in streams on the volcanic island of Basse-Terre in the Guadeloupe archipelago (Lloret et al., 2013). At such low baseflow DOC concentrations, small changes in DOM inputs associated with rainfall or increased discharge should have a dramatic impact on DOC concentration and perhaps DOM composition.

Spikes in DOC concentrations at TEM followed rainfall during both seasons (Fig. 2a), suggesting limitations in the ability of soils in the Río Tempisquito watershed to keep DOM from entering the stream. During rainfall in the dry season, the role of allophane soils in restricting carbon movement could be bypassed in the riparian zone canopy by direct interception, throughfall, or blow-in of leaf litter from the forest floor (Meyer & Wallace, 1998;Raymond & Saiers, 2010; Mei et al., 2014; Osburn et al., 2018; Ryan et al., 2021). In the wet season, the binding of

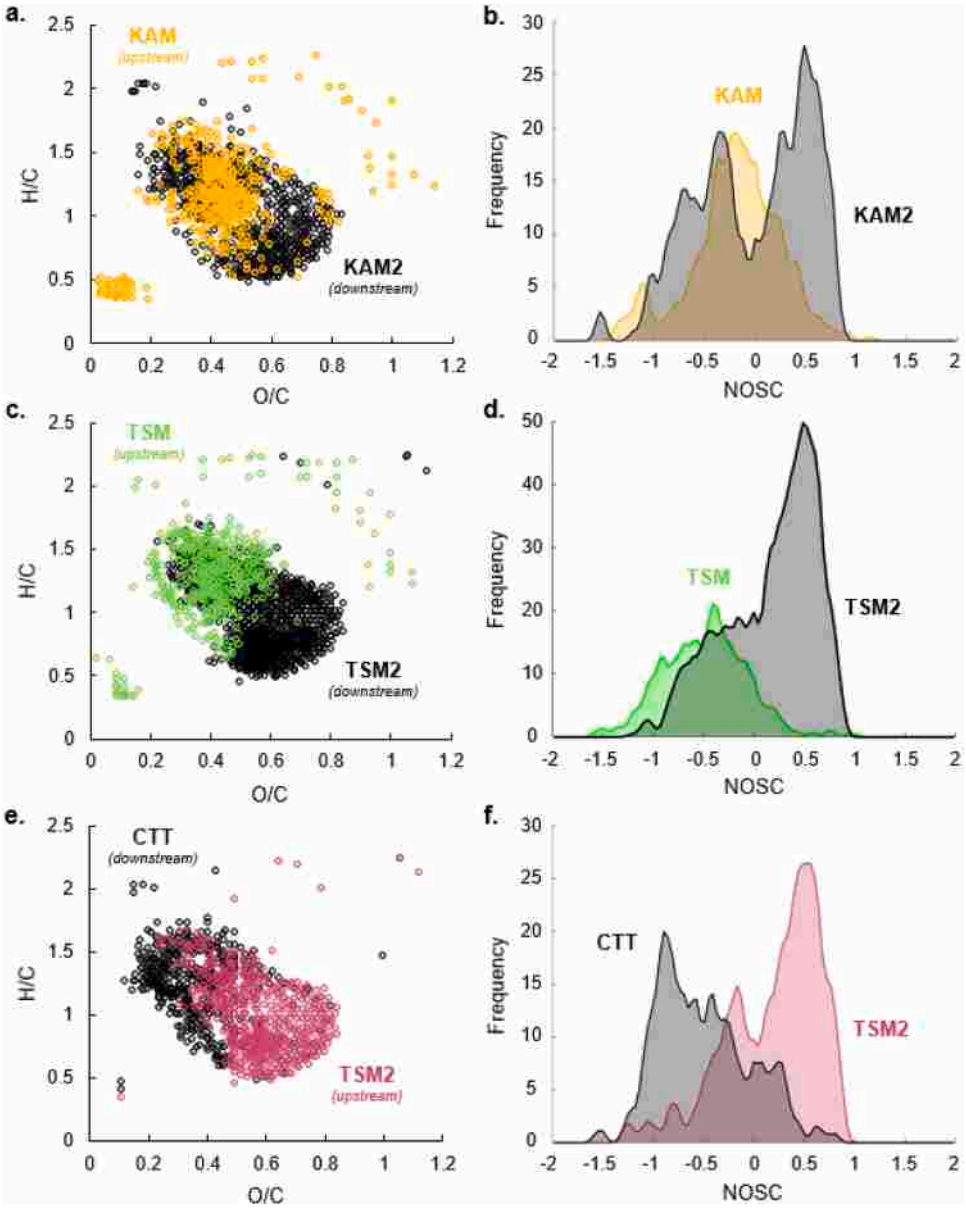

Fig. 6. Upstream versus downstream shifts in DOM molecular composition. (a,c,e) Van Krevelen diagrams and (b,d,f) frequency distributions of the nominal oxidation state of carbon (NOSC) for the DOM formulae unique to each sampling site along a longitudinal gradient ($U = 1.2\times10^5$, 7.1×10^4, and 3.7×10^4 following Mann-Whitney U tests for (b), (d), and (f), respectively, and $p < 0.001$). The DOM formulae unique to each sampling site were identified across all water samples collected at each site across the two seasons ($n = 2$ or 3).

DOM in soils could be overwhelmed during periods of intensive rainfall, possibly resulting in carbon movement via overland flow (McLaughlin & Kaplan, 2013; Mei et al., 2014; Neu et al., 2016). In these ways, meteorological conditions on the day of sampling, perhaps more than seasonal differences in precipitation, could have influenced the seasonal comparisons in DOC concentration across sampling sites. For example, when dry season sampling occurred on a day without rainfall (23 and 24-February; Fig. 2a), including intermediate-sized streams MAM, JOM, and ZOM, DOC concentrations were consistently lower than wet season concentrations (Fig. 3a). Conversely, when dry season sampling occurred on days with rainfall (21 and 28-February; Fig. 2a), which was the case for the largest downstream sites KAM2, TSM2, and CTT, DOC concentrations were consistently higher than wet season concentrations (Fig. 3a).

Molecular-level analyses suggest that, following rainfall, rising groundwater levels within riparian soils impact the composition of DOM entering the stream. The higher abundances of humic-like C2 FDOM, aromatic-like CDOM (i.e., $SUVA_{254}$; Fig. 2b-c), and CRAM-like molecular formulae (Fig. 2d, S2) consistently observed following rainfall suggest the delivery of DOM from a similar terrestrial source to streams during both seasons. Groundwater levels rise during rainfall and this rise increases the connectivity