

Molecular links between whitesand ecosystems and blackwater formation in the Rio Negro watershed

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17 Highlights

- 18 • Tropical blackwater catchments are a hotspot of aquatic C export
- 19 • Exported C represents a major portion of NEP, and stores molecular information
- 20 • Robust Rio Negro basin ecosystem markers identified by molecular and isotopic tools
- 21 • Ecosystem markers linked to specific upland site but not widespread riparian system
- 22 • If properly calibrated, markers may serve as proxies of NEP or terrestrial C export

23 Abstract

24 Tropical rivers constitute a major portion of the global aquatic C flux entering the ocean, and the
25 Rio Negro is one of the largest single C exporters with a particularly high export of terrestrial C.
26 We investigated the role of whitesand ecosystems (WSEs) in blackwater formation in the Rio
27 Negro basin to develop novel constraints for the terrestrial carbon export from land to the aquatic
28 continuum. To this end, we used ultrahigh resolution mass spectrometry (FT-MS, Orbitrap) to
29 identify markers in dissolved organic carbon (DOC) from ground- and surface waters of two
30 contrasting WSEs feeding Rio Negro tributaries, and compared them with known Rio Negro
31 marker from two openly available FT-MS datasets. Tributaries were fed by a whitesand riparian
32 valley connected to *terra firme* plateau, and a typical upland whitesand *Campina*. WSE-DOC
33 molecular composition differed by 80% from plateau DOC, which was characterized by
34 reworked, highly unsaturated N- and S-containing molecules. WSE-DOC contained mainly
35 condensed aromatics and polyphenols. WSE samples differed by 10% in molecular DOC
36 composition and also by their isotopic content (^{14}C , ^{18}O , ^2H). Upland WSE-DOC was exported by
37 fresh precipitation and had maximum age of 13 years, being five years older than riparian valley
38 WSE-DOC. Unexpectedly, only markers from the upland WSE, which cover a small proportion
39 of the landscape, were identical to Negro markers. Markers of the riparian valley WSE, which
40 are widespread and known for high DOC export, surprisingly showed lower coverage with
41 Negro markers. Analysis of robust matching WSE markers between FT-MS datasets by
42 Pubchem suggested well-known plant metabolites (chromenes and benzofurans) as promising
43 candidates for targeted approaches and calibration. Our results suggest that terrestrial DOC from
44 upland WSEs is a main source of specific blackwater molecules missing in the regional
45 ecosystem C balance, whereas C export from the riparian valley and especially from *terra firme*
46 plateaus represents mainly recycled and transformed carbon not directly affecting the ecosystem

C balance. Our study highlights the potential of high-resolution techniques to constrain carbon balances of ecosystems and landscapes. Comparisons of FT-MS datasets and complementary isotopic information shows high potential to identify robust molecular markers that link forests, soils, aquifers and aquatic systems, and are needed for a deeper understanding of the regional C cycle in tropical blackwater catchments.

Keywords: Dissolved organic matter, critical zone, hydrochemistry, chemodiversity, soil organic matter, biomarker, land-to-ocean continuum, watershed, river basin

1 Introduction

The riverine export of terrestrial dissolved organic carbon (DOC) constitutes a major flux within the boundless carbon cycle that connects land and ocean (Regnier et al., 2013; Drake et al., 2018b; Webb et al., 2018). Global estimates indicate a total of 208 ± 28 Tg DOC exported by rivers each year (Dai et al., 2012), mainly coming from large tropical and circumboreal watersheds (Raymond and Spencer, 2014; Li et al., 2019). Tropical rivers account for a major portion of the flux, being equivalent to 62-66 % of global DOC export (Dai et al., 2012; Huang et al., 2012). Moreover, recent modelling efforts show that tropical annual exports have been rising over the past 65 years, with an increase of 10 Tg C compared to 1960 in case of South America (Li et al., 2019). The three tropical rivers with highest discharge, the Amazon (incl. Tocantins), the Congo and the Orinoco, alone deliver 18% of global riverine DOC (Raymond and Spencer, 2014). Due to within-river DOC transformations, actual export of terrestrial organic carbon expected to be even higher (Drake et al., 2018b), and novel markers are needed to quantify the original terrestrial part of the carbon export.

70 Blackwater river basins stand out as hotspots of DOC release in the tropics (Junk et al., 2011).
71 The Rio Negro basin for example, which covers roughly 10% of the area of the Amazon river
72 basin, accounts for an annual DOC export of 5.2 – 6.7 Tg C (Coynel et al., 2005; Guinoiseau et
73 al., 2016) equivalent to 17-23% of the Amazon's total DOC export (Raymond and Spencer,
74 2014). Tropical blackwater rivers such as the Rio Negro are thus pivotal in understanding the
75 global carbon cycle and its response to environmental change (Alvarez-Cobelas et al., 2012;
76 Raymond and Spencer, 2014; Webb et al., 2018).

77 Qualitative analyses of DOC composition have shown great potential to track processes and
78 source contributions on the watershed scale (Creed et al., 2015; Riedel et al., 2016; Hutchins et
79 al., 2017; Drake et al., 2019; Spencer et al., 2019). Processes such as deforestation (Drake et al.,
80 2019; James et al., 2019), drainage (Moore et al., 2013) or warming (Drake et al., 2018a) all
81 affect the molecular composition or age of leached DOC. Progress in qualitative DOC measures
82 could thus effectively complement global and regional modelling efforts based on quantitative
83 DOC export data (Alvarez-Cobelas et al., 2012; Lv et al., 2019). However, we do know
84 surprisingly little about potential molecular markers in DOM and their environmental fate.
85 Traditional targeted approaches aiming at terrestrial source markers, namely lignin phenols, can
86 be questioned due to findings suggesting their fast turnover in soils (Gleixner et al., 2002; Hernes
87 et al., 2007; Marschner et al., 2008) and within the land-to-ocean continuum (Cao et al., 2018),
88 and potential autochthonous sources in marine systems (Powers et al., 2019). Only a few authors
89 have addressed the traceability and stability of ecosystem imprints within the aquatic land-to-
90 ocean continuum, and its conditions, in a non-targeted way by means of ultrahigh resolution
91 mass spectrometry to identify novel markers (FT-MS; Roth et al., 2014; Medeiros et al., 2016;
92 Hutchins et al., 2017; Wagner et al., 2019). This gap is due to the limited availability of these

93 molecular-level analytical tools. FT-MS techniques allow unprecedented molecular insight by
94 resolving thousands of signals within a single DOM sample, which are assigned molecular
95 formulae based on exact mass (Hertkorn et al., 2013). FT-MS techniques have now added
96 important detail in character and transformation of ecosystem imprints at all stages of the aquatic
97 continuum (Hutchins et al., 2017; Raeke et al., 2017; Kellerman et al., 2018; Lynch et al., 2019;
98 Roth et al., 2019; Wagner et al., 2019). Dedicated sets of novel, traceable ecosystem markers are
99 however rare and need to be calibrated to complement high-resolution DOC flux data (Roth et
100 al., 2014; Medeiros et al., 2016; Cao et al., 2018). Robust sets of molecular markers could
101 promote better understanding of ecosystem-resolved DOM export dynamics and its drivers. This
102 knowledge is pivotal to evaluate and predict the vulnerability and biogeochemical functionality
103 of watersheds under environmental change scenarios (McGuire et al., 2014; Abbott et al., 2018;
104 Bernhardt et al., 2018; Jehn et al., 2020).

