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Proton interactions with soil organic matter

Proton interactions with soil organic matter; the importance of aggregation and the weak acids of humin

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Summary

Samples of three organic-rich soils (ombrotrophic peat, podzol H-horizon, humic ranker) were extensively washed with dilute nitric acid, dialysed against deionised water, and then subjected to acid-base titrations over the pH range 3 – 10, in 0.3 – 300 mM $NaNO_3$, and with soil concentrations in the range 2 to 150 g l^{-1}. The results for the three soils were quantitatively similar. Comparison of the titration data with previously published results for humic acids isolated from the same soils showed the soil organic matter to have a greater ionic strength dependency of proton binding and to possess relatively greater buffering capacity at high pH, attributable to weak acid groups (c. 2 - 5 mmol g^{-1}) in the humin fraction of the soils. To describe the soil titration data quantitatively, we modified Humic Ion-Binding Model VI-FD, which utilises a fixed Donnan volume to describe counterion accumulation, by increasing the content of weak acid groups. When artefacts in pH measurement due to the suspension effect were taken into account, the resulting Model VI-FD2 provided good or fair simulations of all the titration data. The results suggest that soil structure, specifically aggregation, plays a significant role in cation binding by organic soils *in situ*. The lack of dependence of the titration results on soil suspension concentration suggests that the findings can be applied to soils *in situ*.

Introduction

The binding of protons and other cations by organic matter is of interest for a variety of reasons, including pH buffering, metal binding and transport, and nutrient control. To predict the cation binding reactions involved in these processes correctly, it is necessary to have an accurate description of the acid-base properties of soil organic matter (Tipping, 2002). Soil organic matter consists of a variety of entities, among which humic substances dominate its cation binding properties. Two well-established models of cation binding by humic substances are Humic Ion-Binding Model VI (Tipping, 1998) and the NICA-Donnan model (Kinniburgh *et al*., 1999). These comprehensive models account for two key characteristics of humic substances, namely the heterogeneity of the cation binding functional groups (carboxylic acids and weaker acid groups such as phenolic-OH) and the ionic strength dependency of cation binding (arising from the electrostatic properties of humic molecules).

The cation binding properties of humic substances isolated from soils under well-defined laboratory conditions are now relatively well understood. However, recent research has highlighted the fact that isolated humic substances may not be a good representation of soil organic matter in terms of the ionic strength dependency of cation binding (Smith *et al*., 2004; Gustafsson & Berggren Kleja, 2005). Suspensions of unaltered soils can be used to study the acid-base properties of soil organic matter, but interpretation of results is by no means straightforward. Uncertainties exist about (i) the dissolution of mineral soil components when the pH is changed, and (ii) the original extent and speciation of metal binding in the soil. Instead, a number of authors have used suspensions of peat, to minimise interference from mineral soil components, pre-treated by extensive acid-washing, to replace bound metals with protons. Smith *et al*. (2004) performed acid-base titrations using acid-washed peat at concentrations of 10 – 30 g l^{-1}. When modelling their own data, and those of previous studies on acid-washed peat (Bloom & McBride, 1979; Marinsky *et al.*, 1980), Smith *et al.* found that the ionic strength dependency of proton binding was stronger than predicted by Model VI. To simulate the acid-washed peat results more accurately, the electrostatic submodel used in Model VI – based on a diffuse electrical layer surrounding an impermeable sphere – was replaced with one based on a fixed Donnan volume. The revised model was called Model VI-FD (Smith *et al.*, 2004).

In a recent study by Cooke *et al.* (2007), acid-base titrations of an initially aggregated humic acid showed a diminishing dependence on ionic strength as the pH was raised (i.e. as the aggregates dissolved), indicating that the ionic strength dependency is related to the degree of aggregation. It was suggested that in humic aggregates the electrical layers of individual particles overlap to form a counter-ion accumulation zone which varies little in volume with ionic strength, which would explain why an electrostatic submodel based on a fixed Donnan volume provides a better description of the acid-base properties of acid-washed peat.

Based on the results of Smith *et al.* (2004), it can be assumed that the acid-base properties of the soil organic matter in peat are different to those of isolated humic substances. However, Smith *et al.* provided no direct comparisons between peat and isolated humic substances, and the experiments were performed over a limited pH range (2.4 – 5.5). In the work reported here, we investigated the proton binding properties of acid-washed organic soils in more detail. We carried out potentiometric titrations over wide ranges of pH and at different ionic strengths on acid-washed preparations of three different organic soil types, and compared the results to those already reported for humic acids isolated from the same soils (Cooke et al., 2007). We determined the concentration-dependence of titration behaviour, in order to investigate (a) the effects of possible variations in the state of aggregation of the organic matter, and (b) the reliability of extrapolation to field conditions. We interpreted our results with Humic Ion-Binding Model VI-FD, and modified the model to account for the new information.

Materials and methods

Samples of organic-rich soil horizons (Ranker, Podzol, Peat) were collected from three contrasting temperate-region soils. The Ranker sample was from the A-horizon of a humic ranker (FAO Histic Leptosol) under mat grass (*Nardus stricta*) in the Duddon Valley, Lake District, UK (54°41' N, 03°14' W). The Podzol sample was from the O_H-horizon of a Cambic Podzol (FAO) under Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*) and birch (*Betula pubescens*) at Birkenes, Southern Norway (58°22' N, 08°13' E). The Peat sample (FAO Dystric Histosol) was from the surface of blanket peat under sphagnum moss (*Sphagnum spp.*), heather (*Calluna vulgaris*) and cotton grass (*Eriophorum spp.*) at Moor House, North Pennines, UK (54°68' N, 02°38' W); the degree of humification was 5 on the von Post scale. Each sample was sieved (10 mm mesh) to remove stones and roots and stored in a refrigerator.

The soils were acid-washed following the method of Smith *et al*. (2004) as follows. Sub-samples were suspended in deionised water and adjusted to pH 1 with concentrated HNO_3. The samples were shaken for *c.* 1 min and then left in the dark at room temperature for at least 16 hours, allowing the suspended particles to settle out. Supernatants were removed using a peristaltic pump, the container refilled with deionised water and the solution returned to pH 1. The acid-washing procedure was repeated seven times. Samples were then subjected to exhaustive dialysis against deionised water using cellulose tubing with a molecular weight cut-off of 12,000 (Sigma D-9402). After dialysis, the acid-washed soils were stored as stock suspensions in a refrigerator.

Soil analysis

The organic matter content of the untreated soils was estimated by determining loss-on-ignition of oven dried samples in a laboratory furnace (550°C for 2 hours). Samples of acid-washed soil were analysed for total C and total N (Universal CHNS-O Vario EL elemental analyser). The humic and fulvic acid contents of the acid-washed soils were determined using a method based on that of Smith *et al.* (2004). To measure the degree to which the acid-washing successfully removed metals from the soils, samples of untreated soil and acid-washed soil were extracted with 0.1 M HNO_3 and metal concentrations determined by inductively coupled plasma-mass spectrometry (Perkin-Elmer Elan DRC II).

