

On the Acid–Base Properties of Humic Acid in Soil

JAMES D. COOKE,^{*,†}
JOHN HAMILTON-TAYLOR,[†] AND
EDWARD TIPPING[‡]

*Environmental Science Department, Lancaster University,
Lancaster LA1 4YQ, United Kingdom, and Centre for Ecology
and Hydrology (Lancaster), Library Avenue, Lancaster LA1
4AP, United Kingdom*

Humic acid was isolated from three contrasting organic-rich soils and acid–base titrations performed over a range of ionic strengths. Results obtained were unlike most humic acid data sets; they showed a greater ionic strength dependency at low pH than at high pH. Forward- and back-titrations with the base and acid revealed hysteresis, particularly at low pH. Previous authors attributed this type of hysteresis to humic acid aggregates—created during the isolation procedure—being redissolved during titration as the pH increased and regarded the results as artificial. However, forward- and back-titrations with organic-rich soils also demonstrated a similar hysteretic behavior. These observations indicate (i) that titrations of humic acid in aggregated form (as opposed to the more usual dissolved form) are more representative of the acid–base properties of humic acid in soil and (ii) that the ionic strength dependency of proton binding in humic acid is related to its degree of aggregation. Thus, the current use of models based on data from dissolved humic substances to predict the acid–base properties of humic acid in soil under environmental conditions may be flawed and could substantially overestimate their acid buffering capacity.

Introduction

The interactions of humic substances with cations exert a powerful chemical influence in the environment, being important in processes such as pH buffering, metal binding and transport, and nutrient control. The interactions involve both solid-phase material in soils and sediments and dissolved material in natural waters. Humic acid and fulvic acid are usually considered to be the major humic substances involved in cation binding. Broadly speaking, humic acid occurs mostly in soils and sediments as part of the solid phase, while fulvic acid, being more soluble, accounts for a major part of the dissolved organic matter in natural waters (1).

Acidic functional groups on humic and fulvic acids (mainly carboxylic and phenolic) determine their binding characteristics for protons and other major cations. Therefore, the acquisition of detailed information about these functional groups is a primary task in the study of cation–humic interactions. Such information is most commonly obtained from acid–base titrations of humic and fulvic acids that have

been isolated from environmental samples, thus enabling their acid–base properties to be studied without interference from other soil and water components.

Mathematical models have been developed to describe cation binding equilibria with isolated humic and fulvic acids. Currently, the two most established models are the Humic Ion Binding Model VI (2) (a component of WHAM—a general equilibrium chemical speciation model (3)) and the NICA-Donnan model (4). These comprehensive models account for two of the key characteristics of humic substances, namely, the heterogeneity of the cation binding functional groups and the ionic strength dependency of cation binding (arising from the electrostatic properties of humic molecules). Both models have been fitted to many acid–base titration data sets (2, 5), and the default or generic parameters can be considered to represent the average proton binding properties of isolated humic or fulvic acids.

Although most acid–base titration data sets for isolated humic or fulvic acids are reasonably well-described by Model VI and the NICA-Donnan model, a number of authors have reported observations that are difficult to interpret or which complicate the simple picture of molecules undergoing reversible proton dissociation (1). A common problematic observation is hysteresis—where a forward-titration path with a base is not followed by a back-titration with acid (6–11).

Much is known about the cation binding properties of isolated humic and fulvic acids under well-defined laboratory conditions, and ideally, we would like to use such knowledge to predict the cation binding properties of soil humic substances *in situ*. However, this is by no means straightforward for a number of reasons. In laboratory experiments, much of the information is derived from isolated humic substances that are fully hydrated, which may not always be the case in soils *in situ* (1). Humic substances undergo a number of physical interactions in soils such as aggregation. The most familiar example of this is the precipitation of humic acid at low pH, a phenomenon that is exploited in its isolation—at low pH, the charge carried by the molecules is much reduced, and the hydrophobic effect causes aggregation to take place.

In an attempt to achieve more realistic conditions, a few authors have used organic-rich soils to investigate the acid–base properties of humic substances in the solid phase (12–14). Preferably, the organic-rich soil will be free of inorganic cations and anions (except for H⁺), a state that can be achieved by acid-washing and exhaustive dialysis. This acid-washing approach has been used successfully by a number of authors (15–17). However, forward- and back-titrations have not been performed with these organic-rich soils, and their hysteretic behavior is unknown, although hysteresis has been noted in an acid sandy soil (18).

In this paper, we investigate the acid–base properties of three isolated soil humic acids and relate their observed hysteretic behavior to previous reports of hysteresis in the literature and to that of fulvic acid and organic-rich soil both acid-washed and untreated, to demonstrate that aggregation has a significant effect on the acid–base properties of humic acid in soil.