105 As described above, the Rio Negro basin is one of the world's largest DOC emitters in terms
106 of estimated annual flux (6.7 Tg DOC) and yield (9.7 g DOC m⁻²), making it a classic
107 "blackwater" river (Coynel et al., 2005; Dai et al., 2012). Scientists early noted the co-
108 occurrence of tropical whitesand ecosystems (WSEs) and blackwater streams, and hypothesized
109 a link between them (Sioli, 1954; Janzen, 1974; Leenheer, 1980; Goulding et al., 1988; Junk et
110 al., 2011). The most characteristic feature of WSEs in these landscapes is their sandy soil,
111 classified as either podzol (2% of Amazon basin area) or arenosol (3%) by the World Reference
112 Base (Quesada et al., 2011). These soils differ largely from the more widespread clayey tropical
113 soils such as ferralsols (32%), acrisols (29%), or plinthosols (9%) that are typically found on
114 plateaus and their slopes (Do Nascimento et al., 2004; Quesada et al., 2011; Lucas et al., 2012).
115 Due to the low water-holding capacity of sand, WSEs are also characterized by specific types of

116 vegetation that differ from highly diverse terra firme rainforests which are dominated by large
117 trees. In the central Amazon, these are sclerophyllous, shrubby and smaller-tree dominated
118 Caatinga, Campina, and Campinarana forests, known for their unique plant secondary
119 metabolites and adapted decomposer communities (Janzen, 1974; Klinge and Medina, 1979;
120 Zanchi et al., 2015; Demarchi et al., 2018; Vasco-Palacios et al., 2018). WSEs occur as local
121 upland depressions on plateaus, as intersected valleys forming large riparian corridors at the foot
122 of plateau slopes, or in low-elevation terrain in the form of wide peneplains (Do Nascimento et
123 al., 2004; Montes et al., 2011). Roughly, upland and riparian valley WSEs are dominant in the
124 lower Rio Negro basin while peneplains are widely distributed in the upper Rio Negro basin
125 (Montes et al., 2011). It is likely that the molecular composition of DOM resolves the different
126 environmental conditions, allowing for proper landscape-based DOC source identification and
127 export calculations.

128 Previous studies that assessed terrestrial sources of Rio Negro DOM largely supported the
129 older hypotheses that WSEs, and more specifically the widely distributed riparian corridors are
130 responsible for the highest amount of carbon export (Junk, 1993; Remington et al., 2007; Melack
131 and Hess, 2010; Bardy et al., 2011). However, this DOC export is mainly controlled by
132 precipitation amount and flooding events (McClain et al., 1997; Remington et al., 2007; Zanchi
133 et al., 2015). This is in line with the finding that in riparian settings, DOC generally shows
134 transport-limited behavior, meaning that its absolute flux scales with discharge (Musolff et al.,
135 2017; Zarnetske et al., 2018). As a result, water passing through the riparian zone continuously
136 leaches existing reserves of processed organic matter (Ledesma et al., 2015; Laudon and
137 Sponseller, 2018; Tiegs et al., 2019). In line with the older hypothesis, McClain and coworkers
138 reported low annual DOC yields for widespread plateau areas (terra firme – ferralsol; 2 g DOC

139 m-2 yr-1) but large yields for a relatively small Campina WSE catchment (40 g DOC m-2 yr-1)
140 in the lower Negro basin, north of Manaus. The authors predicted that a WSE molecular DOM
141 signal would thus be easily detectable in higher order rivers within the Rio Negro catchment
142 (McClain et al., 1997), and later studies conducted in the same region corroborated this
143 hypothesis (Remington et al., 2007). DOM from well-developed podzols reflected best the
144 chemical properties of DOM isolates from local groundwater and nearby rivers, showing the fast
145 transit of DOM in well-drained sandy soils with low sorption potential (Remington et al., 2007;
146 Bardy et al., 2011). However, no subsequent markers were identified or tracked in the lower
147 reaches of the stream network to assess their environmental fate or marker potential (Bardy et al.,
148 2011). Such novel markers are however needed to study variations in DOM export and
149 spatiotemporal dynamics in riverine DOM sources within a catchment (Hutchins et al., 2017;
150 Bernhardt et al., 2018; Laudon and Sponseller, 2018).

151 Recently, watershed-specific molecular DOM signatures of the Rio Negro and other Amazon
152 tributaries (Tapajos, Madeira, Solimões) were reported (Gonsior et al., 2016; Simon et al., 2019)
153 that could serve as potential markers of ecosystem DOC exports due to their largely conservative
154 behavior during mixing (Simon et al., 2019). We here make use of these openly available FT-MS
155 DOM datasets and compare them to groundwater, surface, and soil water DOM measured by
156 Orbitrap FT-MS. We hypothesized that the overall large export of DOM from riparian WSEs in
157 the Rio Negro basin would allow for the retrieval of Rio Negro-specific markers as assumed by
158 previous studies (McClain et al., 1997; Remington et al., 2007; Bardy et al., 2011). We therefore
159 compared an upland Campina forest, and a riparian valley system dominated by Campinarana
160 forest, both typical for WSE-podzol systems within elevated terra firme plateaus north of
161 Manaus. We hypothesized that both WSEs and plateaus would differ in terms of water chemistry

and DOC properties, and that the DOM molecular composition would reflect these differences as well, yielding new sets of unique ecosystem markers. We further hypothesized that riparian valley WSE markers would indicate clear overlap with known Rio Negro markers, and could thus serve as complementary proxies of land-derived primary production in the Rio Negro basin.

2 Materials and Methods

2.1 Field sites and sampling procedures

Soil water samples were taken in early November 2017 at the onset of the rainy season in two protected forest reserves under the responsibility of the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil (Figure 1; Supporting Information Figure S1). Both reserves, the Reserva Biológica do Cuieiras – ZF2 (2°36'32.67" S, 60°12'33.48" W, at 40–110 m above sea level) and the Reserva Biológica de Campina (2°35'30.26" S, 60°01'48.79" W, at 93–101 m a.s.l.) are located about 60 - 70 km north of Manaus (Zanchi et al., 2014; Marques et al., 2016). We conducted sampling from 31st October – 2nd of November, and all lab procedures followed within three days. The geological setting, landscape structure, forest composition, and soil characteristics have been described in detail by Zanchi et al. (2014). Broad swampy valleys surrounded by elevated plateaus cover about half of the Cuieiras reserve's area (in total, 22735 ha) (Zanchi et al., 2014). The valley soils (podzols, gleysols) differ markedly from the clayey plateau and slope soils (oxisols, ultisols). Bleached quartz sand and high amounts of phenolics characterize the valley's podzols (Monteiro et al., 2014; Zanchi et al., 2014; Marques et al., 2016), and the presence of *Mauritia flexuosa* (Luizão et al., 2004), a palm species indicative of hydromorphic conditions, suggests poor drainage (Junk, 1993). A second-order blackwater stream (Rio Açu) drains the area, meeting with Rio Cuieiras, Rio Branquinho, and Rio Negro downstream (Monteiro et al., 2014). The catchment has an area of 660 ha (Monteiro et al., 2014).

185 Monteiro and coworkers report a total annual rainfall of 2806 mm in 2002 and 2004 mm in 2003.
186 Stream discharge was $0.08 - 5.59 \text{ m}^3 \text{ s}^{-1}$ (average, $0.18 \text{ m}^3 \text{ s}^{-1}$) and DOC levels ranged from
187 $3.2 - 15.2 \text{ mg C l}^{-1}$ (average, $8.7 \pm 3.0 \text{ mg C l}^{-1}$) during that period. The watershed's annual
188 total carbon flux was estimated as 13.3 g C m^{-2} in 2003 (Waterloo et al., 2006) and Monteiro
189 and coworkers estimated a stream DOC flux (Rio Açu) of 8.7 g C m^{-2} for the same year. Zanchi
190 and coworkers estimated the annual DOC export of the Açu watershed to lie within the range 9.3
191 $- 22.7 \text{ g C m}^{-2}$ (Zanchi et al., 2015). Water samples were taken from piezometers installed
192 across a valley transect (Monteiro et al., 2014) which is maintained and sampled regularly.
193 Piezometers were emptied once before final sampling. The stream was sampled manually, with
194 nitrile gloves, against the direction of flow, using pre-cleaned (acidified ultrapure water, pH2,
195 HCl, Merck EMSURE®, p.a., ACS grade) Nalgene™ polycarbonate bottles (Fisher Scientific,
196 Schwerte, Germany) that were cleaned with the respective sample before final sampling. We also
197 sampled two deep wells on the plateau (35 m and 39 m depth) by lowering an empty, clean
198 sampling bottle on a string until water was reached.

