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Temporal changes of headwater river surface microlayer characteristics during the dry to wet season transition in the tropical rainforest of Guyana

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1 **Temporal Changes of Headwater River Surface Microlayer**

2 **Characteristics during the Dry to Wet Season Transition**

3 **in the Tropical Rainforest of Guyana**

4

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22 **Note:** We deeply regret to inform that one of the authors, Walter Hill, has

23 passed away during the preparation of this manuscript. His contributions

24 were invaluable, and he will be greatly missed.

25

26 **Abstract**

27

28 The surface microlayer (SML) is a fundamental control of energy transfer,

29 biogeochemical processes, and climate-active gases flux at the water-

30 atmosphere interface. Organic matter in the SML has an important role in

31 marine environments but inland waters have received far less attention.

32 This results in large unknowns of SML variability and its impact on

33 boundary layer exchanges. In this study we investigate variations in
34 dissolved organic carbon (DOC) and dissolved organic matter (DOM)
35 composition in SML and subsurface water (SSW) using size exclusion
36 chromatography in a Northern Amazonia rainforest headwater during the
37 dry to wet season transition. During the dry season, we observed higher
38 DOC concentrations with greater DOM compositional variability,
39 characterized by lower humic substance (HS) abundance and elevated
40 contributions of biopolymers (BP), building blocks (BB), and low-
41 molecular-weight neutrals and acids (LMWN, LMWA). River discharge and
42 DOC flux increased in the wet season. However, the overall DOC
43 concentration decreased accompanied by DOM homogenisation, a
44 dominance of HS, and the disappearance of LMWA.

45

46 The SML and SSW exhibited similar DOC concentrations but distinct
47 compositional differences, evidenced by divergent HS-DOC relationships
48 and HS nominal molecular weight (M_n) characteristics. In the dry season,
49 high M_n variability in the SML suggested preferential accumulation of
50 higher-molecular-weight HS, which diminished as wet-season conditions
51 stabilized DOM distribution. Enrichment factors (EFs) for all DOM
52 compound groups between the SML and SSW displayed strong temporal
53 variability. These patterns likely reflect shifts in hydrodynamic regimes,
54 terrestrial inputs, and microbial or photochemical processing. Notably,
55 rainfall events did likely not directly trigger EF pulses but indirectly
56 influenced DOM composition from local sources through terrestrial
57 mobilization. Our results demonstrate that tropical river DOM dynamics
58 are fundamentally controlled by seasonal hydrological regimes, with
59 discharge-driven processes exerting stronger influence on molecular
60 composition and vertical heterogeneity (particularly in the SML) than
61 direct rainfall effects. This hydrologic dominance shapes DOM distribution
62 through three interconnected mechanisms: source variability during low-
63 flow periods, transport-mediated homogenisation during high discharge,
64 and in-situ processing that differentially affects boundary layer
65 composition.

66

1. Introduction

The interface between the atmosphere and hydrosphere, known as the surface microlayer (SML), covers about 70% of the Earth's surface. The SML is an important biological habitat and a collection point for buoyant particles, and dissolved components from the underlying water [1-5]. Compared to subsurface water (SSW), the SML is subject to a broad set of unique physical and chemical properties [6-8] that are vulnerable to environmental and climatic variations [1]. However, to date, the majority of SML characterizations have been focussed on marine systems with relatively few investigations of freshwater systems [9-11] and a notable paucity in tropical rivers (Raymond et al., 2013). Organic matter compositional difference between SML and SSW have been shown to play a key role in the water-atmosphere transfer of climate active gases including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in marine systems [3, 12]. However, SML processes are poorly understood and rarely represented in marine and freshwater interactions with the atmosphere in numerical models [6, 13, 14].

Although the hydrodynamic energy of rivers and creeks is generally higher than that of marine systems, several mechanisms can still sustain a discrete microlayer at the air–water interface. In marine systems the accumulation of surfactants, exopolymeric substances, and surface-active organic matter can dampen small-scale turbulence and create surface-tension gradients that stabilise the SML even under moderate flow [15, 16]. Evidence from both natural and engineered freshwater systems further indicates that distinguishable microlayers can persist in reservoirs, and constructed wetland channels where surfactant and organic matter accumulation reduce near-surface turbulence [17]. However, to the best

99   of our knowledge, no previous studies have explicitly characterised the
100   SML in headwater river systems or explored the potential for distinct SML
101   to form in headwaters.

102

103   Material known to accumulate in the SML of marine, lake and wetland
104   waters includes a range of naturally occurring organic matter, such as
105   carbohydrates, proteins, lipids [5] and the key nutrients including nitrogen
106   and phosphorous [18]. The enrichment factor (EF), defined as the ratio of
107   the concentration of a specific substance or property in the SML to its
108   corresponding value in the underlying SSW is a widely utilized metric for
109   assessing the accumulation of substances and the distinct characteristics
110   of the SML relative to the SSW [1, 19-22]. The primary sources of material
111   to the SML are phytoplankton exudates, transported to the surface via
112   rising   bubbles   and   diffusion   [23].   Additionally,   hydrophilic
113   polysaccharides, high molecular polysaccharides and complex glucans,
114   major excretion productions of phytoplankton, can become enriched in the
115   SML [5]. In rivers, the surfactant compositional variation between the
116   SML and SSW has been shown in estuary of the Delaware Bay and the,
117   Broadkill River [2] , the estuarine of Selangor River [24] and the Tyne
118   Estuary [25]. An investigation of the Guangzhou segment of the Pearl River
119   further confirmed the enrichment of nutrients (nitrogen, and phosphorus),
120   heavy metals (Fe, Mn, Ni, Cr, and Pb), bacteria, and increased chemical
121   oxygen demand [9, 11] in the SML. However, to our best knowledge, the
122   organic matter SML properties in headwater tropical rainforest rivers is
123   unknown.

124

125   The characteristics of dissolved organic matter (DOM) in aquatic
126   environments vary depending on its source (terrestrial or aquatic) and
127   diagenetic state [26]. Freshwater, estuarine, and marine ecosystems
128   receive large quantities of dissolved organic carbon (DOC) from terrestrial
129   ecosystems, altering their physical and chemical qualities and affecting
130   their metabolic functioning [27]. Instream DOC mineralisation is a notable
131   source of riverine carbon dioxide (CO_2) emission, particularly in tropical

rivers [e.g. 28, 29] with compositional changes of DOM widely observed using a variety of approaches including coloured and fluorescence DOM (CDOM and FDOM) techniques [e.g. 30, 31] liquid chromatography organic carbon and nitrogen detection [22, 32-34], high resolution mass spectrometry [6, 35] and nuclear magnetic resonance [36, 37].

Tropical rivers are a major component of the global biogeochemical cycling [38], with headwaters estimated to account for 70-80% of the total river network [39] and connect the terrestrial and aquatic ecosystems as they are in direct contact with adjacent soils [40, 41]. The transition between dry and wet periods in these ecosystems can affect the water and atmospheric composition [42, 43]. Despite their importance, little is known about the temporal dynamics of DOM. Given the relatively high organic matter fluxes of these systems [44] this study questioned whether partitioning between SML and SSW exists in tropical headwaters. Therefore, this study aims to characterise temporal variations in DOC and DOM composition during the dry-to-wet season transition of the SML and SSW in a tropical rainforest headwater catchment within the Iwokrama Forest, Guyana. Specifically, we:

- Assess the temporal variability of DOC and DOM compound groups during the dry-to-wet season transition, to determine how hydrological and biogeochemical changes (e.g., hydrological mobilisation, microbial or photochemical transformation) influence DOM composition and molecular characteristics.
- Examine the relationships between DOC/DOM metrics, rainfall, and river discharge to identify how hydrological forcing and rainfall events regulate DOM sources, transport pathways, and compositional dynamics in tropical headwaters.
- Quantify the differences in DOC concentration and DOM composition between the SML and SSW to evaluate enrichment processes and compositional differentiation at the air-water interface.

165 Through this approach, we explore how tropical headwater systems
166 regulate carbon dynamics at the air–water interface and influence aquatic
167 carbon cycling.
168
169 **2. Methodology**
170
171 **2.1. Study Area**
172
173 This study selected a previously characterised headwater of the Essequibo
174 River located in the Iwokrama rainforest (Figure 1) in the deep rainforest
175 interior of Guyana, South America [43-45] The Iwokrama forest is in the
176 heart of the Guiana Shield part of northern Amazonia, in tropical South
177 America. The forest is bounded and drained by the Essequibo River to the
178 East and the Siparuni River, a tributary of the Essequibo, to the west and
179 north (See Pereira et al., 2014a for more details). Briefly, Iwokrama is
180 located relatively isolated intact rainforest near a transition in the climate
181 regime from the north (coastal) to south (savannah). The coastal region
182 experiences two wet seasons (a primary wet season from May to July and
183 a secondary wet season from December to January) and two dry seasons
184 (a primary dry season centred around October and a secondary dry season
185 around March). In contrast, the southern savannah region has a single wet
186 season from May to August and a long dry season extending from
187 September to March [45, 46]. Blackwater Creek (BC), a second-order
188 headwater of the Essequibo River was chosen to study the temporal
189 variability of riverine DOM concentration and composition in the SML and
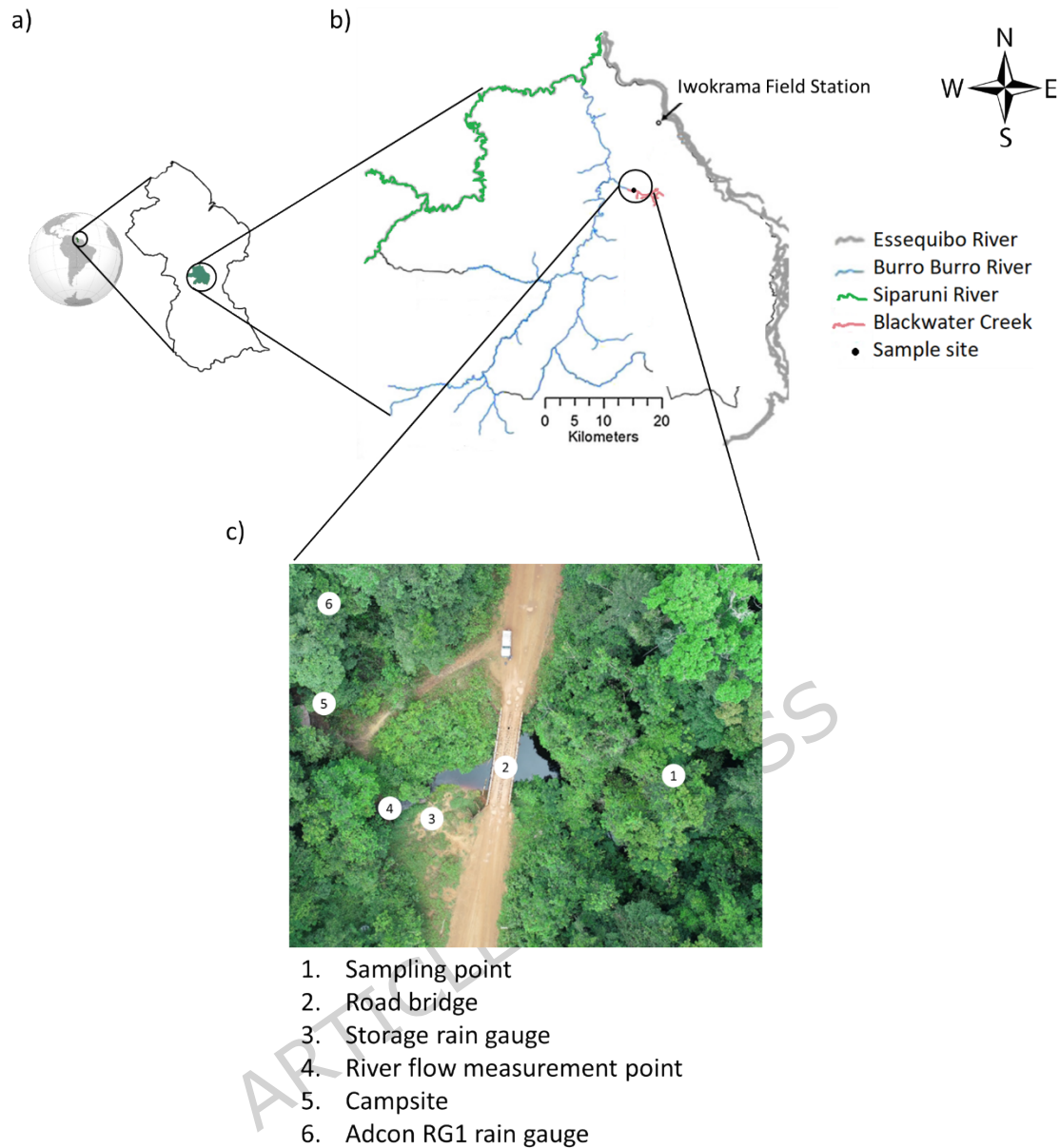190 SSW to further our understanding on a dynamic headwater river systems.
191

