



HAL
open science

Estimating the Acid–Base Properties and Electrical Charge of Organic Matter Using Spectrophotometry

Marawit Tesfa, Aline Dia, Fabrice Mahé, Noémie Janot, Rémi Marsac

► **To cite this version:**

Marawit Tesfa, Aline Dia, Fabrice Mahé, Noémie Janot, Rémi Marsac. Estimating the Acid–Base Properties and Electrical Charge of Organic Matter Using Spectrophotometry. *Environmental Science and Technology*, 2023, 57 (32), pp.12053-12062. 10.1021/acs.est.3c04965 . insu-04182319

HAL Id: insu-04182319

<https://insu.hal.science/insu-04182319>

Submitted on 28 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Estimating the acid-base properties and electrical charge of organic matter using spectrophotometry

Marawit TESFA^{1*}, Aline DIA¹, Fabrice MAHE², Noémie JANOT³, Rémi MARSAC^{1*}

¹ Univ. Rennes, CNRS, Géosciences Rennes - UMR 6118, F-35000, Rennes, France

² Univ. Rennes, CNRS, IRMAR - UMR 6625, F-35000 Rennes, France

³ INRAE Bordeaux Sciences Agro, ISPA - UMR 1391 - 33882 Villenave d'Ornon, France

*Corresponding authors: marawittesfa.research@yahoo.com and remi.marsac@cnrs.fr

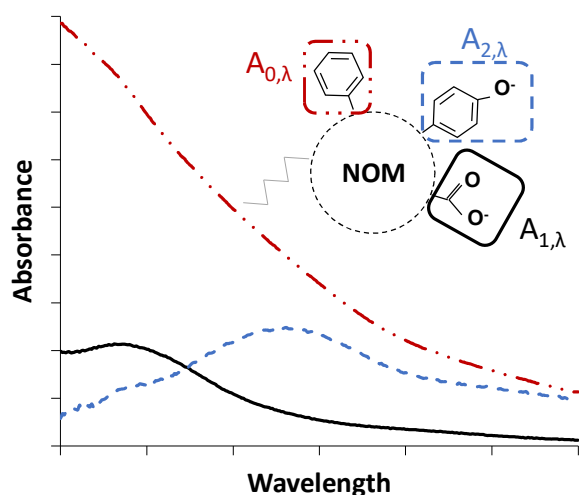
Abstract

Spectrophotometric acid-base titration is a simple and powerful technique to evaluate the properties of proton binding sites of natural organic matter (NOM) at environmentally relevant concentrations. However, it is challenging to quantify the chemical charges (Q) carried by NOM at these concentrations. Based on a previous work, which relates the variation of Q with the specific UV-vis differential absorbance ($\Delta A_{\lambda, \text{pH}}$) at a given wavelength (λ) and pH of a dissolved NOM sample, the present work proposes a method to investigate any NOM sample. It determines specific features in the absorbance spectra attributed to proton-inert chromophores ($A_{0, \lambda}$), and to the deprotonation processes of carboxylic ($A_{1, \lambda}$) and phenolic groups ($A_{2, \lambda}$). It enables to select sample-specific wavelength (λ_{mid}), where both these functional groups significantly contribute to the variation of absorbance with pH. The linear regression analysis of $A_{\lambda_{\text{mid}}, \text{pH}}$ vs. Q for various NOM reference samples evidenced that the sample-specific slope (S_{NOM}) and intercept (I_{NOM}) were related to the intrinsic spectroscopic properties of the sample ($A_{0, \lambda_{\text{mid}}}$, $A_{1, \lambda_{\text{mid}}}$ and $A_{2, \lambda_{\text{mid}}}$). This approach can thus be used to approximate the Q values of the NOM samples at environmentally relevant concentrations: a pre-requisite for predicting the fate and behavior of metal ions in natural systems.

Synopsis. This article provides an empirical approach to approximate the charge of natural organic matter at environmentally relevant concentrations using a novel deconvolution method for absorbance spectra.

Keywords: Natural organic matter, acid-base functional groups, proton titration, absorbance spectra

32 Graphical abstract



33
34

35 Introduction

36

37 Natural organic matter (NOM) is a ubiquitous component in the Critical Zone that profoundly
38 influences the biogeochemical cycles of various contaminants and nutrients, by exerting a
39 strong control on their transport, speciation, (bio)availability and eventually biotoxicity.¹⁻³

40 Natural organic matter is known as a major trace cation vector in the environment, as it can
41 bind significant amounts of diverse dissolved metal ions.⁴⁻⁹ The interaction of NOM with
42 natural and anthropogenic nanoparticles, either organic or inorganic, often increases their
43 colloidal stability,¹⁰⁻¹³ therefore enhancing their mobility. Conversely, the binding of NOM to
44 mineral surfaces may affect its chemical stability, reactivity and transport and hence, the
45 biogeochemical cycle of carbon in soils and aquatic systems, as well as that of associated
46 elements and contaminants.¹³⁻¹⁹

47 The common link between all the above-listed mechanisms is the involvement of NOM
48 functional groups exhibiting acid-base properties.²⁰⁻²⁴ These functional groups are generally
49 divided into carboxylic and minor groups often referred to as phenolic groups, which can form
50 complexes with metal ions, in solution or at the (nano)particles' surfaces.^{19,20,23,25} Therefore, it
51 is essential to accurately determine the concentration of the NOM acid-base functional groups
52 in natural systems in order to predict the behavior and fate of contaminants with geochemical
53 speciation modeling.^{18,21,22,25,26} However, because the carboxylic and phenolic site densities
54 vary broadly with the origin of the NOM, this crucial model input data is rarely available. As a
55 panacea, the generic values proposed by Milne et al.²⁷ are commonly used,^{9,28-30} which might
56 have detrimental consequences on the performance of predictive geochemical models. The most
57 accurate approach to quantify NOM functional groups involves potentiometric acid-base

58 titration. By direct measurement of the electrical charge carried by humic substances, this
59 titration enables the determination of NOM proton-binding site concentrations, along with their
60 protonation constant and their chemical heterogeneity.^{4,23,24,27} Unfortunately, although highly
61 accurate, potentiometric titration cannot be applied to environmental samples, given that the
62 NOM concentrations rarely exceed 100 milligrams of dissolved organic carbon per liter (mgC
63 L⁻¹): such concentrations are too low for optimal electrode measurements requiring several
64 hundreds of mgC L⁻¹.

65 More recently, the NOM acid-base properties were determined using UV-spectrophotometry, a
66 method requiring lower NOM concentrations similar to natural conditions.³¹⁻³⁵ In addition, this
67 approach is a simple, rapid and low-cost analysis of the NOM acid-base properties. It is based
68 on the observation that the UV-vis absorption of a dissolved NOM sample increases with
69 increasing pH. Differential absorbance ($\Delta A_{\lambda,pH}$) spectra are calculated by the subtraction of a
70 reference absorbance value ($A_{\lambda,pH_{ref}}$), usually recorded at low pH (e.g. $pH_{ref} = 3$), from the
71 absorbance value measured for the same sample at higher pH:

$$\Delta A_{\lambda,pH} = A_{\lambda,pH} - A_{\lambda,pH_{ref}} \quad (1)$$

72
73
74
75 This operation aims to remove the typical featureless absorbance spectra of NOM
76 chromophores exhibiting no acid-base properties and, hence, to identify the small variations in
77 absorbance related to the deprotonation of the NOM functional groups of interest.^{31,32,36-38} The
78 differential absorbance spectra of NOM generally show two peaks: a first peak, with a
79 maximum at ~270 nm for which the intensity notably increases between pH 3 and 6 and a
80 second one, with a maximum at 320-380 nm, which appears at pH values above 6. On the basis
81 of their acid-base properties, these peaks can be attributed to the deprotonation processes of the
82 carboxylic and phenolic groups, respectively. By optimizing the parameters of a modified
83 NICA-Donnan model in order to fit the values of $\Delta A_{\lambda,pH}$, it then becomes possible to determine
84 the protonation constants (pK_{a1} and pK_{a2}) of the NOM carboxylic (1) and phenolic (2) groups
85 and their heterogeneity parameters (m_1 and m_2), as well as the specific contribution of the
86 deprotonation process of these groups to the variation of the absorbance ($A_{1,\lambda}$ and $A_{2,\lambda}$).^{31,32,35}
87 The choice of wavelength selected is not very important, however it might be preferable to
88 select the value of λ corresponding to the maximum differential absorbance.^{32,39}
89 Although the currently used UV-spectrophotometric acid-base titration approaches provide
90 reliable information on the reactivity of the NOM acid-base functional groups, they do not
91 directly provide charge values from which the concentrations of the carboxylic and phenolic

92 groups can be estimated. Therefore, other techniques must also be used. To obtain this
93 information, Janot et al.³² combined potentiometric and spectrophotometric titration of purified
94 Aldrich humic acid (PAHA) to establish a linear relationship between $\Delta A_{\lambda,pH}$ and the variation
95 of charge (ΔQ). This allowed to investigate the impact of PAHA adsorption and fractionation
96 at the alumina surface on the PAHA acid-base properties.³⁹ Unfortunately, before now, this
97 relationship has not been tested on different NOM samples, which differ from PAHA in their
98 chemical properties, and thus, might not yet be applicable in environmental conditions.
99 In the present study, we propose to expand the investigation of the relationship between $\Delta A_{\lambda,pH}$
100 and ΔQ previously observed by Janot et al.³² to other NOM samples with various origins and
101 chemical properties. This work attempts to generalize a relationship between $A_{\lambda,pH}$ and Q in
102 order to fully estimate the proton-binding properties of NOM using spectrophotometric data,
103 avoiding the often-impossible collection of potentiometric data. To do so, in this work, recent
104 potentiometric acid-base titration results obtained for seven of the samples provided by the
105 International Humic Substances Society (IHSS) were used.⁴⁰ In addition, a novel data treatment
106 of spectrophotometric titrations is proposed to (i) directly deconvolute absorbance $A_{\lambda,pH}$ instead
107 of the differential absorbance $\Delta A_{\lambda,pH}$, to limit potential biases linked to NOM aggregation which
108 might occur at low pH_{ref} ,^{37,41} and to (ii) take advantage of the complete absorbance dataset
109 collected at λ ranging from 200 to 800 nm or to select the most relevant λ value to derive the
110 NOM-proton binding parameters and NOM charge from the absorbance data.