Hysteresis. Close examination of the published data for isolated humic substances reveals that hysteresis can be divided into two types. In most examples, the difference between the forward-titrations (base) and the back-titrations (acid) is greatest at high pH, and the sample returns to near its original pH on completion of the back-titration. This high pH hysteresis occurs in humic acids (10) and fulvic acids (6–9) and is considered by some authors to be due to

* Corresponding author. E-mail: j.cooke1@lancaster.ac.uk; phone: +44 1524 595800; fax: +44 1524 61536.

[†] Lancaster University.

[‡] Centre for Ecology and Hydrology (Lancaster).

TABLE 1. Soil Site Details

soil	dominant flora	location
ranker	mat grass (<i>Nardus stricta</i>)	54°41' N, 03°14' W
podzol	Norway spruce (<i>Picea abies</i>) Scots pine (<i>Pinus sylvestris</i>) birch (<i>Betula pubescens</i>)	58°22' N, 08°13' E
peat	sphagnum moss (<i>Sphagnum</i> spp.) heather (<i>Calluna vulgaris</i>) cotton grass (<i>Eriophorum</i> spp.)	54°68' N, 02°38' W

TABLE 2. Analysis of Isolated Humic Acid Samples

	ranker	podzol	peat
total C (%)	50.2	56.0	55.7
total N (%)	3.7	2.5	3.0

secondary reactions consuming the base (8, 19). Other authors consider it to be a result of slow protonation and deprotonation reactions (10), which is supported by the fact that the sample returns to its original pH at the end of the back-titration. Where this type of hysteresis is observed, it is usually the case that the titrations have been performed relatively quickly, and so the measurements were probably not at equilibrium.

In the second type of hysteresis, the difference in the forward- and back-titrations is greatest at low pH, and the sample does not return to its original pH at the end of the back-titration but instead to a significantly lower value. In titrations where this low pH hysteresis has been observed, care was taken to ensure that the pH measurements were at equilibrium, and it was noted that repeated titration to high and low pH eliminated the hysteretic effect (11). Low pH hysteresis has been seen in humic acids but not fulvic acids. Fulvic acid is soluble at all pH values in aqueous solutions; however, humic acid is only soluble at high pH. Most authors raise the solution pH to dissolve the (usually lyophilized) humic acid prior to lowering the pH before the start of a forward base-titration (20–23). Others have used humic acid that has not been lyophilized and titrated it in an aggregated form from low pH (11). It is in these titrations of aggregated humic acid that low pH hysteresis is observed.

Materials and Methods

Samples of organic-rich soil horizons were collected from three contrasting temperate-region soils: (i) A-horizon from a humic ranker, Duddon Valley, Lake District, UK; (ii) O_H-horizon from a podzol, Birkenes Forest, Southern Norway; and (iii) O-horizon from a blanket peat, Moor House, North Pennines, UK. Further soil site details are provided in Table 1. The samples—termed ranker, podzol and peat—were sieved to remove any stones or roots and stored in a refrigerator.

Humic acid was isolated from sub-samples of each soil using a method based on the International Humic Substances Society (IHSS) method (24); however, the HCl/HF treatment was omitted as the samples already had low ash contents. Samples were subjected to exhaustive dialysis against deionized water using cellulose tubing with a molecular weight cutoff of 12 000 (Sigma D-9402). After dialysis, the humic acids were not lyophilized but stored as stock suspensions in a refrigerator. A sample of freeze-dried Suwannee River fulvic acid (1S101F) was also purchased from the IHSS as a reference.

Soil sub-samples were acid-washed by suspending in deionized water and adjusting the pH to 1 with concentrated HNO₃. The samples were shaken for ca. 1 min and then left in the dark at room temperature for at least 16 h, allowing the suspended particles to settle out. Supernatants were

removed using a peristaltic pump, the container refilled with deionized water and the solution returned to pH 1. The acid-washing procedure was repeated seven times. Samples were then subjected to exhaustive dialysis in an equivalent manner to the humic acids. After dialysis, the acid-washed soils were stored as stock suspensions in a refrigerator.

Analysis. The organic matter content of the soils was estimated by determining loss-on-ignition of oven dried samples in a laboratory furnace (550 °C for 2 h). Samples of isolated humic acid were analyzed for total C and total N (Universal CHNS-O Vario EL elemental analyzer).

Titration. Acid–base titrations were carried out manually in continuous mode at a comparatively large scale (500 mL). Measurements of pH were made with a combination glass electrode and meter (Radiometer GK2401C and PHM82), which underwent a regular quality control procedure (25). Performance of the experimental setup was verified by carrying out acid–base titrations of KH₂PO₄. Humic acid and acid-washed soil titration solutions were prepared from stock suspensions by weighing out well-mixed aliquots—stock suspension concentrations were determined by drying triplicate aliquots to a constant weight at ca. 110 °C in a laboratory oven. An untreated soil titration solution was prepared by weighing out moist soil. Fulvic acid titration solution was prepared by weighing out freeze-dried material. All titrations were carried out in a water bath at 10 °C. Fulvic acid titrations were performed at 2 g L⁻¹, humic acid titrations at 4 g L⁻¹, and acid-washed soil and untreated soil titrations at 20 g L⁻¹. The relatively high concentrations of humic material ensured good precision in the pH measurements.

