

1 **Characterization of suboxic groundwater colloids using a multi-method approach**

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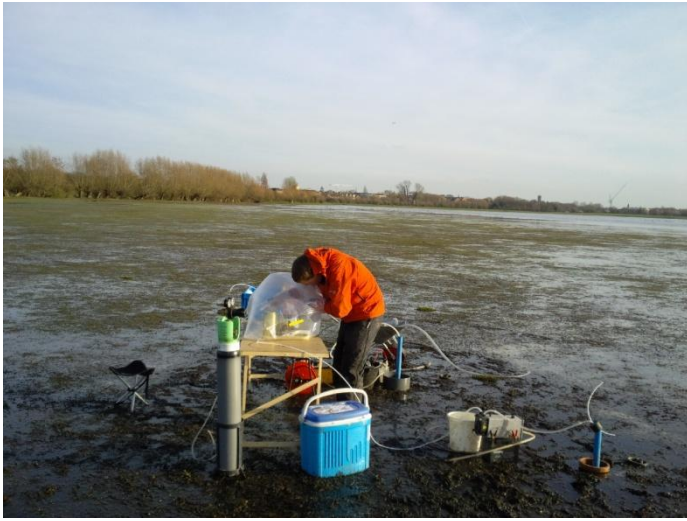
1 **Abstract**

2

3 Anoxic groundwater colloid properties were measured using a minimally perturbing procedure for  
4 sampling, processing and analysis. Analytical methods included atomic force microscopy (AFM),  
5 flow field flow fractionation (FIFFF), transmission and scanning electron microscopy (TEM and  
6 SEM). Shallow groundwater samples showed abundant iron rich nanoparticles (NP) with diameters of  
7 10-30 nm as well as a smaller heterogeneous polydisperse dissolved organic matter (DOM) fraction.  
8 AFM results showed NP with average heights of  $10 \pm 2$  nm, which was corroborated by high  
9 resolution TEM and SEM. FIFFF with UV254 nm detection found particles with number average  
10 diffusion coefficients of  $2-3 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$  and hydrodynamic diameters between 1.5-2 nm, probably  
11 representing smaller organic macromolecules. Aeration of the samples resulted in extensive  
12 agglomeration of NP to form larger (>50 nm) colloids, and a reduction of UV-absorbing material in  
13 the 0.5-4 nm range. The complementary methods described have potential applications for  
14 investigating the fate and transport of NP in suboxic hotspots such as leachate plumes, waste water  
15 treatment plants and within the hyporheic mixing zone.

16

1 **Abstract Art**



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## 2 **Introduction**

3 Natural nanoparticles (NP), particles with one dimension between 1-100 nm, and colloidal material,  
4 particles with one dimension between 1 nm and 1 $\mu$ m, are important vectors for contaminants in the  
5 environment, and include iron or silica mineral particles, complex organic molecules such as humic-  
6 like substances, or material derived from bacterial sources. <sup>[1-3]</sup> Particles spanning these dimensions  
7 can often have a dominant role in controlling speciation and enhancing the mobility of contaminants  
8 in aquatic environments. <sup>[3]</sup> They can also attenuate contaminant transport in some cases, and are  
9 known to have an important role in biogeochemical cycling, and bioavailability of toxic substances. <sup>[4,</sup>  
10 <sup>5]</sup>

11 Many studies have used operationally-defined techniques such as cross-flow-filtration to investigate  
12 element association to colloidal material in environmental samples <sup>[6-8]</sup> including in suboxic  
13 environments. <sup>[9]</sup> Recent research on characterising groundwater NP has also focussed on  
14 understanding radionuclide fate and transport <sup>[10, 11]</sup> due to the potential risk of pollution from long-  
15 term underground radioactive waste storage facilities. Quantifying the size, structure and surface  
16 chemistry of colloids is important for understanding pollutant-colloid interactions. <sup>[12-14]</sup>