of stream water with riparian and vadose zone soils (McLaughlin & Kaplan, 2013; Mei et al., 2014). It follows that rising groundwater levels may enable the delivery of soil-derived DOM to the stream despite the strong carbon binding capacity of the allophane soils. The higher abundances of humic and CRAM-like DOM in the stream following rainfall suggests that the DOM delivered from rising groundwater levels is made up of alicyclic carboxylic acids (DiDonato et al., 2016;Peter et al., 2020; Gamage et al., 2024). Because humic and CRAM-like DOM are expected to be degradation products of lignin (DiDonato & Hatcher, 2017), the soil-derived DOM entering the stream during rainfall may come from more degraded terrestrial organic matter. These results suggest that as the amount of rainfall increases, there may be a convergence in the composition of stream DOM toward the more

humic and CRAM-like.

4.2. Seasonal litterfall enhances DOM export to streams

The seasonality of precipitation within the dry tropics generates dry season water stress that results in a peak in litterfall during the dry season compared to the wet season (Lawrence, 2005; de Queiroz et al., 2019; Morffi-Mestre et al., 2020; Marod et al., 2023). A study that included an old-growth forest in the Cordillera de Talamanca, Costa Rica, found that litterfall correlated negatively with monthly precipitation with highest litterfall in a dry season from January to April (Köhler et al., 2008). When litterfall peaks in the dry season, the accumulation of fresh, easily leached litter on the forest floor and as leaf packs in the stream may result in large amounts of DOM entering the streamwater. Given that litter decomposition can be rapid, with a >95 % mass loss within a year in most tropical sites and decomposition rates that highly correlate with mean annual precipitation (Powers et al., 2009), it follows that terrestrial litter stocks on the forest floor likely become depleted by the middle of the wet season. Elevated discharge during the wet season as well as decomposition remove leaf packs from the streams. Thus, in the wet season, declining litterfall and degraded litter stocks on the forest floor and in the stream may result in small amounts of more degraded DOM entering the stream compared to the less degraded DOM entering the stream during the dry season. These differences in the amount and degradation state of litter in the dry season versus the wet season likely explain the magnitudes of increased stream water DOC concentration in response to precipitation across the seasons. For example, 7 mm of precipitation falling on more abundant and fresher litter during the dry season (27-February) and 92 mm of precipitation falling on less abundant and more degraded litter in the wet season (10-July) generated similar ~500 μg C/L increases in DOC concentration at TEM (Fig. 2a).