Titrations were performed at four initial background electrolyte concentrations; 0.3, 3, 30, and 300 mM NaNO₃. No base was added prior to any of the titrations to help dissolve the samples, and all titrations were started from the initial low pH of the solution. Particulate material was kept in suspension by continual stirring with a Teflon-coated magnetic stirrer bar.

Because of the surfactant properties of humic substances, the suspensions were not degassed during the titrations but were bubbled with oxygen-free nitrogen for ca. 5 min prior to the titrations and then kept under a nitrogen blanket. The nitrogen was bubbled through a ca. 0.2 M NaOH, followed by deionized water, to ensure it was CO₂-free and water-saturated. The pH probe was calibrated immediately before and after each titration using pH 4, 7, and 10 IUPAC buffers, pre-equilibrated at 10 °C. Any drift in response over the course of a titration was taken into consideration in the calculation of pH by linear interpolation.

Approximately 0.5 M carbonate-free NaOH was prepared and standardized against acid by Gran titration. For forward-titrations, the NaOH was dispensed from a calibrated autoburette (Radiometer ABU80) fitted with a trap containing Carbosorb, to minimize the sorption of CO₂ by the NaOH solution. For back-titrations, 0.5 M HNO₃ was prepared from ConvoL solutions and dispensed by calibrated pipet. Using concentrated solutions of base and acid kept the volume change on addition of titrant to a minimum. Care was taken to ensure that the system had reached equilibrium before measurements were taken and further titrant added. This was only done when the drift on the electrode was less than 1 mV over a 10 min period (≈0.002 pH min⁻¹). Titrations typically took between 7 and 12 h to complete. The manual nature of the titrations and the care taken to ensure equilibrium resulted in approximately six data points for each titration.

Results and Discussion

Loss-on-ignition results gave estimates for the organic matter content of the soils as; ranker 71%, podzol 71%, and peat 97%. Table 2 shows results of the isolated humic acid analysis.

