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

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#### 11 Abstract

12 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 13 14 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 15 16 the specific UV-vis differential absorbance ( $\Delta A_{\lambda,pH}$ ) at a given wavelength ( $\lambda$ ) and pH of a 17 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 18 19  $(A_{0,\lambda})$ , and to the deprotonation processes of carboxylic  $(A_{1,\lambda})$  and phenolic groups  $(A_{2,\lambda})$ . It 20 enables to select sample-specific wavelength ( $\lambda_{mid}$ ), where both these functional groups 21 significantly contribute to the variation of absorbance with pH. The linear regression analysis 22 of  $A_{\lambda \text{mid}, pH}$  vs. Q for various NOM reference samples evidenced that the sample-specific slope  $(S_{\text{NOM}})$  and intercept  $(I_{\text{NOM}})$  were related to the intrinsic spectroscopic properties of the sample 23  $(A_{0,\lambda \text{mid}}, A_{1,\lambda \text{mid}} \text{ and } A_{2,\lambda \text{mid}})$ . This approach can thus be used to approximate the Q values of the 24 25 NOM samples at environmentally relevant concentrations: a pre-requisite for predicting the fate and behavior of metal ions in natural systems. 26

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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.

31 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 influences the biogeochemical cycles of various contaminants and nutrients, by exerting a 38 strong control on their transport, speciation, (bio)availability and eventually biotoxicity.<sup>1-3</sup> 39 Natural organic matter is known as a major trace cation vector in the environment, as it can 40 bind significant amounts of diverse dissolved metal ions.<sup>4-9</sup> The interaction of NOM with 41 natural and anthropogenic nanoparticles, either organic or inorganic, often increases their 42 colloidal stability,<sup>10–13</sup> therefore enhancing their mobility. Conversely, the binding of NOM to 43 mineral surfaces may affect its chemical stability, reactivity and transport and hence, the 44 biogeochemical cycle of carbon in soils and aquatic systems, as well as that of associated 45 elements and contaminants.<sup>13–19</sup> 46

The common link between all the above-listed mechanisms is the involvement of NOM 47 functional groups exhibiting acid-base properties.<sup>20-24</sup> These functional groups are generally 48 49 divided into carboxylic and minor groups often referred to as phenolic groups, which can form complexes with metal ions, in solution or at the (nano)particles' surfaces.<sup>19,20,23,25</sup> Therefore, it 50 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 speciation modeling.<sup>18,21,22,25,26</sup> However, because the carboxylic and phenolic site densities 53 vary broadly with the origin of the NOM, this crucial model input data is rarely available. As a 54 panacea, the generic values proposed by Milne et al.<sup>27</sup> are commonly used.<sup>9,28–30</sup> which might 55 have detrimental consequences on the performance of predictive geochemical models. The most 56 57 accurate approach to quantify NOM functional groups involves potentiometric acid-base titration. By direct measurement of the electrical charge carried by humic substances, this titration enables the determination of NOM proton-binding site concentrations, along with their protonation constant and their chemical heterogeneity.<sup>4,23,24,27</sup> Unfortunately, although highly accurate, potentiometric titration cannot be applied to environmental samples, given that the NOM concentrations rarely exceed 100 milligrams of dissolved organic carbon per liter (mgC L<sup>-1</sup>): such concentrations are too low for optimal electrode measurements requiring several hundreds of mgC L<sup>-1</sup>.

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

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- 73

$$\Delta A_{\lambda,pH} = A_{\lambda,pH} - A_{\lambda,pHref} \tag{1}$$

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75 This operation aims to remove the typical featureless absorbance spectra of NOM chromophores exhibiting no acid-base properties and, hence, to identify the small variations in 76 absorbance related to the deprotonation of the NOM functional groups of interest.<sup>31,32,36–38</sup> The 77 differential absorbance spectra of NOM generally show two peaks: a first peak, with a 78 79 maximum at ~270 nm for which the intensity notably increases between pH 3 and 6 and a second one, with a maximum at 320-380 nm, which appears at pH values above 6. On the basis 80 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 NICA-Donnan model in order to fit the values of  $\Delta A_{\lambda,pH}$ , it then becomes possible to determine 83 the protonation constants ( $pK_{a1}$  and  $pK_{a2}$ ) of the NOM carboxylic (1) and phenolic (2) groups 84 and their heterogeneity parameters  $(m_1 \text{ and } m_2)$ , as well as the specific contribution of the 85 deprotonation process of these groups to the variation of the absorbance  $(A_{1,\lambda} \text{ and } A_{2,\lambda})$ .<sup>31,32,35</sup> 86 The choice of wavelength selected is not very important, however it might be preferable to 87 select the value of  $\lambda$  corresponding to the maximum differential absorbance.<sup>32,39</sup> 88