Titrations

Acid-base titrations were carried out manually in continuous mode using a suspension volume of 500 cm^3 in most cases, but 50 cm^3 when high soil concentrations were employed. Measurements of pH were made with a combination glass electrode and meter (Radiometer GK2401C and PHM82) which underwent a regular quality control procedure (Davison, 1990). The manufacturer quotes a typical outflow rate of 10 $\mu l\ h^{-1}$ for the saturated KCl solution used as the salt bridge of the electrode. Performance of the experimental set-up was verified by carrying out acid-base titrations of KH_2PO_4. 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 *c.* 110°C in a laboratory oven. Additional untreated soil titration solutions were prepared by weighing out moist soil directly. All titrations were carried out in a water bath at 10°C. Soil titrations were performed at 2, 20 and 150 $g\ l^{-1}$, the last of these concentrations being the highest practicably attainable. During the titrations labelled "150 g l^{-1}" in the following account, the actual concentration fell to about 120 g l^{-1} due to dilution, but this was taken into account in calculations.

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. Titrations were performed at four initial background electrolyte concentrations; 0.3, 3, 30 and 300 mM $NaNO_3$. In model applications, account was taken of the presence in the suspension of KCl that had leaked from the pH electrode. The KCl concentration due to leakage typically reached 0.7 mM by the end of titrations with a volume of 500 cm^3, and 5 mM when a volume of 50 cm^3 was used.

Because of the surfactant properties of humic substances, the suspensions were not degassed during the titrations, but were bubbled with oxygen-free nitrogen for *c*. 5 min. prior to the titrations and then kept under a nitrogen blanket. The nitrogen was passed through 0.2 M NaOH, followed by deionised water, to ensure it was CO_2-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 standardised against acid by Gran titration. The NaOH was dispensed from a calibrated auto-burette (Radiometer ABU80) fitted with a trap containing Carbosorb, to minimise the sorption of CO_2 by the NaOH solution. Using a concentrated solution of base 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 ($\approx$ 0.002 pH min^{-1}). Titrations typically took between 7 and 12 hours to complete. The manual nature of the titrations and the care taken to ensure equilibrium resulted in approximately six data points for each titration. To increase the number of data points, independent repeat titrations were performed on the acid-washed soils at 20 g l^{-1} and various ionic strengths with different additions of base. This also served to confirm the reproducibility of the experimental results. The repeat titrations were performed once the first set of titrations were complete, so variability due to time-related factors (e.g. new batches of NaOH) were effectively included.

Calculation of charge

Charge on the organic matter was calculated on the basis of charge balance (i.e. the total system is electrically neutral). Thus, the net charge in equivalents per gram of dry soil is given by

$$Z = ([NO_3^-] + [OH^-] - [Na^+] - [H^+]) / [soil] \qquad (1)$$

where square brackets indicate concentrations. Concentrations of H^+ and OH^- were calculated from their activities, using the Davies equation (with $B = 0.2$) to derive activity coefficients. The H^+ activity was calculated from pH measurements, and the OH^- activity from the temperature-compensated ion product of water.

Suspension effect experiment

Three Peat suspensions were prepared in duplicate, by adding the equivalent of 46 g dry solids per litre to 0.3 mM, 3mM and 100 mM $NaNO_3$. The suspensions (350 cm^3) were stirred overnight and their pH values determined. They were then centrifuged at 18 000 *g* for 3 hours, the supernatants were removed and their pH values measured. The centrifuge pellets and supernatants were recombined to check that no change in pH had occurred as a result of the centrifugation. The degree of dispersion of the solid material, which includes both dissolved and colloidal suspended organic matter, was determined by drying the supernatants from separate experiments and weighing the solid residue, correcting for the presence of added salt.

Ion-binding models

Humic Ion-Binding Model VI has been described in full elsewhere (Tipping, 1998). In brief, it is a discrete site model in which binding is modified by electrostatic interactions. The model contains eight functional groups of different acid strengths, each characterised by an intrinsic equilibrium constant (proton binding with no electrostatic effects), the negative logarithms of which are denoted pK_1 – pK_8. The four most acidic groups are referred to as type A and represent mainly carboxylic acid groups, while the remaining four groups are referred to as type B and represent weaker acids, such as phenolic groups. The eight groups are described by four constants; pK_A and pK_B define the mean intrinsic pK values of the type A and type B groups respectively, while ΔpK_A and ΔpK_B define the (uniform) spread of the pK values around the means. The ΔpK values, in a simple way, represent the chemical heterogeneity of the humic material. The total number of type A groups is given by n_A. On the basis of published data for isolated humic substances, and to avoid over-parameterisation, the total number of type B groups is fixed at n_A / 2. The imposed regularity of the groups facilitates the formation of multi-dentate binding sites for metals, however, this does not affect the description of proton binding as it is exclusively monodentate. Table 1 shows the default parameters of Model VI.

In Model VI, the effect of electrostatic interactions are accounted for by treating the molecules as impermeable spheres, each surrounded by a diffuse layer of counter-ions. The intrinsic equilibrium constants of binding sites are modified with an empirical term that is dependent on ionic strength, I. The Donnan expression is then used to calculate counterion accumulation within a zone around each molecule. The size of the counter-ion accumulation zone is partly determined by the diffuse layer thickness, which is taken to be the reciprocal of the Debye-Hückel parameter, κ. As κ is proportional to $I^{\frac{1}{2}}$, the diffuse layer thickness is inversely related to ionic strength. For aqueous solutions at 25°C, $1 / \kappa \approx 10^{-8}$ m at $I = 0.001$ M, and $1 / \kappa \approx 10^{-9}$ m at $I = 0.1$ M (Tipping, 2002). When this electrostatic submodel is applied to soils *in situ*, the concentration of humic substances can be such that the theoretical volume of the diffuse layer becomes impossibly large. Model VI gets round this problem by restricting the diffuse layer volume to 25% of the total volume of water in the system.

In soils *in situ*, and in suspensions, the humic matter is obviously highly aggregated. In Model VI-FD (Smith *et al.*, 2004), these aggregates are treated as basic units. The electrostatic submodel of Model VI is replaced by one based on an aggregate of impermeable spheres, surrounded by a fixed Donnan volume in which counter-ions can accumulate. Electrostatic effects are accounted for with an unadjusted Donnan formulation. Binding or dissociation reactions within a Donnan phase are described by conventional equilibrium expressions (Tipping, 2002). Thus, for the binding of a proton or metal cation by a humic compound:

$$\mathrm{R}^{Z} + \mathrm{M}^{z} = \mathrm{RM}^{Z+z} \tag{2}$$

where R is the humic compound of charge Z (invariably negative) and M is the cation of charge z, the equilibrium constant for the reaction in the Donnan volume, K_{D}, is

$$K_{\mathrm{D}} = \frac{[\mathrm{RM}^{Z+z}]_{\mathrm{D}}}{[\mathrm{R}^{Z}]_{\mathrm{D}}[\mathrm{M}^{z}]_{\mathrm{D}}} \tag{3}$$

where $[\mathrm{RM}^{Z+z}]_{\mathrm{D}}$, $[\mathrm{R}^{Z}]_{\mathrm{D}}$ and $[\mathrm{M}^{z}]_{\mathrm{D}}$ are the reactant concentrations in the Donnan volume. The concentration of a cation in the Donnan volume is related to its concentration in the bulk solution by the Boltzmann law

$$[\mathrm{M}^{z}]_{\mathrm{D}} = [\mathrm{M}^{z}]\exp\left(-\frac{ze\psi_{\mathrm{D}}}{kT}\right) \tag{4}$$

where Ψ_{D} is the Donnan potential, k is Boltzmann's constant, T is the absolute temperature and e is the charge on a single ion (1.6×10^{-19} C). Charge balance means the following electroneutrality condition must apply

$$\frac{Z}{V_{\mathrm{D}}} + \sum[\mathrm{M}_{i}]_{\mathrm{D}} + \sum[\mathrm{A}_{j}]_{\mathrm{D}} = 0 \tag{5}$$

where V_{D} is the Donnan volume (l g^{-1}) and A are anions (which, for an anionic molecule, have a lower concentration in the Donnan volume than in the bulk solution). If V_{D} is known or assumed, and the values of $[\mathrm{M}^{z}]$ are known, the values of $[\mathrm{M}^{z}]_{\mathrm{D}}$ and Ψ_{D} can be calculated.