1. Sampling point
2. Road bridge
3. Storage rain gauge
4. River flow measurement point
5. Campsite
6. Adcon RG1 rain gauge

192
193
194   **Figure 1:** *Study site map. The map of (a) Guyana, (b) the Iwokrama*
195   *rainforest (modified from [45]), and (c) aerial view of Blackwater Creek*
196   *study site (modified after [43]).*

197
198   **2.2.   Hydrometeorological data collection**
199
200   Rainfall was measured at the site using an Adcon RG1 rain gauge equipped
201   with a 0.2 mm tipping bucket with a 200 cm^2 diameter from 3rd to 28th May
202   2019 (no data was recorded in the last 10 days of the study period due to

203   the rain gauge failure) and a storage rain gauge with a 127 mm diameter
204   placed on the ground was used to record daily rainfall from 14th May to 7th
205   June 2019 [45].

206

207   River stage was measured using sealed Solinst Levelogger Edge pressure
208   transducers located in a stilling well recording every 1 minute from 3rd
209   May to 7th June 2019. River stage was corrected for atmospheric pressure
210   changes using a Solinst Barologger. River discharge for the whole study
211   period was calculated from an empirical relationship between the
212   measured discharge and river stage (rating curve) as follows:

$$Q = 2.309 \, (S - 0.57)^{1.575} \hspace{4cm} (1)$$

213   where Q is river discharge ($m^3 \, s^{-1}$) and S is the river stage (m). (For further

214   details and complete data set used for rating curves please refer to [43].)

215

216   **2.3.   Sample Collection, Preservation and Analysis**

217

218   SSW grab samples were collected [47] into 60 ml HDPE bottles from the
219   top 50 cm of the river water column from 4th May until 7th June 2019 during
220   four intensive sampling phases from 4th May 12:00 to 10th May 05:00 (First
221   Phase), 13th May 14:00 to 18th May 05:00 (Second Phase), 21st May 11:00
222   to 28th May 05:00 (Third Phase) and 31st May 10:00 to 7th June 05:00
223   (Fourth Phase) 2019. SML samples were collected using a Garrett Screen
224   [48] at the same time as SSW samples. The screen (mesh 16, 0.36 mm wire
225   diameter with an opening of 1.25 mm, a frame width of 1.25 cm) had an
226   effective surface area of 101 cm^2. A 1.5 m stainless steel rod was attached
227   onto the frame to minimise disturbing the SML. The screen was gently
228   placed on the water's surface allowing it to capture the SML and then
229   withdrawn slowly and drained directly into 60 ml HDPE bottles [49]. These
230   steps were repeated until two sample bottles were filled (3.5 dips per
231   bottle on average). Samples were collected every 6 hours at 05:00, 11:00,
232   17:00 and 23:00 (baseline samples) to capture diurnal variation and extra
233   water samples were collected every 30 minutes during rainstorm events

234 in response to observed hydrological changes such as rainfall and/or
235 increased river discharge (rainstorm event samples). In total, 238 SML
236 samples (94 baseline and 144 rainstorm event samples) were collected
237 from BC. The sampling team recorded whether it was raining at the time
238 of each sample collection, categorizing samples into rain event samples
239 (48 samples) collected during rainfall, and dry condition samples (190
240 samples) collected under non-rainy conditions. The identification of rain
241 event samples was then cross-checked against rainfall data from the Adcon
242 RG1 rain gauge, using a threshold of >5 mL min^{-1}.

243

244 All water samples were collected following standard protocols outlined by
245 previous work for Liquid chromatography-organic carbon detection-
246 organic nitrogen detection (LC-OCD-OND) determination [50, 51]. Briefly,
247 water samples were filtered immediately using pre-combusted GF-F filters
248 (450 °C for 8 h) collected in 60 ml high density polyethylene (HDPE) bottles
249 which were acid-washed (10 % HCl) and rinsed with deionised water (18.2
250 M Ω cm-1, carbon-free) prior to use. All samples were transported to
251 mobile laboratory in the Iwokrama river lodge under dark and cold
252 conditions using a portable chiller (4–6 °C).

253

254 **2.4.   Field analysis and mobile laboratory**

255

256 DOC analyses were completed on the same day as sample collection using
257 a Sievers M5310C Portable TOC Analyzer, with attached GE Autosampler
258 with a 0.03 to 50 mg L^{-1} carbon range in the mobile laboratory in the
259 Iwokrama River Lodge. DOC results were within the specification of $< 1\%$
260 RSD precision and $\pm$ 2% accuracy. Deionised water was used as an
261 analytical blank.

262

263 DOC EF were calculated as follows:

264     $$\text{DOC EF} = \frac{\text{DOC}_{\text{SML}}}{\text{DOC}_{\text{SSW}}}$$ Equation (1)

265 where DOC_{SML} (mg L^{-1}) is concentration of the substance or property in
266 the SML and DOC_{SSW} (mg L^{-1}) is concentration of the substance or

267   property in the SSW. EF higher than 1 indicates enrichment of the

268   substance or property in the SML relative to the SSW. EF less than 1

269   indicates enrichment of the substance or property in the SSW relative to

270   the SML.

271

272   **2.5. Liquid chromatography-organic carbon detection-organic**

273   **nitrogen detection (LC-OCD-OND)**

274

275   A second set of 60 ml HDPE bottles were sealed at the field sites, and then

276   kept at -20 °C until analysed at the Lyell Centre at Heriot-Watt University,

277   UK, for further DOM compositional analysis [34, 51, 52].

278

279   The DOM composition was measured using LC-OCD-OND for SML and

280   SSW water samples. LC-OCD-OND is a size exclusion chromatography

281   (SEC) technique, providing quantitative information regarding organic

282   carbon compound groups [53]. LC-OCD-OND allows 1 mL of whole water

283   to be injected onto a size exclusion column (2 mL min-1; HW50S, Tosoh,

284   Japan) with a phosphate buffer (potassium dihydrogen phosphate 1.2 g L-

285   1 plus 2 g L-1 di-sodium hydrogen phosphate dihydrate, pH 6.58) and

286   separated into five DOM compound groups. These include biopolymers

287   (BP; high molecular weight polysaccharides and proteins), humic

288   substances (HS), building blocks (BB; lower molecular weight HS), low

289   molecular weight acids (LMWA), and low molecular weight neutrals

290   (LMWN; amphiphilic/neutral compounds including alcohols, aldehydes,

291   ketones, and amino acids) were measured [32, 53].

292

293   LC-OCD-OND can be used to determine the nominal molecular weight of

294   HS (M_n) in natural waters [53], which can describe the apparent size or

295   weight of the HS relative to the known molecular weight of IHSS HA and

296   FA. This technique has been used in study of organic matter in SML and

297   SSW of freshwater or marine systems [44, 54, 55]. The resulting compound

298   groups were identified using detectors for organic carbon (OC), UV-

299   amenable carbon and organic nitrogen (ON) [53]. All peaks were identified

and quantified with bespoke software (Labview, 2013) normalized to International Humic Substances Society (IHSS) humic and fulvic acid (HA and FA, respectively) standards, potassium hydrogen phthalate and potassium nitrate.

EFs were calculated for BP, HS, BB, LMWN, LMWA concentration and M_n following Equation (1). These were denoted as BP EF, HS EF, BB EF, LMWN EF, LMWA EF, and Mn EF, respectively.

2.6. Statistical analysis

All statistical analyses were performed using R software (version 4.3.3) and Microsoft Excel (version 365). Prior to analysis, Rosner's test for outliers was applied to identify and remove extreme values, ensuring the robustness of the results. Group differences were assessed using independent samples t-tests assuming unequal variances , with the t-statistic used to determine statistical significance at a threshold of $\alpha = 0.05$. Specifically, t-tests were used to compare (i) DOC concentrations between SML and SSW, (ii) DOC concentrations and DOM EFs between rain event and dry-period samples, and (iii) DOM compound group EFs (BP, HS, BB, LMWN, and LMWA) between rising and falling river discharge conditions.

To evaluate relationships between variables, linear regression analyses were conducted. These included (i) relationships between HS and DOC concentrations for SML and SSW during the drier (phases 1–2) and wetter (phases 3–4) periods, and (ii) relationships between DOC and other DOM compound groups (BP, BB, LMWN, and LMWA). The slopes and intercepts of regression models were compared using analysis of covariance (ANCOVA) to determine statistically significant differences between groups (e.g. between SML and SSW, and between dry and wet transition periods). The coefficient of variation (CV) was calculated to assess relative variability within each dataset and across sampling phases.