199 The Reserva Campina is a 900 ha reserve that shows only small relief; poor sandy soils (up to
200 99% sand) that co-occur with typical but specific forest types, so-called Campina and
201 Campinarana forests (heath forests; Demarchi et al., 2018) characterize the area. In contrast to
202 the highly diverse plateau (terra firme) forests, Campinas show much lower species diversity and
203 low canopy (~10 m vs. 25-40 m at Reserva Cuieiras). Bare patches of sand cover ~11% of the
204 area (Zanchi et al., 2014). The headwater area is drained by a single headwater stream that is less
205 than 1m wide and often less than 30 cm deep (McClain et al., 1997) and feeds the Rio Tãruma
206 Açu in the southward direction, meeting with the Rio Negro close by Manaus (Figure 1). The
207 catchment area is estimated by 6.5 ha and thus ~100 times smaller than the Rio Açu catchment

208 (Zanchi et al., 2015). According to the same authors, annual rainfall levels are comparable
209 among both reserves. Surface runoff (from the stream) and estimated groundwater outflow
210 amounted to 485 and 1071 mm in that period, respectively (in sum 1556 mm; as compared to
211 Açu 1362 mm; Waterloo et al., 2006). Zanchi and coworkers estimated the total watershed's
212 DOC export with 49.2 g C m⁻², including groundwater outflow and rainfall, of which the stream
213 exported 15.3 g C m⁻². An annual streamflow of 485 mm equals an estimated discharge of 1.0 x
214 10⁻³ m³ s⁻¹, which is about 180 times lower than average discharge at Rio Açu in 2002 – 2003
215 (Monteiro et al., 2014). McClain and coworkers reported annual DOC exports of 40 g C m⁻² yr
216 (McClain et al., 1997; Zanchi et al., 2015) from the same catchment in the period 1993 – 1994.
217 We took samples at the side slopes of the stream from piezometers installed in 1993, as detailed
218 in McClain et al. (1997). For this purpose, wells were emptied three times and sampled afterward
219 (Zanchi et al., 2015). The stream was sampled as described above.

220 2.2 *Water chemistry: TOC, pH, electrical conductivity*

221 Aliquots of the samples were subjected to TOC analysis in the water laboratory of the
222 Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil (Laboratório de Águas
223 do INPA/ CPRHC – Coordenação de Pesquisas em Recursos Hídricos e Clima). Samples were
224 measured on a total organic carbon analyzer (TOC-VCPH model, Shimadzu, Kyoto, Japan)
225 (Monteiro et al., 2014). Before extraction, we analyzed samples for pH and electrical
226 conductivity (EC) with a Multi 340i probe system (WTW, Weilheim, Germany).

227 2.3 *Solid-phase extraction of DOM*

228 DOM samples were solid-phase extracted (SPE) shortly after sampling at INPA, Manaus
229 (Laboratório de Ecossistemas Aquáticos) using an established protocol (Dittmar et al., 2008).
230 The solid-phase sorbent was a modified styrene-divinylbenzene polymer (PPL Bond Elut™,

Agilent, Santa Clara, CA, USA). Samples were acidified to pH 2 with 37% hydrochloric acid (Merck EMSURE®, p.a., ACS grade) before extraction. Solvents used for extraction were ultrapure water, acidified ultrapure water (pH 2, HCl), and ultrapure methanol (Biotec Reagentes Analíticos, p.a., ACS grade). We loaded columns with maximal amounts of 3 mg C. The extraction efficiency (EE) of samples with high DOC concentrations ($> 2 \text{ mg l}^{-1}$, $n = 11$) was always $> 60\%$ and on average $71 \pm 8\%$, at a loading ratio of 443 ± 143 (average \pm standard deviation; PPL: DOC in mg/ mg). Sample PT6 was an exception (high DOC, low EE: 33%). Samples with low DOC concentrations ($< 2 \text{ mg l}^{-1}$, $n = 4$) showed lower extraction efficiency ($23 \pm 15\%$), and loading ratios were generally higher (2400 ± 960) (Supporting Information Table S1).

2.4 Water isotopes

Isotopic signatures of water ($\delta^2\text{H}$ - and $\delta^{18}\text{O}$ -values) were analyzed by high-temperature conversion-isotope ratio mass spectrometry (HTC-IRMS) in the stable isotope laboratory of the Max Planck Institute for Biogeochemistry (BGC-IsoLab). For method details, we refer the reader to the literature (Gehre et al., 2004). In short, measurements were conducted on a Delta+ XL coupled to a high-temperature furnace via a ConFlow III interface (Thermo Fisher Scientific, Bremen, Germany). One μl of water was injected using an A200S autosampler (CTC Analytics AG, Zwingen, Switzerland), and the furnace temperature was held at 1350°C . The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values are reported on the VSMOW scale, which is realized by parallel analysis of samples against in-house standards. In-house standards are routinely calibrated against internationally accepted water standards. Daily standard deviations for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurements are usually better than 1 and 0.1 ‰, respectively. We accessed regional isotope ratio estimates of rain for October and November with the help of the online isotopes in precipitation calculator (OIPC2.2,

version 3.1, <http://wateriso.utah.edu/waterisotopes/>; Bowen and Revenaugh, 2003). The values were similar for both sites; -7 ‰ and -17 ‰ (V-SMOW) for $\delta^2\text{H}$, and -2.4‰ and -3.8‰ (V-SMOW) for $\delta^{18}\text{O}$ in October and November, respectively. We estimated the average between both monthly values for each isotope, considering our sampling date at the end of October/beginning of November.

2.5 Radiocarbon analysis of solid-phase extracted DOC

We transferred an aliquot of methanolic PPL extract equivalent to 0.25 mg C to tin capsules (8 mm diameter, 20 mm height; IVA Analysentechnik, Meerbusch, Germany). The methanol was left to evaporate. Air-dried capsules were combusted in an elemental analyzer and graphitized for radiocarbon analysis on a 3 MV Tandetron 14C-AMS (HVEE, Amersfoort, Netherlands) at the Max Planck Institute for Biogeochemistry in Jena, Germany. Modern (Oxalic Acid II) and ^{14}C -depleted standard materials were carried along for quality control and data corrections (Steinhof et al., 2017; Benk et al., 2018). Graphitization is conducted with Duran glass tubes at a temperature of 550°C in the presence of hydrogen and with an iron catalyst (Steinhof et al., 2017). Radiocarbon concentrations are given as fraction modern ($F^{14}\text{C}$), which is the fraction of the standard concentration normalized for $\delta^{13}\text{C}$ of the oxalic acid standard measured at the same time (with $\delta^{13}\text{C}$ of -19‰; Trumbore et al., 2016). $\Delta^{14}\text{C}$ values also take into account radioactive decay of the oxalic acid standard since 1950, which demarks the start of bomb tests that increased the ^{14}C content of the atmosphere (Trumbore et al., 2016). We calibrated our background-corrected $F^{14}\text{C}$ data with atmospheric data published in Graven (2015) to derive DOC age. We used values for the inner-tropical convergence zone (zone SH3). To cover the time point of sampling (2017), we predicted $\Delta^{14}\text{C}$ values for 2016 and 2017 based on data from 1997-2015 by linear regression ($R^2 = 0.996$) and then estimated DOC mean

calendar age. The measurement error was < 1 year and < 2 years for repeated measurements (standard deviation).