17 Developments in analytical methods e.g. flow field flow fractionation (FIFFF), atomic force  
18 microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy  
19 (TEM), have enabled the detailed characterisation of natural colloidal material. <sup>[15-18]</sup> There has been  
20 particular attention paid to the importance and role of small (<20 nm) NP in trace element occurrence  
21 due to their relatively high specific surface area. <sup>[19]</sup>

22 FIFFF has been successfully coupled with a range of detectors (e.g. fluorescence, UV-vis, light  
23 scattering, <sup>[20]</sup> AFM, <sup>[18]</sup> and inductively coupled plasma-mass spectroscopy <sup>[21-23]</sup>), to characterise  
24 fractionated particles. Importantly, FIFFF and AFM can cover the same size-range, provide detailed  
25 structural information on size, shape, diffusion coefficient and softness, and work under ambient  
26 redox conditions.

1 Characterising suboxic environmental NP requires careful protocols for both sampling and analysis to  
2 preserve the native redox status. For example, some aquifers or waste water systems may be partly or  
3 completely lacking dissolved oxygen (DO). Subsequent introduction of O<sub>2</sub> can lead to the oxidation of  
4 ferrous iron to form colloidal iron oxide.<sup>[24, 25]</sup> In addition, sampling at low flow rates (ca. 100 mL<sup>-1</sup>  
5 min) following borehole purging is required to collect representative groundwater samples.<sup>[26]</sup> Due to  
6 the challenges of maintaining suboxic conditions, in both sampling and analysing NP, very few  
7 studies to date have successfully used modern state-of-the-art techniques to characterise nano-scale  
8 particles under environmental conditions. However, many NP contaminant hot spots e.g. parts of  
9 sewerage treatment processes, landfill leachate plumes and the hyporheic zone, can be classified as  
10 suboxic environments. There is therefore a clear need to develop robust methods to characterise and  
11 investigate the physiochemical properties and fate of both natural and introduced NP within these  
12 environments.

13 In this study we have developed and piloted a procedure for sampling and characterizing  
14 physiochemical properties of nano-scale particles in natural suboxic waters using a range of  
15 complimentary methods: FIFFF and AFM, TEM and SEM. The procedure was used to investigate  
16 organic and iron-rich nanoscale particles (<30 nm) within a shallow alluvial groundwater in the  
17 Thames floodplain, United Kingdom, locally impacted by a landfill leachate plume. These techniques  
18 also have potential wider applications for studying the occurrence and fate of natural and engineered  
19 nano-scale particles within other suboxic pollution hotspots.

20

## 1 **Experimental**

2 **Sampling.** The field site (Figure S1 supplementary information) is located down gradient of a  
3 landfill site in the floodplain of the River Thames, Oxford, United Kingdom [51° 46'5.18''N, 1° 16',  
4 47.67''W]. A pilot sampling round in December 2010 was used to test the sampling protocol and  
5 assess the stability of the samples prior to characterisation by AFM. Once the sampling and storage  
6 procedure had been tested in the December round and evaluated using AFM, the suboxic sampling  
7 methodology was extended to other characterisation techniques. In subsequent rounds (February  
8 2011) samples were sampled and analysed by AFM, SEM and TEM; in the April 2011 round samples  
9 were analysed by FIFFF.

10 At two piezometer nests (sites 26 and 28) groundwater from the floodplain Terrace Gravels was  
11 sampled from 1.5 m (piezometer c) and 3.5 m (piezometer d) below ground level. In the December  
12 round piezometric heads in the piezometers were all below ground level, in the February round the  
13 piezometric head in 28c and 28d was above ground level by 10 mm, and the area around this nest  
14 flooded. In April 2011 they were below ground level at both sites. NP suboxic sampling was carried  
15 out in a portable chamber with an inert atmosphere (BOC™ oxygen free N<sub>2</sub> gas). Piezometers were  
16 purged (minimum of 3 borehole volumes) and sampled using a peristaltic pump at a flow rate of 100  
17 mL/minute. DO concentrations in both the pumped groundwater and the chamber were monitored  
18 continuously prior and during sampling. Samples were stored in sealed high density polyethylene  
19 bottles. All containers were acid-washed (dilute nitric acid, Aristar™ grade) and rinsed with ultra-pure  
20 water ( $R > 18.2 \text{ M}\Omega\text{cm}^{-1}$ ) prior to sampling. A surrounding jacket of airtight suboxic groundwater was  
21 used to protect the sample from DO diffusion prior to analysis, in a similar manner to methods used  
22 for groundwater dating using chlorofluorocarbons<sup>[27]</sup> (See Figure 1). Samples were filtered using two  
23 45 mm diameter in-line 0.45  $\mu\text{m}$  (Milipore™) membrane filters in the field to remove larger  
24 particulate material, which is known to result in rapid aggregation.<sup>[28, 29]</sup> More detail on the sampling  
25 process and groundwater chemistry are provided in Supplementary Information.