333 Temporal trends in DOC concentration, HS Mn, and DOM composition
334 were evaluated using the non-parametric Mann–Kendall test, which
335 detects monotonic trends in time-series data without assuming normality.
336 A significance threshold of α = 0.05 was applied to all tests.
337
338 **3. Results**
339
340 **3.1. Medium-term Climate and hydrology**
341
342 The total monthly rainfall in the study site for May 2019 was 421 mm. The
343 river discharge ranged from 0.05 to 5.59 m^3 s^{-1} and the river water level
344 ranged from 0.66 m to 2.32 m (SD=0.34) ((Figure 2 a and Figure 4 a). The
345 distinction between wet and dry periods in this study follows the climatic
346 characterisation reported elsewhere [43]. Briefly, monthly rainfall data
347 recorded at the Iwokrama Field Station from January 2016 to December
348 2019 show a bimodal precipitation pattern with two wet and two dry
349 seasons typical of the Guianas region [46]. Based on the Köppen-Geiger system
350 for the Guianas (rainfall in the driest month is ≥ 60 mm or < [100 mm – (mean
351 annual rainfall/25)]), 2019 started with a wet season that began in April
352 2018.[56].
353
354 **3.2. Temporal dynamics of DOC and DOM composition**
355
356 **3.2.1. Temporal variability of DOC concentration**
357
358 DOC concentrations ranged from 9.33 to 26.41 mg L^{-1} (20.01 ± 2.92 mg L^{-1})
359) for the SSW (Figure 2 c) and from 9.82 to 26.08 mg L^{-1} (20.19 ± 2.89
360 mg L^{-1}) for the SML (Figure 2 b) over the study period. The DOC
361 concentration during rainstorm event SML samples (n=144, ranging from
362 13.87 to 25.28 mg L^{-1}, 20.02 ± 2.91 mg L^{-1}) does not deviate from baseline
363 samples (n=94, ranging from 9.82 to 26.08 mg L^{-1}, 20.36 ± 1.81 mg L^{-1})
364 (t-test: two-sample assuming unequal variances, t static =-1.08, t critical
365 = 1.66). The enrichment of SML DOC concentration relative to the SSW,
366 EF DOC ranged from 0.78 to 1.33 (1.00 ± 0.04) over the study period

367   (Figure 2d). The majority of samples (96 % of the total sample set) reported

368   a DOC EF between 0.92 and 1.08 (1 ± 2 × SD (=0.04)). As both SML and

369   SSW DOC were normally distributed, t-tests suggest that the SML DOC

370   and SSW DOC are not statistically different (t static=0.36, t critical=1.97).

371   The Mann–Kendall test further indicated a downward temporal trend in

372   DOC concentration for both the SML and SSW ($z = -2.48$ and -2.75,

373   respectively; $p < 0.05$) (Table S9), suggesting a gradual decrease over the
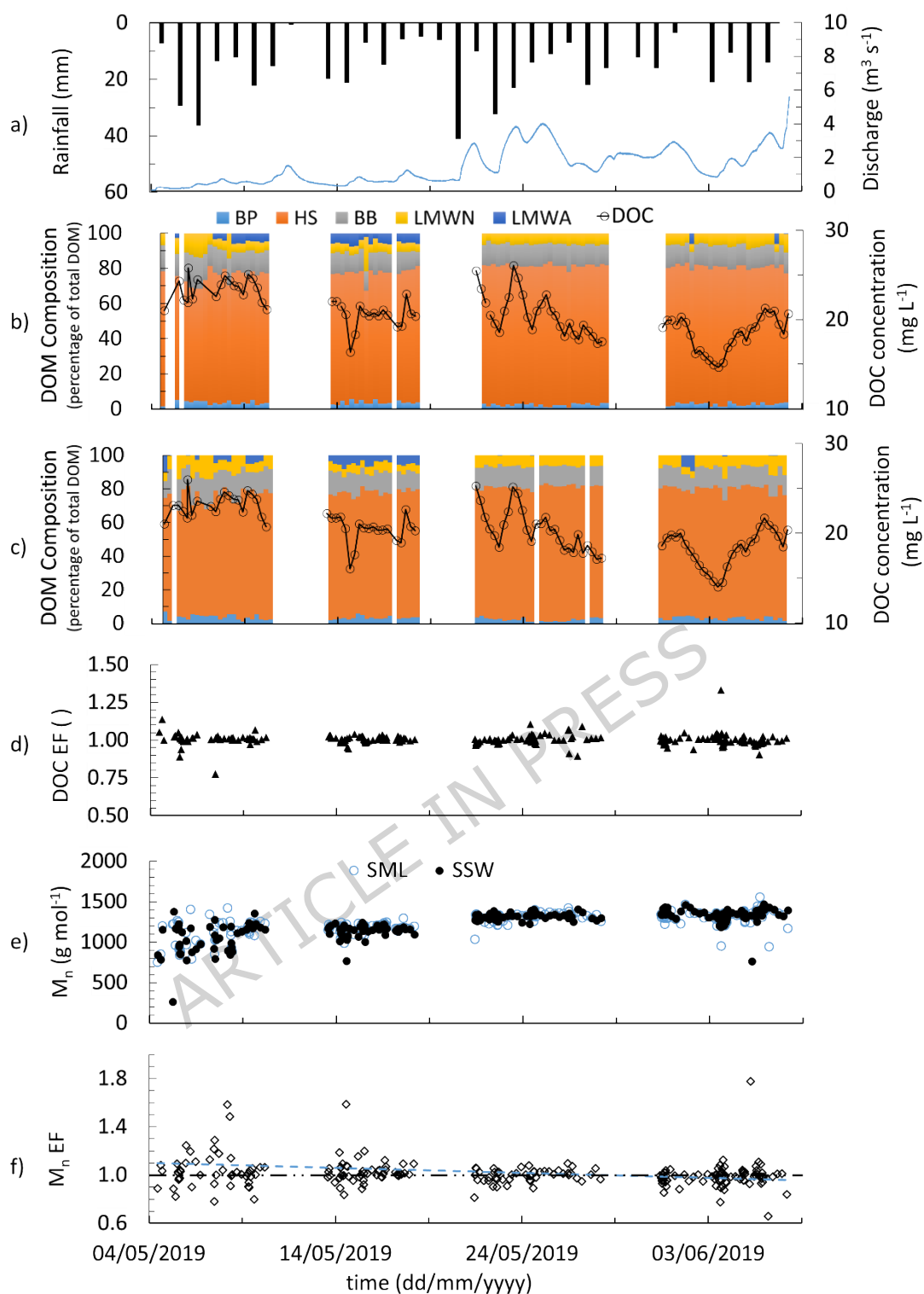
374   study period.

Figure 2: *BC daily rainfall (black bar) and river discharge (blue line) (a),*

temporal variability of the percentage of the total DOM pool and DOC

concentration in baseline SML (b) and SSW samples (c), DOC EF (d), M_n

of HS in the SML and SSW (e), and the HS M_n EF (f). The black dashed

line in (e) shows the general increase of M_n in the SML and SSW. The

blue dashed line shows the general trend of M_n EF and the black dashed

382   *line shows EF=1 in (f).*

383 **3.2.2.   Temporal variability of DOM compound groups**

384

385 The BP concentration ranged from 0.06 to 1.44 mg L^{-1} (0.56 ± 0.30 mg L$^-$

386 ^{1}) in SML and from 0.02 to 1.44 mg L^{-1} (0.56 ± 0.29 mg L^{-1}) in the SSW.

387 BP accounted for 2.8 ± 1.1 % of the total DOM present in the SML (0.6 to

388 5.8 %) (Figure 2 b) and 2.91 ± 1.1 % of the DOM in the SSW (0.5 to 6.7 %)

389 (Figure 2 c). HS, which is the dominant compound group observed, ranged

390 from 3.25 to 20.69 mg L^{-1} (14.30 ± 3.50 mg L^{-1}) in the SML and from 0.27

391 to 21.66 mg L^{-1} (14.08 ± 3.99 mg L^{-1}) in the SSW. Generally, the HS

392 fraction increases in both SML and SSW waters ranging from 54.6 to 89.3

393 % in the SML (73.7 ± 6.3%; Figure 2 b) and 54.9 to 84.2 % in the SSW

394 during the study period (73.5 ± 6.0 %; Figure 2 c). The BB concentrations

395 ranged from 0.51 to 4.54 mg l^{-1} (2.34 ± 0.67 mg L^{-1}) in the SML and from

396 0.07 to 4.50 mg L^{-1} (2.31 ± 0.76 mg L^{-1}) in the SSW. The BB accounted for

397 9.1 to 18.5 % (12.4 ± 1.7 %) of DOM in the SML (Figure 2 b) and 7.2 to

398 18.7 % (12.1 ± 1.9 %) in the SSW (Figure 2 c). The LMWNs were found at

399 concentrations of 0.08 to 5.09 mg L^{-1} (1.30 ± 0.63 mg L^{-1}) in the SML and

400 0.01 to 3.82 mg L^{-1} (1.29 ± 0.65 mg L^{-1}) in the SSW. The LMWNs

401 contributed 0.0 to 22.0 % (6.6 ± 2.8 %) to the total DOM in the SML (Figure

402 2 b) and 0.0 to 26.1 % (6.8 ± 3.1 %) in SSW (Figure 2 c). The BP, BB and

403 LMWN overall concentration and contributions to the total DOM pool

404 generally decreased over the study period in both SML and SSW waters.

405 The LMWAs concentration ranged from below detection to 1.48 mg L^{-1}

406 (0.92 ± 0.45 mg L^{-1}) and 1.59 mg L^{-1} (0.80 ± 0.47 mg L^{-1}) in the SML and

407 SSW, respectively. When present, LMWAs contributed 0.4 to 7.5 % (4.2 ±

408 2.2 %) to DOM in SML (Figure 2 b) and 7.2 to 10.2 % (4.9 ± 1.8 %) in SSW

409 (Figure 2 c).

410

411 The LMWAs were detected inconsistently during the first phase of the

412 study, appearing in 22 SML and 24 SSW samples (out of 50 total sample

413 pairs). LMWAs were found in 11 SSW samples without corresponding

414 detection in the SML and in 9 SML samples below detection in the SSW.

415 During the second phase, LMWAs were detected in all waters (100 samples
416 in total). In the third and fourth phases, LMWAs were detected in both the
417 SML and SSW for 5 sample pairs.

418

419 The Mann–Kendall trend analysis revealed statistically significant
420 temporal patterns in several DOM compound groups (Table S8). In the
421 SML and SSW, significant increasing trends were observed for HS
422 percentage of total DOM ($z = 4.05$, $p < 0.01$ and $z = 4.27$, $p < 0.01$,
423 respectively), whereas BB and LMWN percentages exhibited significant
424 decreasing trends ($z = -3.07$, $p < 0.01$ and $z = -3.29$, $p < 0.01$,
425 respectively), and LMWN percentage of total DOM ($z = -3.03$, $p < 0.01$,
426 and $z = -3.03$, $p < 0.01$, respectively). BP showed a weak decreasing
427 tendency in the SML ($z = -1.85$, $p = 0.06$) and a significant decline in the
428 SSW ($z = -2.82$, $p < 0.01$), while LMWA showed no significant temporal
429 change ($p > 0.9$) (Table S9).