2.6 Orbitrap measurements of DOM and data processing

We conducted Orbitrap measurements at the Max Planck Institute for Biogeochemistry, as described elsewhere (Simon et al., 2018), at a nominal resolution setting of 480,000. Allowable numbers of atoms in formula calculation were as follows: ¹²C, 0-60; ¹H, 0-120; ¹⁴N, 0-2; ³²S, 0-1; ¹⁶O, 1-60, ¹³C, 0-1. The assignment was done at ± 1 ppm tolerance. We defined noise as the smallest peak in the sample set, not including zeros. We then calculated the maximal signal-to-noise ratio (S/N_{max}) of a peak across all samples (without blanks and reference material) and only kept peaks with a value > 5. We calculated the number of matches of each peak across all samples (without blanks and reference) and only kept peaks with more than one match. To exclude contaminants, we calculated a signal-to-noise ratio of blank peaks (only blank samples) and discarded all peaks with values > 20. Finally, we excluded those peaks that were only present in less than 20% of all samples (i.e., < 9 matches) and small (S/N_{max} < 20). After the exclusion of peaks outside the usual mass defect range of natural organic compounds (nominal m/z in Da, -0.05 mDa ... +0.3 mDa), 13658 peaks remained in the dataset in total. Of those, 9893 had a formula assigned, and 3672 included one ¹³C atom. Formulae having H/C ratios > 2 or DBE-O values (double bond equivalent minus oxygen atoms) > 15 (modulus) or containing the elemental combination N₂S were discarded due to their unlikely occurrence in DOM (Hawkes et al., 2016). In the case of ambiguous peaks with more than one assigned molecular formula, only suggested CHO formulae with a DBE-O of < 10 (modulus) were kept (Herzprung et al., 2014). Other formula suggestions or ambiguous hits were excluded from the formula pool and kept as “no reference” peaks. ¹³C-containing formulae that were missing their equivalent

300 monoisotopic (only ^{12}C) formula were excluded. As a last measure, we only considered peaks
301 detected twice in two separate runs for further analysis (Riedel and Dittmar, 2014). The final
302 dataset contained 7705 formulae (of those, 1963 containing a ^{13}C). For comparison of samples,
303 we normalized all mass spectra to the sum of their peak intensities (including all peaks $> \text{S/N} =$
304 5, also those with no assigned formula). The further analysis of the data focused on the subset of
305 peaks with an assigned monoisotopic formula ($n = 5709$). The crosstab is available from
306 <https://doi.org/10.1594/PANGAEA.922606> (Supporting Information Data Set S1).

307 2.7 Statistical analyses: Ecosystem fingerprints and molecular links

308 We analyzed the molecular formula data by Principal Coordinate Analysis (PCoA, cmdscale
309 function, stats package, v3.5.1) and post-ordination gradient fitting analysis (envfit function,
310 vegan package, v2.5-2) within the statistical computation environment R Studio (v1.1.453, ©
311 2009-2018 RStudio, Inc.). PCoA was based on Bray-Curtis dissimilarities obtained by the
312 function vegdist from package vegan (Oksanen, 2010). The method allows the comparison of
313 samples based on their formula populations. Redundancy within the dataset, i.e., formulae
314 showing similar trends in ion abundance across samples, is effectively reduced and yields a set of
315 coordinates that summarize the variability of the dataset best (Osterholz et al., 2016). We then
316 analyzed the distribution of samples in coordinate space for clustering (indicating similarity
317 among samples) and correlations with specific DOM indices by the envfit function (at 999
318 permutations). DOM indices aggregate properties of the molecular formula population of each
319 sample (Supporting Information Table S2). We further assessed molecular formulae with a
320 significant ($p = 0.05$) association to clusters of samples derived from PCoA analyses by
321 Student's t-test of averaged relative ion abundances across samples of each group. We conducted
322 two-sided tests assuming unequal variances.

To link headwaters and downstream signals, we compared sets of specific molecular formulae to known Rio Negro-specific markers available from two open-access FT-MS datasets (Gonsior et al., 2016; Simon et al., 2019). The dataset from 2016 compared samples from the Rio Negro and its adjacent lakes (close to Novo Airão, 120 km northwest of Manaus) to distant large rivers (Rio Tapajos, Rio Madeira) to reveal large-scale differences in chemodiversity, i.e., unique signals of each river basin. We extracted the robust Rio Negro fingerprint by selecting unique formulae of the Rio Negro detected across all measurements, with an average ion abundance of at least 5×10^7 (or $\sim 0.01\%$ relative intensity; $n = 225$; Gonsior et al., 2016). The dataset published in 2019 assessed differences of riverine DOM at the confluence of the Amazon near Manaus (Encontro das Águas). To obtain a robust DOM fingerprint, we extracted formulae that showed a significant positive correlation (Pearson's r , $p = 0.05$) to the fraction of Rio Negro during mixing in each of the studies' three experiments (Simon et al., 2019; $n=299$). The datasets were also compared on a general level. Information on this aspect is presented in the Supporting Information (Supporting Information Text S1). The combined data from all three studies is available in presence/absence format and with ion abundance information from <https://doi.org/10.1594/PANGAEA.922606> (Supporting Information Data Set S2).

3 Results

3.1 Water chemistry, water isotopic composition and carbon isotopic composition of DOC

Samples were clearly differentiated by water chemistry, revealing two major endmembers related to fine- and coarse-textured soils ("Plateau" vs. "Upland"; Figure 2, Supporting Information Table S1), and one intermediate site ("Valley"). Acidity (pH), electrical conductivity (EC) and concentrations of dissolved organic carbon (DOC) were 3.7 ± 0.1 , $49 \pm 6 \mu\text{S cm}^{-1}$ and $37.7 \pm 7.54 \text{ mg C l}^{-1}$ in Campina samples (Figure 2a – c), and some samples at Reserva Cuieiras

346 indicated the same tendency (PR7, PR8, PR9), suggesting common WSE water properties.
347 Samples from the plateau environment showed slightly higher pH, and low EC and DOC levels
348 (4.5 ± 0.2 , $12 \pm 2 \mu\text{S cm}^{-1}$ and $0.9 \pm 0.6 \text{ mg C l}^{-1}$). Three samples from Reserva Cuieiras (PR10,
349 PT06, and stream RA, shown by open symbols) indicated “intermediate” levels of all three
350 parameters (pH 3.9 – 4.3, EC 14 – 29 $\mu\text{S cm}^{-1}$ and 2.3 – 31.3 mg C l^{-1}). Magnitudes and
351 correlations of acidity (pH), electrical conductivity (EC), and dissolved organic carbon (DOC)
352 concentration agree with previous reports from similar environments, showing a strong positive
353 correlation of EC, proton concentration, and DOC (Do Nascimento et al., 2008; Bardy et al.,
354 2011; Monteiro et al., 2014). Plateau samples showed higher pH values and stronger variability,
355 and the absence of acidic DOC (Do Nascimento et al., 2004; Do Nascimento et al., 2008). DOC
356 concentrations of Rio Açu (RA) were low compared to surrounding valley piezometers. In
357 contrast, DOC concentration of the stream (“Rio Campina”, RC) draining the upland site
358 revealed similarly high DOC levels as the surrounding piezometers. Despite similarities in water
359 chemistry of WSE samples (non-intermediate “Valley” and “Upland” samples, Figure 2a-c),
360 differences were most apparent in water isotopic composition, and radiocarbon content of DOC
361 (Figure 2d-e, Supporting Information Figure S2, Supporting Information Figure S3). All samples
362 plotted on the local meteoric water line, resembling the range of expected isotopic composition
363 found in the region. However, water was overall lighter as compared to local precipitation in
364 previous years (Supporting Information Figure S2). Despite regional climatological effects, sites
365 differed significantly in water composition. Campina samples showed a very homogenous water
366 isotopic composition except for the most upslope sample P2. In comparison, samples from
367 Reserva Cuieiras showed consistently lighter (more negative) $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values; plateau
368 samples were lightest and showed a very homogenous water isotopic composition. The valley

369 samples indicated variation in isotopic composition within the range of the other samples. While
370 the $\delta^{13}\text{C}$ composition of all DOC extracts was roughly similar (average \pm standard deviation: -
371 29.40 ± 0.45 ‰, $n=13$) and reflected a typical C_3 source, their calibrated radiocarbon ages were
372 young (less than 2 to 13 years old at maximum) and differed markedly by five years between
373 both sites, with the upland site showing older DOC (Figure 2e).