Although the currently used UV-spectrophotometric acid-base titration approaches provide reliable information on the reactivity of the NOM acid-base functional groups, they do not 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. <sup>32</sup> 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 ( $\Delta Q$ ). This allowed to investigate the impact of PAHA adsorption and fractionation 96 at the alumina surface on the PAHA acid-base properties.<sup>39</sup> 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  $\Delta A_{\lambda,pH}$ and  $\Delta Q$  previously observed by Janot et al.<sup>32</sup> to other NOM samples with various origins and 100 chemical properties. This work attempts to generalize a relationship between  $A_{\lambda,pH}$  and Q in 101 order to fully estimate the proton-binding properties of NOM using spectrophotometric data, 102 avoiding the often-impossible collection of potentiometric data. To do so, in this work, recent 103 potentiometric acid-base titration results obtained for seven of the samples provided by the 104 International Humic Substances Society (IHSS) were used.<sup>40</sup> In addition, a novel data treatment 105 106 of spectrophotometric titrations is proposed to (i) directly deconvolute absorbance  $A_{\lambda,pH}$  instead of the differential absorbance  $\Delta A_{\lambda,pH}$ , to limit potential biases linked to NOM aggregation which 107 might occur at low pHref,<sup>37,41</sup> and to (ii) take advantage of the complete absorbance dataset 108 109 collected at  $\lambda$  ranging from 200 to 800 nm or to select the most relevant  $\lambda$  value to derive the 110 NOM-proton binding parameters and NOM charge from the absorbance data.
- 111

#### 112 Materials & Methods

113

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

Sample preparation. A 250 mL NOM mother solution (1 g L<sup>-1</sup>) was prepared by dissolving 126 25 mg of NOM powder using NaOH (0.1 mol L<sup>-1</sup>) in ultrapure water. Complete dissolution was 127 ensured by maintaining the pH of the NOM solution at around 9 upon the addition of NaOH. 128 All pH measurements were performed with a HANNA, HI5221 pH meter. The pH of the NOM 129 mother solution was monitored for 24 hours, with adjustments performed at 3, 6 and 12 hours 130 after the start of the NOM dissolution process. The NOM mother solution was left to mix on a 131 shaking table in-between these pH adjustments. Subsequently, the dissolved organic carbon 132 concentration (DOC) was determined through a Shimadzu TOC-L analyzer. The NOM samples 133 134 destined to be analyzed by spectrophotometric acid-base titration were prepared by diluting the mother solution to a carbon concentration of 25 mgC  $L^{-1}$ , and by adjusting their ionic strength 135 (IS) to 0.01 mol  $L^{-1}$ , with NaCl. 136 The preparation of the buffer solutions followed the protocol described in a previous article.<sup>34</sup> 137 Briefly, buffer salt was dispersed in a total of 50 mL volume of ultrapure water at final 138 concentration of 1 mol L<sup>-1</sup>. The pH of the buffer solution was adjusted to match the pKa value 139

140 of the buffer salt, or values within pKa - 1 < pH < pKa + 1 with acid (HCl, 1, 0.1 mol L<sup>-1</sup>) or

141 base (NaOH, 1, 0.1 mol L<sup>-1</sup>) 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 was also carried out as described in a previous study.<sup>34</sup> Each of the studied NOM samples was 146 divided into 13 aliquots of 4 mL (25 mgC L<sup>-1</sup> and 0.01 mol L<sup>-1</sup> NaCl). The pH of the 11 aliquots 147 of the NOM solution, ranging from pH 3.8 to 9, was fixed using the corresponding buffer, with 148 a final buffer concentration of 10<sup>-3</sup> mol L<sup>-1</sup>. The pH of the two remaining aliquots was adjusted 149 to pH 3 and 3.4 using HCl. The pH was monitored for 15 minutes to ensure it was stable before 150 proceeding to the absorbance measurement. Subsequently, the raw UV-vis absorbance of each 151 aliquot was recorded for wavelengths ( $\lambda$ ) between 200-800 nm, using a UV-vis Shimadzu 152 UV2600 spectrophotometer and a 1-cm-wide quartz cuvette. In this study, the raw absorbance 153  $(A_{\lambda,\text{pH,raw}})$  in this study was normalized by the DOC data and by the optical pathlength, in order 154 to determine the specific UV absorbance spectra, as detailed in the following equation (Eq. 2) 155 156