430

431 **3.2.3.   Variations in HS and molecular characteristics**

432

433 DOM data from this study were categorized into two groups: phase 1 and
434 2 (first half of the study period) and phase 3 and 4 (second half of the
435 study period) based on earlier observations of DOM and river discharge
436 by [43]. Notably HS represents a greater proportion of the DOC in the
437 first half of the study compared to the latter half in both the SML and the
438 SSW (Figure 3).

439

a) SML   b) SSW
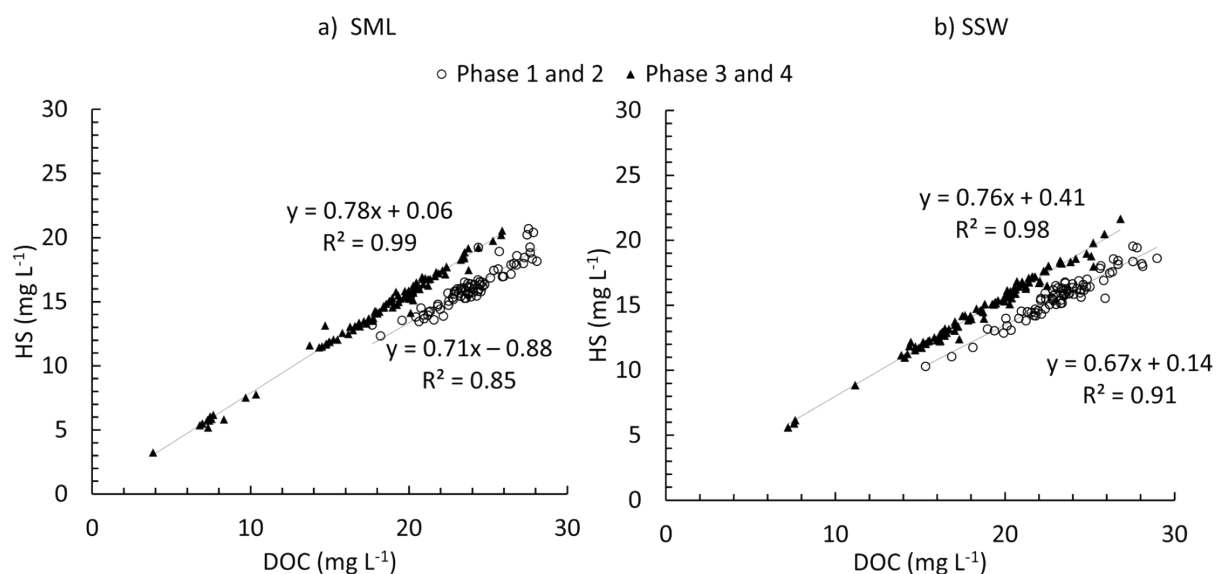
○ Phase 1 and 2   ▲ Phase 3 and 4

Figure 3: *BC HS vs DOC concentration for the SML (a) and SSW (b) in the first (circles) and second (triangles) half of the study period.*

Figure 3 further shows that the relationship of HS to the total DOC pool for phases 1 and 2 (first half of the study period and drier) and phases 3 and 4 (second half of the study period and wetter) follow different trends evidenced by two different sets of slopes and intercepts. The HS and DOC concentrations show a strong correlation in SML (R^2=0.99, n=103, p < 0.0001 in phase 1 and 2 and R^2=0.85, n=135, p < 0.0001 in phase 3 and 4) and SSW (R^2=0.98 n=103, p < 0.0001 in phase 1 and 2 and R^2=0.91, n=135, p < 0.0001 in phase 3 and 4). The comparative analysis of the linear regression slopes of HS and DOC relationship for two groups of data (group 1: phase 1 and 2, group 2: phase 3 and 4) suggests a significant but variable relationship between HS and DOC between these two groups (SML: p < 0.0001, α = 0.05, SSW: p<0.0001, α = 0.05) despite the intercept not being significantly different in these two groups (SML: p = 0.31, SSW: p = 0.23). In addition, the interaction term (i.e. the statistical test of whether the slope of the HS-DOC relationship differs significantly between SML and SSW) suggests statistically significant differences in the second half of the study period (p = 0.004, α = 0.05). However, no statistically significant difference was observed between the intercepts (p = 0.21, α = 0.05) and no statistically significant difference was observed

463 between the intercept and interaction term in the first half of the study
464 period (intercept: p = 0.84, α = 0.05, interaction term p = 0.48, α = 0.05).
465 In addition, the linear relationship between DOC and other DOM
466 compound groups (BP, BB, LMWN, and LMWA) were tested. For all
467 groups, the correlations were weak ($R^2 < 0.8$) except for BB in the first half
468 of the study period ($R^2 = 0.86$ for SML and $R^2 = 0.95$ for SSW).

469

470 HS M_n increases over the study period (ranges from 747 to 1553 (1236 ±
471 134) g mol^{-1} in the SSW and from 763 to 1465 (1232 ± 151) g mol^{-1} in the
472 SML (Figure 2 e). In the first phase of the study period, HS M_n has a high
473 SML and SSW variability (CV=15 and 15 % for the first phase,
474 respectively), which decreases during the study period (for example, CV=7
475 and 7 % for the fourth phase, respectively). The M_n EF ranges from 0.66
476 to 1.70 (1.01 ± 0.11) with maximum variability in the first phase (CV=14
477 %, second phase CV=10 %, third phase CV=5 % and fourth phase CV=11
478 %) (Figure 2 f). The comparison of Humic substances diagram (HS-
479 diagram, the SAC/ OC ratio (aromaticity) of aquatic humic substances
480 plotted against M_n) [53] in different phases shows a gradual shift of the
481 samples to higher molecularity and lower aromaticity from phase 1 to
482 phase 4 (SI section H, Figure S28 to 32).

483

484 **3.3. SML–SSW Compositional Differences and Enrichment**
485 **Factors**

486

487 The EF of the DOM compound groups is used for the compositional
488 comparison of the SML and SSW. The BP EF ranges from 0.41 to 8.03 (1.24
489 ± 0.99) (Figure 4 b), HS EF ranges from 0.21 to 7.38 (1.16 ± 0.76) (Figure
490 4 c), BB EF ranges from 0.21 to 7.61 (1.16 ± 0.75) (Figure 4 d), LMWN
491 fraction EF ranges from 0.05 to 5.97(1.19 ± 0.78) (Figure 4 e) and LMWA
492 fraction EF ranges from 0.09 to 2.63 (1.14 ± 0.43) (Figure 4 f). BP EF
493 shows the maximum variability (CV=80 %) among DOM compound groups
494 (HS EF (CV=66 %, BB EF CV=64 %, LMWN EF CV=66 %, and LMWA 38
495 %). BP, HS, BB, and LMWN EF are more variable in the second half of the

496   study period (CV=55, 48, 42, and 58 % in phase 1 and 2 and CV=91, 74,

497   73, and 70 % in phase 3 and 4, respectively). Weak linear relationships

498   were observed between the EF of all DOM compound groups ($R^2<0.7$,

499   n=238, p <0.0001), except for BB and HS, which showed a strong

500   correlation ($R^2=0.92$, n=238, p <0.0001).

501

502   As such, the DOM EFs of these regimes were investigated to assess

503   whether the rising or falling phases exert control over the compositional

504   differences between the SML and SSW. Two categories of water samples

505   collected during rising and falling river discharge in the second half of the

506   study period (as it has distinct rising and falling period) were statistically

507   compared. No statistically significant variation of DOM composition was

508   observed in relation to river discharge trends (t critical =2.00 and t static=

509   0.83, 0.82, 0.68, and 0.55 and 1.51 for BP EF, HS EF, BB EF, LMWN EF,

510   and LMWA EF, respectively.).

511

512   To evaluate the relationship between rain events and compositional

513   variations in the water column, rain event samples and dry condition

514   samples were compared. Given the normal distribution of the data and the

515   unequal variance between the groups, t-tests were performed. In all cases,

516   the calculated t-statistics were lower than the corresponding critical t-

517   values, indicating no statistically significant compositional variation in the

518   water column between the samples collected during rain events and those

519   collected during dry periods. (t critical =1.98 and t static= 1.11, 0.94, 1.14,

520   and 1.32 for BP EF, HS EF, BB EF, and LMWN, respectively.). To

521   determine whether high EF values (spikes) are associated with rain events,

522   EF values greater than two were filtered, and the proportion of samples

523   with high EF values collected during rain events was compared to the total

524   number of such samples. The percentages of high EF samples collected

525   during rain events were 21% (6 out of 29 samples) for BP EF, 29% (4 out

526   of 14 samples) for HS EF, 27% (4 out of 15 samples) for BB EF, 20% (4 out

527   of 20 samples) for LMWN EF, and 62% (5 out of 8 samples) for LMWA EF.