The Boltzmann term, $\exp(-\varepsilon\Psi_{\mathrm{D}} / kT)$, can be eliminated from equation (4) to give

$$\left(\frac{[\mathrm{M}_{1}^{z1}]_{\mathrm{D}}}{[\mathrm{M}_{1}^{z1}]}\right)^{\frac{1}{z1}} = \left(\frac{[\mathrm{M}_{2}^{z2}]_{\mathrm{D}}}{[\mathrm{M}_{2}^{z2}]}\right)^{\frac{1}{z2}} = \left(\frac{[\mathrm{M}_{3}^{z3}]_{\mathrm{D}}}{[\mathrm{M}_{3}^{z3}]}\right)^{\frac{1}{z3}} = \mathrm{K} \tag{6}$$

where $z1$, $z2$, $z3$ etc. are the charges borne by the different cations, so that it is not necessary to know or derive Ψ_{D} in order to solve the equations for species distributions. Equation (6) shows that the ratio of the cation concentration in the Donnan volume to that in the bulk solution depends only upon its charge (in the absence of selectivity).

If it is assumed that the Donnan volume is fixed (i.e. it does not vary with ionic strength) the apparent pK values of humic binding sites, calculated from bulk solution pH, vary by approximately one log unit per tenfold change in ionic strength – except when the degree of humic dissociation is low (Tipping, 2002). Thus, if the ionic strength of a humic solution is increased from 0.01 to 0.1 M, the bulk solution pH would decrease by *c*. 1 pH unit.

Suspension effect correction

The measurement of proton binding in concentrated suspensions at low ionic strength will be subject to artefacts due to the "suspension effect of the first kind" as defined by Oman *et al.* (2007). This arises because the ion-sensitive probe (the glass electrode in this case) experiences counterions associated with particulate and colloidal material as well as those in the bulk solution. The effect increases with suspension concentration but decreases with ionic strength, as the H^+ concentration difference between the diffuse layer and bulk solution decreases. When using Model VI-FD alone, we estimated the magnitude of the suspension effect using the equation

$$[H^+]_{app} = \alpha_{SE}\, V_D[\mathrm{soil}]\, [H^+]_D + (1 - V_D[\mathrm{soil}])\, [H^+] \qquad (7)$$

Here $[H^+]_{app}$ is the hydrogen ion concentration experienced by the probe, $[H^+]_D$ refers to the Donnan volume, and $[H^+]$ to the bulk solution. The term $V_D[\mathrm{soil}]$ is the fraction of the total liquid volume due to the Donnan volume. The parameter α_{SE} is the fraction of the counterion H^+ experienced by the probe, and lies between zero and unity.

Calculations for systems that contained both aggregated and dispersed soil employed both Model VI-FD and the standard Model VI. In these circumstances, the Donnan volume of the aggregates and the diffuse volumes of the dispersed humic and fulvic acids were combined to estimate the total volume containing counterions, and the average H^+ concentration in this volume was used to quantify the suspension effect.

Results

Table 2 shows the results of the soil analysis. The Peat is almost entirely composed of organic matter, while the Ranker and Podzol each contain about 30% mineral matter. Given the high functional group content of natural organic matter, it was expected that the proton binding properties of all three soils would be dominated by the organic matter. The values for total C, humic acid C and fulvic acid C, along with the organic matter estimates, highlight the fact that the majority of organic matter in these soils is not extractable humic or fulvic acids, but material with insufficient ionisable groups to be rendered soluble by the high concentrations of base used in the humic substances extraction. The proportions of humic and fulvic acids for the three soils are similar to previous results obtained by the same procedure (Tipping et al., 1995; Smith et al., 2004). The acid washing brought about substantial decreases in Al, Ni and Cd and more modest changes in Fe, Cu and Pb. It can be assumed that the weakly-binding base cationic metals (Na, Mg, K, Ca) were effectively removed.

Titration results for acid-washed soils

Experimental results for titrations of acid-washed Ranker, Peat and Podzol samples, at 20 g l^{-1} and four different concentrations of background electrolyte, are shown by the points in Figure 1. Results for all three soils are alike; the titration curves exhibit a similar shape and spacing in each case. This indicates that the organic matter in each of these soils has comparable acid-base properties, and thus a similar distribution of acidic functional groups and a similar ionic strength dependency of proton binding. The Podzol and Peat samples have nearly identical buffering capacity over the range titrated, while the buffering capacity of the Ranker sample is slightly less (the titration curves are steeper), indicating that the Ranker possesses fewer acidic functional groups than the other samples.

Figure 2 compares the calculated net charge on the acid-washed soils (Figure 2a-c) with the net charge on the humic acids isolated from the same soils (Figure 2d-f), taken from the results of Cooke *et al*. (2007), at the same four concentrations of background electrolyte. A precise comparison of the two sets of results is not possible because the isolated humic acids have higher contents (mol g^{-1}) of ionisable groups than the soils, but for our present purposes it is sufficient to adjust the charge scales so that the titration plots at acid pH appear similar. There are two major differences between the acid-washed soils and the humic isolates. Firstly, the soils have steeper charge curves at high pH, indicating higher contents of weak acid groups (greater site densities). Secondly, proton binding by the soils displays a greater dependence on ionic strength, as shown by the greater spacing of the charge curves.

We checked that the differences between acid-washed soil and humic acid were relevant to the field situation, i.e. that the properties of the former were not artefacts of the acid washing procedure, by performing additional experiments with an untreated Peat sample. The titration results with untreated Peat were reasonably similar to those for acid-washed material (data not shown). In particular, the ionic strength dependence of proton binding was the same, as judged by comparison of the titration results at low and high ionic strength. Figure 3 shows the pH differences between titrations in 0.3 and 300 mM $NaNO_3$ for the untreated and acid-washed Peat samples to be essentially identical, and markedly different from the differences for isolated humic acid.

The data shown in Figures 1 and 2 were supplemented by the results of titrations carried out with different soil concentrations, 2 and 150 g l^{-1}. These results are presented below.

Modelling

We adapted Model VI-FD in the light of the evidence from Figure 2 that soil organic matter is relatively richer in weak acid groups than the corresponding humic acids. The site density in the model was modified by increasing n_B, the total number of type B groups in humic acid. The site contents of fulvic acid were maintained at the default values. To fit the experimental data, we maintained the default parameter values of Table 1, except that we optimised n_B and pK_B for humic acid. The value of n_B was constrained to bear a simple relationship to n_A in anticipation of the need to formulate a regular array of multidentate binding sites for metals; thus n_B was allowed to be either $2n_A$ or $4n_A$. The values of pK_B were adjusted because the additional weak acid sites might not have the same properties as those of humic acid. To avoid over-fitting we did not optimise ΔpK_B as well, although this might also be done on the same grounds. We also optimised f_{HS}, the fraction of the soil dry weight that is due to humic substances (humic and fulvic acids, in the proportions found by extraction; Table 2), and α_{SE}, the parameter that corrects for the suspension effect (equation 7).