374 3.2 DOM characterization by ultrahigh-resolution mass spectrometry

375 The analysis of molecular DOM composition reflected the separation of samples based on
376 water and DOC properties presented in the previous section (Figure 3a). The PCoA separated
377 samples into two main clusters (“plateau” and “WSE”) on the first coordinate, which held 78%
378 of molecular variation. Plateau-derived DOM was thus most dissimilar from WSE-DOM, in line
379 with a major control of DOC properties and abundance by soil texture (Remington et al., 2007).
380 Consequently, PCoA 1 was linked to significant (Pearson’ r , $p < 0.05$) trends in pH, EC, and
381 DOC (not shown). In line with more subtle differences between WSE samples, the overall
382 explained variability of the second coordinate was smaller (10%). However, WSE sites were
383 clearly separated, and molecular trends thus paralleled differences in water isotopes and
384 radiocarbon age. Moreover, the separation of WSE-DOM by sites suggested differing trajectories
385 of DOM processing that seemed to converge to a common DOM composition (note the close
386 proximity of samples P7 and PR7 in Figure 3a). Several significant trends in molecular indices
387 derived from the DOM data paralleled the PCoA separation (Figure 3b-d). The used descriptors
388 – a-priori defined molecular groups and formula classes that aggregate molecular composition
389 information – are described in Supporting Information Table S2, and data is available online
390 from <https://doi.org/10.1594/PANGAEA.922606> (Supporting Information Data Set S1). WSE-
391 DOM was more oxidized (higher O/C and NOSC), less saturated (lower H/C, higher DBE) and

392 more aromatic (higher AImod) than plateau DOM (Figure 3b). Consequently, a-priori-defined
393 molecular groups reflected those trends (Figure 3c): Aromatic and oxidized groups (polyphenols,
394 black carbon-like, carbohydrate-like, and O-rich highly unsaturated formulae) were more
395 abundant in WSE samples, while aliphatic, less oxidized groups (Oxygen-poor highly
396 unsaturated markers, unsaturated aliphatics, and peptide-like compounds) were dominant in
397 plateau DOM. Average numbers of C, O, and N atoms per formula followed these major trend as
398 well (Figure 3b, d). Simple oxidized formulae (CHO) dominated the upland WSE cluster while
399 the percentage of N- and S- containing formulae (CHNO, CHOS, CHNOS) were more abundant
400 in plateau and plateau-influenced (“intermediate”) valley WSE samples. The percentage of
401 CHNO formulae also differentiated WSE-DOM from upland and valley sites on PCoA2. Besides
402 the effect of N-containing formulae, upland WSE-DOM was also heavier in terms of molecular
403 weight (MW, Figure 3b) due to more C and O atoms per molecular formula. These findings are
404 in line with known bulk characteristics of DOC endmembers from soils, groundwater, and rivers
405 in the region (Leenheer, 1980; McClain et al., 1997; Remington et al., 2007). In a next step we
406 thus extracted the molecular markers that caused the separation of DOM from different plateau
407 and whitesand environments.

408 As expected from the results of the gradient analysis, we found clear sets of markers for each
409 DOM type that paralleled trends of weight-averaged molecular indices (Figure 3b-d). To this
410 end, we separated unique from shared (“common”) markers, and thus excluded non-informative
411 formulae (Figure 4, Supporting Information Figure S4, and Supporting Information Figure S5). It
412 is important to note that our definition of “unique” and “common” relates to significant
413 differences in abundance. In fact, most molecular formulae were shared based only on presence
414 (30% of all formulae shared among all three ecosystems, and 83% among WSEs; Supporting

Information Figure S6). However, besides subsets of formulae indicating less overlap (CHNO, CHOS: Supporting Information Figure S6; BC, PP, HU: Supporting Information Figure S7), ecosystems differed significantly in abundances of formulae, giving rise to individual “molecular fingerprints” (Supporting Information Figure S5). Markers of plateau DOM showed a narrow mass and chemical space distribution as assessed by van Krevelen diagrams (formulae centered at m/z 425, $O/C < 0.5$, and $H/C > 1$). They were also characterized by high numbers of N- and S-containing formulae (63% of all markers) as compared to “simpler” CHO formulae (Figure 4a-c, and Supporting Information Figure S5a). Unsaturated, relatively less oxidized nitrogen-containing formulae classified as “highly unsaturated” compounds (“HU”, i.e., lignin-like formulae, or carboxyl-rich alicyclic molecules, CRAM) were the most dominant group of markers, representing ~50% of all plateau markers. In contrast, molecular markers common to both WSE-DOM types were dominated by CHO formulae and showed a broad chemical space distribution with molecular weights up to m/z 800, and a distinct center at low m/z (~ 200 Da; Supporting Information Figure S4a-c). The most distinct WSE-DOM markers were oxidized, phenolic/ aromatic CHO formulae (74% belonging to molecular groups “orHU”, “PP”, or “BC”; and 64% belonging to “CHO” class).

We observed clear molecular differences between WSE-DOM from the Cuieiras valley and upland Reserva Campina, as suggested by the explorative PCoA (PCoA 2 in Figure 3a), and unique markers of each WSE reflected this divergence (Figure 4d-f, and g-i). This differentiation was most apparent through a sharp “cutoff” at ~ m/z 400 (Figure 4d, g). Reserva Cuieiras valley samples were characterized by lower-molecular-weight N-containing formulae which represented 62% of all valley markers (weighted average mass ~ m/z 300; Figure 4d, f, and Supporting Information Figure S5b). Despite their low mass, these formulae showed wide

distribution in chemical space, mostly belonging to the classes of highly unsaturated and polyphenolic compounds (“HU”, 47% of all markers, and “PP”, 20%; Figure 4e). Upland WSE-DOM, on the other hand, was differentiated by a confined cluster of CHO formulae with higher average mass ($\sim m/z$ 425). Indicative upland CHO formulae concentrated in a chemical space defined by $O/C > 0.3$ and $H/C < 1$ and showed distribution across a wide mass range, high aromaticity, and high oxidation state (Figure 4h, i). All in all, 57% of upland WSE markers belonged to the molecular groups of “orHU”, “PP”, or “BC”, and 83% were classified “CHO” formulae.

3.3 *Molecular links between terrestrial ecosystems and the aquatic continuum*

We compared the sets of distinct DOM markers of the valley and upland WSEs to known Rio Negro markers in order to gain qualitative insight into the contribution of headwater aquifer and stream DOM to the “integrated” watershed signal of the lower Rio Negro (Figure 5). Although many CHNO and CHOS formulae were part of the specific sets of signals in both WSEs, the overlap to Rio Negro markers was restricted to CHO formulae. The two WSEs differed in terms of potential molecular links. The cluster of heavy, highly oxidized, and aromatic upland WSE markers indicated consistent overlap with known Rio Negro markers (Figure 5). Overlapping sets of formulae appeared in a confined area of the Van Krevelen plot (boxes in Figure 5b, d; ranges: H/C 0.4 – 0.8, O/C 0.4 – 0.8), suggesting robust matching. However, the overlap was caused by different sets of Rio Negro markers, as indicated by the formula’s m/z (Figure 5c, f). One study (Gonsior et al., 2016) revealed molecular links with markers of higher mass (m/z 350 – 650, Figure 5c), while another (Simon et al., 2019) found them in the lower mass range (m/z 200 – 500, Figure 5f). Nevertheless, all these markers were specific to the upland WSE and

restricted to a common type of chemistry, and valley WSE markers contributed to a much lower degree.