528   Since 26% of all samples were collected during rain events, these

529 percentages are generally close to or below the overall sampling
530 proportion, except for LMWA. This distribution indicates that high EF
531 values occurred in both rain and dry conditions at similar frequencies,
532 suggesting no consistent or systematic increase in EF during rain events.
533 Therefore, the data do not support a strong association between rain
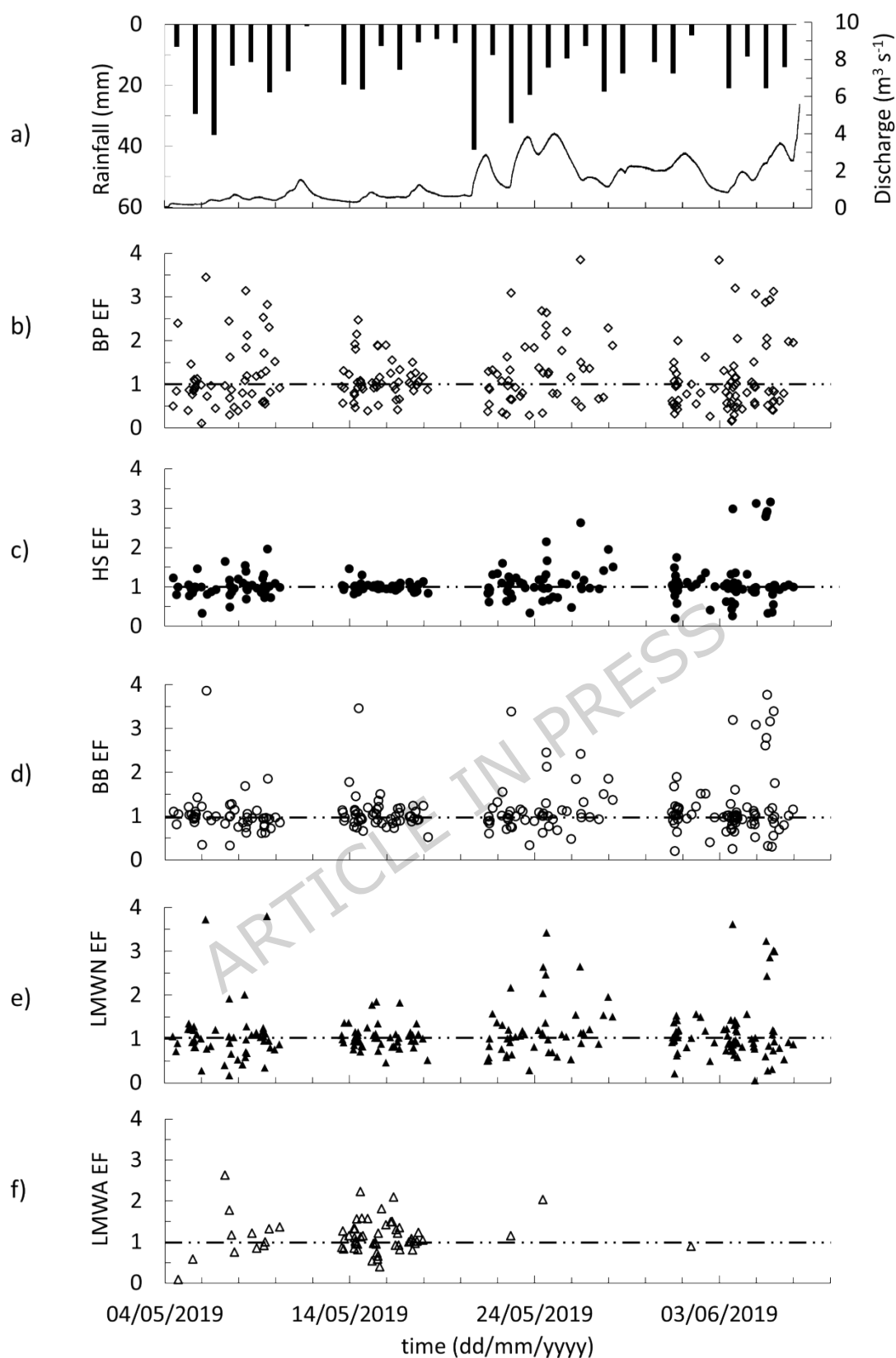534 conditions and the occurrence of high EF values.
535

Figure 4: *BC daily rainfall (black bar) and river discharge (line) (a) and the temporal variability of EF of DOM compound groups (b) to (f) (dashed lines mark EF=1).*

540   **4. Discussion**

541

542   **4.1. Seasonal hydrological connectivity and implications for DOM**
543   **transport**

544

545   The dry season typically occurs from February to April, and the wet season from
546   May to August, with transitional periods in between. The present study was
547   conducted in May 2019, during the onset of the major wet season,
548   corresponding to the dry-to-wet transitional period [43]. To briefly place
549   the study period in a broader context, monthly precipitation totals for the
550   month of May at the Iwokrama field station from 2016 to 2019 were 283,
551   425, 898, and 421 mm, respectively [43]. The 2019 value therefore
552   represents a typical early-wet-season rainfall for the region, neither
553   unusually high nor low relative to interannual variability. This suggests
554   that the catchment was transitioning from dry to wet season during the
555   35-day sampling period. The Iwokrama headwater catchment exhibits a
556   clear and a rapid hydrological response to rainfall [43], which our study
557   period captures. At the onset of the wet season, increasing rainfall likely
558   mobilises shallow groundwater and near-surface flow paths, producing
559   short-lived discharge peaks as the catchment becomes progressively
560   hydrolically connected. During drier periods, river flow is likely sustained
561   primarily by groundwater inputs, resulting in lower and more stable
562   baseflow. This seasonal alternation between surface-dominated and
563   groundwater-dominated conditions strongly influences DOC–discharge
564   relationships and the type of organic matter exported from the catchment
565   [57].

566

567   **4.2. DOC and DOM through the dry to wet season transition**

568

569   In the transition from the dry to wet season, we observed SSW DOC
570   concentrations at the lower range of DOC values previously reported for
571   this river that captured intense rain events [44]. However, here we observe
572   that during the first phase of our study period, where drier conditions
573   prevail, DOC concentrations are generally higher. As river discharge

increases, reflecting wet season conditions, DOC is less concentrated albeit with a greater overall flux that is responsive to fluctuations in river discharge [43]. Interestingly, when the river discharge is at its lowest in the dry season the variability in the composition of DOM in the SSW is at its greatest. At this time, LC-OCD-OND analysis demonstrates that DOM exhibits a lower presence of HS and a higher abundance of BP, BB, LMWN, and LMWA. However, as more water enters the catchment, the range in DOC concentration increases, with very little variability in DOM composition, a notable absence of LMWA, and a greater proportion of HS. The high variability of DOM composition during lower discharge indicates contributions from a more heterogeneous mix of organic matter sources within the catchment. This compositional variability is particularly evident in the HS fraction, with M_n showing higher variability early in the study period before declining over time and shift in the position of the samples in HS-diagram. As the catchment becomes wetter with higher river discharge it is likely that DOM sources become homogenised [43]. That earlier study demonstrated that during the onset of the wet season, DOC concentrations can decline even as total DOM flux increases, due to dilution and the mobilisation of more distal or humic-rich sources. Consistent with this, the observed decline in M_n variability supports the hydrological mobilisation of dry-season DOM sources by rainfall, followed by the increasing influence of a more dominant source, likely high-molecular-weight HS delivered via wet-season dynamics. However, DOM and in particular HS can absorb solar radiation, leading to significant reduction in abundance, and changes in composition and molecular size [58, 59]. Photochemical reactions mainly occur in the photic zone of the upper water column and have been observed to increase the molecular weight of DOM [60], while microbial degradation is ubiquitous and can consume up to 60% of DOM [61]. While HS is traditionally considered chemically stable and resistant to microbial degradation [62] there is evidence that bacteria can utilise DOM as a substrate [63]. The observed changes in HS molecular weight during the study are therefore consistent

606 with concurrent photochemical activity and microbial reworking processes
607 acting on DOM within the water column.

4.3.   SML and SSW Compositional Differences

609

610 We observe that turbulent river flow homogenises the water column in
611 terms of DOC concentration between the SML and the SSW (DOC EF
612 CV=10 %). The observation that these compound groups are not uniformly
613 distributed between SML and SSW likely indicate a change in either the
614 hydrodynamic regime of the river system, a change in the supply of organic
615 matter to the river, compositional variation of the compound groups, or a
616 change in the degradation pathway or rate of organic matter in the river.

617

618 The high temporal variability of EFs for all DOM compound groups
619 demonstrates the dynamic nature of a headwater tropical river. The
620 changing pattern of DOM compound enrichments in the SML throughout
621 our time series is notable. The variability of EFs for BP, HS, BB, and LMWN
622 is greater in the wet transition period with BP and LMWN compounds
623 more frequently observed to fluctuate between depleted to enriched in the
624 SML Figure 4 b to e).
625 The weak EF relationships observed for LMWAs, LMWNs, and BPs
626 suggests that these compound groups migrate independently between the
627 SML and SSW. This may suggest that they are derived from different
628 sources or are subjected to differing transformation process. It may also
629 suggest that the chemical composition of these compound groups have
630 notable differences in their relative buoyancy. Intriguingly the observed
631 relationship with HS and BB EFs suggests these DOM compound groups
632 may be coupled together when transported in the either the SML or SSW.

633

634 The significantly different slope and intercept of the relationship between
635 HS and DOC in the SML and SSW, indicates compositional differences of
636 HS further supported by M_n dynamics. The EFs demonstrate a higher
637 variability early in the study period indicating preferential accumulation

638 of high-molecular-weight HS in the SML at this time. Towards the wet-
639 season phase, HS variability decreases coincident with more uniform SML-
640 SSW composition. Combined this may suggest that the supply, processing
641 and/or migration of HS between the SML and SSW are in a quasi-steady
642 state.

643

644 Our results show no immediate or consistent association between
645 individual rain events and high EF values, indicating that direct rainfall
646 does not lead to an EF pulse. Thus, the observed EF pulses may result from
647 complex, rainfall-initiated processes or instream autochthonous processes
648 rather allochthonous supply or direct rain effects. This is consistent with
649 the idea that discrete and frequent rain events, such as those associated
650 with tropical rainforests, deliver organic substances with variable
651 buoyancy or surface activity potential to the river, as proposed for the
652 Delaware basin [2]. While differentiating individual mechanisms of SML
653 enrichments between organic matter supply and degradation is
654 challenging due to intertwined and competing processes, the rising trend
655 of higher molecular weight humic substances combined with a reduction
656 in SML enrichment over our study period does suggest a change in source
657 composition. Whether this is reflective of the other components of the
658 DOM pool is unknown. The absence of LMWA in the second half of study
659 may suggest reactions with humic substances in the SML generating
660 volatile organic compounds [64]. The stabilization of HS EFs in the second
661 phase, when LMWA was consistently present, further highlights the
662 linkage between LMWA and HS in driving SML-SSW compositional
663 differences. Alternatively, the increasing variability in the EFs of BP and
664 LMWN coupled with the notable absence of LMWA in the wet transition
665 period may indicate an increased microbial presence and activity in the
666 SML [9, 49, 65]. Assuming that the compounds are predominantly derived
667 from microbial sources, the absence of acids may be a result of higher
668 demand than supply for this compound group as observed in microbial
669 incubation systems [66].

670

5. Implications

In the paucity of freshwater studies, we note that marine systems have been shown to have SML enrichments of DOM in upwelling regions likely derived from autochthonous microbial release of DOM as a response to light exposure in this environment [67]. High-resolution observations of DOM in the coastal and open ocean have demonstaretd that increasing wind speeds, solar radiation and photochemical degradation lower SML enrichments of organic material [20, 25]. However, organic enrichments within the SML can persist under high turbulence conditions [68] with bubbles in particular enriching the microlayer further [2]. Additionally, the presence of surfactants and humic substances in the SML may attenuate turbulence [69] with reductions in the exchange of gases including oxygen [70] and CO_2 [3, 12]. Importantly, the variable suppression of gas exchange at similar concentrations of surfactants in the SML suggests organic composition has a notable impact. However, whether these mechanisms are directly comparable to headwater river systems remains unknown [2, 67, 68]

Our study reveals compositional variation in the water column, even when DOC concentrations remain similar. The interplay between these compositional DOM changes demonstrates that rivers are never truly well-mixed with the surface microlayer a distinct chemical layer even in headwater river systems. The dynamic nature of the SML at the water-atmosphere interface likely contributes to highly variable gas exchange rates, similar to observations in marine environments [3, 12]. However, the uncertainty surrounding riverine greenhouse gas outgassing highlights the need for research across various scales of river catchments. In particular, mechanisms driving water column mixing in small headwaters may differ fundamentally from those in larger downstream systems. Expanding such studies in tropical rivers and other aquatic systems will enhance our understanding of the SML's role in global biogeochemical cycles.