Differences in stream DOM composition between the two seasons (as shown in Fig. 3,4) further suggest that, in the Río Tempisquito watershed, dry season precipitation falls on fresher and more labile terrestrial litter stocks compared to the more degraded litter encountered by precipitation in the middle of the wet season. Fresh litterfall in tropical forests generally contains proteins, cellulose, carbohydrates, and simple phenolic compounds that become depleted over the timescales of days to weeks from their preferential degradation over lignin or tannin/oxidized lignin-like compounds (Fonte and Schowalter, 2004; Wieder et al., 2009). The higher abundances of biologically labile tyrosine-like C5 FDOM (Cory and Kaplan, 2012) and aliphatic-like molecular formulae within stream DOM during the dry season compared to the wet season (Fig. 3c,4) suggests that fresh litterfall delivers biologically labile DOM to streams in the dry season. As precipitation increases toward the wet season, litter decomposition increases, resulting in the delivery of more degraded DOM with a higher relative abundance of aromatic-rich and tannin/oxidized lignin-like compounds to the watershed (Figs. 3b,4). With the nearly constant stream water temperatures between the two seasons (Table S6), it is unlikely that the observed seasonal differences in stream DOM composition would be due to differences in rates of stream ecosystem metabolism.

4.3. Longitudinal increases in DOM oxidation state reflect microbial metabolism

The absence of seasonally specific longitudinal changes in DOC concentration across the Río Tempisquito watershed may indicate a balance of DOM sources and sinks within the watershed. However, this finding could also result from the relatively short distances between sampling sites (200 m to 2.5 km; Fig. 1) compared to the uptake lengths (Elwood et al., 1981) over which heterotrophic microorganisms remove DOM from the water column. In streams with high flow velocities, rapid water turnover times can limit DOM uptake associated with in-stream metabolism to only the most biologically labile compounds (Li et al., 2020). With increasing water depth, velocity, and discharge with downstream distances - all factors that further increase uptake lengths for DOM (Newbold, 1992) - there should be relatively more downstream transport of DOM than in-stream processing between sampling locations (McLaughlin & Kaplan, 2013; Hall et al., 2013). Because the most biologically labile compounds make up a small fraction of the DOM pool on a mass basis (Kaplan et al., 2008), it is likely that measurements more sensitive than DOC concentration are needed to decipher the DOM shifts attributed to microbial metabolism across streams in the Río Tempisquito watershed.

The molecular-level FT-ICR-MS analyses revealed a broader range of compounds and oxidation states within van Krevelen space in the small seeps upstream compared with a narrower range of compounds in larger streams downstream (Table S4-S5), which could be indicative of the rapid metabolism of labile compounds and the persistence of more recalcitrant DOM along the reach (Creed et al., 2015; Mosher et al., 2015). Complete mineralization and removal of compounds to CO_2 during microbial metabolism could result in a narrower range of compounds observed in van Krevelen space for downstream DOM if lateral inputs generally declined downstream. The selective removal of compounds during in-stream metabolism, coinciding with the persistence of more recalcitrant DOM, may be supported by the longitudinal increases in the humic-like C1 FDOM relative to humic-like C2 and C3 observed across seasons (Fig. 5, S5). Although the abundances of humic-like FDOM C1-C3 all decreased downstream as overall concentrations of DOC decreased along the longitudinal sequence (Fig. S5, Table S3), the percentage of FDOM that came from C1 increased along the longitudinal sequence while C2 and C3 decreased (Fig. S5). A longitudinal increase in the percentage of humic-like C1 has only been previously observed in one other basin, the Orinoco basin (Yamashita et al., 2010), with no detectable downstream changes observed in other watersheds (Yamashita et al., 2010; Williams et al., 2010). However, a growing number of studies of stream DOM have attributed humic-like C1 to products of microbial metabolism (Yamashita et al., 2010; Yamashita et al., 2011; Osburn et al., 2018; Attermeyer et al., 2022) or pools that are generally more recalcitrant to degradation compared to other humic-like FDOM components (Williams et al., 2010; Lambert et al., 2017). Downstream increases in the percentage of humic-like C1 following the decreases in percentages of humic-like C2 and C3 suggest that terrestrially derived humic-like DOM may be the source of C1 to the FDOM pool. Alternatively, the higher percentages of humic-like C1 downstream could be due to the selective degradation of humic-like FDOM C2 and C3 over a more recalcitrant humic-like C1.