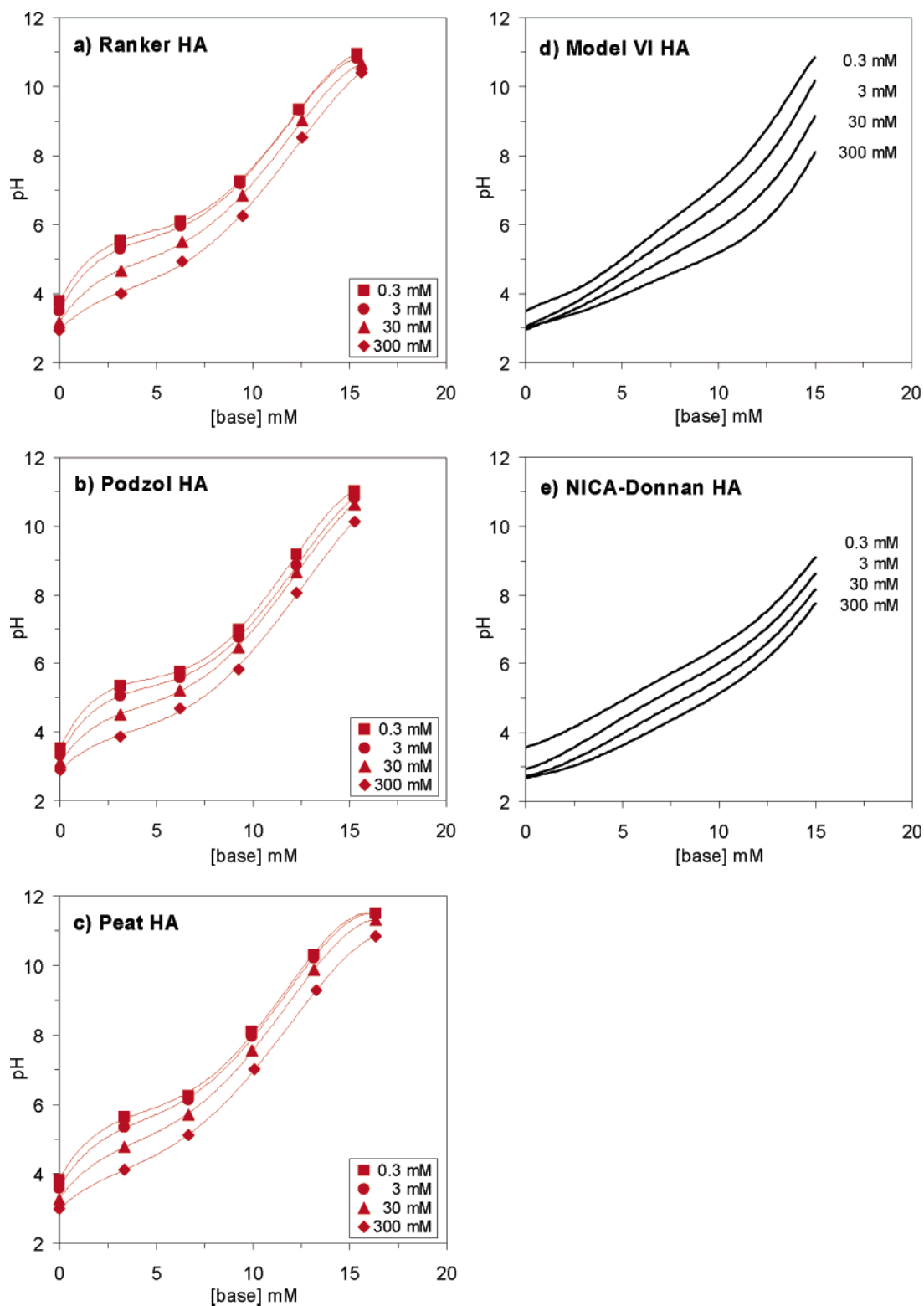


FIGURE 1. Forward-titrations (base) of 4 g L^{-1} humic acid (HA) in 0.3, 3, 30, and 300 mM background electrolyte (NaNO_3) at 10°C . (a–c) Titrations of initially aggregated humic acids, isolated from ranker, podzol, and peat soils (points are experimental values joined by trend lines). (d and e) Model VI and NICA-Donnan predictions, using default or generic parameters for humic acid.

The total C and total N values for the isolated humic acids are within the normal range observed in the literature (26).

Humic Acid Titrations. Experimental results for forward-titrations (base) of humic acid isolated from the ranker, podzol, and peat samples, at four different concentrations of background electrolyte, are shown by the points in Figure 1a–c. Results for the three samples are alike, showing that all the humic acids have comparable acid–base properties

and thus similar acidic functional groups. Results show the usual dependence upon ionic strength (i.e., titrations at higher ionic strength required a larger volume of base to reach a given pH). However, they indicate that the ionic strength dependency increases to a maximum at ca. pH 5 and then decreases again, seen as a bulge in the spacing of the titration curves at low pH. The results show comparatively little buffering at low pH and low ionic strength and differ

significantly from the results of most humic acid titrations—represented in Figure 1d,e by the predictions of Model VI and the NICA-Donnan model using default or generic parameters for humic acid. However, similar results have been seen before with humic acid in the results of Milne et al. (11) and Barak and Chen (27).

Milne et al. further investigated the effect by performing consecutive forward-titrations (base) and back-titrations (acid), at 4 g L^{-1} and low ionic strength, to make two complete cycles. Considerable hysteresis of the low pH type was evident in the first cycle but was much reduced in the second cycle. Similar hysteresis was also evident at 0.4 g L^{-1} . For these experiments, the humic acid preparation method was comparable to that used in the present study; the titration solutions were prepared from stock suspensions, and the humic acid was not dissolved by raising the pH prior to titration. Milne et al. attributed the hysteretic effects to a rapid disaggregation of humic acid on the first forward leg (increasing pH, increasing negative charge) but concluded that the reverse aggregation reaction going from high pH to low pH was so slow that it did not occur to any great extent during the course of the experiment. They proposed that the hysteresis was possibly an experimental artifact arising from the method of preparation of the humic acid since the final steps in the isolation and purification procedure involve H^+ -saturation and centrifugation, which causes aggregates to form.

Hysteresis in Humic and Fulvic Acid. Experimental results for forward-titrations (base) and back-titrations (acid) of humic acid isolated from the ranker sample, at low ionic strength, are shown by the points in Figure 2a. They demonstrate the same low pH hysteresis as observed by Milne et al. The results imply that during the forward-titration, the humic acid has a lower (negative) charge per gram than during the back-titration, particularly at low pH.

It was possible that this hysteresis was apparent rather than real, being due to the ionic strength of the solution increasing during the course of a titration because of base and acid additions. Therefore, we estimated the effect that this change in ionic strength might have on the humic acid charge, based on the ionic strength predictions of Model VI. Results indicated that the size of this effect is small.

Experimental results for the ranker humic acid are compared to the predictions of Model VI for the back-titration, using default parameters for humic acid, in Figure 2b. The results show that during the back-titration, the humic acid behaved as predicted by the model and hence as reported by most other authors. Generally, other authors have dissolved humic acid prior to titration. Thus, this result supports the conclusions of Milne et al.—that, for this type of hysteresis, the humic acid is in an aggregated form at the start of the forward-titration, but dissolves as the pH is raised, and remains in solution for the back-titration.