Firstly we used only Model VI-FD, i.e. we assumed that the Donnan volume remains fixed over the entire range of experimental conditions, and does not change due to dispersion at higher pH. The root-mean-squared-deviation (RMSD) between observed and calculated pH was used as the criterion of fit. Results for the Peat were best with $n_B = 2n_A$ and f_{HS} in the range 0.28 – 0.30, but a number of combinations of pK_B (range 8.0 - 8.8) and α_{SE} (0.1 – 0.5) were possible. The same range of f_{HS} applied to the Podzol, but the results depended less on n_B (although a value of $4n_A$ was slightly better), and again various combinations of pK_B (8.0 - 8.8) and α_{SE} (0.1 – 0.5) gave good results. The Ranker gave poorer fits than the other two soils, but the parameters were better defined with $f_{HS} = 0.18$ or 0.19, $n_B = 4n_A$ and low values of pK_B (8.0 – 8.2), although the results were comparatively insensitive to α_{SE}. Given that the data do not provide unique parameter sets, we forced the values of pK_B and α_{SE} to be the same for each soil sample, with values of 8.0 and 0.2 respectively. Then the value of f_{HS} was optimised with $n_B = 2n_A$ for the Peat and $n_B = 4n_A$ for the Podzol and the Ranker. The final results are shown in Table 3, and the fitted data in Figure 4. We refer to this modified version of the model, i.e. with additional weak acid sites, as Model VI-FD2.

The possible effects of dispersion on the titration data were explored using a combination of Model VI-FD2 with the standard Model VI for the dispersed fraction. The extent of dispersion was approximated with the equation;

$$\text{fraction dispersed} = 10^{-\beta}\,\text{pH}^{\beta} \qquad (8)$$

This causes the fraction dispersed to increase from zero to 1 in the pH range 0 to 10. With $\beta = 3$, the fraction dispersed is 0.05 at pH 3.7, 0.2 at pH 5.8 and 0.5 at pH 7.9. These values are qualitatively consistent with the observed behaviour of soil organic matter during the extraction of humic substances by treatment with base, fulvic and humic acids being brought into solution (or at least a finely divided colloidal state) at high pH, and humic acid being fully precipitated at low pH, and also with the progressive dispersion of humic matter with pH demonstrated by Tipping and Ohnstad (1984). In the suspension effect experiment (see Materials and methods), the dispersed fraction at the lowest ionic strength, with an observed supernatant pH of 3.99, was 0.01. Therefore setting β to 3 overestimates dispersion at acid pH, and the true dispersion behaviour of the system is likely to fall between the simulations for $\beta = \infty$ (corresponding to the pure Model VI-FD2) and $\beta = 3$. With $\beta = 0$, the system is fully dispersed at all pH values. Figure 5 shows simulated pH titrations for these three values of β. There is hardly any difference between the results for $\beta = 3$ and $\beta = \infty$, but full dispersion gives rise to quite different simulated titration behaviour at low pH, which agrees with the experimental findings of Cooke et al. (2007).

The parameterised model was used to simulate the results of an experiment with peat to investigate the suspension effect (see Materials and methods). In 0.3 mM $NaNO_3$, the observed supernatant pH of 3.99 was 0.26 greater than the pH of the homogeneous suspension, while the calculated difference (with $\alpha_{SE} = 0.2$) was 0.42. The corresponding differences in 3 mM $NaNO_3$ (observed supernatant pH = 3.76) were 0.14 (observed) and

0.08 (calculated), while no difference was observed or calculated in 100 mM $NaNO_3$ (observed supernatant pH = 3.11). While the observations and model are not in full agreement, these results support the assumption that the suspension effect is operating in these systems, and influencing observed electrode response.

Discussion

Although the three soil samples are similar in their high contents of organic matter, they differ in their circumstances of formation. Therefore the very similar titration behaviours suggest that the results obtained in this work can be taken as representative, at least of temperate soil organic matter. Moreover, the similar ionic strength dependences of the acid washed Peat and the untreated material support the assumption that results obtained with acid washed material are relevant to the field situation.

The results show that the soil samples differ in their functional group contents from the corresponding isolated humic acids. This can be taken into account in modelling by the inclusion of additional weak acid sites, and by optimising their average p*K* value. By adopting this approach, we imply that the additional sites are due to organic matter that is not extractable by 0.1 M NaOH, i.e. is not isolated as humic and fulvic acids. The non-extractable organic matter corresponds to the humin fraction, assuming the mass contribution of identifiable organic compounds (not strictly part of humic acid, fulvic acid or humin) to be negligible. To calibrate the model we have assumed all the COOH are on the extractable humic matter, but it is also possible that some are on the humin. In either event, the humin must be much richer in weak acid groups than carboxylic acids. The deduced preponderance of weak acid groups in the humin accords with the findings of Zelanzny & Carlisle (1974) who reported humin to contain a higher proportion of phenolic groups than fulvic or humic acids, possibly because it is rich in lignin. Stevenson (1994) referred to “inherited humin”, by which is meant “altered lignin-like polymers and/or microscopic (subcellular) particles of plant origin….”. This type of material would be likely in the organic-rich soils studied here, as opposed to the humin of mineral soils which is dominated by organic matter tightly-sorbed to mineral particulates. From the data in Tables 2 and 3, we estimate that the weak acid group contents of the humin fraction are 1.8, 5.3 and 3.1 mmol g^{-1} for the Peat, Podzol and Ranker respectively. These cannot be considered precise because they depend on the modelling assumptions, but it is certain that the soils contain significant numbers of weak acid groups that are not estimated from the extractable humic matter.

Other possible contributors to the weak acid contents of the Ranker and Podzol samples are silica and/or aluminosilicates, which must account for most or all of the mineral fractions of these soils, and which possess surface hydroxyl groups that would ionize at high pH in the titration experiments. Their possible contributions can be estimated from the typical hydroxyl group content (c. 8 μmol m^{-2}; Lofts and Tipping, 1998) and density (2.7 g cm^{-3}; Iler, 1979) of silica. We calculate that for silica particles at the upper end of the clay size range (i.e. about 2 μm diameter), the mineral matter would contribute c. 0.002 mmol of weak acid groups per gram of soil sample, which is negligible compared to the estimated total contents from the titrations of c. 1 mmol g^{-1} (see Results). For the silica weak acid groups to be significant, a particle size of about 10 nm would be required, which is highly unlikely; indeed visual inspection of the mineral matter in the Podzol and Ranker samples suggests the predominance of silt and sand-sized mineral matter. For the same reasons, it is unlikely that interactions between the organic and mineral components would significantly modify the charging behaviour of the organic matter. Therefore we conclude that the acid functional groups in the acid-washed soils are due almost entirely to the organic matter.