111

112 Materials & Methods

113

114 **Reagents.** All NOM samples used in this study were purchased from the IHSS. Along with
115 their IHSS reference code, there are three samples from Suwannee River: one fulvic acid (FA),
116 one humic acid (HA) and one natural organic matter (NOM) named SRFA (3S101F), SRHA
117 (3S101H) and SRNOM (2R101N), respectively; two samples from Pahokee Peat (PPFA
118 (2S103F) and PPHA (1S103H)); and two HA soil samples: one from Leonardite (LHA,
119 1S104H) and one from Elliot Soil (ESHA, 4S102H). Data for SRFA were taken from our
120 previous study.³⁴ Reagents such as HCl, NaOH, NaCl, and the pH-buffers
121 tris(hydroxymethyl)aminomethane (TRIS), 3-(N-morpholino)propanesulfonic acid (MOPS), 2-
122 (N-morpholino)ethanesulfonic acid (MES) and acetic acid (AA) (see Appendix A, Table S1, in
123 the Supplementary Information (SI)) were purchased from Sigma Aldrich. All samples were
124 prepared with ultrapure water provided through a Milli-Q purification system.

125

126 **Sample preparation.** A 250 mL NOM mother solution (1 g L^{-1}) was prepared by dissolving
127 25 mg of NOM powder using NaOH (0.1 mol L^{-1}) in ultrapure water. Complete dissolution was
128 ensured by maintaining the pH of the NOM solution at around 9 upon the addition of NaOH.
129 All pH measurements were performed with a HANNA, HI5221 pH meter. The pH of the NOM
130 mother solution was monitored for 24 hours, with adjustments performed at 3, 6 and 12 hours
131 after the start of the NOM dissolution process. The NOM mother solution was left to mix on a
132 shaking table in-between these pH adjustments. Subsequently, the dissolved organic carbon
133 concentration (DOC) was determined through a Shimadzu TOC-L analyzer. The NOM samples
134 destined to be analyzed by spectrophotometric acid-base titration were prepared by diluting the
135 mother solution to a carbon concentration of 25 mgC L^{-1} , and by adjusting their ionic strength
136 (IS) to 0.01 mol L^{-1} , with NaCl.

137 The preparation of the buffer solutions followed the protocol described in a previous article.³⁴
138 Briefly, buffer salt was dispersed in a total of 50 mL volume of ultrapure water at final
139 concentration of 1 mol L^{-1} . The pH of the buffer solution was adjusted to match the pKa value
140 of the buffer salt, or values within $\text{pKa} - 1 < \text{pH} < \text{pKa} + 1$ with acid (HCl, $1, 0.1 \text{ mol L}^{-1}$) or
141 base (NaOH, $1, 0.1 \text{ mol L}^{-1}$) addition. A total of 11 buffer solutions, with pH values ranging
142 from 3.8 to 9, were prepared with four initial buffer salts TRIS, MOPS, MES and AA (Appendix
143 A, Table S1 in the SI).

144

145 **Spectrophotometric acid-base titration.** The spectrophotometric acid-base titration
146 was also carried out as described in a previous study.³⁴ Each of the studied NOM samples was
147 divided into 13 aliquots of 4 mL (25 mgC L^{-1} and 0.01 mol L^{-1} NaCl). The pH of the 11 aliquots
148 of the NOM solution, ranging from pH 3.8 to 9, was fixed using the corresponding buffer, with
149 a final buffer concentration of $10^{-3} \text{ mol L}^{-1}$. The pH of the two remaining aliquots was adjusted
150 to pH 3 and 3.4 using HCl. The pH was monitored for 15 minutes to ensure it was stable before
151 proceeding to the absorbance measurement. Subsequently, the raw UV-vis absorbance of each
152 aliquot was recorded for wavelengths (λ) between 200-800 nm, using a UV-vis Shimadzu
153 UV2600 spectrophotometer and a 1-cm-wide quartz cuvette. In this study, the raw absorbance
154 ($A_{\lambda,\text{pH,raw}}$) in this study was normalized by the DOC data and by the optical pathlength, in order
155 to determine the specific UV absorbance spectra, as detailed in the following equation (Eq. 2)

156

157

$$A_{\lambda,\text{pH}} = \frac{1}{l_{\text{cell}}} \times \frac{A_{\lambda,\text{pH,raw}}}{\text{DOC}} \quad (2)$$

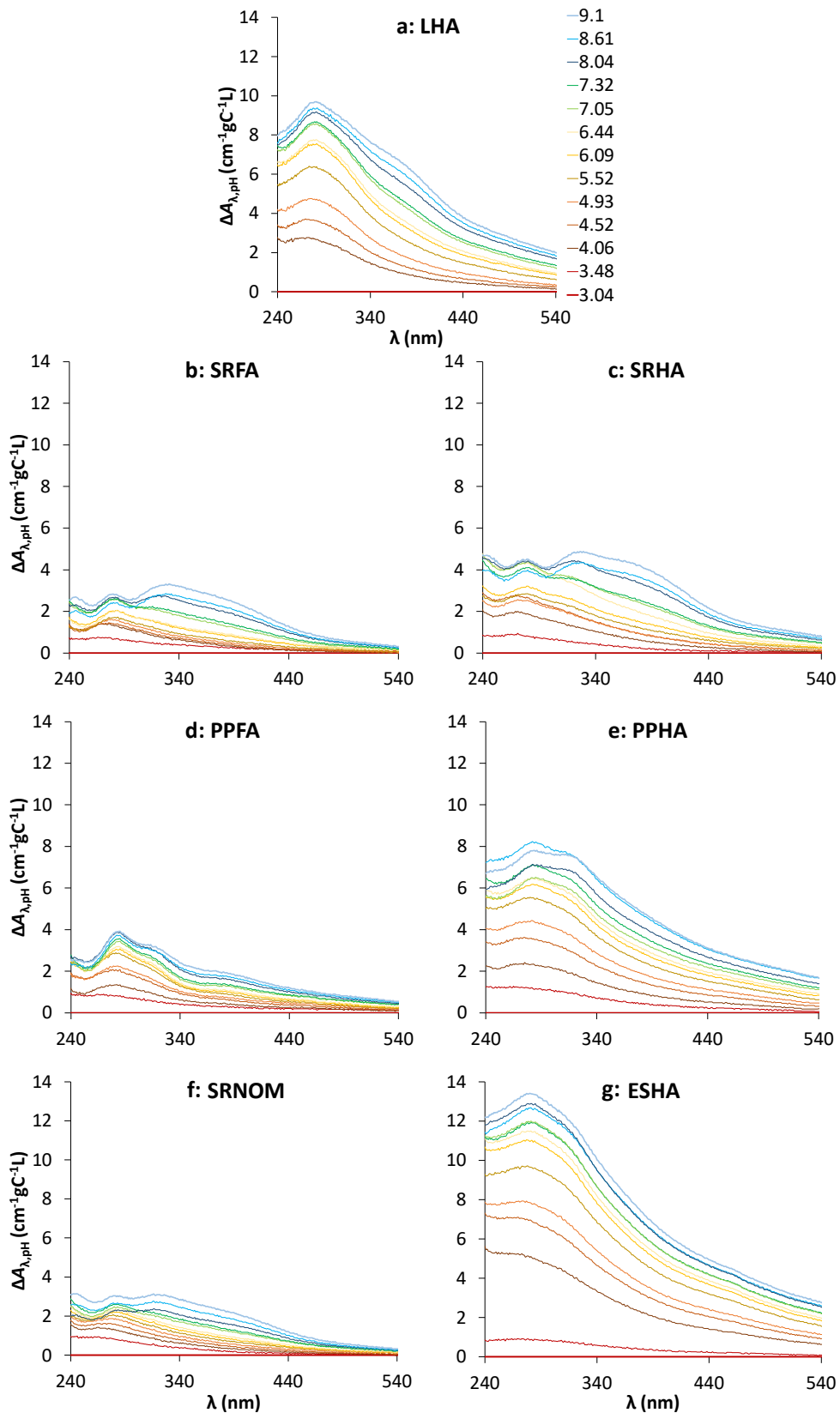
158 The specific UV absorbance spectra, here denoted “ $A_{\lambda,\text{pH}}$ ” (in $\text{cm}^{-1} \text{gC}^{-1} \text{L}$) for simplicity, is
159 commonly referred to as “SUVA” in the literature. The specific absorbance spectra, above 540
160 nm, contain little information and, as demonstrated in a previous study,³⁴ the absorbance of the
161 pure buffer solutions is limited to $\lambda < 240$ nm. Consequently, all data treatments were conducted
162 within a λ range of 240 nm – 540 nm.