Experimental results for forward- and back-titrations of the IHSS Suwannee River fulvic acid, at low ionic strength, are shown by the points in Figure 2c. They indicate that fulvic acid does not exhibit any significant hysteresis; the forward- and back-titration curves are similar to each other and to the predictions of Model VI for the back-titration using default parameters for fulvic acid. This is presumably because fulvic acid is soluble at all pH values and thus in a similar physical form throughout the titrations. The results also indicate that our experimental setup was giving similar results to previous studies, providing further validation of the measurements.

Hysteresis in Acid-Washed and Untreated Soil. Experimental results for forward-titrations (base) and back-titrations (acid) of the acid-washed ranker sample are shown by the points in Figure 3a. They indicate that humic substances in soil also exhibit low pH hysteresis. However, during their

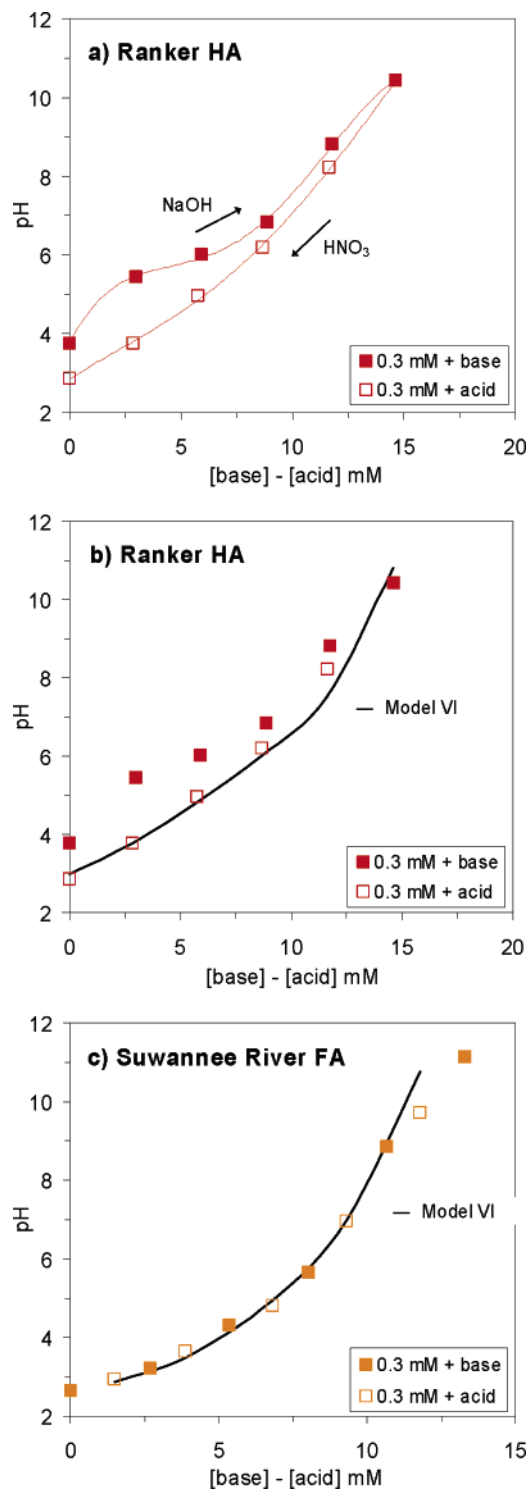


FIGURE 2. Forward-titrations (base) and back-titrations (acid) of 4 g L^{-1} ranker humic acid (HA) and 2 g L^{-1} Suwannee River fulvic acid (FA) in 0.3 mM background electrolyte (NaNO_3) at 10°C . (a) Acid–base hysteresis in ranker humic acid (points are experimental values joined by trend lines). (b and c) Comparison of observations for ranker humic acid and Suwannee River fulvic acid with Model VI predictions for back-titrations, using default parameter values.

preparation, the soils were only subjected to settling under gravity; thus, this hysteresis is not an artifact caused by centrifugation. To investigate if the hysteretic effect was caused by acidification during acid-washing, forward- and back-titrations were performed with the acid-washed peat sample, but the back-titration was continued until the pH