The inclusion of extra type B (weak acid) binding sites in Model VI-FD2 means that we have effectively defined a soil component that is a combination of humic acid and humin. For soil in the aggregated state, with electrostatic properties modelled in terms of the fixed-Donnan volume, the presence of the extra type B sites does not compromise the underlying theory of the model, as long as only protons are considered. However, the humic acid and humin must, by definition, differ with respect to dispersion (including solubility), and so strictly speaking the two materials should be modelled separately when the soil is dispersed. But as shown by the results in Figure 5, this is not of any great significance because the soil titration properties differ little from the fully-aggregated case when dispersion follows equation (8) with β values greater than 3. Another point that arises from the humic acid / humin component is the formulation of multidentate sites for metals, which is accomplished in Model VI in terms of proximity factors, calculated from molecular dimensions, and assuming that the ligand atoms are present on the same molecule. In previous work (Smith et al., 2004) we assumed that the proximity factors also apply to aggregated soil, although there might be scope for additional multidentate sites to be formed by ligand atoms on different adjacent molecules. With the inclusion of the humin type B sites, this possibility increases. More data on metal binding, and modelling work, are needed to resolve this issue.

The parameters derived by applying the model to the titration data are not all precisely defined, because different combinations can provide comparable fits. Therefore we restricted some of the allowable values. Since the suspension effect is assumed to be due to interactions between soil particles and the pH electrode, a common value of α_{SE} is clearly warranted, and the best value of 0.2 implies that the electrode experiences 20% of the protons residing in the Donnan volume or diffuse zone. The relatively low value of pK_{HB}, again forced to apply to each soil, means that the average additional (humin-associated) type B site is a somewhat stronger acid than the average humic acid type B site. We restricted the value of n_B to be either 2 n_A or 4 n_A because of the anticipated need to incorporate multidentate site formulations into Model VI-FD2 when it is applied to metals (see above), but it was necessary to permit the different soils to have different n_B / n_A relationships. Finally, the fitted values of f_{HS} for the Peat and Podzol are in good agreement with the soil contents of NaOH-extractable carbon (Table 2), which correspond to f_{HS} values of 0.35 and 0.28 respectively if carbon is assumed to account for 50% of the humic matter. Agreement is less good for the Ranker, for which the NaOH-extractable carbon content corresponds to f_{HS} = 0.30, about 50% greater than the fitted value. This discrepancy cannot be accounted for by differences in the humic acids aming the soils, because they give closely similar titiration behaviour (Cooke et al., 2007; Figure 1).

We are unaware of any previous work in which the suspension effect has been taken into account in the interpretation of titration data for organic soils, and this at least partly reflects the fact that under most experimental conditions its influence is negligible. But we have found it to be important in concentrated suspensions at low ionic strength, and therefore have included a correction term (equation 7), the use of which is supported by the results of a small experiment. After making this correction, we find that there is no discernible concentration effect on the pH titration behaviour of any of the soils, within the concentration range 2 to 150 g l^{-1} (Figure 4). This result supports the extrapolation of the experimental results and modelling to field situations. Indeed, in the case of peat, it is not uncommon to encounter field water contents in excess of 80%, which would correspond to a solids concentration of a few hundred grams per litre, similar to the highest concentration that we used in our experiments.

The use of a fixed Donnan volume to model ion-binding by organic soils is based on the idea that counterions accumulate in a zone that is defined by the aggregate structure of the soil, rather than in a diffuse layer surrounding the soil particles. This picture gives rise to a much higher dependence on ionic strength than is found for samples of either isolated humic substances or organic soils that have been dispersed at high pH (Cooke et al., 2007). The different behaviour is due to the electrostatic properties of the aggregates under low ionic strength conditions, as illustrated by the simulations of Figure 5. The lack of dependence of the titration results on suspension concentration (see above) shows that dispersion cannot be brought about by dilution, which suggests that the aggregates are fairly stable at acid pH values. For these acid soils *in situ* the organic matter must be in the aggregated form, since it will not have been exposed to high pH conditions. More generally, it is probably not pH *per se*, but the net organic matter charge or potential, determined by interactions with cations such as Al^{3+} and Ca^{2+} as well as H^+, that governs aggregation (Tipping and Woof, 1991; Weng et al., 2002).

During the titration experiments, changes in the degree of dispersion of the organic matter which occur as the pH is increased are not taken into account by Model VI-FD2, which assumes a fixed-Donnan volume under all circumstances. Using a plausible pH-dependence of dispersion (equation 8), we found that, as long as the main dispersion process takes place at pH values above the acid range, the simulations are not greatly affected (Figure 5). This is because the fixed-Donnan volume and impermeable-sphere electrostatic models differ less in their predictions of ionic strength dependence when the soil bears a relatively high charge. Therefore as the pH increases, the choice of model becomes less important, and the degree of dispersion has less influence.

We propose that Model VI-FD2 is an improved description of ion binding by solid-phase soil organic matter, but we advocate the use of the conventional Humic Ion-Binding

Model VI to describe interactions in solution. Therefore to simulate partitioning and transport in the field, a combination of the models would be needed. Similarly, the NICA-Donnan model (Kinniburgh et al., 1999) deals well with solution reactions, using a Donnan volume that depends upon ionic strength. But Gustafsson & Berggren Kleja (2005) have shown that the current NICA-Donnan parameterisation does not account for the ionic strength dependency observed in acid-washed peat, i.e. it fails in the same way as Model VI. To apply the NICA-Donnan model to soil solids, we suggest the adoption of a fixed Donnan volume, together with modification of the soil organic matter binding site content.

The underlying reason for conducting this and similar work is to create models that can describe field soils and catchments. For example, Model VI is at the heart of the CHemistry of the Uplands Model (CHUM), which has been used to simulate the effects of acidifying pollutants and heavy metals on soil-water systems (Tipping et al., 2006a,b). The incorporation of Model VI-FD2 into CHUM would improve the description of low ionic strength soil systems, which dominate most natural and semi-natural habitats of the UK and elsewhere. Moreover, while field soils do not experience ionic strength ranges as large as those employed in our experiments, storm events can deliver high concentrations of sea salts to upland catchments in countries with maritime climates, and cause episodic acidification (e.g. Langan, 1989), which might be better explained with Model VI-FD2. Furthermore, although the additional weak acid groups that we have identified from our titration studies are not expected to have much influence on proton binding at field pH values, they could be involved in metal binding, and again use of Model VI-FD2 might be provide better predictions than the current version. It is therefore worthwhile to continue the development and testing of ion-binding models based on data for acid-washed organic soils, and to evaluate their predictions with field data.

Conclusions

1. Soil organic matter samples from three different soil types (Peat, Podzol H layer, Ranker) display similar proton-binding characteristics.
2. The soil organic matter possesses proportionally more weak acid groups than do humic acids isolated from the same soils. The additional weak acid groups reside in the humin fraction, at a content of c. 2 - 5 mmol g^{-1}.
3. The electrostatic properties of the soils are governed by their aggregated state, especially at low ionic strength, and this gives rise to a high pH dependence of proton binding.
4. The acid-base titration behaviour of the soils over wide ranges of pH and ionic strength can be modeled successfully, if the additional weak acid groups are taken into account, and with the assumption of counterion accumulation in a fixed-Donnan volume.
5. Proton binding is independent of soil concentration in the range 2 – 150 g l^{-1}, which supports the extrapolation of the findings to the field.

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References

Bloom, P. R. and McBride, M. B. 1979. Metal ion binding and exchange with hydrogen ions in acid-washed peat. *Soil Science Society of America Journal*, **43,** 688-692.