704

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References

1. Hardy, J.T., *The sea surface microlayer: biology, chemistry and anthropogenic enrichment.* Progress in Oceanography, 1982. **11**(4): p. 307–328.
2. Burdette, T.C., et al., *Organic Signatures of Surfactants and Organic Molecules in Surface Microlayer and Subsurface Water of Delaware Bay.* ACS Earth and Space Chemistry, 2022. **6**(12): p. 2929–2943.
3. Pereira, R., K. Schneider-Zapp, and R. Upstill-Goddard, *Surfactant control of gas transfer velocity along an offshore coastal transect: results from a laboratory gas exchange tank.* Biogeosciences, 2016. **13**(13): p. 3981–3989.
4. Södergren, A., *Role of aquatic surface microlayer in the dynamics of nutrients and organic compounds in lakes, with implications for their ecotones.* Nutrient Dynamics and Retention in Land/Water Ecotones of Lowland, Temperate Lakes and Rivers, 1993: p. 217–225.
5. Wurl, O., et al., *Formation and global distribution of sea-surface microlayers.* Biogeosciences, 2011. **8**(1): p. 121–135.
6. Lechtenfeld, O.J., et al., *The influence of salinity on the molecular and optical properties of surface microlayers in a karstic estuary.* Marine Chemistry, 2013. **150**: p. 25–38.
7. Wu, M.-h., et al., *Semivolatile organic compounds in surface microlayer and subsurface water of Dianshan Lake, Shanghai, China: implications for accumulation and interrelationship.* Environmental Science and Pollution Research, 2017. **24**(7): p. 6572–6580.
8. Wurl, O. and J.P. Obbard, *A review of pollutants in the sea-surface microlayer (SML): a unique habitat for marine organisms.* Marine pollution bulletin, 2004. **48**(11-12): p. 1016–1030.
9. Liu, Q., et al., *Comparison of the water quality of the surface microlayer and subsurface water in the Guangzhou segment of the Pearl River, China.* Journal of Geographical Sciences, 2014. **24**(3): p. 475–491.
10. Meyers, P.A. and O.E. Kawka, *Fractionation of hydrophobic organic materials in surface microlayers.* Journal of Great Lakes Research, 1982. **8**(2): p. 288–298.
11. Yu, J., et al., *Study on water quality and genotoxicity of surface microlayer and subsurface water in Guangzhou section of Pearl River.* Environmental monitoring and assessment, 2011. **174**(1): p. 681–692.
12. Pereira, R., et al., *Reduced air–sea CO 2 exchange in the Atlantic Ocean due to biological surfactants.* Nature Geoscience, 2018: p. 1.
13. Engel, A., et al., *The ocean's vital skin: Toward an integrated understanding of the sea surface microlayer.* Frontiers in Marine Science, 2017. **4**: p. 262783.
14. Raymond, P.A., et al., *Global carbon dioxide emissions from inland waters.* Nature, 2013. **503**(7476): p. 355.
15. Jenkinson, I.R., et al., *Biological modification of mechanical properties of the sea surface microlayer, influencing waves, ripples, foam and air-sea fluxes.* Elem Sci Anth, 2018. **6**: p. 26.
16. Barthelmeß, T. and A. Engel, *How biogenic polymers control surfactant dynamics in the surface microlayer: insights from a coastal Baltic Sea study.* Biogeosciences Discussions, 2022. **2022**: p. 1–42.
17. Rippy, M.A., et al., *Microlayer enrichment in natural treatment systems: linking the surface microlayer to urban water quality.* Wiley Interdisciplinary Reviews: Water, 2016. **3**(2): p. 269–281.
18. Hillbricht-Ilkowska, A. and I. Kostrzewska-Szlakowska, *Surface microlayer in*

765   *lakes of different trophic status: nutrients concentration and accumulation.*
766   Polish Journal of Ecology, 2004. **52**(4): p. 461–478.
767   19.   Elzerman, A.W. and D.E. Armstrong, *Enrichment of Zn, Cd, Pb, and Cu in the*
768   *surface microlayer of Lakes Michigan, Ontario, and Mendota 1.* Limnology
769   and Oceanography, 1979. **24**(1): p. 133–144.
770   20.   Mustaffa, N.I.H., et al., *High-resolution observations on enrichment*
771   *processes in the sea-surface microlayer.* Scientific reports, 2018. **8**(1): p.
772   13122.
773   21.   van Pinxteren, M., et al., *The influence of environmental drivers on the*
774   *enrichment of organic carbon in the sea surface microlayer and in*
775   *submicron aerosol particles–measurements from the Atlantic Ocean.*
776   Elementa: Science of the Anthropocene, 2017. **5**.
777   22.   Triesch, N., et al., *Sea spray aerosol chamber study on selective transfer*
778   *and enrichment of free and combined amino acids.* ACS Earth and Space
779   Chemistry, 2021. **5**(6): p. 1564–1574.
780   23.   Žutić, V., et al., *Surfactant production by marine phytoplankton.* Marine
781   Chemistry, 1981. **10**(6): p. 505–520.
782   24.   Alsalahi, M.A., et al., *Distribution of surfactants along the estuarine area of*
783   *Selangor River, Malaysia.* Marine pollution bulletin, 2014. **80**(1-2): p. 344–
784   350.
785   25.   Rickard, P.C., G. Uher, and R.C. Upstill-Goddard, *Photo-Reactivity of*
786   *Surfactants in the Sea-Surface Microlayer and Subsurface Water of the Tyne*
787   *Estuary, UK.* Geophysical Research Letters, 2022. **49**(4): p.
788   e2021GL095469.
789   26.   Helms, J.R., et al., *Absorption spectral slopes and slope ratios as indicators*
790   *of molecular weight, source, and photobleaching of chromophoric dissolved*
791   *organic matter.* Limnology and Oceanography, 2008. **53**(3): p. 955–969.
792   27.   Lennon, J.T., et al., *Relative importance of CO2 recycling and CH4 pathways*
793   *in lake food webs along a dissolved organic carbon gradient.* Limnology and
794   Oceanography, 2006. **51**(4): p. 1602–1613.
795   28.   Richey, J.E., et al., *Outgassing from Amazonian rivers and wetlands as a*
796   *large tropical source of atmospheric CO2.* Nature, 2002. **416**(6881): p. 617–
797   620.
798   29.   Davidson, E.A., et al., *Dissolved CO2 in small catchment streams of eastern*
799   *Amazonia: A minor pathway of terrestrial carbon loss.* Journal of Geophysical
800   Research: Biogeosciences, 2010. **115**(G4).
801   30.   Spencer, R.G., K.D. Butler, and G.R. Aiken, *Dissolved organic carbon and*
802   *chromophoric dissolved organic matter properties of rivers in the USA.*
803   Journal of Geophysical Research: Biogeosciences, 2012. **117**(G3).
804   31.   Carter, H.T., et al., *Freshwater DOM quantity and quality from a two-*
805   *component model of UV absorbance.* Water research, 2012. **46**(14): p.
806   4532–4542.
807   32.   Pereira, R., et al., *Investigating the role of hydrological connectivity on the*
808   *processing of organic carbon in tropical aquatic ecosystems.* Frontiers in
809   Earth Science, 2024. **11**: p. 1250889.
810   33.   Van Pinxteren, M., et al., *The influence of environmental drivers on the*
811   *enrichment of organic carbon in the sea surface microlayer and in*
812   *submicron aerosol particles–measurements from the Atlantic Ocean.* Elem
813   Sci Anth, 2017. **5**: p. 35.
814   34.   Aukes, P.J. and S.L. Schiff, *Composition Wheels: Visualizing dissolved*
815   *organic matter using common composition metrics across a variety of*
816   *Canadian ecozones.* Plos one, 2021. **16**(7): p. e0253972.
817   35.   Dittmar, T., et al., *Tracing terrigenous dissolved organic matter and its*
818   *photochemical decay in the ocean by using liquid chromatography/mass*

819   *spectrometry.* Marine Chemistry, 2007. **107**(3): p. 378–387.

820   36.   Li, S., et al., *Dearomatization drives complexity generation in freshwater*
821   *organic matter.* Nature, 2024. **628**(8009): p. 776–781.

822   37.   Vemulapalli, S.P.B., C. Griesinger, and T. Dittmar, *A fast and efficient tool*
823   *for the structural characterization of marine dissolved organic matter:*
824   *Nonuniform sampling 2D COSY NMR.* Limnology and Oceanography:
825   Methods, 2023. **21**(7): p. 401–413.

826   38.   Huang, T.-H., et al., *Fluvial carbon fluxes in tropical rivers.* Current Opinion
827   in Environmental Sustainability, 2012. **4**(2): p. 162–169.

828   39.   Gomi, T., R.C. Sidle, and J.S. Richardson, *Understanding processes and*
829   *downstream linkages of headwater systems: headwaters differ from*
830   *downstream reaches by their close coupling to hillslope processes, more*
831   *temporal and spatial variation, and their need for different means of*
832   *protection from land use.* BioScience, 2002. **52**(10): p. 905–916.

833   40.   Argerich, A., et al., *Comprehensive multiyear carbon budget of a temperate*
834   *headwater stream.* Journal of Geophysical Research: Biogeosciences, 2016.
835   **121**(5): p. 1306–1315.

836   41.   Benstead, J.P. and D.S. Leigh, *An expanded role for river networks.* Nature
837   Geoscience, 2012. **5**(10): p. 678–679.

838   42.   Bela, M., et al., *Ozone production and transport over the Amazon Basin*
839   *during the dry-to-wet and wet-to-dry transition seasons.* Atmospheric
840   Chemistry and Physics, 2015. **15**(2): p. 757–782.

841   43.   Norouzi, S., et al., *Dissolved organic matter quantity and quality response*
842   *of tropical rainforest headwater rivers to the transition from dry to wet*
843   *season.* Scientific Reports, 2024. **14**(1): p. 3270.

844   44.   Pereira, R., et al., *Mobilization of optically invisible dissolved organic matter*
845   *in response to rainstorm events in a tropical forest headwater river.*
846   Geophysical Research Letters, 2014. **41**(4): p. 1202–1208.

847   45.   Pereira, R., et al., *Seasonal patterns of rainfall and river isotopic chemistry*
848   *in northern Amazonia (Guyana): From the headwater to the regional scale.*
849   Journal of South American Earth Sciences, 2014. **52**: p. 108–118.

850   46.   Bovolo, C.I., et al., *Fine-scale regional climate patterns in the Guianas,*
851   *tropical South America, based on observations and reanalysis data.*
852   International Journal of climatology, 2012. **32**(11): p. 1665–1689.

853   47.   Facchi, A., C. Gandolfi, and M. Whelan, *A comparison of river water quality*
854   *sampling methodologies under highly variable load conditions.*
855   Chemosphere, 2007. **66**(4): p. 746–756.