1 **Evaluation of Sampling and Storage Methodology.** In suboxic waters, where dissolved Fe and Mn  
2 are present in reduced Fe (II) and Mn (II) forms, contact with oxygen result in these species being  
3 oxidised, via Fe(III) and Mn (III, IV), to form colloidal Fe and Mn oxide phases.<sup>[30]</sup> This will  
4 potentially result in a shift in the distribution of Fe/Mn NP to larger sizes due to aggregation. To test  
5 the robustness of suboxic sampling and analytical procedure, an aliquot of sample was aerated by  
6 gently bubbling with compressed air for 10 minutes. This was carried out under laboratory conditions  
7 to investigate the evolution of NP during aeration and compare NP size distributions using AFM and  
8 FIFFF with aliquots that had been sampled using the suboxic methodology. As well as during  
9 sampling, DO was monitored in the samples before and after carrying out the preparation for AFM to  
10 ensure that samples were not contaminated with atmospheric oxygen during storage and transport.

11 **Atomic Force Microscopy.** AFM determines the height of NP, after sorption to a suitable flat  
12 substrate (mica in this case), by exploiting the repulsive and attractive forces between the sample and  
13 the silicon cantilever mounted instrument tip . All sample preparation and analysis with AFM were  
14 carried out within 36 hours of groundwater sampling to minimise any changes in NP composition  
15 during sample storage. The groundwaters (suboxic and aerated aliquots) were transferred to 5 mL  
16 vials under a nitrogen atmosphere. This method specifically focuses on: a) small diffusible NP and b)  
17 NP which strongly bind to the mica. The mica sheets were then rinsed by immersing in suboxic  
18 ultrapure water for a few seconds (to remove slats and loosely adhered materials) followed by drying  
19 under 60 % humidity in nitrogen atmosphere for 30 minutes. With the AFM-instrument (Park System  
20 XE-100) placed in a laminar glove box with nitrogen-atmosphere, AFM-images were acquired over  
21 different areas (e.g. 0.5×0.5 μm, 2×2 μm, 20×20 μm) of the mica, in non-contact mode with a 42 N m<sup>-1</sup>  
22 force constant and 330 kHz frequency. For each sample, the NP height distribution of >190 particles  
23 was determined by measuring the maximum NP height above the mica of 25-35 NP on at least 6  
24 different 2×2 μm images. The relationships  $\sum n_i z_i^2 / \sum n_i z_i$  and  $\sum n_i z_i / \sum n_i$  were used to calculate the weight  
25  $S(z)$  and number  $N(z)$  average particle heights respectively for each sample where  $n_i$  is the number and  
26  $z_i$  is the height of each particle measured. The polydispersity ( $P$ ) of the samples was evaluated using  
27 the relationship  $S(z)/N(z)$ .<sup>[15]</sup>