Additionally, the shift in DOM composition toward a higher oxidation state with downstream transport in KAM and TSM (Fig. 6) suggests that the DOM compounds selectively removed during in-stream metabolism of the most biologically labile pool were more reduced (Weigelhofer et al., 2020). The partial degradation of reduced DOM compounds through aerobic metabolism involving enzymatic reactions could result in the release of relatively more oxidized and possibly more refractory end products (DiDonato et al., 2024). One exception to the downstream shift towards more oxidized DOM composition occurred at the most downstream site, CTT, where the wider stream channel allows a larger area of the streambed to be unshaded by the riparian forest, bringing in visible light that could support in-stream photosynthesis by algae and mosses. There, the release of reduced, aliphatic-like DOM during autotrophic production (Kaplan & Bott, 1982) could be responsible for the less oxidized DOM composition observed at CTT compared to the upstream sites (Fig. 6e-f, S8).

It is unlikely that the downstream shifts in DOM composition toward a higher oxidation state are attributed to photo-oxidation. Rates of DOM photo-oxidation generally increase with increasing photon fluxes reaching the water surface and with increasing concentrations of light-absorbing CDOM in the water (Cory et al., 2015). Although photon fluxes are high year-round at tropical latitudes, streams in the Río Tempisquito watershed are well-shaded during both the wet and dry

seasons. We found that fluxes of ultraviolet light were 95 % lower in a shaded area of the Río Tempisquito stream (TEM) compared to a sunnier spot on one day during the dry season, which was consistent with the ~80–90 % lower photon fluxes measured in other shaded streams compared to clear-sky model predictions (Kelly et al., 2003; Frost et al., 2006). In addition to low photon fluxes, CDOM concentrations in streams in the Río Tempisquito watershed are on the lower end (average a_{305} of ~5 m^{-1}, Table S3) of those previously reported in streams where photo-oxidation has been found to occur at rates comparable to in-stream metabolism of DOM (a_{305} = ~5 to 500 m^{-1}; Cory et al., 2014, Bowen et al., 2020a; Bowen et al., 2024). Thus, low photon fluxes at the water surface combined with low CDOM concentrations and high flow velocities (Newbold et al., 1995) result in rates of light absorption by CDOM that are likely too low to result in substantial photo-oxidation of DOM during downstream transport between small seeps and larger streams downstream (< 0.1 mol photon m^{-2} d^{-1}; Bowen et al., 2020a).

Furthermore, the minimal if any photo-oxidation occurring is more likely to result in a net decrease in the average oxidation state of DOM than a net increase. Some of the most photochemically labile compounds within terrestrially derived DOM include carboxylic acids (Ward and Cory, 2016, Bowen et al., 2020b). It has been shown that the photo-oxidation of carboxylic acids to CO_2 can result in a net decrease in the average oxidation state of DOM (Ward and Cory, 2016), despite photo-oxidation being an oxidative process (Ward and Cory, 2020). Consistent with this observation, the oxidation state of DOM compounds have been shown to decrease following short light exposures of stream and riverine DOM (< 24 hours; Gonsior et al., 2009; Bowen, 2021). Thus, with the relatively short distances between sampling sites, photo-oxidation is likely limited to small losses of the most photochemically labile compounds, and if these compounds include carboxylic acids as in other freshwaters, then photo-oxidation would not lead to a downstream shift in DOM composition toward a higher oxidation state.