Cooke, J. D., Hamilton-Taylor, J., and Tipping, E. 2007. On the acid-base properties of humic acid in soil. *Environmental Science Technology*, **41,** 465-470.

Davison, W. 1990. A practical guide to pH measurement in freshwaters. *Trends in Analytical Chemistry*, **9,** 80-83.

Gustafsson, J. P. and Berggren Kleja, D. 2005. Modeling salt-dependent proton binding by organic soils with the NICA-Donnan and Stockholm humic models. *Environmental Science Technology*, **39,** 5372-5377.

Iler, R.K. 1979. *The Chemistry of Silica.* Wiley, New York.

Kinniburgh, D. G., van Riemsdijk, W. H., Koopal, L. K., Borkovec, M., Benedetti, M. F., and Avena, M. J. 1999. Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **151,** 147-166.

Langan, S. J. 1989. Sea-salt induced streamwater acidification. *Hydrological Proc*esses, **3**, 25-41.

Lofts, S and Tipping, E. (1998) An assemblage model for cation binding by natural particulate matter. *Geochimica et Cosmochimica Acta*, **62**, 2609-2625.

Marinsky, J. A., Wolf, A., and Bunzl, K. 1980. The binding of trace amounts of lead(II), copper(II), cadmium(II), zinc(II) and calcium(II) to soil organic matter. *Talanta*, **27,** 461-468.

Oman, S. F., Camões, M. F., Powell, K. J., Rajagopalan, R. and Spitzer, P. (2007) Guidelines for potentiometric measurements in suspensions Part A. The suspension effect (IUPAC Technical Report) *Pure and Applied Chemistry*, 79, 67–79.

Smith, E. J., Rey-Castro, C., Longworth, H., Lofts, S., Lawlor, A. J., and Tipping, E. 2004. Cation binding by acid-washed peat, interpreted with Humic Ion-Binding Model VI-FD. *European Journal of Soil Science*, **55,** 433-447.

Stevenson, F.J. 1994. *Humus Chemistry*. Wiley, New York.

Tipping, E. 1998. Humic ion-binding model VI: an improved description of the interactions of protons and metals ions with humic substances. *Aquatic Geochemistry*, **4,** 3-48.

Tipping, E. 2002. *Cation Binding by Humic Substances*. Cambridge University Press.

Tipping, E., Berggren, D., Mulder, J. & Woof, C. 1995. Modelling the solid-solution distributions of protons, aluminium, base cations and humic substances in acid soils. *European Journal of Soil Science,* **46**, 77-94.

Tipping, E., Lawlor, A.J. and Lofts, S. 2006a. Simulating the long-term chemistry of an upland UK catchment: major solutes and acidification. *Environental Pollution,* 141, 151-166.

Tipping, E., Lawlor, A.J., Lofts, S. and Shotbolt, L. 2006b. Simulating the long-term chemistry of an upland UK catchment: heavy metals. *Environental Pollution,* **141**, 139-150.

Tipping, E. and Ohnstad, M. 1984. Aggregation of aquatic humic substances. *Chemical Geology,* **44**, 349-357.

Tipping, E. and Woof, C. 1991. The distribution of humic substances between the solid and aqueous phases of acid organic soils; a description based on humic heterogeneity and charge-dependent sorption equilibria. *Journal of Soil Sciience,* **42**, 437-448.

Weng, L., Temminghoff, E.J.M. and van Riemsdijk, W.H. 2002. Interpretation of humic acid coagulation and soluble soil organic matter using a calculated electrostatic potential. *European Journal of Soil Science*, **53,** 575-587.

Zelazny, L.W. and Carlisle, V.W. 1974. Physical, chemical, elemental, and oxygen-containing functional group analysis of selected Florida histosols. In: *Histosol: Their characteristics, classification, and use* (eds Aandahl, A. R., Buol, S. W., Hill, D. E., and Bailey, H. H.), Soil Science Society of America Special Publication Number 6, Madison, WI.

Table 1 Parameters for proton binding to humic acid in Humic Ion-Binding Models VI (Tipping, 1998) and VI-FD (Smith et al., 2004).

parameter	HA	explanation
M	15000	molecular weight
r (nm)	1.72	radius
n_A (mol g^{-1})	3.3×10^{-3}	number of type A groups
n_B (mol g^{-1})	1.65×10^{-3}	$= 0.5 \times n_A$
pK_A	4.1	median proton dissociation constant for type A groups
pK_B	8.8	median proton dissociation constant for type B groups
ΔpK_A	2.1	range factor for pK_A
ΔpK_B	3.6	range factor for pK_B
P	-330	electrostatic parameter in Model VI
$V_{D,HS}$ (cm^3 g^{-1})	6.0	specific Donnan volume in Model VI-FD

Table 2 Results of soil analyses

	Peat	Podzol	Ranker
Sieved raw sample LOI %	97	71	71
Acid-washed sample			
C %	57.5	44.8	40.6
N %	1.9	1.5	2.7
humic acid C %	15.7	11.6	12.4
fulvic acid C %	1.9	2.2	2.7
Soil metal contents	raw / acid-washed		
Al mmol kg^{-1}	19 / 2	25 / 5	184 / 13
Fe mmol kg^{-1}	5.1 / 1.0	nd* / 3.9	32 / 22
Ni µmol kg^{-1}	14 / 3	69 / 15	26 / 6
Cu µmol kg^{-1}	32 / 22	45 / 24	76 / 35
Cd µmol kg^{-1}	6.0 / 0.1	4.6 / 0.2	3.9 / 0.1
Pb µmol kg^{-1}	846 / 100	307 / 81	888 / 193

* not determined

Table 3 Parameter sets for Model VI-FD2

	Peat	Podzol	Ranker
f_{HS}	0.28	0.28	0.19
n_B / n_A [a]	2	4	4
pK_{HB} [b]	8.0	8.0	8.0
α_{SE} [b]	0.2	0.2	0.2
RMSD in pH	0.11	0.10	0.27

[a] constrained to be either 2 or 4

[b] forced to be the same for each soil

FIGURE CAPTIONS

Figure 1. Titrations of 20 g l^{-1} acid-washed soil suspensions with base in 0.3 (squares), 3 (circles), 30 (triangles) and 300 (diamonds) mM background electrolyte ($NaNO_3$) at 10°C. Open and filled symbols represent data from separate experiments. Trend lines are included for guidance.

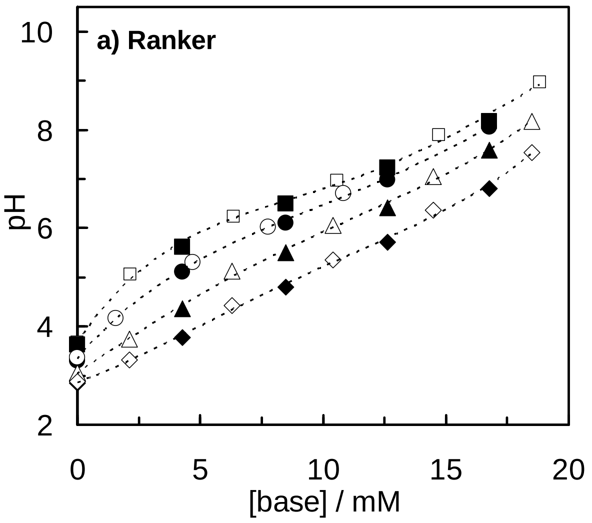
Figure 2. Comparison of net charge, Z, for 20 g l^{-1} acid-washed soil suspension and 4 g l^{-1} humic acid titrations with base in 0.3, 3, 30 and 300 mM background electrolyte ($NaNO_3$) at 10°C. The points (see Figure 1 for key) are experimental values joined by trend lines.