856   48.   Garrett, W.D., *Collection of slick-forming materials from the sea surface.*
857   Limnology and Oceanography, 1965. **10**(4): p. 602–605.

858   49.   Cunliffe, M., et al., *Sea surface microlayers: A unified physicochemical and*
859   *biological perspective of the air–ocean interface.* Progress in Oceanography,
860   2013. **109**: p. 104–116.

861   50.   Aukes, P.J.K., *Use of liquid chromatography-Organic carbon detection to*
862   *characterize dissolved organic matter from a variety of environments.* 2012,
863   University of Waterloo.

864   51.   Heinz, M., et al., *Comparison of organic matter composition in agricultural*
865   *versus forest affected headwaters with special emphasis on organic*
866   *nitrogen.* Environmental science & technology, 2015. **49**(4): p. 2081–2090.

867   52.   Fonvielle, J.A., et al., *Assessment of sample freezing as a preservation*
868   *technique for analysing the molecular composition of dissolved organic*
869   *matter in aquatic systems.* RSC advances, 2023. **13**(35): p. 24594–24603.

870   53.   Huber, S.A., et al., *Characterisation of aquatic humic and non-humic matter*
871   *with size-exclusion chromatography–organic carbon detection–organic*
872   *nitrogen detection (LC-OCD-OND).* Water research, 2011. **45**(2): p. 879–

873   885.

874   54.   van Pinxteren, M., et al., *Marine organic matter in the remote environment of the Cape Verde islands–an introduction and overview to the MarParCloud campaign.* Atmospheric Chemistry and Physics, 2020. **20**(11): p. 6921–6951.

878   55.   Wang, X.-X., et al., *Characterization of algal organic matter as precursors for carbonaceous and nitrogenous disinfection byproducts formation: comparison with natural organic matter.* Journal of Environmental Management, 2021. **282**: p. 111951.

882   56.   Peel, M.C., B.L. Finlayson, and T.A. McMahon, *Updated world map of the Köppen-Geiger climate classification.* 2007.

884   57.   Norouzi, S., *Constraining the global climate-active gases flux between atmospheric and water reservoirs*, in *Energy, Geoscience, Infrastructure and Society*. 2023, Heriot-Watt University: Edinburgh, Scotland.

887   58.   Cory, R.M., et al., *Sunlight controls water column processing of carbon in arctic fresh waters.* Science, 2014. **345**(6199): p. 925–928.

889   59.   Kellerman, A.M., et al., *Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology.* Nature communications, 2014. **5**(1): p. 3804.

892   60.   Spray, J.F., et al., *Unravelling Light and Microbial Activity as Drivers of Organic Matter Transformations in Tropical Headwater Rivers.* Biogeosciences Discussions, 2021. **2021**: p. 1–26.

895   61.   Hur, J., M.-H. Park, and M.A. Schlautman, *Microbial transformation of dissolved leaf litter organic matter and its effects on selected organic matter operational descriptors.* Environmental science & technology, 2009. **43**(7): p. 2315–2321.

899   62.   Allard, B., et al., *Degradation of humic substances by UV irradiation.* Environment International, 1994. **20**(1): p. 97–101.

901   63.   Lipczynska-Kochany, E., *Effect of climate change on humic substances and associated impacts on the quality of surface water and groundwater: A review.* Science of the total environment, 2018. **640**: p. 1548–1565.

904   64.   Stirchak, L.T., et al., *Differences in photosensitized release of VOCs from illuminated seawater versus freshwater surfaces.* ACS Earth and Space Chemistry, 2021. **5**(9): p. 2233–2242.

907   65.   Cunliffe, M., et al., *Dissolved organic carbon and bacterial populations in the gelatinous surface microlayer of a Norwegian fjord mesocosm.* FEMS microbiology letters, 2009. **299**(2): p. 248–254.

910   66.   Shi, X., et al., *Microbial stratification and DOM removal in drinking water biofilters: Implications for enhanced performance.* Water Research, 2024. **262**: p. 122053.

913   67.   Galgani, L. and A. Engel, *Changes in optical characteristics of surface microlayers hint to photochemically and microbially mediated DOM turnover in the upwelling region off the coast of Peru.* Biogeosciences, 2016. **13**(8): p. 2453–2473.

917   68.   Sabbaghzadeh, B., et al., *The Atlantic Ocean surface microlayer from 50 N to 50 S is ubiquitously enriched in surfactants at wind speeds up to 13 m s−1.* Geophysical Research Letters, 2017. **44**(6): p. 2852–2858.

920   69.   Esters, L., et al., *Turbulence scaling comparisons in the ocean surface boundary layer.* Journal of Geophysical Research: Oceans, 2018. **123**(3): p. 2172–2191.

923   70.   Goldman, J.C., M.R. Dennett, and N.M. Frew, *Surfactant effects on air-sea gas exchange under turbulent conditions.* Deep Sea Research Part A. Oceanographic Research Papers, 1988. **35**(12): p. 1953–1970.

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Authors' Contribution Statement

937

938 S. Norouzi (SN) led the writing of the manuscript. SN interpreted the
939 results with the help of R. Pereira (RP), T. Wagner (TW), and A. Macdonald
940 (AMM). SN, J. Bischoff (JB1), J. Brasche (JB2), S. Trojahn (ST), J. Spray (JS)
941 and RP collected water samples on the field trip. SN, JB2, ST, and JS ran
942 field measurements. SN, ST, JB1, JS, and Walter Hill (WH) performed the
943 analytic calculations of LC-OCD-OND. SN calculated rainfall, river flux and
944 discharge data. JB1, RP, and ST ran mobile lab experiments. JS collected
945 hydrological data. RP supervised the field trip and the findings of this
946 work. SN, TW, AMM, JB1, JB2, ST, JS, and RP provided critical feedback
947 on the manuscript.

948

Data Availability Statement

950

951 The data utilized in this research study is available as supplemental
952 material documents within this manuscript. Furthermore, these data is
953 deposited in Mendeley repository (here) with the title of this manuscript
954 and will be published upon acceptance of this manuscript for publication.
955 For any further queries regarding the dataset, please contact the corresponding
956 authors.

957

958 **Competing Interests Statement**

959

960 The author(s) declare no competing interests.

961

962 **Figure Legends**

963

964 Figure 1: Study site map. The map of (a) Guyana, (b) the Iwokrama
965 rainforest (modified from Pereira et al. 2014), and (c) arial view of
966 Blackwater Creek study site (modified after Norouzi et al. 2024).

967

968 Figure 2: BC daily rainfall (black bar) and river discharge (blue line) (a),
969 temporal variability of the percentage of the total DOM pool and DOC
970 concentration in baseline SML (b) and SSW samples (c), DOC EF (d), Mn
971 of HS in the SML and SSW (e), and the HS Mn EF (f). The black dashed
972 line in (e) shows the general increase of Mn in the SML and SSW. The blue
973 dashed line shows the general trend of Mn EF and the black dashed line
974 shows EF=1 in (f).

975

976 Figure 3: BC HS vs DOC concentration for the SML (a) and SSW (b) in the
977 first (circles) and second (triangles) half of the study period.