4 Discussion

4.1 Site characterization: Biogeochemistry of water and carbon

The general analysis of water chemistry showed clear differences among samples, revealing two major endmembers and one intermediate environment (Figure 2). Strongest differences were apparent between fine- and coarse-textured soils (“Plateau” vs. “Upland”; Figure 2, Supporting Information Table S1). Valley samples PR10, PT06, and stream RA indicated “intermediate” levels in terms of pH, electrical conductivity and DOC concentration and were likely plateau-influenced, i.e., affected by mixing due to plateau water supply, or dilution from precipitation in case of stream RA at the time point of sampling. In contrast, similarly high DOC concentrations of all samples from Reserva Campina draining the upland site indicated direct contact between stream and surrounding soils at the time point of sampling (McClain et al., 1997; Zanchi et al., 2015). Local differences in ecohydrology can explain the heavier isotopic composition of groundwater and stream water in the drier upland Reserva Campina (Supporting Information Figure S2; Zanchi et al., 2014; Zanchi et al., 2015), and the lighter isotopic composition of water in the valley and its adjacent plateaus (Leopoldo et al., 1982; Kunert et al., 2017). While isotopically heavy samples like upland P2 (most upslope) and valley PR8/ PR9 (high water levels) likely reflected the imprint of recent precipitation, groundwater replenished during previous wet seasons can explain the lighter water isotopic composition (Leopoldo et al., 1982; Tomasella et al., 2007; Zhang et al., 2009; Miguez-Macho and Fan, 2012; Jasechko and Taylor, 2015; Zanchi et al., 2015; Supporting Information Figure S3). Radiocarbon content differed among sites but indicated very young DOC age. Young radiocarbon ages of tropical DOC are

483 frequently reported from aquatic (Mayorga et al., 2005; Moyer et al., 2013; Ward et al., 2013)
484 and soil systems (James et al., 2019) and are explained by strong linkages between C fixation,
485 DOC release, and nutrient recycling (Mayorga et al., 2005). The slight but consistent differences
486 in DOC radiocarbon ages between the drier upland and the wetter valley system likely relate to
487 processes that govern the short-term (years to decades) turnover of organic matter, for example
488 litter turnover and soil respiration rates (Zanchi et al., 2011; Zanchi et al., 2014; Zanchi et al.,
489 2015).

490 *4.2 DOM properties and fingerprints reflect biogeochemical differences among ecosystems*

491 Molecular analysis of DOM strongly reflected both the major distinction between ecosystem
492 types by water chemistry (plateau vs. WSE-type ecosystems) and the minor distinction between
493 WSE sites by water isotopic composition and ^{14}C age (Figure 3). This allowed us to extract sets
494 of unique markers for each environment (Figure 4, Supporting Information Figure S4). The
495 distinct markers of plateau and WSE settings documented a major texture effect on DOM
496 properties: Fine-textured soils can cause longer water retention and contact times between
497 minerals, microbes, and water (Marques et al., 2004; Remington et al., 2007) and may thus favor
498 overall lower DOC levels due to intensified decomposition (Marques et al., 2010), explaining
499 low DOC concentrations. Clay particles are also often associated with N-containing compounds
500 (Chassé et al., 2015; Newcomb et al., 2017), and newly synthesized, larger and N-containing
501 microbial compounds can also become dominant during decomposition (Roth et al., 2019), thus
502 leading to higher CHNO compound abundances and diversity. Previous reports on narrow C/N
503 ratios of DOC (~10) in plateau soils support these general findings (McClain et al., 1997). In
504 turn, higher DOC concentration and the dominance of mainly CHO type, aromatic, and oxidized
505 WSE markers may thus represent initial stages of decomposition that also agree with reports on

506 wider C/N ratios in WSE sites (>15 , up to 60; McClain et al., 1997). These results demonstrate
507 the overall importance of WSEs for the amount and quality of exported terrestrial DOC in the
508 lower Rio Negro basin.

509 Distinct differences in WSE-DOM composition between sites may reflect ecohydrological
510 differences linked to the frequency of drying/ rewetting events (non-saturated/ saturated
511 conditions), which was also suggested by water isotopic composition and radiocarbon data. We
512 expected that valley WSE-DOM fingerprints would reflect in part the lateral flows from adjacent
513 plateau environments, and the presence of large amounts of indicative N-containing markers
514 with relatively high saturation, low oxidation and low molecular weight supports this assumption
515 (compare Figure 4b,c and e,f). Large differences in DOM composition, however, indicate a loss
516 of the plateau-derived DOM signature upon transit through the valley WSE system before
517 groundwater enters the stream. This finding is remarkable because the riparian zone concentrates
518 the water flux that is sourced from the surrounding plateaus (Miguez-Macho and Fan, 2012a).
519 Simultaneously, this water flux drives the continuous export of young dissolved organic matter
520 from the riparian valleys (Ledesma et al., 2015). However, permanent saturation, i.e., stagnating
521 waters, seem to contribute to the preservation of a wide suite of organic compounds as opposed
522 to the well-drained upland Campina site, and could thus explain the presence of unique, and
523 mainly N-containing, valley WSE-DOM markers. Oxygen depletion in stagnating groundwater
524 limits the turnover of reduced organic matter (Boye et al., 2017). The prevalence of low
525 molecular weight ($m/z < 450$) N- and S-containing molecular formulae with a relatively high
526 degree of saturation ($H/C > 1$) and a low degree of oxygenation ($O/C < 0.6$) could thus also be
527 due to limited DOM uptake caused by unfavorable environmental conditions (low oxygen, low
528 pH, high concentrations of phenolics; Bardy et al., 2011). In contrast, upland WSE-DOM

contained unique, highly oxidized, aromatic, and phenolic CHO formulae (Figure 4g-i). These molecules likely represent the initial decomposition products of plant material containing large portions of lignin, cellulose, tannin, flavonoids, and terpenoids. Under non-stagnating, well-drained conditions, sandy soils favor the escape of such surface signals to streams because of the low sorption capacity of soil (Remington et al., 2007). However, fast export contrasts with older calibrated radiocarbon ages of DOC at the upland WSE (~11 years) as compared to the valley WSE site. This delay can be explained by slower litter turnover and reduced CO₂ efflux rates as discussed above (Zanchi et al., 2011; Zanchi et al., 2014). Highly acidic conditions, periods of drought, and an adapted plant community emerge as main drivers of such ecosystem-level differences. It is of special interest that fungi are known to remain active under dry and acidic conditions (Rousk et al., 2010; Vasco-Palacios et al., 2018). Fungal enzymes can potentially alter DOM towards higher-molecular weight, aromatic, and oxidized structures (Waggoner et al., 2015; Zavarzina et al., 2018), all of which seem to be occurring uniquely in upland WSE soils.

4.3 Upland Campina WSEs are a potential source of indicative Rio Negro markers

We found a clear distinction between potential sources of known sets of Rio Negro markers that could be suitable for further efforts to quantify terrestrial transfer of organic carbon to the regional aquatic continuum and beyond (Figure 5). While the matching with plateau markers was negligible, the two WSE environments clearly differed in terms of matching markers. Independent of the two sets of known Rio Negro DOM markers, a cluster of relatively heavy, highly oxidized, and aromatic upland WSE markers indicated matching (“overlap”) with known Rio Negro markers (Figure 5b, e). Differences in overlap among the both available Rio Negro datasets (Gonsior et al., 2016; Simon et al., 2019) does not diminish the consistent match in terms of chemical space (black boxes in Figure 5b, e). Fine-tuning of measurement conditions

552 can easily affect the instrument response in terms of ion abundance patterns and thus, m/z range
553 (Hawkes et al., 2016; Simon et al., 2018; Hawkes et al., 2020). Despite sample set and lab/
554 instrument effects, measurement settings were relatively similar among the two available studies
555 and ours, thus supporting the general overlap of the upland WSE marker and Rio Negro marker
556 sets in chemical space (oxidation and saturation state; Supporting Information Text S1).
557 Accumulation time of ions before FT-MS analysis is one factor that explains m/z shifts (Hawkes
558 et al., 2016; Simon et al., 2018). However, sampling criteria such as location, season, scale, and
559 coverage will also have a strong influence on the resulting sets of markers. While it is not
560 surprising to see large differences in marker sets (Supporting Information Figure S8 and
561 Supporting Information Figure S9), it is remarkable to find small but consistent overlap between
562 studies, pointing toward subsets of markers with specific oxidation and saturation states. It is
563 known that different instruments capture gradients of variation across a sample set even at very
564 slight compositional differences (Hawkes et al., 2016), as may be expected when samples
565 originate from similar environmental contexts as in our study. It is thus highly encouraging to
566 find consistent and robust molecular overlap in three independent FT-MS datasets, being in line
567 with long-standing hypotheses of landscape functioning in the Rio Negro basin (Leenheer, 1980;
568 Goulding et al., 1988), namely that whitesand ecosystems are potential sources of indicative Rio
569 Negro markers. Counter-intuitively, the highly indicative set of upland WSE markers were not
570 only found to overlap with Rio Negro samples from the proximity of its draining higher-order
571 river, the Rio Tarumã Açu (Simon et al., 2019) but also in samples upstream (Gonsior et al.,
572 2016; compare Figure 1). This observation implies that similar high-molecular-weight oxidized
573 aromatic compounds are exported upstream of Novo Airão, possibly by other upland, Campina-

covered WSE systems. The DOM fingerprints of these other (extensive) WSE systems, especially in the upper Rio Negro basin (Adeney et al., 2016), remain to be revealed.