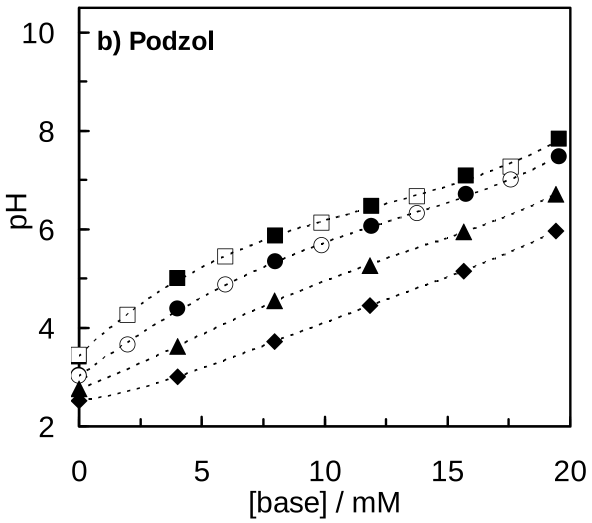
Figure 3. Comparison of ionic strength dependency in untreated Peat (open circles), acid-washed Peat (closed circles) and Peat humic acid (closed squares), expressed as the difference in pH between titrations in 0.3 and 300 mM $NaNO_3$. The points are experimental values joined by trend lines.

Figure 4. Experimental titration data fitted with Model VI-FD2 using parameter values from Table 3. Starting concentrations of $NaNO_3$ in mM were 0.3 (open circles), 3 (closed circles), 30 (open squares) and 300 (closed squares).

Figure 5. Simulated titration curves of the Podzol (20 g l^{-1}) in 0.3 mM $NaNO_3$. The points are experimental results and the lines simulations. Solid line, Model VI-FD2 without dispersion; short dashes Model VI-FD2 + Model VI with $\beta = 3$; long dashes Model

VI (full dispersion). Note that the plot for $\beta = 3$ almost coincides with the no-dispersion plot.


a) Ranker
pH
10
8
6
4
2
0
5
10
15
20
[base] / mM


b) Podzol
pH
10
8
6
4
2
0
5
10
15
20
[base] / mM

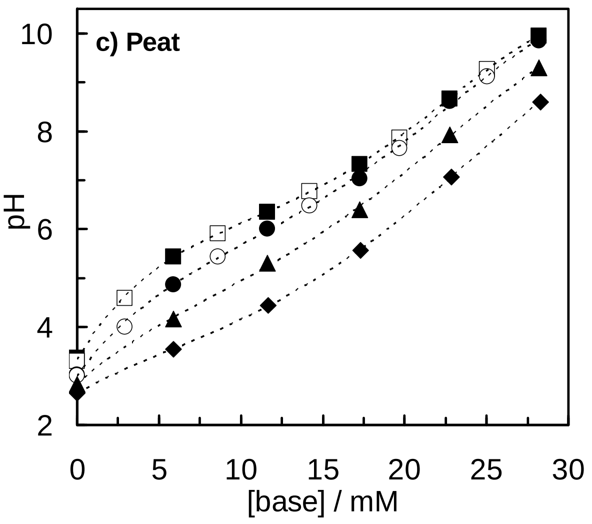

c) Peat
pH
10
8
6
4
2
0
5
10
15
20
25
30
[base] / mM

Figure 1

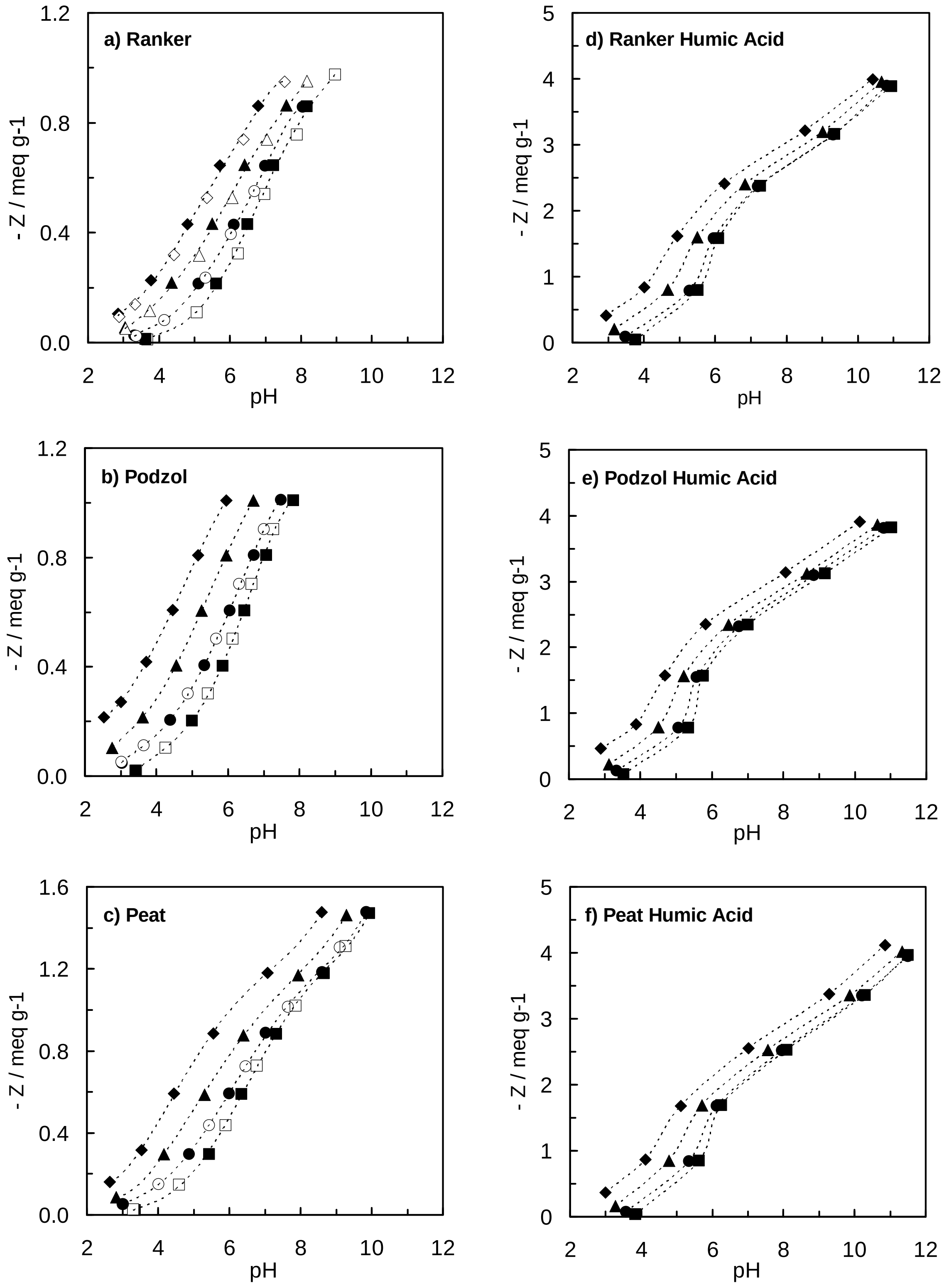

a) Ranker
b) Podzol
c) Peat
d) Ranker Humic Acid
e) Podzol Humic Acid
f) Peat Humic Acid
- Z / meq g-1
pH
0.0
0.4
0.8
1.2
1.6
0
1
2
3
4
5
2
4
6
8
10
12