978

979 Figure 4: BC daily rainfall (black bar) and river discharge (line) (a) and the
980 temporal variability of EF of DOM compound groups (b) to (f) (dashed lines
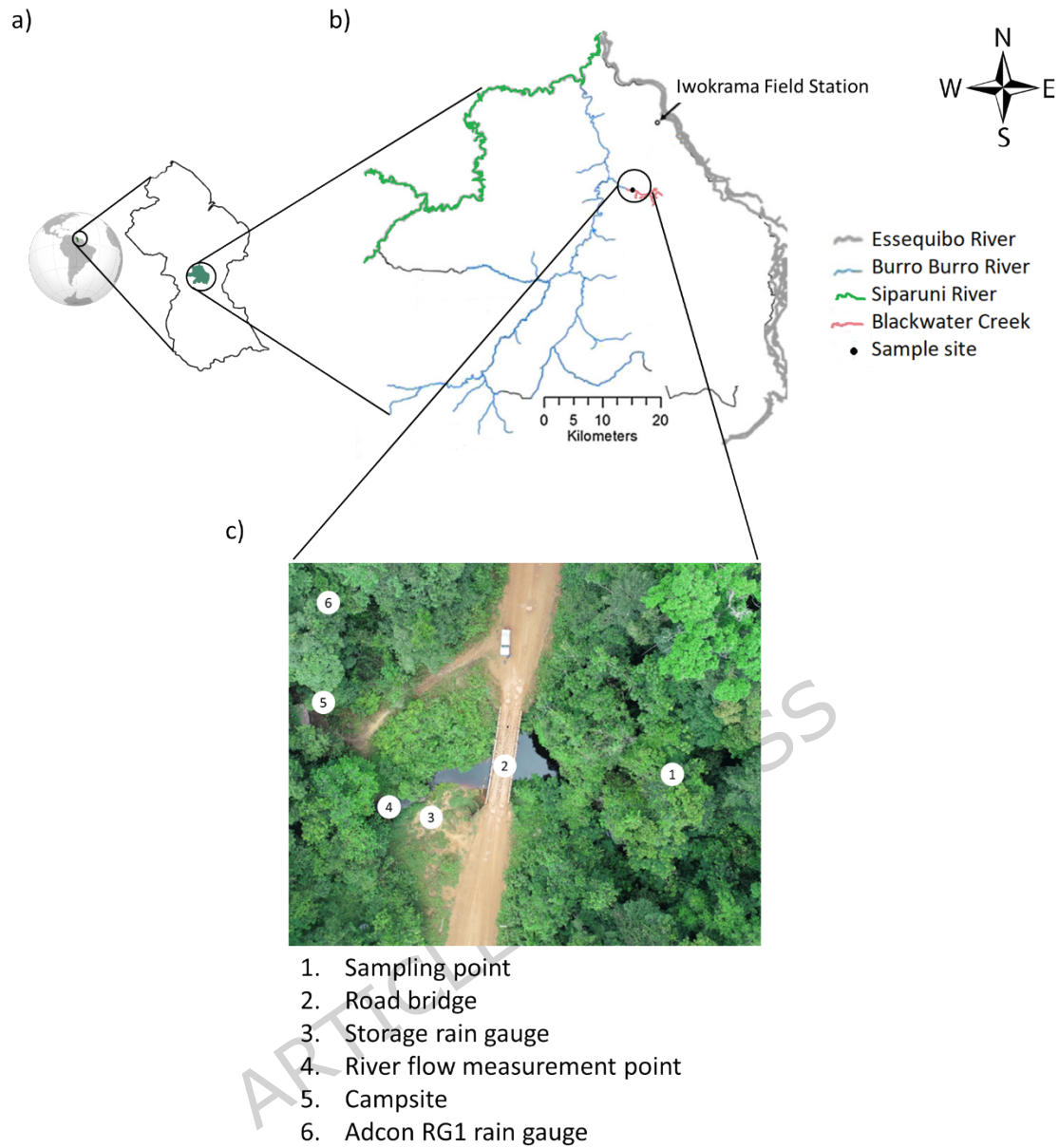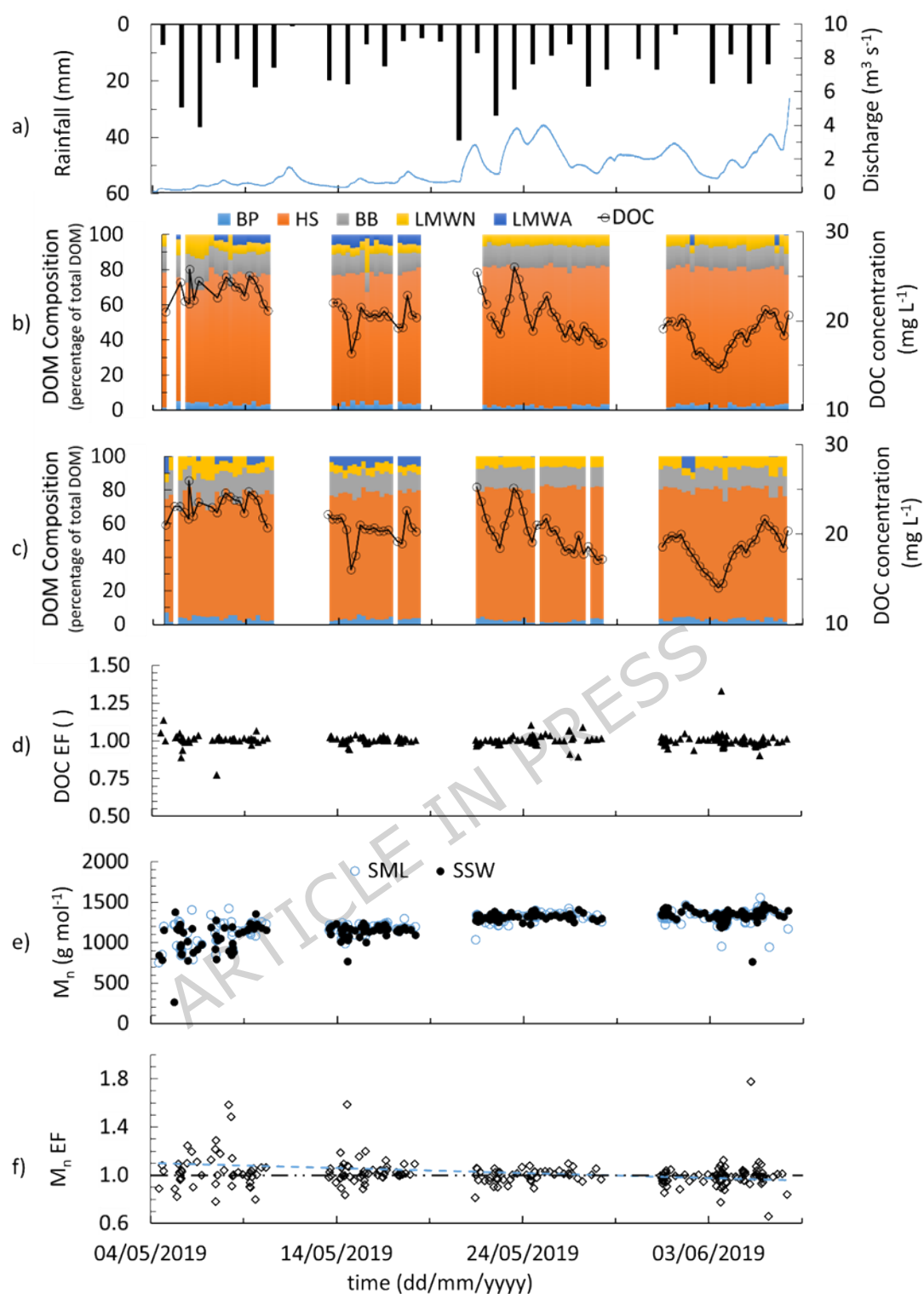981 mark EF=1).

Figure 1: *Study site map. The map of (a) Guyana, (b) the Iwokrama rainforest (modified from [45]), and (c) aerial view of Blackwater Creek study site (modified after [43]).*

F Figure 2: *BC daily rainfall (black bar) and river discharge (blue line) (a), temporal variability of the percentage of the total DOM pool and DOC concentration in baseline SML (b) and SSW samples (c), DOC EF (d), M_n of HS in the SML and SSW (e), and the HS M_n EF (f). The black dashed line in (e) shows the general increase of M_n in the SML and SSW. The blue dashed line shows the general trend of M_n EF and the black dashed*
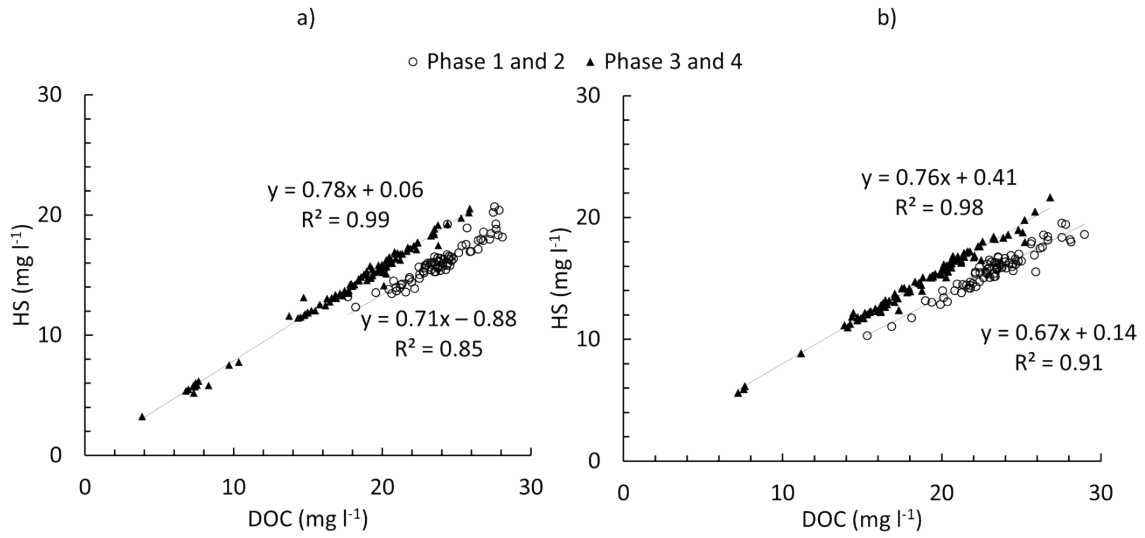
line shows EF=1 in (f).

Figure 3: *BC HS vs DOC concentration for the SML (a) and SSW (b) in the first (circles) and second (triangles) half of the study period.*
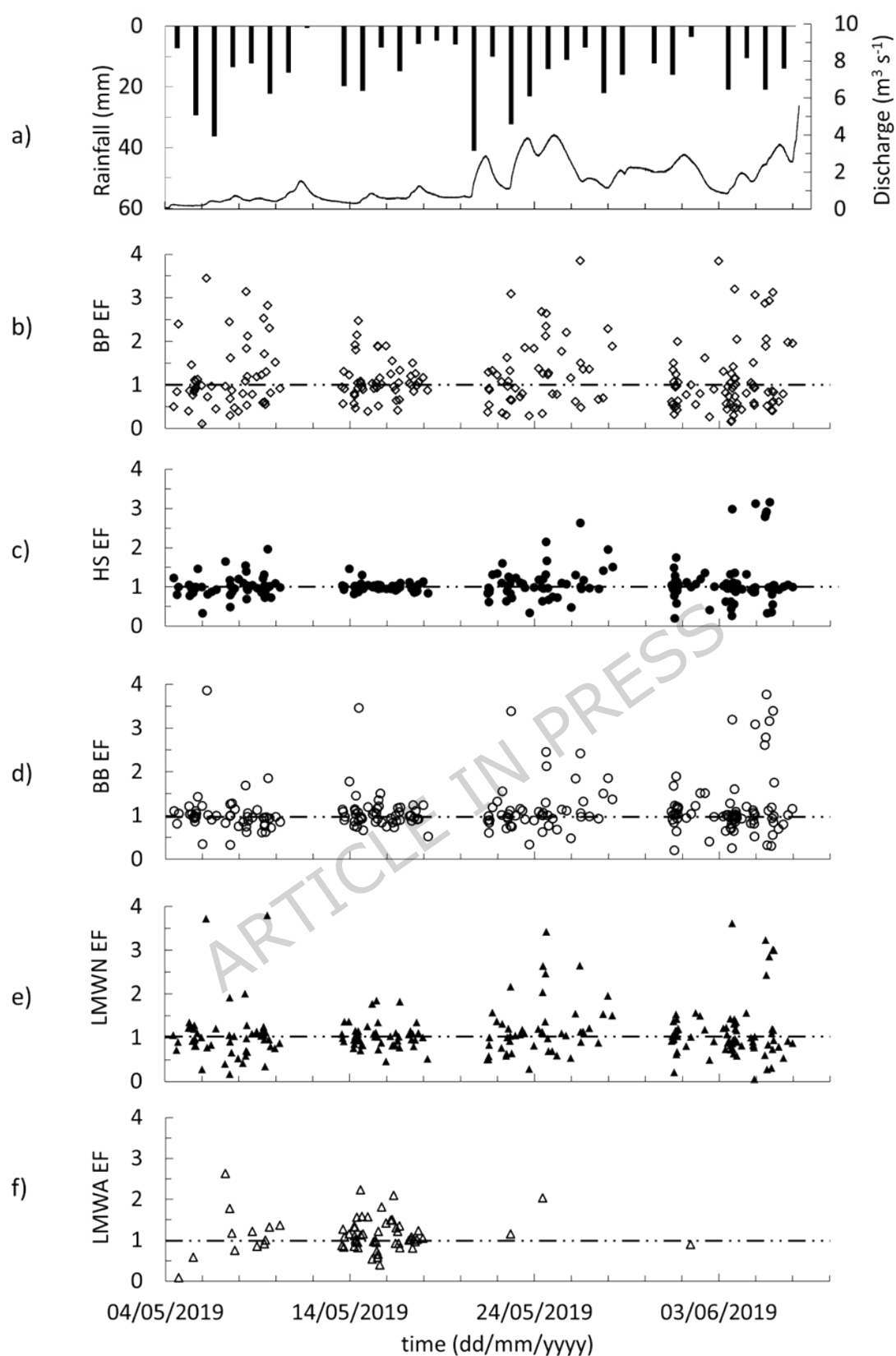
Figure 4: *BC daily rainfall (black bar) and river discharge (line) (a) and the temporal variability of EF of DOM compound groups (b) to (f) (dashed lines mark EF=1).*