157 
$$A_{\lambda,pH} = \frac{1}{l_{cell}} \times \frac{A_{\lambda,pH,raw}}{DOC}$$
(2)

158 The specific UV absorbance spectra, here denoted " $A_{\lambda,pH}$ " (in cm<sup>-1</sup> gC<sup>-1</sup> 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,<sup>34</sup> the absorbance of the 161 pure buffer solutions is limited to  $\lambda < 240$  nm. Consequently, all data treatments were conducted 162 within a  $\lambda$  range of 240 nm – 540 nm.

163

#### 164 Results & Discussion

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



192Figure 1. Differential specific absorbance spectra ( $\Delta A_{\lambda,pH}$  in cm<sup>-1</sup>gC<sup>-1</sup>L) of (a) LHA, (b) SRFA<sup>34</sup>, (c)193SRHA, (d) PPFA, (e) PPHA, (f) SRNOM and (g) ESHA derived from the absolute specific194absorbance spectra ( $A_{\lambda,pH}$ ). The spectra were recorded at different pH values, from 3.04 to 9.10 (the195same colors are used in all figures).196

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

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- 211

$$A_{\lambda,pH} = A_{0,\lambda} + A_{1,\lambda} \times f_{1,pH} + A_{2,\lambda} \times f_{2,pH}$$
(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 linear combination of three components  $(A_{0,\lambda}, A_{1,\lambda} \text{ and } A_{2,\lambda})$ , two of which  $(A_{1,\lambda} \text{ and } A_{2,\lambda})$  are 215 associated with their pH-dependent coefficients ( $f_{i,pH}$ ). The component  $A_{0,\lambda}$  mainly represents 216 the proton-inert chromophores within the investigated pH range. According to the calculations, 217  $A_{0,\lambda}$  also includes the specific absorbance of the proton-reactive chromophores under their 218 219 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  $A_{I,\lambda}$ and  $A_{2,\lambda}$  represent the difference in specific absorbance between the deprotonated and 221 protonated species of the acid-base functional groups. The coefficients  $f_{1,pH}$  and  $f_{2,pH}$  are the 222 fractions of deprotonated chromophores at the corresponding pH. Consequently,  $A_{0,\lambda}$  is not 223 associated with a pH-dependent coefficient  $f_{i,pH}$ . All of the parameters  $(A_{0,\lambda}, A_{1,\lambda}, f_{1,pH}, A_{2,\lambda}, f_{2,pH})$ 224 225 were optimized to best fit the experimental  $A_{\lambda,pH}$  values using an iterative optimization method. However, the optimization problem used to estimate the parameters of Eq.3 has multiple 226 solutions but a reasonable a priori estimation of the values of the parameters could be obtained 227 228 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 explanation of the optimization process, including the choice of starting values, is given in the 232 SI (Appendix D in the SI). Figure 2 shows the results of the deconvolution process for all IHSS 233 234 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.  $\lambda$  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 (Appendix B, Figure S1 in SI). The characteristic components of the carboxylic and phenolic 238 239 groups  $(A_{1,\lambda} \text{ and } A_{2,\lambda})$  display a peak shape with a maximum at  $\lambda \approx 270$  nm and  $\lambda$  ranging from 320 to 370 nm, in agreement with previous investigations involving a differential absorbance 240 analysis.<sup>31,33</sup> The fractions of deprotonated carboxylic ( $f_{1,pH}$ ) and phenolic ( $f_{2,pH}$ ) groups as a 241 function of pH for all samples are depicted in Figure S2 (Appendix E in the SI). Using the 242 243 Langmuir-Freundlich equation (Eq. 4) and a least-square fitting procedure, the  $f_{1,pH}$  and  $f_{2,pH}$  vs. pH curves obtained can be further processed to determine the apparent protonation constants 244  $(K_1 \text{ and } K_2)$  and heterogeneity parameters  $(m_1 \text{ and } m_2)$  of the proton binding sites: 245

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

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



Figure 2. Results of the deconvolution of the acid-base spectrophotometric titration data with equation 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) PPFA, (f) PPHA, (g) SRNOM and (h) ESHA.