163

164 Results & Discussion

165

166 **Spectrophotometric titration results.** The specific absorbance spectra (A vs. λ) were
167 measured at different pH values for each IHSS sample (Appendix B, Figure S1 in the SI). The
168 resulting spectra are typical of NOM, i.e., featureless decreasing specific absorbance spectra
169 with increasing wavelength.^{31,32,36,37} The specific absorbance also increases with increasing pH.
170 This effect can be observed by inspecting the variation of the specific absorbance spectra at a
171 given wavelength ($\Delta A_{\lambda,\text{pH}}$) as a function of pH with respect to a reference spectrum.^{31,32,37} The
172 corresponding $\Delta A_{\lambda,\text{pH}}$ values, calculated using equation 1 and the specific absorbance spectrum
173 recorded at $\text{pH} = 3$ as a reference, are plotted in Figure 1 for the seven IHSS samples
174 investigated. The present data are in excellent agreement with previous acid-base
175 spectrophotometric titrations of the IHSS samples.^{31–33} All samples display two characteristic
176 peaks of the deprotonation process of the carboxylic (with a maximal absorbance at ~ 270 nm)
177 and phenolic groups (with a maximal absorbance at ~ 350 nm), the intensity of which increases
178 with increasing pH over two distinct pH-ranges. Note that, if each type of acid-base functional
179 groups exhibits a maximal absorbance values, they can absorb photons on large λ -ranges
180 because a large diversity of chromophores is included in each group. The intensity of the peak
181 at 270 nm increases between approximately pH 3 and 7, whereas the peak at 350 nm only
182 appears at pH values above 6. The variation range of $\Delta A_{\lambda,\text{pH}}$ as a function of pH is different
183 depending on the sample. For instance, compared to humic samples (SRFA vs. SRHA and
184 PPFA vs. PPFA), fulvic samples seem to have lower variation in the absorbance range.
185 Additionally, the specific absorbance intensity of the carboxylic and phenolic deprotonation
186 processes varies depending on the origin of the sample. The samples from Suwannee River
187 (SRFA, SRHA and SRNOM) display a more intense $\Delta A_{\lambda,\text{pH}}$ peak around 350 nm compared to
188 the peak at 270 nm. This trend is inverted in the other samples where the 270 nm peak is always
189 more intense than the peak at ~ 350 nm. For this reason, it is crucial to consider both carboxylic
190 and phenolic deprotonation processes in the procedure for specific absorbance deconvolution.



191

192 **Figure 1.** Differential specific absorbance spectra ($\Delta A_{\lambda,pH}$ in $\text{cm}^{-1}\text{gC}^{-1}\text{L}$) of (a) LHA, (b) SRFA³⁴, (c)
 193 SRHA, (d) PPFA, (e) PPHA, (f) SRNOM and (g) ESHA derived from the absolute specific
 194 absorbance spectra ($A_{\lambda,pH}$). The spectra were recorded at different pH values, from 3.04 to 9.10 (the
 195 same colors are used in all figures).
 196

197 **Deconvolution of absolute specific absorbance spectra.** The approach described
 198 above outlines the methods used so far in previous studies to characterize NOM through UV-
 199 vis spectroscopy.^{31–33} This approach might present a potential bias related to NOM aggregation
 200 at low pH,^{37,41} which may arise when using the data collected at pH 3 as a reference for the
 201 calculation of $\Delta A_{\lambda,pH}$. In addition, the subtraction process removes part of the information
 202 contained in the data because, even at pH 3, a significant fraction of the carboxylic groups might
 203 be deprotonated.^{24,27,40} Lastly, when further interpreting the data, the focus on a single
 204 wavelength discards the information throughout the rest of the data at different λ . As an
 205 alternative approach used in the present work, the deconvolution of the specific absorbance
 206 spectra was inspired from previous studies but carried out on the specific absorbance spectra
 207 throughout the entire wavelength range (240 nm – 540 nm). This operation was conducted using
 208 the Matlab™ software, in which a model (Eq. 3) describing the specific absorbance spectra has
 209 been implemented:

210

$$211 \quad A_{\lambda,pH} = A_{0,\lambda} + A_{1,\lambda} \times f_{1,pH} + A_{2,\lambda} \times f_{2,pH} \quad (3)$$

212

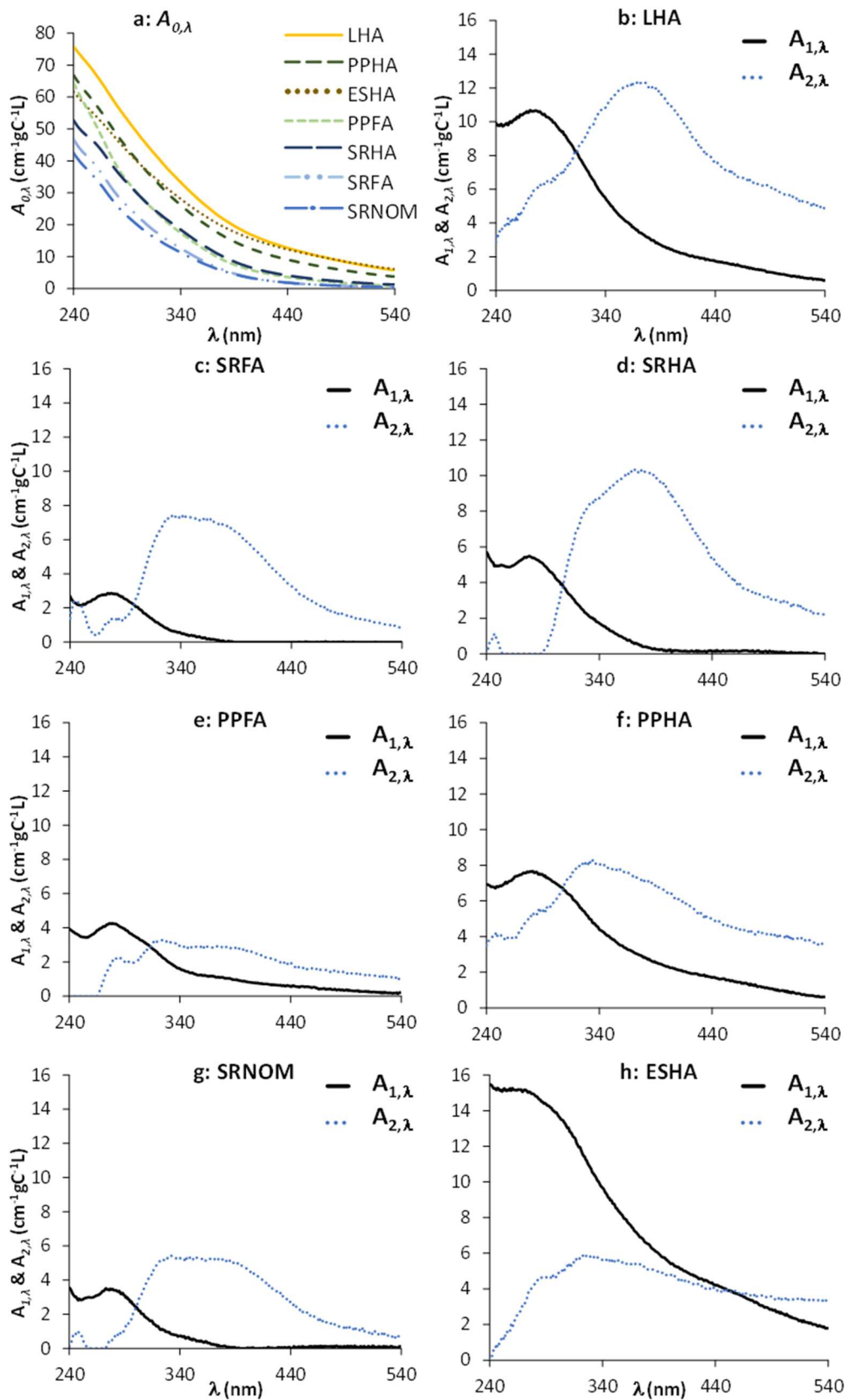
213 The mathematical development leading to equation 3 is given in Appendix C in the SI. With
 214 equation 3, we hypothesize that the experimentally measured specific absorbance ($A_{\lambda,pH}$) is a
 215 linear combination of three components ($A_{0,\lambda}$, $A_{1,\lambda}$ and $A_{2,\lambda}$), two of which ($A_{1,\lambda}$ and $A_{2,\lambda}$) are
 216 associated with their pH-dependent coefficients ($f_{i,pH}$). The component $A_{0,\lambda}$ mainly represents
 217 the proton-inert chromophores within the investigated pH range. According to the calculations,
 218 $A_{0,\lambda}$ also includes the specific absorbance of the proton-reactive chromophores under their
 219 protonated form (Appendix C, Eq. S5 in the SI). The $A_{1,\lambda}$ and $A_{2,\lambda}$ components are attributed to
 220 the deprotonation process of the carboxylic and phenolic groups, respectively. Thus, both $A_{1,\lambda}$
 221 and $A_{2,\lambda}$ represent the difference in specific absorbance between the deprotonated and
 222 protonated species of the acid-base functional groups. The coefficients $f_{1,pH}$ and $f_{2,pH}$ are the
 223 fractions of deprotonated chromophores at the corresponding pH. Consequently, $A_{0,\lambda}$ is not
 224 associated with a pH-dependent coefficient $f_{i,pH}$. All of the parameters ($A_{0,\lambda}$, $A_{1,\lambda}$, $f_{1,pH}$, $A_{2,\lambda}$, $f_{2,pH}$)
 225 were optimized to best fit the experimental $A_{\lambda,pH}$ values using an iterative optimization method.
 226 However, the optimization problem used to estimate the parameters of Eq.3 has multiple
 227 solutions but a reasonable *a priori* estimation of the values of the parameters could be obtained
 228 on the basis of chemical constraints. The local convergence of the optimization algorithm was
 229 used to improve these starting values. The values of $f_{1,pH}$ and $f_{2,pH}$ were constrained to (i) vary