1 **Flow Field Flow Fractionation.** FIFFF is a technique that determines the hydrodynamic diameter  
2 distribution of NP based on their interaction with a cross flow field while they are eluted along a flow  
3 channel.<sup>[15]</sup> The asymmetrical FIFFF instrument (AF 2000, Postnova Analytics) had a channel defined  
4 by a 0.35 mm spacer and a 1 kDa nominal cut-off ultrafiltration membrane of regenerated cellulose  
5 (Postnova Analytics). The suboxic FIFFF-carrier solution was made up of 10 mM NaCl at pH 8. 5 mL  
6 of the groundwater samples (suboxic and aerated aliquots) were injected and focused in the channel  
7 for 30 min with a tip flow of 0.5 mL min<sup>-1</sup> and a focus flow of 2.5 mL min<sup>-1</sup>, followed by elution and  
8 fractionation with a crossflow of 3 mL min<sup>-1</sup> and detector flow of 0.5 mL min<sup>-1</sup>. NP eluting from the  
9 FIFFF-channel were detected on-line with UV-absorbance at 254, 350, 400, 575 and 700 nm and  
10 fluorescence at excitation-emission pair 350/450 nm which is specific to humic material. The  
11 continuous size distributions of NP were determined by converting retention time into diffusion  
12 coefficient. Equivalent hydrodynamic diameter distribution ( $d_H$ ) was calculated using the FIFFF-  
13 theory based on the Stokes-Einstein relationship,<sup>[31]</sup> after calibrating the FIFFF channel thickness  
14 using two proteins (bovine serum albumin and ferritin) with known diffusion coefficients. Number  
15 average diffusion coefficients  $D_N$  for particles in the 0-5 nm range were calculated using the  
16 relationship  $\sum m_i D_i^2 / \sum m_i D_i$  where  $m$  is the mass based signal obtained by UV254 absorbance,  
17 proportional to concentration of organic matter, and  $D$  is the diffusion coefficient calculated using  
18 FIFFF theory.

19 **Transmission and Scanning Electron Microscopy.** SEM and TEM are imaging techniques based on  
20 the electrons emitted/backscattered from (SEM) or transmitted through (TEM) the sample exposed to  
21 an electron beam.<sup>[32]</sup> TEM and SEM are able to distinguish certain types of NP in complex samples,  
22 quantify their size and morphology, and have been used to calculate fractal dimensions of e.g. humic  
23 substances.<sup>[17]</sup> In combinations with energy dispersive X-ray (EDX), measuring the energy of  
24 characteristic X-rays emitted from the sample under the electron beam, TEM and SEM can be used to  
25 determine the major element composition of individual NP.<sup>[11]</sup> Samples for TEM and SEM were  
26 prepared under nitrogen atmosphere, by placing droplets of the samples (sub-oxic and aerated  
27 aliquots) on Formvar/carbon coated 300 mesh Cu TEM grids placed horizontally on a clean surface.



1 After 30 min, the grids were rinsed by immersing in suboxic ultrapure water for a few seconds,  
2 followed by drying in a nitrogen atmosphere. TEM (JEOL 1200EX) images were acquired at 80 keV,  
3 on five different areas of the grids using a range of magnifications (30 000-500 000).. SEM images  
4 (JEOL 7000) with associated EDX spectra were acquired on three different particles for each sample  
5 as well as on the background grids. All microscopy was carried out within two weeks of sampling.  
6 Although the TEM grids were prepared shortly after sampling, and dried under nitrogen to minimise  
7 oxidation, some grids were stored in air after drying for several days prior to analysis. The formation  
8 of NP and colloids as a result iron and manganese oxidation of the dried samples is therefore possible  
9 but minimal.

10

## 1 **Results and Discussion**

2 **Assessment of suboxic sampling protocol.** Figure 2 shows examples of AFM scans ( $1 \times 1 \mu\text{m}$ ) of NP  
3 from each site with and without aeration. The AFM NP size range for suboxic samples were found to  
4 be between 1-20 nm, while those which were aerated had NP had mean heights of  $>50\text{nm}$ , and in  
5 some cases these reached sizes of  $>100 \text{ nm}$ . DO concentrations in samples prior to preparation were  
6  $<0.5 \text{ mg/L}$ , and had not altered during storage. These results show the rapid formation and  
7 agglomeration of NP following contact with air, resulting in a dramatic shift in the NP size  
8 distribution. This demonstrates that maintaining suboxic conditions during storage and preparation is  
9 important. The shift in particle size and properties was also corroborated by FIFFF and SEM data and  
10 provides strong evidence that the NP distributions in aliquots that were sampled and processes under  
11 suboxic conditions are representative of true environmental conditions. Filtering the samples prior to  
12 NP characterisation is important to reduce aggregation, microbiological activity, and the potential  
13 breakdown of organic and mineral phases, as well as sorption/desorption reactions on particulate  
14 phases.