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

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

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

308



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

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

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323

$$A_{\lambda mid} = I_{NOM} + Q \times S_{NOM} \tag{5}$$

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

334

$$S_{NOM} = S_b + S_a \times (A_{1,\lambda mid} + A_{2,\lambda mid})$$

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

(6)



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345 **Figure 4**. (a) Intercept I<sub>NOM</sub> and (b) slope S<sub>NOM</sub> of linear regression between  $A_{\lambda mid}$  and Q (equation 5) as a function of  $A_{0,\lambda mid}$  and  $A_{1,\lambda mid}$  +  $A_{2,\lambda mid}$ , respectively for all IHSS samples (SRFA, SRHA, 346 347 SRNOM, PPFA, PPHA, LHA and ESHA), as well as the literature data (PAHA 0.01-0.5; values refer to the ionic strength;<sup>32</sup>), for comparison. Linear regressions do not take the PAHA data into account. 348 349 Data from the literature on PAHA<sup>32</sup> were used to corroborate the above-proposed approach 350 using an independent dataset. Because PAHA was titrated by both spectrophotometric and 351 potentiometric acid-base titration data at three different background electrolyte concentrations 352  $(0.01, 0.1 \text{ and } 0.5 \text{ mol } L^{-1} \text{ in NaClO}_4)$ , the method described here could be tested on a larger 353 range of physico-chemical conditions than the concentration used to establish the method (0.01 354 mol L<sup>-1</sup> of NaCl). Hereafter, the three PAHA samples will be referred to as "PAHA 0.01", 355 "PAHA 0.1" and "PAHA 0.5" according to their respective background electrolyte 356 357 concentrations. As described above for the seven IHSS samples studied here, the three PAHA specific absorbance datasets were deconvoluted using equation 3. After a close inspection of 358 their  $A_{1,\lambda mid}$  and  $A_{2,\lambda mid}$  values (Figure 5), a  $\lambda_{mid}$  value for each NaClO<sub>4</sub> concentration was 359 selected (Appendix G, Table S3 in the SI). Using the potentiometric titration, data from Janot 360

et al.,<sup>32</sup>  $A_{\lambda \text{mid}}$  were plotted as a function of Q in Figure 3 and specific  $I_{PAHA}$  and  $S_{PAHA}$  were 361 extracted and plotted in Figures 4a and 4b, respectively. The three PAHA samples are in very 362 good agreement with the linear relationship established using the seven different IHSS samples, 363 showing an increase in both I<sub>NOM</sub> and S<sub>NOM</sub> with increasing electrolyte concentration. The 364 background electrolyte concentration affects proton-NOM binding and, hence, Q as it impacts 365 the electrostatic contribution to the overall interaction between HS and protons.<sup>40</sup> This was 366 previously shown to affect NOM absorbance.<sup>38</sup> Furthermore, ionic strength has more impact on 367 absorbance than on charge measurement. This can be explained by the aggregation of NOM 368 369 molecules at higher ionic strength, due to charge screening, increasing the interactions between chromophores.<sup>32</sup> Thus, for a same sample, I<sub>NOM</sub> and S<sub>NOM</sub> values increase with ionic strength, 370 which is accompanied by an increase in  $A_{0,\lambda \text{mid}}$  and  $A_{1,\lambda \text{mid}} + A_{2,\lambda \text{mid}}$ , respectively. The 371 consistency between the observations made on an independent sample in various ionic strength 372 conditions and on our reference samples validates the approach developed here. 373

As such, we propose a condensed empirical equation enabling the estimation of Q directly from the specific absorbance data. This empirical equation combines equation 3 with the individual equations relating  $I_{NOM}$  to  $A_{0,\lambda mid}$  ( $I_{NOM} \approx A_{0,\lambda mid}$ ) and linearly relating  $S_{NOM}$  to  $A_{1,\lambda mid} + A_{2,\lambda mid}$ (Figure 4; eq. 6).