230 from 0 to 1 (as they represent fractions) and to (ii) increase with rising pH. These constraints
 231 facilitated the convergence of $f_{1,pH}$ and $f_{2,pH}$ to chemically realistic values. A detailed
 232 explanation of the optimization process, including the choice of starting values, is given in the
 233 SI (Appendix D in the SI). Figure 2 shows the results of the deconvolution process for all IHSS
 234 samples. Based on the $\Delta A_{\lambda,pH}$ calculations carried out in the previous section, the variation of
 235 specific absorbance related to the increase in pH (i.e. the deprotonation process) represents less
 236 than 1% of the total variation found in the $A_{\lambda,pH}$ vs. λ curve. As a consequence, the $A_{0,\lambda}$ for all
 237 seven IHSS samples (Figure 2a) is similar to their own raw specific absorbance spectra
 238 (Appendix B, Figure S1 in SI). The characteristic components of the carboxylic and phenolic
 239 groups ($A_{1,\lambda}$ and $A_{2,\lambda}$) display a peak shape with a maximum at $\lambda \approx 270$ nm and λ ranging from
 240 320 to 370 nm, in agreement with previous investigations involving a differential absorbance
 241 analysis.^{31,33} The fractions of deprotonated carboxylic ($f_{1,pH}$) and phenolic ($f_{2,pH}$) groups as a
 242 function of pH for all samples are depicted in Figure S2 (Appendix E in the SI). Using the
 243 Langmuir-Freundlich equation (Eq. 4) and a least-square fitting procedure, the $f_{1,pH}$ and $f_{2,pH}$ vs.
 244 pH curves obtained can be further processed to determine the apparent protonation constants
 245 (K_1 and K_2) and heterogeneity parameters (m_1 and m_2) of the proton binding sites:

$$246 \quad f_{i,pH} = \frac{1}{1+(K_i [H^+])^{m_i}} \quad (4)$$

247 These apparent protonation constants and heterogeneity parameters are detailed in Table S2
 248 (Appendix F in the SI). The values calculated for the carboxylic and phenolic groups are
 249 generally in line with those found in the literature, i.e., $\log K_1$ ranging from 3.26 to 4.10, $\log K_2$
 250 ranging from 9.66 to 10.07,^{24,42} and the associated heterogeneity parameter with average values
 251 of 0.45 for m_1 and 0.20 for m_2 .²⁴

252



253

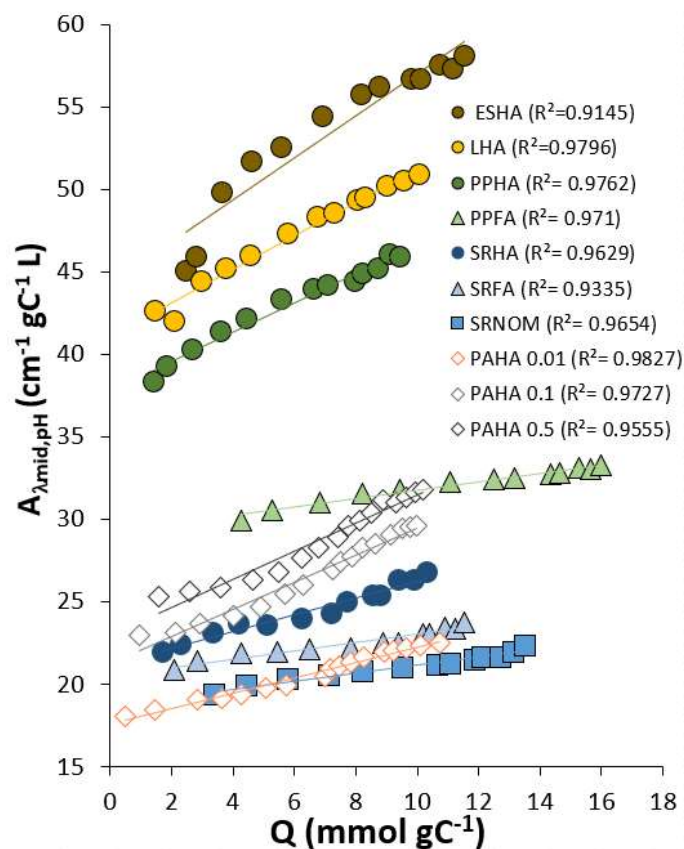
254 **Figure 2.** Results of the deconvolution of the acid-base spectrophotometric titration data with equation
 255 3: (a) component $A_{0,\lambda}$ for all samples, $A_{1,\lambda}$ and $A_{2,\lambda}$ components for (b) LHA, (c) SRFA, (d) SRHA, (e)
 256 PPFA, (f) PPHA, (g) SRNOM and (h) ESHA.

257

258 **Relationship between charge and specific absorbance.**

259 As mentioned in the introduction section, it is a major challenge to measure the concentration
260 of the acid-base functional groups in NOM at environmentally-relevant concentrations.³²
261 Although spectrophotometric methods do provide valuable quantitative information with
262 respect to the acid-base properties of the NOM functional groups, they cannot be used to
263 determine the electrical charge of NOM, from which the functional group density can be
264 deduced. This requires additional measurements such as potentiometric titrations or other
265 spectroscopic techniques (e.g. Infra-Red (IF), Nuclear Magnetic Resonance (NMR)).^{24,43} A
266 linear relationship between the differential specific absorbance $\Delta A_{\lambda,pH}$ and the differential
267 charge ΔQ of PAHA was determined by Janot et al.,³² which allowed subsequent investigations
268 of the PAHA acid-base properties after its fractionation on the surface of aluminum oxide at
269 environmentally relevant NOM concentrations.³⁹ However, this relationship was specific to
270 PAHA and it might not be possible to extrapolate it to other NOM with different spectroscopic
271 characteristics as shown above (Figures 1 and 2). Since the seven IHSS samples analyzed in
272 the present study were previously titrated by potentiometry,⁴⁰ the relevance of such a
273 relationship between the specific absorbance and the charge is tested for various NOM samples.
274 Setting up a link between $A_{\lambda,pH}$ and Q requires the selection of a representative wavelength.
275 However, the values of $A_{270,pH}$ ³² or $A_{360,pH}$ ³¹ selected in the literature are not suitable for the
276 entire set of samples studied in the present work. Close inspection of the specific absorbance
277 spectra of the carboxylic and phenolic groups for all the presently investigated samples (Figure
278 2) shows that these functional groups can be almost spectroscopically silent at certain λ ranges.
279 Specifically, $A_{2,\lambda}$ is very low for $\lambda < 300$ nm and $A_{1,\lambda}$ is very low for $\lambda > 320$ nm for SRFA,
280 SRHA, SRNOM and PPFa. As a solution, we propose to determine and use a sample specific
281 λ , at which both carboxylic and phenolic deprotonation processes significantly contribute to the
282 UV-vis absorption. For this purpose, the λ value at the mid-section (λ_{mid}) of the peak of both
283 carboxylic and phenolic specific absorbance spectra was chosen and is listed in Table S3
284 (Appendix G in the SI) for each NOM sample. A schematic representation of the method used
285 to select the λ_{mid} is shown with an example in Figure S3 (Appendix H in the SI). Typically, the
286 λ_{mid} ranges from 291 to 330 nm (Appendix G in the SI). The specific absorbance at the selected
287 λ_{mid} ($A_{\lambda_{mid}}$) is represented as a function of the measured Q extracted from a previous work⁴⁰ in
288 Figure 3. Note that the Q in this present study was considered in mol gC⁻¹ unlike the previous
289 study⁴⁰ in which Q was measured in mol kg_{NOM}⁻¹. Details of the unit conversion are given in
290 equation S12 (Appendix I in the SI). Figure 3 shows $A_{\lambda_{mid}}$ for all seven of the studied IHSS