To identify potentially robust candidate markers, we queried the nine upland whitesand Rio Negro markers that consistently matched with the two independent lists of Rio Negro markers (“Upland overlap”, red squares in Figure 5) in PubChem to obtain potential structure suggestions (Supporting Information Table S3). A query with the ChEBI (chemical entities of biological interest) database yielded only two suggestions that were also found in PubChem. The structural suggestions thus represent only a first indication of potential structures and not necessarily structures that were identified in DOM or organisms. Pubchem yielded up to 108 hits for formula $C_{10}H_6O_6$ and five at minimum for formula $C_{11}H_6O_8$ (numbers after exclusion of twelve ions, one hydrate and six stereoisomers from the list). In general, structures were highly oxidized, showing on average 2.5 carbonyl groups, 0.97 ether bonds, and 0.65 lactone groups per molecule across formulae. All formulae were classified as Black-Carbon like or polyphenol-like (BC, PP). On average, 68% of suggested structures per formula featured at least one heterocyclic ring, with five and six-membered rings dominating (each 30% on average). Functional group count was strongly correlated with mass ($R^2 = 0.84$, range = 5.2 – 9.7), same as numbers of carbon double bonds (0.86, 3.2 – 8.4) aromatic rings per molecule (0.87, 0.6 – 2.6) and thus, DBE (0.85, 8 – 16). Suggestions of smaller molecules (< 270 Da, with less than 15 C atoms) were by tendency dominated by naphthalene and chromene structures that are oxygen-poor scaffolds consisting of two rings. Larger molecules > 320 Da (with >15 C atoms) in contrast showed more hits involving at least one benzofuran, phenol or benzenecarboxylic scaffold, which all show a higher degree of oxidation and functionalization. Chromenes and benzofurans are well known photo- and bioactive plant metabolites (Towers and Hudson, 1987). Knowledge of these trends and

597 tendencies in molecular properties may help to develop tailored chromatographic techniques
598 (LC-MS) for the targeted analysis of these biological relevant ecosystem markers in future
599 (Petras et al., 2017). Structural data used for the above analyses is openly available from
600 <https://doi.org/10.1594/PANGAEA.922606> (Supporting Information Data Set S1, Supporting
601 Information Data Set S3).

602 Groundwater DOM markers revealed a potential direct link between ecosystem-specific
603 headwater and ecosystem-integrated downstream signals in the Rio Negro basin. The consistent
604 overlap of upland WSE and Rio Negro markers was unexpected because our initial hypothesis,
605 namely that typical valley WSE's will show stronger, or at least similar, matching with Rio
606 Negro markers, had to be refused. Highly specific, tannin-like aromatic DOM compounds of the
607 smaller upland WSE watershed represent a potential molecular link between headwaters and the
608 Rio Negro. This finding opposes the assumed role of riparian valley WSEs as main sources of
609 DOM in the Rio Negro watershed (McClain et al., 1997; Bardy et al., 2011). Although Bardy
610 and coworkers showed that upland Campina forests areas produce a marked DOM signal
611 traceable to rivers, others found that riparian and wetland WSEs along rivers contributed mainly
612 to DOC exports of Amazonian and other watersheds (Dosskey and Bertsch, 1994; McClain et al.,
613 1997; Remington et al., 2007). Our results suggest that there is more detail to add to this simple
614 model. Small watersheds with strong terrestrial-lotic linkages may leave a more significant
615 downstream imprint as expected from absolute annual discharge, areal extent, or DOC export.
616 Our results show that highly specific forest ecosystems potentially leave a distinct imprint within
617 the Rio Negro's exported DOM, although contributing only secondarily to the overall fluxes of
618 water, and maybe, DOC. The smaller upland WSE watershed at Reserva Campina shows a two
619 times lower average annual stream discharge (based on the catchment area) but a two-three times

higher annual DOC export (Monteiro et al., 2014; Zanchi et al., 2015). This discrepancy demonstrates that water and carbon cycles may be decoupled on the molecular level and the watershed scale, despite the generally accepted transport-limited, or chemostatic, DOC behavior (Musolff et al., 2017; Zarnetske et al., 2018). Ecosystems may show different behavior in terms of discharge and DOC export (Webb et al., 2018), or release of ecosystem markers (Wagner et al., 2019). Taking into account that hydromorphic soils in this region are thought to cover more than 40% of area (Junk, 1993), their missing imprint is evidence for the importance of unique ecosystems in control of indicative DOM characteristics within the land-to-ocean continuum. Riparian valley corridors likely contribute the major part of the annual DOC export in this region due to constant water supply from adjacent plateaus and their wider spatial distribution (Remington et al., 2007; Miguez-Macho and Fan, 2012a; Miguez-Macho and Fan, 2012b), but do only contribute general DOM markers that likely reflect processes operating in many watersheds. In conclusion, our results indicate that riparian valleys may contribute less to the specific signal that discerns the Rio Negro from other watersheds on the molecular level. These specific markers show the potential for qualitative ecosystem recognition further downstream, and may thus serve as new proxies of land-derived primary production in the Rio Negro basin if properly calibrated.

5 Conclusion

This study investigated potential molecular links between indicative DOM markers at two main stages of the land-to-ocean-continuum, headwater catchments and river basins. New sets of markers are needed to better constrain variations in land-derived DOM exports and losses within aquatic systems, especially in the tropics, which account for 62% of riverine DOC exports. We provide molecular evidence of long-assumed hypothetical links drawn from the simultaneous

643 occurrence of tropical whitesand ecosystems (WSEs) and blackwater rivers at the example of the
644 Rio Negro basin (Goulding et al., 1988, and references therein). Unique whitesand ecosystems
645 contribute mainly to the fast export of DOC, while it is retained and decomposed in the highly
646 productive, widespread plateau systems. Regardless of the wide occurrence of whitesand
647 ecosystems and the known variation in WSE characteristics across the Amazon, there is little
648 appreciation of this knowledge in models of DOC export and information content. This gap is
649 due to a lack of qualitative DOC data. By using FT-MS techniques, we provide unique markers
650 of whitesand ecosystems that can serve as future constraints on the terrestrial portion of aquatic
651 DOC export. However, for such applications further calibration studies are necessary.

652 Against initial expectation, large valley WSEs may contribute only little to the flux of soluble
653 ecosystem markers found at the river basin scale. In contrast, highly specific upland Campina
654 WSEs emerged as their potential source. The twofold larger annual discharge and hundred-fold
655 larger size of the Cuieiras watershed seemingly do not result in the transfer of DOM markers
656 downstream, as expected from conservative (chemostatic) DOC leaching of dominant source
657 layers. Instead, estimates suggest that the two-three times higher annual DOC export of the
658 smaller upland watershed leaves a stronger imprint in terms of traceable WSE markers. This
659 discrepancy stresses the importance of specific ecosystems for DOM information content at
660 higher-order stages of the aquatic continuum, and how it may inform hydrological models that
661 include qualitative DOC data. Future research needs to assess the influence of the large
662 bandwidth of WSE types across the Rio Negro basin, and relate their environmental
663 characteristics (climate, soil, vegetation) and geographical extent with information on quantity
664 (DOC) and quality (molecular composition) of the exported DOM.