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

380

By using a least-square fitting procedure, and all the available data in the present work for the 381 IHSS and PAHA samples, we estimated  $S_a = 0.089 \pm 0.003$  and  $S_b = -0.228 \pm 0.016$  (root-mean-382 square error, RMSE = 1.29). The assumption of a linear relationship instead of equality between 383  $A_{0,\lambda mid}$  and  $I_{NOM}$  was not statistically justified because the decrease in RMSE associated with the 384 optimization of two additional parameters was very small (RMSE = 1.30). The accuracy of the 385 present approach to predict Q is shown in Figure S5 (Appendix L in the SI), where 86% of the 386 experimentally determined values can be calculated ( $Q_{calc}$ ) within an error range of  $\pm 2$  mol 387 kgC<sup>-1</sup>. Table S5 (Appendix M in the SI), shows the RMSE values for the individual datasets, 388 and demonstrates that the largest error is attributed to SRHA and PAHA 0.01 (RMSE = 2.24389 390 and 2.38, respectively), whereas, for the other NOM samples, 0.48 (PAHA 0.1)  $\leq$  RMSE  $\leq$  1.13 391 (ESHA).



Figure 5. Results of the deconvolution of the acid-base spectrophotometric titration data with equation
394 3 for the PAHA samples in (a) 0.01, (b) 0.1 and (c) 0.5 M NaClO<sub>4</sub>.

# 395 Environmental implications396

Although many important mechanisms involve NOM acid-base functional groups, it is difficult 397 to quantify them in natural samples, especially since it is still a major challenge to determine 398 the NOM charge at environmentally relevant concentrations. To overcome this, we developed, 399 400 in the present study, a novel UV-vis spectrophotometric method based on a previously established relationship between the  $\Delta A$  and  $\Delta Q$  of a NOM sample (PAHA).<sup>32</sup> By extending 401 this finding to various NOM samples, using a recently collected potentiometric titration 402 dataset,<sup>40</sup> it was possible to provide an empirical approach to estimate the charge values of 403 NOM samples using UV-vis absorption data only. The parameters defining the linear 404 relationship between A and Q, i.e. I<sub>NOM</sub> and S<sub>NOM</sub>, were themselves linearly related to the proton 405 inert chromophores  $A_{0,\lambda mid}$  and to the sum of both proton sensitive chromophores  $A_{1,\lambda mid}$  and 406  $A_{2,\lambda mid}$ , respectively. The advantage of the empirical approach proposed here to estimate Q is 407 that it (i) does not require to perform a potentiometric acid-base titration, (ii) is based on sample-408 409 specific spectra parameters ( $A_{0,\lambda mid}$   $A_{1,\lambda mid}$  and  $A_{2,\lambda mid}$ ) and (iii) is also suitable at different 410 background electrolyte concentrations (in NaClO<sub>4</sub>) compared to the studied concentrations. Because spectroscopy is suitable for NOM sample characterization in terms of their 411 412 environmental concentration, the novel spectrophotometric deconvolution method developed 413 in the present study, and sample specific empirical spectra analysis could be used to approximate the Q of unknown natural samples and subsequently estimate their carboxylic and 414 phenolic functional groups' concentrations. However, in contrast to Na<sup>+</sup>, other cations such as 415 Cu<sup>2+</sup> or Al<sup>3+</sup> can bind to NOM and affect the absorbance signal.<sup>47</sup> The present approach might 416 be applicable to natural samples containing cations other than monovalent ones if the 417 418 interferences on the absorbance spectra they generate remain insignificant. Otherwise, it might be necessary to remove these cations from the sample using a simple purification procedure. 419

420 The present approach further allows the estimation of the NOM charge in various aquatic systems, which is critical to understanding the role of NOM in environmental studies. 421 422 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 provides important keys for understanding and predicting the biogeochemical cycle of NOM 426 427 and its associated elements.

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#### 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 ( $\lambda_{mid}$ ) 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  $A_{1,\lambda mid}$  and  $A_{2,\lambda mid}$  are selected, shown in two steps
- 453 Appendix I: Equation S12 to convert Q from mol kgNOM<sup>-1</sup> to mol gC<sup>-1</sup>
- 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

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