291 samples as a function of Q and displays a linear relationship between $A_{\lambda_{\text{mid}}}$ and Q for each
292 sample, which is in agreement with a previous work on PAHA.³² It is possible to discern three
293 clusters of samples: a first one containing ESHA, LHA and PPHA with high $A_{\lambda_{\text{mid}}}$ and low Q
294 values, a second one assembling SRHA, SRFA and SRNOM with low $A_{\lambda_{\text{mid}}}$ and low Q values
295 and, finally, a third cluster containing PFFA only, with intermediate $A_{\lambda_{\text{mid}}}$ values and the highest
296 Q values. The separation between the humic and fulvic samples is in line with the UV
297 spectroscopy indicators, such as A_{250}/A_{365} (Figure S4, Appendix J in the SI),⁴⁴ which are often
298 considered as an indicator of the NOM molecular weight (M_w). The same separation can also
299 be observed when considering the aromaticity of the IHSS samples determined by NMR.⁴³
300 Additionally, M_w and the particle radius (r_p) determined in previous studies by using Soft
301 Poisson Boltzmann-based titration (SPBT) formalism were examined.⁴⁰ A first clustering
302 between the humic and fulvic samples was observed, in addition to a clustering between the
303 soil samples (with $1.71 < r_p < 3.67$ nm; $3.64 < M_w < 11.10$ kg mol⁻¹) and water samples (with
304 $0.8 < r_p < 1.8$ nm and $1.14 < M_w < 4.02$ kg mol⁻¹). These observations are in agreement with
305 other studies that associated samples with higher weight, size and aromaticity content with
306 plant-sourced terrestrial refractory NOM, as opposed to lighter, smaller and less aromatic
307 NOM, which are often linked to labile water or bacterial sourced NOM.^{40,45,46}
308
309



310

311

312 **Figure 3.** Specific absorbance ($A_{\lambda_{\text{mid}}}$ in $\text{cm}^{-1} \text{gC}^{-1} \text{L}$) at λ_{mid} , as a function of the charge (Q in mmol gC^{-1})
 313 ¹) for the seven IHSS samples. Charge values are from Tesfa et al.,⁴⁰ solid symbols represent the
 314 experimental measurements and corresponding lines represent linear regressions (R^2 in brackets). Data
 315 for PAHA were derived from Janot et al.³². The slope and intercept values of each linear regression are
 316 detailed in Table S4 (Appendix K in the SI).

317

318 Each of the established relationships between $A_{\lambda_{mid}}$ and Q exhibits a slope (S_{NOM}) and intercept
 319 (I_{NOM}) specific to the corresponding NOM sample, which consequently cannot be extrapolated
 320 to other NOM samples:

321

$$322 \quad A_{\lambda_{mid}} = I_{NOM} + Q \times S_{NOM} \quad (5)$$

323

324 However, it is proposed here to estimate S_{NOM} and I_{NOM} for any NOM using its UV-vis optical
 325 properties. In fact, I_{NOM} can easily be calculated using a combination of equations 3 and 4, and
 326 through a comparison with equation 5. In extreme acidic conditions ($\text{pH} \rightarrow -\infty$), Q , f_1 and f_2
 327 tend to 0 because all acid-base groups are fully protonated. As a result, $A_{\lambda_{mid}} \rightarrow A_{0,\lambda_{mid}}$ (Eq. 3)
 328 and $A_{\lambda_{mid}} \rightarrow I_{NOM}$ (Eq. 5). Thus, $A_{0,\lambda_{mid}}$ can be approximated to I_{NOM} , which is verified with
 329 Figure 4a, where I_{NOMS} vs. $A_{0,\lambda_{mid}}$ displays a linear relationship ($R^2 = 0.9988$) with a slope
 330 approximatively equal to 1, and a negligible intercept value if compared to $A_{0,\lambda_{mid}}$ or I_{NOM} . To
 331 provide an estimation of S_{NOM} , we hypothesized it to be related to the sum $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$ as
 332 described in equation 6, because these parameters concomitantly control the variation of the
 333 specific absorbance with pH (Eq. 3).

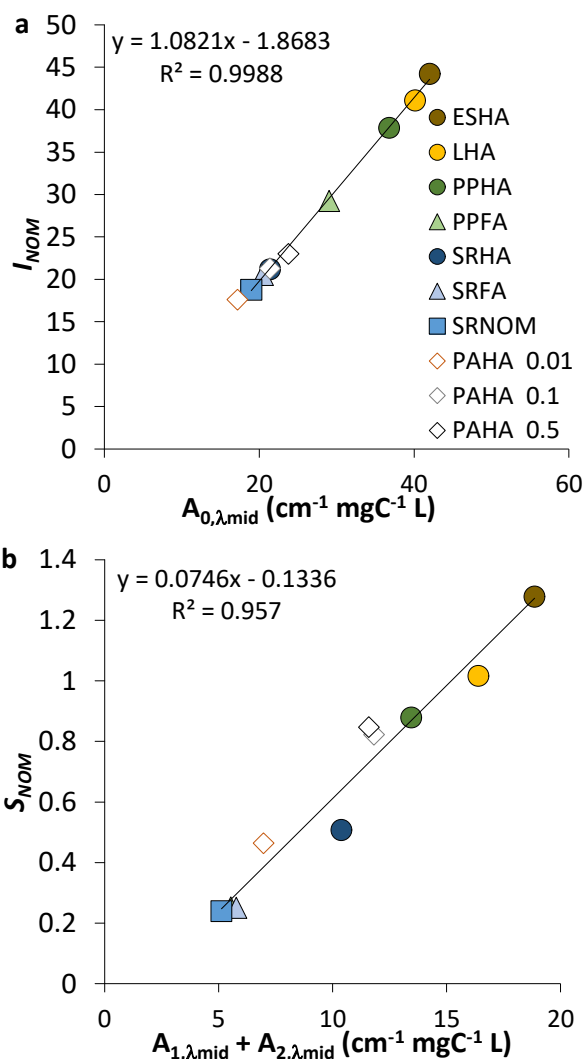
334

$$335 \quad S_{NOM} = S_b + S_a \times (A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}) \quad (6)$$

336

337 In equation 6, S_a represents the slope and S_b represents the intercept of a linear relationship
 338 between S_{NOM} and $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$. Accordingly, Figure 4b exhibits aligned values of S_{NOM} vs.
 339 $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$ for all samples, thereby establishing a linear relationship between these
 340 parameters ($R^2 = 0.9858$). Consequently, considering the specific parameters of equation 3 (i.e.
 341 $A_{0,\lambda}$, $A_{1,\lambda}$ and $A_{2,\lambda}$) and the carefully selected λ_{mid} might allow to approximate Q for unknown
 342 NOM samples using only absorbance data.

343



344
 345 **Figure 4.** (a) Intercept I_{NOM} and (b) slope S_{NOM} of linear regression between $A_{\lambda_{mid}}$ and Q (equation 5)
 346 as a function of $A_{0,\lambda_{mid}}$ and $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$, respectively for all IHSS samples (SRFA, SRHA,
 347 SRNOM, PPFA, PPHA, LHA and ESHA), as well as the literature data (PAHA 0.01-0.5; values refer
 348 to the ionic strength;³²), for comparison. Linear regressions do not take the PAHA data into account.
 349

350 Data from the literature on PAHA³² were used to corroborate the above-proposed approach
 351 using an independent dataset. Because PAHA was titrated by both spectrophotometric and
 352 potentiometric acid-base titration data at three different background electrolyte concentrations
 353 (0.01, 0.1 and 0.5 mol L⁻¹ in NaClO₄), the method described here could be tested on a larger
 354 range of physico-chemical conditions than the concentration used to establish the method (0.01
 355 mol L⁻¹ of NaCl). Hereafter, the three PAHA samples will be referred to as “PAHA 0.01”,
 356 “PAHA 0.1” and “PAHA 0.5” according to their respective background electrolyte
 357 concentrations. As described above for the seven IHSS samples studied here, the three PAHA
 358 specific absorbance datasets were deconvoluted using equation 3. After a close inspection of
 359 their $A_{1,\lambda_{mid}}$ and $A_{2,\lambda_{mid}}$ values (Figure 5), a λ_{mid} value for each NaClO₄ concentration was
 360 selected (Appendix G, Table S3 in the SI). Using the potentiometric titration, data from Janot