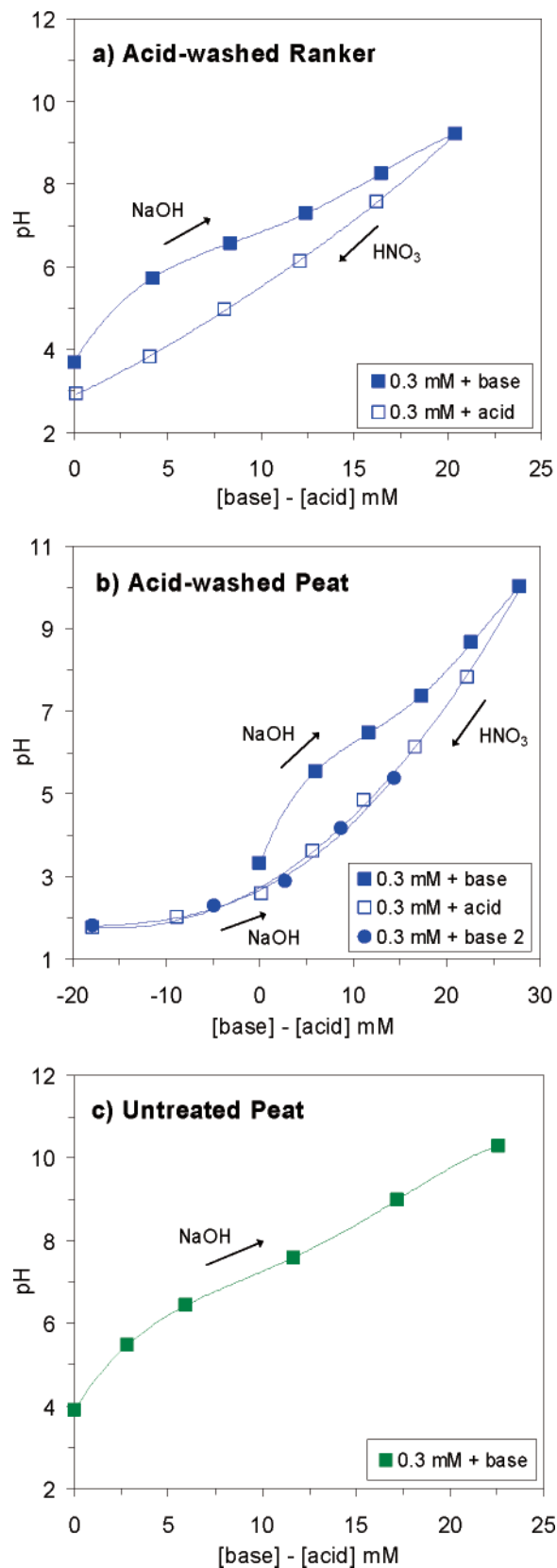


FIGURE 3. Forward-titrations (base) and back-titrations (acid) of 20 g L^{-1} acid-washed and untreated organic-rich soil in 0.3 mM background electrolyte (NaNO_3) at 10°C (points are experimental values joined by trend lines). (a) Acid–base hysteresis in acid-washed ranker. (b) Acid–base hysteresis in acid-washed peat, demonstrating that hysteresis is not caused by acidification. (c) Forward-titration of untreated peat, indicating similarity to acid-washed soil forward-titrations.

was less than 2. The sample was left at this pH overnight and then forward-titrated again. Experimental results, shown by the points in Figure 3b, demonstrate that the second forward-titration curve follows the back-titration curve—indicating that acidification alone does not cause this type of hysteresis.

As a final test of whether this hysteresis is an artifact caused by the preparation procedure, a forward-titration was performed with an untreated soil sample. Peat was chosen for this because, as compared to the other soil types, it contains less bound metal to interfere with the acid–base titration. Experimental results, shown by the points in Figure 3c, clearly indicate that the shape of the untreated peat forward-titration curve is similar to the two acid-washed soil forward-titration curves, particularly at low pH where the hysteretic effect is greatest. Any difference in the position of the untreated and acid-washed peat titration curves is most probably due to other cations contributing to the overall ion balance.

From these results, we conclude that the unusual ionic strength dependency noted in our isolated humic acid data sets (Figure 1a–c) (i.e., the bulge in the spacing of the titration curves at ca. pH 5) is due to the humic acid being in the aggregated form at low pH and dissolved form at high pH. We propose that the ionic strength dependency of proton binding in humic acid is related to its degree of aggregation—when humic acid is in an aggregated form at low pH, it has a greater ionic strength dependency than when in the dissolved form at high pH. Our results also indicate that forward-titrations of humic acid in the aggregated form are more representative of the acid–base properties of humic acid in soil, as compared to titrations in which humic acid is in solution throughout—presumably because the physical state of aggregation brought about by centrifuging during isolation is closer to the physical state of the humic substances in soil.

Mechanisms. There are a number of possible physico-chemical mechanisms by which aggregation may give rise to the observed hysteretic phenomenon (Figure 2a) (i.e., the comparatively little buffering at low pH and low ionic strength in the aggregated state). One possibility is a kinetic effect, due to the slow penetration of ions into the aggregated material. However, to test this hypothesis, titrations would have to be performed over long timescales. A second possibility is an electrostatic effect, in which the acidities of the functional groups change through local electrostatic interactions. As the difference between the forward-titration results (Figure 1a–c) and the prediction of the models (Figure 1d,e) is greatest at low ionic strength—where the electrostatic effect is most influential—we suggest that the underlying cause of the observed hysteretic phenomenon is probably electrostatic.