Figure 2

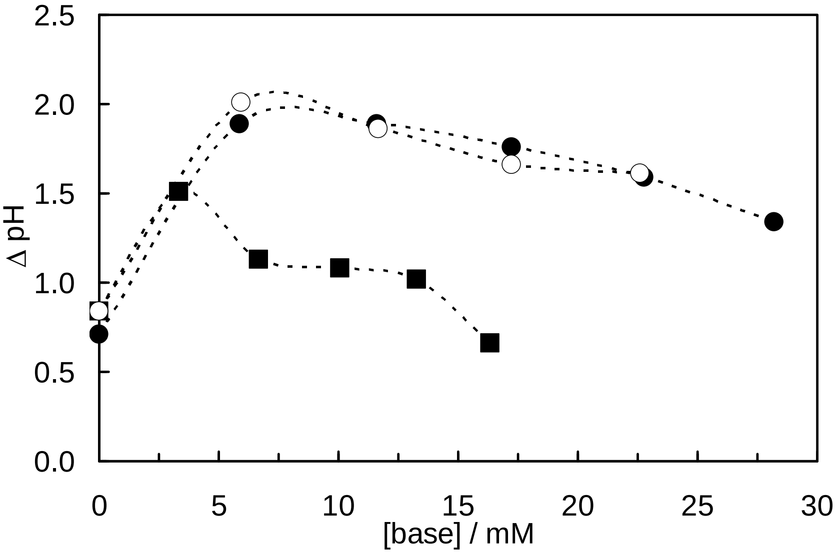


Figure 3

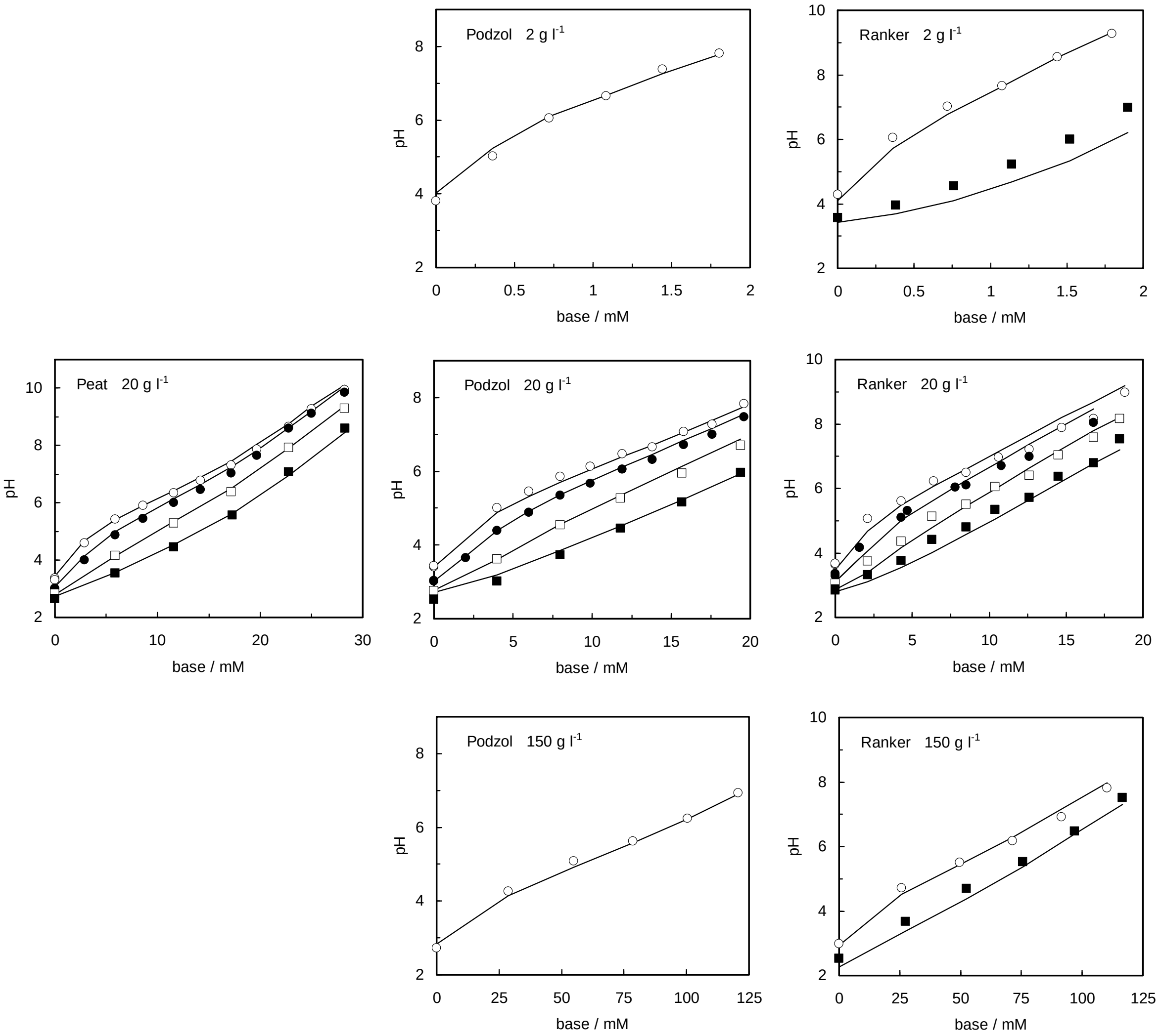

Podzol 2 g l^{-1}
Ranker 2 g l^{-1}
Peat 20 g l^{-1}
Podzol 20 g l^{-1}
Ranker 20 g l^{-1}
Podzol 150 g l^{-1}
Ranker 150 g l^{-1}
pH
base / mM
0
0.5
1
1.5
2
5
10
15
20
25
30
50
75
100
125
4
6
8
10

Figure 4

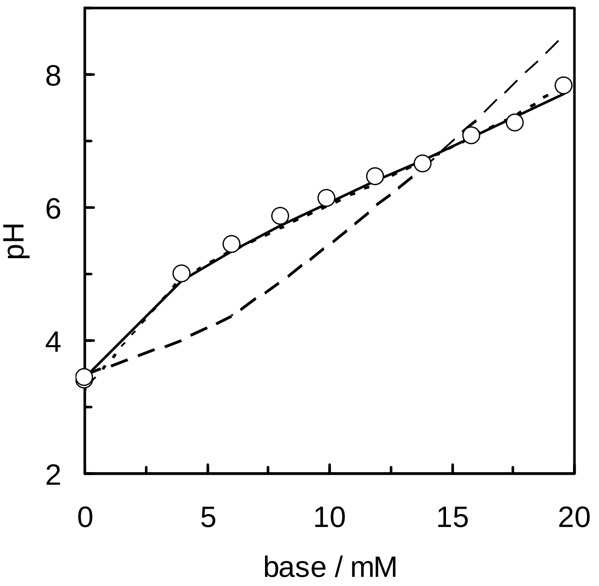


Figure 5