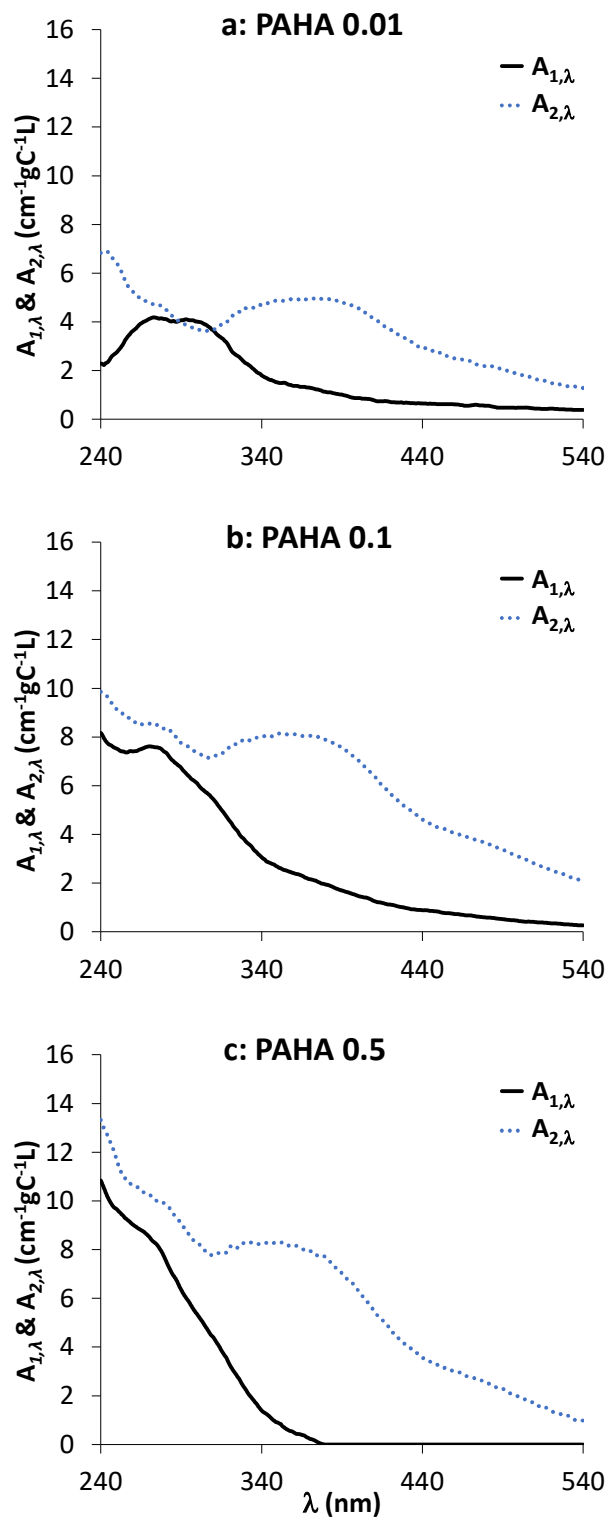
361 et al.,³² $A_{\lambda_{mid}}$ were plotted as a function of Q in Figure 3 and specific I_{PAHA} and S_{PAHA} were
 362 extracted and plotted in Figures 4a and 4b, respectively. The three PAHA samples are in very
 363 good agreement with the linear relationship established using the seven different IHSS samples,
 364 showing an increase in both I_{NOM} and S_{NOM} with increasing electrolyte concentration. The
 365 background electrolyte concentration affects proton–NOM binding and, hence, Q as it impacts
 366 the electrostatic contribution to the overall interaction between HS and protons.⁴⁰ This was
 367 previously shown to affect NOM absorbance.³⁸ Furthermore, ionic strength has more impact on
 368 absorbance than on charge measurement. This can be explained by the aggregation of NOM
 369 molecules at higher ionic strength, due to charge screening, increasing the interactions between
 370 chromophores.³² Thus, for a same sample, I_{NOM} and S_{NOM} values increase with ionic strength,
 371 which is accompanied by an increase in $A_{0,\lambda_{mid}}$ and $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$, respectively. The
 372 consistency between the observations made on an independent sample in various ionic strength
 373 conditions and on our reference samples validates the approach developed here.
 374 As such, we propose a condensed empirical equation enabling the estimation of Q directly from
 375 the specific absorbance data. This empirical equation combines equation 3 with the individual
 376 equations relating I_{NOM} to $A_{0,\lambda_{mid}}$ ($I_{NOM} \approx A_{0,\lambda_{mid}}$) and linearly relating S_{NOM} to $A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}}$
 377 (Figure 4; eq. 6).

378

$$379 \quad Q_{pH} = \frac{A_{\lambda_{mid},pH} - A_{0,\lambda_{mid}}}{(A_{1,\lambda_{mid}} + A_{2,\lambda_{mid}})S_a + S_b} \quad (7)$$

380

381 By using a least-square fitting procedure, and all the available data in the present work for the
 382 IHSS and PAHA samples, we estimated $S_a = 0.089 \pm 0.003$ and $S_b = -0.228 \pm 0.016$ (root-mean-
 383 square error, RMSE = 1.29). The assumption of a linear relationship instead of equality between
 384 $A_{0,\lambda_{mid}}$ and I_{NOM} was not statistically justified because the decrease in RMSE associated with the
 385 optimization of two additional parameters was very small (RMSE = 1.30). The accuracy of the
 386 present approach to predict Q is shown in Figure S5 (Appendix L in the SI), where 86% of the
 387 experimentally determined values can be calculated (Q_{calc}) within an error range of ± 2 mol
 388 kgC^{-1} . Table S5 (Appendix M in the SI), shows the RMSE values for the individual datasets,
 389 and demonstrates that the largest error is attributed to SRHA and PAHA 0.01 (RMSE = 2.24
 390 and 2.38, respectively), whereas, for the other NOM samples, 0.48 (PAHA 0.1) \leq RMSE \leq 1.13
 391 (ESHA).



392

393

394

Figure 5. Results of the deconvolution of the acid-base spectrophotometric titration data with equation 3 for the PAHA samples in (a) 0.01, (b) 0.1 and (c) 0.5 M NaClO_4 .

395 Environmental implications

396

397 Although many important mechanisms involve NOM acid-base functional groups, it is difficult
398 to quantify them in natural samples, especially since it is still a major challenge to determine
399 the NOM charge at environmentally relevant concentrations. To overcome this, we developed,
400 in the present study, a novel UV-vis spectrophotometric method based on a previously
401 established relationship between the ΔA and ΔQ of a NOM sample (PAHA).³² By extending
402 this finding to various NOM samples, using a recently collected potentiometric titration
403 dataset,⁴⁰ it was possible to provide an empirical approach to estimate the charge values of
404 NOM samples using UV-vis absorption data only. The parameters defining the linear
405 relationship between A and Q , i.e. I_{NOM} and S_{NOM} , were themselves linearly related to the proton
406 inert chromophores $A_{0,\lambda_{\text{mid}}}$ and to the sum of both proton sensitive chromophores $A_{1,\lambda_{\text{mid}}}$ and
407 $A_{2,\lambda_{\text{mid}}}$, respectively. The advantage of the empirical approach proposed here to estimate Q is
408 that it (i) does not require to perform a potentiometric acid-base titration, (ii) is based on sample-
409 specific spectra parameters ($A_{0,\lambda_{\text{mid}}}$, $A_{1,\lambda_{\text{mid}}}$ and $A_{2,\lambda_{\text{mid}}}$) and (iii) is also suitable at different
410 background electrolyte concentrations (in NaClO_4) compared to the studied concentrations.
411 Because spectroscopy is suitable for NOM sample characterization in terms of their
412 environmental concentration, the novel spectrophotometric deconvolution method developed
413 in the present study, and sample specific empirical spectra analysis could be used to
414 approximate the Q of unknown natural samples and subsequently estimate their carboxylic and
415 phenolic functional groups' concentrations. However, in contrast to Na^+ , other cations such as
416 Cu^{2+} or Al^{3+} can bind to NOM and affect the absorbance signal.⁴⁷ The present approach might
417 be applicable to natural samples containing cations other than monovalent ones if the
418 interferences on the absorbance spectra they generate remain insignificant. Otherwise, it might
419 be necessary to remove these cations from the sample using a simple purification procedure.

420 The present approach further allows the estimation of the NOM charge in various
421 aquatic systems, which is critical to understanding the role of NOM in environmental studies.
422 Natural organic matter can be a nanoparticle stabilizing agent, a contaminant vector or a
423 reaction catalyzer. Understanding and predicting the geochemical behavior and transport of
424 aquatic NOM thus requires taking its charge into account, because the charge predominantly
425 controls its interaction with other organic or inorganic particles. Therefore, the present work
426 provides important keys for understanding and predicting the biogeochemical cycle of NOM
427 and its associated elements.

428 Acknowledgments

429

430 This work was supported by the C-FACTOR project funded by ANR (project number
431 ANR-18-CE01-0008; coordinator: R. Marsac). Through the support of the GeOHeLiS
432 analytical platform of Rennes University, this publication is also supported by the European
433 Union through the European Regional Development Fund (FEDER), the French ministry of
434 Higher Education and Research, the French Region of Brittany and Rennes Metropole. Lastly,
435 Dr. S. Mullin is acknowledged for post-editing the English content.

436

437 Supporting Information

438 The supporting information section contains 11 pages, 12 equations, 7 references, 5 tables and
439 5 figures. It is organized as follows:

440 **Appendix A: Table S1.** Characteristics of the buffer salts used to prepare the buffer solutions

441 **Appendix B: Figure S1.** Specific absorbance spectra and the corresponding deconvoluted A_0
442 component.