665 The molecular composition of DOM at different stages of the aquatic continuum emerges as a
666 qualitative measure of DOM exports that complements quantitative DOC data. Our study shows
667 the gained information that can be drawn from comparative FT-MS studies when complemented
668 with other types of ecosystem information, especially information on the isotopic composition of
669 water and organic carbon. Integration and nesting of DOM data show great prospects to bridge
670 traditional gaps between soil science, limnology, and hydrology. The interconnected nature and
671 multivariate complexity of DOM shows high potential to allow studying multiple source
672 contributions and processes simultaneously. However, improvements in data integration across
673 FT-MS platforms are required to reveal this information properly, and to reach robust
674 conclusions about sources, fate, and identity of new sets of ecosystem markers in DOM. For this
675 it will also be central to calibrate novel ecosystem markers with environmental variables such as
676 DOC export, discharge, or ecosystem productivity.

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691 **Declaration of potential conflicts of interest**

692 The authors declare that the research was conducted in the absence of any commercial or
693 financial relationships that could be construed as a potential conflict of interest.

694 **Data availability**

695 All data necessary to reproduce the findings reported in this manuscript are available online free
696 of charge via <https://doi.org/10.1594/PANGAEA.922606>.

697 **Supplementary Material**

698 29 pages, containing 9 Tables, 17 Figures and 17 References.

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Captions

Figure 1. Detail of the lower Rio Negro catchment northwest of Manaus showing sampling sites of available FT-MS studies. We sampled at two locations north of Manaus (green dots; “Cuieiras”, “Campina”). Two other FT-MS datasets from blackwater sampling locations are shown, including the Rio Negro and connected lakes (black dots; Gonsior et al., 2016) and the lower reach of the Rio Negro and two of its tributaries close to Manaus (red dots; Simon et al., 2019). We accessed watershed limits and river data as shapefiles from www.ore-hybam.org (Seyler et al., 2009). The headwater stream width is not drawn to scale. Roads and main water bodies were extracted from the OpenStreetMap project (OSM; natural features and roads) and downloaded as shapefiles from www.download.geofabrik.de. Map editor: Marcus Guderle, MPI Jena.

Figure 2. Differences in the water and SPE-DOM properties. Variables shown are a) pH, b) electrical conductivity, c) dissolved organic carbon concentration, d) difference in $\delta^{18}\text{O}$ values of water compared to regional average precipitation (OIPC estimate), e) mean calendar age of DOC in solid-phase extracts. Samples are grouped into biogeochemical environments (upland Campina; riparian valley, and plateau). Symbols denote sample type (squares, piezometer; star, deep well; circle, stream); open symbols mark “intermediate” valley samples (see main text).

Figure 3. Multivariate analysis based on molecular DOM data. a) Separation of samples in a principal coordinate analysis (PCoA) based on Bray Curtis dissimilarity. The plot shows only the first two coordinates, the third coordinate (5% of explained variability, not shown) did not contribute further to separation. Percentages denote the degree of explained variability in DOM molecular composition. Samples are grouped into biogeochemical environments (“Upland”,

1056 “Valley”, “Plateau”). Symbols denote sample type (square, piezometer; star, deep well; circle,
 1057 stream); open symbols mark “intermediate” valley samples (see main text). b - d) Post-ordination
 1058 gradient fit (function envfit of R package vegan, at 999 permutations) of three different sets of
 1059 variables based on PCoA separation (same as in a). Variables sets are b) DOM indices, c)
 1060 Molecular groups, and d) Formula classes. Significant correlations (Pearson, $p < 0.05$) with the
 1061 ordination are shown as arrows. Arrow length corresponds to the strength of correlation and
 1062 arrows head into the direction of the steepest increase of the respective variable, based on the
 1063 ordination pattern of samples. Variable abbreviations in b): C, H, O, N, S (average numbers of
 1064 respective atoms per formula), MW (molecular weight as mass to charge-ratio), AImod
 1065 (Aromaticity index), DBE, DBEmO, DBE.C (double bond equivalents, DBE minus oxygen,
 1066 DBE/C ratio), H/C (atomic ratio of hydrogen to oxygen, “saturation axis”), O/C (atomic ratio of
 1067 oxygen to carbon, “oxidation axis”), NOSC (nominal oxidation state of carbons), Np, Np%, Npi
 1068 % (number, percentage and relative abundance of N-containing peaks), Sp, Sp%, Spi% (same for
 1069 S-containing peaks). Abbreviations in c): BC (Polycyclic, condensed aromatics, such as “Black
 1070 Carbon”), PP (polyphenols), HU (highly unsaturated), PEP (unsaturated, O- and N-containing,
 1071 such as peptides), UA (unsaturated aliphatics), SUG (very high O content, such as sugars). Prefix
 1072 to PP, HU & UA: op ($O/C \leq 0.5$); or ($O/C > 0.5$). Prefix to BC and PP: lw (< 15 C atoms), hw (\geq
 1073 15 C atoms). Abbreviations in d): CHO (average number of molecular formulae containing only
 1074 C, H and O atoms), CHNO, CHN₂O, CHOS, CHNOS (formulae containing one N, two N atoms,
 1075 one S atom, or both one N and S atom).
 1076 Figure 4. Subsets of molecular formulae showing significant “enrichment” (higher ion
 1077 abundance), i.e., ecosystem specificity, in the plateau (panels a-c), valley WSE (d-f) and upland
 1078 WSE samples (g-i). General WSE (valley and upland) markers and non-significant signals

1079 (common to all samples) are shown in Supporting Information Figure S4. Left column panels (a,
1080 d, g) show the average (and max) mass spectrum of each ecosystem. Mid column panels (b, e, h)
1081 show the formula subsets in Van Krevelen space (each dot represents a molecular formula
1082 defined by its atomic ratios of hydrogen, H/C, and oxygen to carbon, O/C). Formulae are colored
1083 according to classes (see legend and numbers of formulae in panels to the right). The plot is
1084 divided by solid lines that mark molecular group categories (see also caption of Figure 3, and
1085 Supporting Information Table S2). Right column panels (c, f, i) show the same formula subsets
1086 from mid-row panels in H/C vs. m/z (mass-to-charge) space. Dotted grey lines are for visual
1087 guidance and comparison (at m/z = 450, O/C = 0.5, and H/C = 1). Left- and right-column plots
1088 share the m/z abscissa (x-axis) while mid- and right-column plots share the H/C ordinate (y-
1089 axis).

1090 Figure 5. Overlap of WSE-specific formulae (significantly enriched formulae) with two
1091 independent sets of Rio Negro DOM markers (a-c: data from Gonsior et al., 2016; d-f: data from
1092 Simon et al., 2019). Left panels (a, d) show the overlap of three sets of markers (Rio Negro,
1093 Valley, Upland) in a Venn diagram created with package “eulerr” in RStudio. Overlap indicates
1094 common formulae, i.e., shared information. Areas are scaled to number of formulae, which are
1095 given for each subset (note color, area may be dissected). Overlap is shown only for CHO
1096 formulae because Rio Negro markers were mainly CHO-type, other formula classes showed no
1097 distinct overlap. Right panels show Rio Negro markers of each study (grey filled squares) in
1098 chemical space as a Van Krevelen plot (b, e) or H/C vs. m/z plot (c, f; similar visualization as in
1099 Figure 4). Colored symbols show the match between datasets (“overlap”) and refer to the bold
1100 ellipse in panels a and d, respectively. Plateau markers are additionally added for comparison.
1101 Red squares denote the small set of WSE markers that were found to match with both sets of Rio

1102 Negro markers. They were uniquely enriched in upland ($n = 6$) or common to upland and valley
1103 WSE's ($n = 3$). Based on the chemistry of these nine formulae, the black box denotes the wider
1104 area of consistent matching in terms of chemical space (H/C 0.4 – 0.8; O/C 0.4 – 0.8).
1105 Independent of the underlying set of Rio Negro markers, the matching rate was higher for upland
1106 WSE markers (lilac).