The comparatively little buffering at low pH and low ionic strength is a consequence of the greater ionic strength dependency of proton binding in the aggregated form, and the ionic strength dependency is indicative of the electrostatic contribution—a point emphasized by Marinsky (28). An explanation of the greater ionic strength dependency of humic acid in an aggregated form can be proposed if we consider two extreme cases: (i) a freely expanding diffuse layer case—where the volume that the diffuse electrical layer around each molecule can expand into is unrestricted and (ii) a fixed-volume Donnan case—where molecular packing causes the volume of the diffuse electrical layer to be fixed, creating a Donnan system. The ionic strength dependency of proton binding is greater in a fixed-volume Donnan case; the apparent pK_a values of binding sites, calculated from bulk solution pH, vary by approximately 1 log unit per decadal change in ionic strength (except when the degree of dissociation is low) (1). If this were the case for aggregated humic acid, the forward-titration curves (Figure 1a–c) would be

separated by approximately 1 pH unit at low pH. The observed ionic strength dependency is not as large as this, but we propose that humic acid in an aggregated form is closer to a fixed-volume Donnan case than humic acid in a dissolved form.

Implications of Results. Our results demonstrate that aggregated and dissolved humic acid have different proton binding properties, and this has implications for understanding the interaction of humic acid with other major cations. However, it is useful to obtain some estimate of the magnitude and environmental significance of the difference in proton binding. The buffering capacity of soil organic matter is traditionally measured in terms of total acidity. One method of determining total acidity is by titration of a soil suspension in a salt solution, using a strong base, to a reference pH (29). This definition of buffering can be applied to our experimental data. In the titration of the acid-washed ranker sample, shown in Figure 3a, the base required to titrate to pH 7 in the dissolved form (lower curve) would be 14.6 mM, while in the aggregated form (upper curve), it would be 10.9 mM (i.e., 25% less). Organic-rich soils are often acidic in their natural state, and the buffering difference between dissolved and aggregated forms is even greater at low pH. The base required to titrate to pH 5 in the dissolved form would be 8.2 mM, while in the aggregated form, it would be 2.2 mM (i.e., 73% less). This situation is mirrored in the ranker humic acid (total acidity to pH 5: 6.1 to cf. 1.6 mM, -74% less).

While these comparisons are only approximations, it would seem reasonable to assume that the current use of models based on data from dissolved humic substances to predict the acid-base properties of humic acid in soil under environmental conditions may be flawed and could substantially overestimate their acid buffering capacity.

Acknowledgments

This research was funded by the U.K.'s Natural Environment Research Council (NERC). Gratitude is expressed to Jan Mulder and Nicholas Clarke for help in obtaining the Norwegian soil sample and to Sarah Thacker for help in sample analysis. We thank each of the three anonymous referees for their insightful comments, which led to improvements in the paper.