443 **Appendix C:** Mathematical development for equation 3

444 **Appendix D:** Script details on the optimization method and constraints used on the estimated
445 parameters for the description of the NOM specific absorbance spectra

446 **Appendix E: Figure S2.** Deconvolution results of the deprotonated fractions of the carboxylic
447 and phenolic sites for all seven IHSS samples

448 **Appendix F: Table S2.** Apparent protonation constants ($\log K_1$ and $\log K_2$) and heterogeneity
449 parameters (m_1 and m_2) of the carboxylic and phenolic groups, respectively

450 **Appendix G: Table S3.** Middle wavelength (λ_{mid}) corresponding to the mid-section between
451 both maxima of the specific spectra for the carboxylic and phenolic groups

452 **Appendix H: Figure S3.** Example of how $A_{1,\lambda_{\text{mid}}}$ and $A_{2,\lambda_{\text{mid}}}$ are selected, shown in two steps

453 **Appendix I:** Equation S12 to convert Q from mol kgNOM^{-1} to mol gC^{-1}

454 **Appendix J: Figure S4.** Comparison of A_{250}/A_{365} for the IHSS samples

455 **Appendix K: Table S4.** Slope (S_{NOM}) and intercept (I_{NOM}) of the linear regression established
456 between specific absorbance and charge

457 **Appendix L: Figure S5.** Calculated Q (Q_{cal}) vs. experimental Q (Q_{exp})

458 **Appendix M: Table S5.** RMSE values for each NOM dataset

459

460 References

461

- 462 (1) Lachaux, N.; Catrouillet, C.; Marsac, R.; Poirier, L.; Pain-Devin, S.; Gross, E. M.; Giamberini, L.
463 Implications of Speciation on Rare Earth Element Toxicity: A Focus on Organic Matter Influence
464 in *Daphnia Magna* Standard Test. *Environ. Pollut.* **2022**, *307*, 119554.
465 <https://doi.org/10.1016/j.envpol.2022.119554>.
- 466 (2) McCarthy, J. F. Bioavailability and Toxicity of Metals and Hydrophobic Organic Contaminants.
467 In *Aquatic Humic Substances*; Suffet, I. H., MacCarthy, P., Eds.; Advances in Chemistry;
468 American Chemical Society: Washington, DC, 1988; Vol. 219, pp 263–277.
469 <https://doi.org/10.1021/ba-1988-0219.ch018>.
- 470 (3) Peng, C.; Sundman, A.; Bryce, C.; Catrouillet, C.; Borch, T.; Kappler, A. Oxidation of Fe(II)–
471 Organic Matter Complexes in the Presence of the Mixotrophic Nitrate-Reducing Fe(II)-Oxidizing
472 Bacterium *Acidovorax* Sp. BoFeN1. *Environ. Sci. Technol.* **2018**, *52* (10), 5753–5763.
473 <https://doi.org/10.1021/acs.est.8b00953>.
- 474 (4) Benedetti, M. F.; Milne, C. J.; Kinniburgh, D. G.; Van Riemsdijk, W. H.; Koopal, L. K. Metal Ion
475 Binding to Humic Substances: Application of the Non-Ideal Competitive Adsorption Model.
476 *Environ. Sci. Technol.* **1995**, *29* (2), 446–457. <https://doi.org/10.1021/es00002a022>.
- 477 (5) Catrouillet, C.; Davranche, M.; Dia, A.; Bouhnik-Le Coz, M.; Marsac, R.; Pourret, O.; Gruau, G.
478 Geochemical Modeling of Fe(II) Binding to Humic and Fulvic Acids. *Chem. Geol.* **2014**, *372*,
479 109–118. <https://doi.org/10.1016/j.chemgeo.2014.02.019>.
- 480 (6) Hiemstra, T.; van Riemsdijk, W. H. Biogeochemical Speciation of Fe in Ocean Water. *Mar. Chem.*
481 **2006**, *102* (3), 181–197. <https://doi.org/10.1016/j.marchem.2006.03.008>.
- 482 (7) Pokrovsky, O. S.; Schott, J.; Dupré, B. Trace Element Fractionation and Transport in Boreal Rivers
483 and Soil Porewaters of Permafrost-Dominated Basaltic Terrain in Central Siberia. *Geochim.*
484 *Cosmochim. Acta* **2006**, *70* (13), 3239–3260. <https://doi.org/10.1016/j.gca.2006.04.008>.
- 485 (8) Tang, J.; Johannesson, K. H. Speciation of Rare Earth Elements in Natural Terrestrial Waters:
486 Assessing the Role of Dissolved Organic Matter from the Modeling Approach. *Geochim.*
487 *Cosmochim. Acta* **2003**, *67* (13), 2321–2339. [https://doi.org/10.1016/S0016-7037\(02\)01413-8](https://doi.org/10.1016/S0016-7037(02)01413-8).
- 488 (9) Tipping, E.; Rey-Castro, C.; Bryan, S. E.; Hamilton-Taylor, J. Al(III) and Fe(III) Binding by
489 Humic Substances in Freshwaters, and Implications for Trace Metal Speciation. *Geochim.*
490 *Cosmochim. Acta* **2002**, *66* (18), 3211–3224. [https://doi.org/10.1016/S0016-7037\(02\)00930-4](https://doi.org/10.1016/S0016-7037(02)00930-4).
- 491 (10) Aiken, G. R.; Hsu-Kim, H.; Ryan, J. N. Influence of Dissolved Organic Matter on the
492 Environmental Fate of Metals, Nanoparticles, and Colloids. *Environ. Sci. Technol.* **2011**, *45* (8),
493 3196–3201. <https://doi.org/10.1021/es103992s>.
- 494 (11) Guénet, H.; Davranche, M.; Vantelon, D.; Gigault, J.; Prévost, S.; Taché, O.; Jaksch, S.; Pédrot,
495 M.; Dorcet, V.; Boutier, A.; Jestin, J. Characterization of Iron–Organic Matter Nano-Aggregate
496 Networks through a Combination of SAXS/SANS and XAS Analyses: Impact on As Binding.
497 *Environ. Sci. Nano* **2017**, *4* (4), 938–954. <https://doi.org/10.1039/C6EN00589F>.
- 498 (12) Pradel, A.; Ferreres, S.; Veclin, C.; El Hadri, H.; Gautier, M.; Grassl, B.; Gigault, J. Stabilization
499 of Fragmental Polystyrene Nanoplastic by Natural Organic Matter: Insight into Mechanisms. *ACS*
500 *EST Water* **2021**, *1* (5), 1198–1208. <https://doi.org/10.1021/acsestwater.0c00283>.
- 501 (13) Vindedahl, A. M.; Strehlau, J. H.; Arnold, W. A.; Penn, R. L. Organic Matter and Iron Oxide
502 Nanoparticles: Aggregation, Interactions, and Reactivity. *Environ. Sci. Nano* **2016**, *3* (3), 494–
503 505. <https://doi.org/10.1039/C5EN00215J>.
- 504 (14) Cheng, W.; Zhou, L.; Marsac, R.; Boily, J.-F.; Hanna, K. Effects of Organic Matter–Goethite
505 Interactions on Reactive Transport of Nalidixic Acid: Column Study and Modeling. *Environ. Res.*
506 **2020**, *191*, 110187. <https://doi.org/10.1016/j.envres.2020.110187>.
- 507 (15) Kleber, M.; Bourg, I. C.; Coward, E. K.; Hansel, C. M.; Myneni, S. C. B.; Nunan, N. Dynamic
508 Interactions at the Mineral–Organic Matter Interface. *Nat. Rev. Earth Environ.* **2021**, *2* (6), 402–
509 421. <https://doi.org/10.1038/s43017-021-00162-y>.
- 510 (16) Kleber, M.; Sollins, P.; Sutton, R. A Conceptual Model of Organo-Mineral Interactions in Soils:
511 Self-Assembly of Organic Molecular Fragments into Zonal Structures on Mineral Surfaces.
512 *Biogeochemistry* **2007**, *85* (1), 9–24. <https://doi.org/10.1007/s10533-007-9103-5>.