15 **Nanoparticle size distributions.** Adsorbed NP height distributions detected by AFM for the suboxic  
16 samples from all four sites are graphically summarised as kernel-density estimates in Figure 3a-d.  
17 Median values for the samples in each case are shown as vertical lines in Figure 3. It is clear from  
18 these plots that none of the samples follow a normal distribution, as confirmed for all samples using a  
19 Shapiro-Wilks test <sup>[33]</sup> for normality ( $p < 0.05$ ). Results of a Kruskal-Wallis <sup>[34]</sup> test were found to be  
20 significant ( $p < 2 \times 10^{-16}$ ); the mean ranks of particle heights are significantly different for the 7  
21 samples. Median values for all sites were close to 10 nm (see Figure 3). However, these comparisons  
22 do not represent the complex polymodal distribution found in all samples. The proportion of adsorbed  
23 particles observed by AFM that were in the 0-5 nm size range, typical sizes for humic-like  
24 macromolecules, varied between 5-50%, and for most samples was only found to be approximately  
25 10%. However, the smaller particles may be over represented due to the relatively short adsorption  
26 time (30 min) used in the sample preparation. Sites from the more shallow locations (26c and 28c)  
27 had bi-modal distributions, and were less skewed compared to the samples from the deeper sites. The

1 three samples from the deeper sites had more complex distributions with a greater proportion of larger  
2 outliers with particle heights >25 nm. Using a Wilcoxon rank sum test <sup>[35]</sup>, samples from the two  
3 shallow sites 26c and 28c were found to have significantly different mean ranks of particle height for  
4 the two different sampling rounds ( $p < 6 \times 10^{-14}$ ), while for sample 26d this was not found to be  
5 significant ( $p = 0.79$ ). This may suggest that the particle distribution in the shallow sites show  
6 comparatively greater temporal variability.

7 Average polydispersity index ( $P$ ) (Table 1) were found to be comparable for the two shallow  
8 groundwaters, ca.1.1, while they were higher for the two deeper sites (1.4 and 2.1 respectively). This  
9 suggests that there is an increase in NP heterogeneity with depth. This is likely due to a range of  
10 processes including microbiological processing of DOC in the leachate plume, redox and pore-water  
11 diffusion controlled processes as well as, seasonally dynamic recharge and mixing of waters.<sup>[36, 37]</sup> In  
12 addition, the groundwater chemistry (Table S1, supplementary information) shows that the deeper  
13 sites are also affected to a greater extent by the landfill leachate plume (e.g. higher DOC, SEC,  
14  $\text{HCO}_3$ ) which is likely to be a major factor driving the geochemical processes and colloid generation  
15 in this shallow aquifer.

16 For all sites there were two dominant populations, one with a median particle height of ca. 5 nm and a  
17 second with a particle height of ca. 15 nm, with overlapping distributions. In the shallow sites these  
18 were found to change in their relative proportions during the two sampling rounds. For example, at  
19 site 26c (Figure 3a), the larger population dominated the December round, while the smaller  
20 population dominated the February round. The reverse was observed for site 28c (Figure 3c). These  
21 results suggests that the NP distributions in the deeper sites are less dynamic compared to those in the  
22 shallow samples which showed marked shifts in distributions between sampling rounds. Overall,  
23 these observed differences are likely to be due to mixing and recharge processes in the shallow  
24 groundwaters influenced in part by regular inundation within the alluvial floodplain. The pre (first  
25 round) and post (second round) inundation conditions at site 28c could perhaps explain the observed  
26 changes in DOM within the shallow aquifer at this site, with a shift to larger NP caused by particle  
27 agglomeration due mixing of suboxic groundwater with soil water and surface water as groundwater

1 levels rise with the onset of inundation. One explanation for these different populations is that the  
2 larger particles represent agglomerations of smaller particles. Alternatively, they are different types of  
3 NP, perhaps the small NP representing humic/fulvic like organic matter while the larger NP represents  
4 iron rich mineral NP.<sup>[15, 38]</sup> These possibilities were further explored by FIFFF coupled to UV and  
5 fluorescence detection.