Literature Cited

- (1) Tipping, E. *Cation binding by humic substances*; Cambridge University Press: New York, 2002.
- (2) Tipping, E. Humic Ion Binding Model VI: an improved description of the interactions of protons and metals ions with humic substances. *Aquat. Geochem.* **1998**, *4*, 3–48.
- (3) Tipping, E. WHAM—a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion binding by humic substances. *Comput. Geosci.* **1994**, *20*, 973–1023.
- (4) Kinniburgh, D. G.; van Riemsdijk, W. H.; Koopal, L. K.; Borkovec, M.; Benedetti, M. F.; Avena, M. J. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry, and thermodynamic consistency. *Colloids Surf., A* **1999**, *151*, 147–166.
- (5) Milne, C. J.; Kinniburgh, D. G.; Tipping, E. Generic NICA-Donnan model parameters for proton binding by humic substances. *Environ. Sci. Technol.* **2001**, *35*, 2049–2059.
- (6) Davis, H.; Mott, C. J. B. Titrations of fulvic acid fractions I: Interactions influencing the dissociation/reprotonation equilibria. *J. Soil Sci.* **1981**, *32*, 379–391.
- (7) Paxéus, N.; Wedborg, M. Acid-base properties of aquatic fulvic acid. *Anal. Chim. Acta* **1985**, *169*, 87–98.
- (8) Bowles, E. C.; Antweiler, R. C.; MacCarthy, P. In *Humic substances in the Suwannee River, Georgia: Interactions, properties, and proposed structures*; U.S. Geological Survey (open-file report 87-557): Reston, VA, 1989; pp 209–229.
- (9) Santos, E. B. H.; Esteves, V. I.; Rodrigues, J. P. C.; Duarte, A. C. Humic substances' proton binding equilibria: Assessment of errors and limitations of potentiometric data. *Anal. Chim. Acta* **1999**, *392* (2–3), 333–341.
- (10) Marshall, S. J.; Young, S. D.; Gregson, K. Humic-acid proton equilibria—A comparison of two models and assessment of titration error. *Eur. J. Soil Sci.* **1995**, *46*, 471–480.
- (11) Milne, C. J.; Kinniburgh, D. G.; de Wit, J. C. M.; Van Riemsdijk, W. H.; Koopal, L. K. Analysis of proton binding by a peat humic acid using a simple electrostatic model. *Geochim. Cosmochim. Acta* **1995**, *59*, 1101–1112.
- (12) Tipping, E.; Berggren, D.; Mulder, J.; Woof, C. Modeling the solid-solution distribution of protons, aluminium, base cations, and humic substances in acid soils. *Eur. J. Soil Sci.* **1995**, *46*, 77–94.
- (13) Lofts, S.; Woof, C.; Tipping, E.; Clarke, N.; Mulder, J. Modeling pH buffering and aluminum solubility in European forest soils. *Eur. J. Soil Sci.* **2001**, *52*, 189–204.
- (14) Gustafsson, J. P.; Berggren, D. Modeling salt-dependent proton binding by organic soils with the NICA-Donnan and Stockholm humic models. *Environ. Sci. Technol.* **2005**, *39*, 5372–5377.
- (15) Bloom, P. R.; McBride, M. B. Metal ion binding and exchange with hydrogen ions in acid-washed peat. *Soil Sci. Soc. Am. J.* **1979**, *43*, 688–692.
- (16) Marinsky, J. A.; Wolf, A.; Bunzl, K. The binding of trace amounts of lead(II), copper(II), cadmium(II), zinc(II), and calcium(II) to soil organic matter. *Talanta* **1980**, *27*, 461–468.
- (17) Smith, E. J.; Rey-Castro, C.; Longworth, H.; Lofts, S.; Lawlor, A. J.; Tipping, E. Cation binding by acid-washed peat, interpreted with Humic Ion Binding Model VI-FD. *Eur. J. Soil Sci.* **2004**, *55*, 433–447.
- (18) Kinniburgh, D. G.; Milne, C. J.; Venema, P. Design and construction of a personal computer-based automatic titrator. *Soil Sci. Soc. Am. J.* **1995**, *59*, 417–422.
- (19) Ritchie, J. D.; Perdue, E. M. Proton binding study of standard and reference fulvic acids, humic acids, and natural organic matter. *Geochim. Cosmochim. Acta* **2003**, *67*, 85–96.
- (20) Stevenson, F. J. Stability constants of Cu²⁺, Pb²⁺, and Cd²⁺ complexes with humic acids. *Soil Sci. Soc. Am. J.* **1976**, *40*, 665–672.
- (21) Westall, J. C.; Jones, J. D.; Turner, G. D.; Zachara, J. M. Models for association of metal ions with heterogeneous environmental sorbants. 1. Complexation of Co(II) by Leonardite humic acid as a function of pH and NaClO₄ concentrations. *Environ. Sci. Technol.* **1995**, *29*, 951–959.
- (22) Robertson, A. P. Geothite/humic acid interactions and their effect on copper(II) binding. Ph.D. Thesis, Stanford University, Stanford, CA, 1996.
- (23) Avena, M. J.; Koopal, L. K.; van Riemsdijk, W. H. Proton binding to humic acids: Electrostatic and intrinsic interactions. *J. Colloid Interface Sci.* **1999**, *217*, 37–48.
- (24) Swift, R. S. Organic matter characterization. In *Methods of Soil Analysis. Part 3. Chemical methods*; Sparks, D. L., Ed.; Soil Science Society of America: Madison, WI, 1996; pp 1018–1020.
- (25) Davison, W. A practical guide to pH measurement in freshwater. *Trends Anal. Chem.* **1990**, *9*, 80–83.
- (26) Tan, K. H. *Principles of soil chemistry*, 2nd ed.; Marcel Dekker: New York, 1993.
- (27) Barak, P.; Chen, Y. Equivalent radii of humic macromolecules from acid-base titration. *Soil Sci.* **1992**, *154*, 184–195.
- (28) Marinsky, J. A. A two-phase model for the interpretation of proton and metal ion interaction with charged polyelectrolyte gels and their linear analogs. In *Aquatic Surface Chemistry*; Stumm, W., Ed; John Wiley and Sons, Inc.: New York, 1987; pp 49–81.
- (29) Bloom, P. R. Soil pH and pH buffering. In *Handbook of Soil Science*; Sumner, M. E., Ed; CRC Press: Boca Raton, FL, 2000; pp 333–352.

Received for review June 14, 2006. Revised manuscript received October 12, 2006. Accepted October 13, 2006.

ES061424H