- 513 (17) Lehmann, J.; Kleber, M. The Contentious Nature of Soil Organic Matter. *Nature* **2015**, *528* (7580),
514 60–68. <https://doi.org/10.1038/nature16069>.
- 515 (18) Reiller, P. E. Modelling Metal–Humic Substances–Surface Systems: Reasons for Success, Failure
516 and Possible Routes for Peace of Mind. *Mineral. Mag.* **2012**, *76* (7), 2643–2658.
517 <https://doi.org/10.1180/minmag.2012.076.7.02>.
- 518 (19) Weng, L.; Van Riemsdijk, W. H.; Hiemstra, T. Effects of Fulvic and Humic Acids on Arsenate
519 Adsorption to Goethite: Experiments and Modeling. *Environ. Sci. Technol.* **2009**, *43* (19), 7198–
520 7204. <https://doi.org/10.1021/es9000196>.
- 521 (20) Buffle, J. Complexation Reactions in Aquatic Systems; Analytical Approach. *Acta Hydrochim.*
522 *Hydrobiol.* **1989**, *17* (2), 230–230. <https://doi.org/10.1002/ahch.19890170220>.
- 523 (21) Christensen, J. B.; Tipping, E.; Kinniburgh, D. G.; Grøn, C.; Christensen, T. H. Proton Binding
524 by Groundwater Fulvic Acids of Different Age, Origins, and Structure Modeled with the Model
525 V and NICA–Donnan Model. *Environ. Sci. Technol.* **1998**, *32* (21), 3346–3355.
526 <https://doi.org/10.1021/es971134o>.
- 527 (22) Gustafsson, J. P. Modeling the Acid–Base Properties and Metal Complexation of Humic
528 Substances with the Stockholm Humic Model. *J. Colloid Interface Sci.* **2001**, *244* (1), 102–112.
529 <https://doi.org/10.1006/jcis.2001.7871>.
- 530 (23) Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena,
531 M. J. Ion Binding to Natural Organic Matter: Competition, Heterogeneity, Stoichiometry and
532 Thermodynamic Consistency. *Colloids Surf. Physicochem. Eng. Asp.* **1999**, *151* (1–2), 147–166.
533 [https://doi.org/10.1016/S0927-7757\(98\)00637-2](https://doi.org/10.1016/S0927-7757(98)00637-2).
- 534 (24) Ritchie, J. D.; Perdue, E. M. Proton-Binding Study of Standard and Reference Fulvic Acids,
535 Humic Acids, and Natural Organic Matter. *Geochim. Cosmochim. Acta* **2003**, *67* (1), 85–96.
536 [https://doi.org/10.1016/S0016-7037\(02\)01044-X](https://doi.org/10.1016/S0016-7037(02)01044-X).
- 537 (25) Tipping, E. Humic Ion–Binding Model VI: An Improved Description of the Interactions of Protons
538 and Metal Ions with Humic Substances. *Aquat. Geochem.* **1998**, *4* (1), 3–47.
539 <https://doi.org/10.1023/A:1009627214459>.
- 540 (26) Koopal, L. K.; Saito, T.; Pinheiro, J. P.; Riemsdijk, W. H. van. Ion Binding to Natural Organic
541 Matter: General Considerations and the NICA–Donnan Model. *Colloids Surf. Physicochem. Eng.*
542 *Asp.* **2005**, *265* (1–3), 40–54. <https://doi.org/10.1016/j.colsurfa.2004.11.050>.
- 543 (27) Milne, C. J.; Kinniburgh, D. G.; Tipping, E. Generic NICA–Donnan Model Parameters for Proton
544 Binding by Humic Substances. *Environ. Sci. Technol.* **2001**, *35* (10), 2049–2059.
545 <https://doi.org/10.1021/es000123j>.
- 546 (28) Gustafsson, J. P.; Persson, I.; Kleja, D. B.; Van Schaik, J. W. J. Binding of Iron(III) to Organic
547 Soils: EXAFS Spectroscopy and Chemical Equilibrium Modeling. *Environ. Sci. Technol.* **2007**,
548 *41* (4), 1232–1237. <https://doi.org/10.1021/es0615730>.
- 549 (29) Marsac, R.; Catrouillet, C.; Davranche, M.; Bouhnik-Le Coz, M.; Briant, N.; Janot, N.; Otero-
550 Fariña, A.; Groenenberg, J. E.; Pédrot, M.; Dia, A. Modeling Rare Earth Elements Binding to
551 Humic Acids with Model VII. *Chem. Geol.* **2021**, *567*, 120099.
552 <https://doi.org/10.1016/j.chemgeo.2021.120099>.
- 553 (30) Neweshy, W.; Planas, D.; Tellier, E.; Demers, M.; Marsac, R.; Couture, R.-M. Response of
554 Sediment Phosphorus Partitioning to Lanthanum-Modified Clay Amendment and Porewater
555 Chemistry in a Small Eutrophic Lake. *Environ. Sci. Process. Impacts* **2022**.
556 <https://doi.org/10.1039/D1EM00544H>.
- 557 (31) Dryer, D. J.; Korshin, G. V.; Fabbicino, M. In Situ Examination of the Protonation Behavior of
558 Fulvic Acids Using Differential Absorbance Spectroscopy. *Environ. Sci. Technol.* **2008**, *42* (17),
559 6644–6649. <https://doi.org/10.1021/es800741u>.
- 560 (32) Janot, N.; Reiller, P. E.; Korshin, G. V.; Benedetti, M. F. Using Spectrophotometric Titrations To
561 Characterize Humic Acid Reactivity at Environmental Concentrations. *Environ. Sci. Technol.*
562 **2010**, *44* (17), 6782–6788. <https://doi.org/10.1021/es1012142>.
- 563 (33) Liu, S.; Benedetti, M. F.; Han, W.; Korshin, G. V. Comparison of the Properties of Standard Soil
564 and Aquatic Fulvic and Humic Acids Based on the Data of Differential Absorbance and
565 Fluorescence Spectroscopy. *Chemosphere* **2020**, *261*, 128189.
566 <https://doi.org/10.1016/j.chemosphere.2020.128189>.

- 567 (34) Tesfa, M.; Dia, A.; Ollivier, M.; Osorio-Leon, I.-D.; Marsac, R. An Easy Spectrophotometric
568 Acid-Base Titration Protocol for Dissolved Organic Matter. *MethodsX* **2022**, *9*, 101721.
569 <https://doi.org/10.1016/j.mex.2022.101721>.
- 570 (35) Yan, M.; Dryer, D.; Korshin, G. V. Spectroscopic Characterization of Changes of DOM
571 Deprotonation–Protonation Properties in Water Treatment Processes. *Chemosphere* **2016**, *148*,
572 426–435. <https://doi.org/10.1016/j.chemosphere.2016.01.055>.
- 573 (36) Conte, P.; Piccolo, A. Conformational Arrangement of Dissolved Humic Substances. Influence of
574 Solution Composition on Association of Humic Molecules. *Environ. Sci. Technol.* **1999**, *33* (10),
575 1682–1690. <https://doi.org/10.1021/es9808604>.
- 576 (37) Korshin, G. V.; Li, C.-W.; Benjamin, M. M. Monitoring the Properties of Natural Organic Matter
577 through UV Spectroscopy: A Consistent Theory. *Water Res.* **1997**, *31* (7), 1787–1795.
578 [https://doi.org/10.1016/S0043-1354\(97\)00006-7](https://doi.org/10.1016/S0043-1354(97)00006-7).
- 579 (38) Yan, M.; Korshin, G. V.; Claret, F.; Croué, J.-P.; Fabbicino, M.; Gallard, H.; Schäfer, T.;
580 Benedetti, M. F. Effects of Charging on the Chromophores of Dissolved Organic Matter from the
581 Rio Negro Basin. *Water Res.* **2014**, *59*, 154–164. <https://doi.org/10.1016/j.watres.2014.03.044>.
- 582 (39) Janot, N.; Reiller, P. E.; Zheng, X.; Croué, J.-P.; Benedetti, M. F. Characterization of Humic Acid
583 Reactivity Modifications Due to Adsorption onto α -Al₂O₃. *Water Res.* **2012**, *46* (3), 731–740.
584 <https://doi.org/10.1016/j.watres.2011.11.042>.
- 585 (40) Tesfa, M.; Duval, J. F. L.; Marsac, R.; Dia, A.; Pinheiro, J.-P. Absolute and Relative Positioning
586 of Natural Organic Matter Acid–Base Potentiometric Titration Curves: Implications for the
587 Evaluation of the Density of Charged Reactive Sites. *Environ. Sci. Technol.* **2022**, *acs.est.2c00828*.
588 <https://doi.org/10.1021/acs.est.2c00828>.
- 589 (41) Cincotta, M. M.; Perdrial, J. N.; Shavitz, A.; Libenson, A.; Landsman-Gerjoi, M.; Perdrial, N.;
590 Armfield, J.; Adler, T.; Shanley, J. B. Soil Aggregates as a Source of Dissolved Organic Carbon
591 to Streams: An Experimental Study on the Effect of Solution Chemistry on Water Extractable
592 Carbon. *Front. Environ. Sci.* **2019**, *7*, 172. <https://doi.org/10.3389/fenvs.2019.00172>.
- 593 (42) Fernandes, A. N.; Giacomelli, C.; Giovanela, M.; Vaz, D. O.; Szpoganicz, B.; Sierra, M. M. D.
594 Potentiometric Acidity Determination in Humic Substances Influenced by Different Analytical
595 Procedures. *J. Braz. Chem. Soc.* **2009**, *20* (9), 1715–1723. [https://doi.org/10.1590/S0103-](https://doi.org/10.1590/S0103-50532009000900021)
596 [50532009000900021](https://doi.org/10.1590/S0103-50532009000900021).
- 597 (43) Ritchie, J. D.; Perdue, E. M. Analytical Constraints on Acidic Functional Groups in Humic
598 Substances. *Org. Geochem.* **2008**, *39* (6), 783–799.
599 <https://doi.org/10.1016/j.orggeochem.2008.03.003>.
- 600 (44) Rodríguez, F. J.; Schlenger, P.; García-Valverde, M. Monitoring Changes in the Structure and
601 Properties of Humic Substances Following Ozonation Using UV–Vis, FTIR and ¹H NMR
602 Techniques. *Sci. Total Environ.* **2016**, *541*, 623–637.
603 <https://doi.org/10.1016/j.scitotenv.2015.09.127>.
- 604 (45) Guggenberger, G.; Zech, W.; Haumaier, L.; Christensen, B. T. Land-Use Effects on the
605 Composition of Organic Matter in Particle-Size Separates of Soils: II. CPMAS and Solution ¹³C
606 NMR Analysis. *Eur. J. Soil Sci.* **1995**, *46* (1), 147–158. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2389.1995.tb01821.x)
607 [2389.1995.tb01821.x](https://doi.org/10.1111/j.1365-2389.1995.tb01821.x).
- 608 (46) Machado, W.; Franchini, J. C.; de Fátima Guimarães, M.; Filho, J. T. Spectroscopic
609 Characterization of Humic and Fulvic Acids in Soil Aggregates, Brazil. *Heliyon* **2020**, *6* (6),
610 e04078. <https://doi.org/10.1016/j.heliyon.2020.e04078>.
- 611 (47) Yan, M.; Wang, D.; Korshin, G. V.; Benedetti, M. F. Quantifying Metal Ions Binding onto
612 Dissolved Organic Matter Using Log-Transformed Absorbance Spectra. *Water Res.* **2013**, *47* (7),
613 2603–2611. <https://doi.org/10.1016/j.watres.2013.02.044>.
- 614