Microbially driven shifts in the distribution of NOSC to more oxidized compounds downstream are not supported by results from a prior study of the Río Tempisquito stream (TEM) that found selective uptake of more oxidized DOM by stream microorganisms (Kim et al., 2006). However, the result of that earlier study is not directly comparable to the field results presented here as it used laboratory-based bioreactors (Kaplan and Newbold, 1995) to capture the degradation of DOM molecules of varying biological labilities, including semi-labile DOM. Unlike the most biologically labile DOM, semi-labile DOM would likely have uptake lengths greater than 4.5 km (Kaplan et al., 2008). Given the short residence time of water in the Río Tempisquito watershed, most of the semi-labile DOM entering small seeps would not be used within the approximately 2 km of stream length between the most upstream spring seep (ROS) and furthest downstream sampling site (CTT; Fig. 1). Thus, the downstream changes in DOM oxidation state detected between sampling locations in the present study were likely dominated by uptake of the most biologically labile compounds with semi-labile compounds instead transported out of the watershed.

Taken together, these findings suggest that the oxidation state of DOM may better track downstream changes in DOM quality from microbial metabolism of labile DOM compounds compared to chemodiversity or aromaticity in dry tropical streams. The minimal change in DOM molecular diversity after the most upstream site (ROS) in the Río Tempisquito watershed (Table S4) contradicts the speculation of a sharp downstream decline proposed in the River Continuum Concept (RCC; Vannote et al., 1980), published prior to the availability of methods for DOM molecular characterization. In contrast to what was proposed by the RCC, the minor downstream change in DOM diversity supports past observations of small changes in DOM molecular or functional diversity with distance downstream in three other watersheds (Mosher et al., 2015; Peter et al., 2020; Danczak et al., 2023). While measures of $SUVA_{254}$ detected a downstream decrease in DOM aromaticity between upstream and downstream sampling locations (KAM/KAM2 and TSM/TSM2), consistent with the findings in Creed et al. (2015) and Hosen et al. (2021), downstream decreases were only observed in the wet season and the opposite pattern was found in the dry season (Fig. S5). The contradicting longitudinal shift in $SUVA_{254}$ observed in the dry season suggests that downstream changes in DOM aromaticity in forested headwaters may be influenced by processes other than heterotrophic metabolism, such as the higher leaf litter quality and subsidy in the dry season compared to the wet season (Fig. 3b). Unlike DOM chemodiversity and aromaticity, the consistent downstream increase in NOSC observed in shaded reaches unaffected by in-stream photosynthesis during both seasons (Fig. 6, S6) suggests that measures of the oxidation state of DOM may uniquely capture in-stream metabolism in forested headwaters independent of changing terrestrial subsidy throughout the year.

5. Conclusions

The seasonal separation of rainfall from litterfall in the dry tropics, processes that both contribute DOM to streams, provides an environmental setting that influences DOM composition with fresher, more reduced DOM introduced through direct interception and blow in of litter in the dry season and more degraded, oxidized DOM introduced in groundwater and runoff over degraded litter stocks in the wet season. Despite differences in the composition of stream DOM between the dry and wet seasons, there were consistent increases in the delivery of humic and CRAM-like DOM to streams during rainfall, which may be attributed to rising groundwater levels within riparian soils. Between the dry and wet seasons, there were also consistent increases in the oxidation state of DOM downstream, which we propose are driven by the preferential microbial metabolism of a pool of labile, reduced DOM compounds. These findings extend the River Continuum Concept by showing that DOM oxidation state can be a sensitive indicator of microbial metabolism in the absence of measurable decreases in downstream DOM concentrations. As the canopy opens downstream, however, in-stream autotrophic production may supply reduced DOM that masks the persistence of a more recalcitrant, oxidized DOM pool in the watershed.

CRediT authorship contribution statement

Samantha N. Sullivan: Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Jennifer C. Bowen:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **Louis A. Kaplan:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rose M. Cory:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Patrick G. Hatcher:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.123668.

Data availability

Spectral loadings of FDOM components from the PARAFAC model of stream DOM can be found in the Supplementary Data file and on OpenFluor. Data used in the manuscript can be found in the Supplementary Material and the Mendeley data repository (DOI: 10.17632/khbnr3mg8c.1).

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