6 **Hydrodynamic diameters of UV-absorbing material.** Continuous 0.1-10 nm hydrodynamic  
7 diameter and diffusion coefficient distributions of UV-absorbing material, determined by FIFFF, are  
8 shown in Figure 4. Diffusion coefficients of suboxic samples were distributed between  $1-5 \times 10^{-10}$   
9  $\text{m}^2\text{s}^{-1}$ , with maximum values between  $2-4 \times 10^{-10} \text{m}^2\text{s}^{-1}$ , see Figure 4a. The hydrodynamic diameter of  
10 UV-absorbance at 254 nm (UV254) was mainly distributed over the 0.5-5 nm size range, with a  
11 maximum at around 1.5 nm (Figure 4c). The hydrodynamic diameter of UV-absorbing material at 575  
12 nm (UV575) in the suboxic samples had its maximum at around 3 nm. UV254 showed a secondary  
13 maximum, coinciding with the 3 nm maximum for samples 26c and 26d, this peak was not detected  
14 for sample 28d. In the aerated samples the UV575-signal was too low to be distinguished from the  
15 background, and the relative amount for UV254 in the aerated samples were less than half of those in  
16 the suboxic samples.

17 The material giving rise to a maximum UV254 at 1.5 nm is most likely fulvic or humic acid-like.<sup>[39]</sup>  
18 This was also confirmed by excitation emission matrix fluorescence spectroscopy of bulk samples  
19 (Figure S2) and FIFFF coupled to fluorescence excitation emission spectroscopy specific to humic  
20 material (see Figure S3).

21 A comparison of number average adsorbed particle heights by AFM and number average  
22 hydrodynamic diameters by FIFFF are shown in Table 1. In all cases average heights by AFM were  
23 larger by a factor of at least 5 compared to the number average values for the hydrodynamic diameters  
24 measured using FIFFF-UV absorbance. To some extent, the different size distributions achieved by  
25 AFM and FIFFF-UV can be explained by the AFM analysed particles being in a semi-dried state,  
26 while FIFFF-UV analysed them in liquid suspension. However, more important is probably the

1 selectivity of FIFFF-UV for particles with strong UV-absorbance, such as humic and fulvic acids,  
2 which are typically found in the same 0.5-5 nm hydrodynamic diameter range as most of the UV254-  
3 absorbing material detected by FIFFF-UV. Coupling other detectors to FIFFF, such as inductively  
4 coupled plasma mass spectrometry (ICP-MS), has shown that inorganic (e.g., iron-rich) colloids in  
5 natural waters are typically distributed towards larger hydrodynamic diameters relative to UV-  
6 absorbance <sup>[7, 19, 40]</sup>, closer to the average particle height determined by AFM in the present study.  
7 Nevertheless, it is surprising that such small fractions (usually around 10%) of the particles detected  
8 by AFM were in the 0-5 nm hydrodynamic diameter range, where most UV254-absorbing matter was  
9 detected by FIFFF-UV, and where fulvic and humic acids are expected to be found. <sup>[39]</sup> One  
10 mechanisms that could explain this result is the selective adhesion of near-neutrally charged particles  
11 (e.g., most iron oxyhydroxides at neutral pH) over particles with strong negative charge (e.g., humic  
12 and fulvic acids) to the negatively charged mica <sup>[41]</sup>. It is possible extensive adhesion of humic and  
13 fulvic acids result in the formation of a surface film on the muscovite surface <sup>[42]</sup> making it impossible  
14 to distinguish these particles in our samples using AFM. Formation of larger particles through the  
15 aggregation of humic and fulvic acids during AFM sample preparation is not likely to have occurred,  
16 because such aggregates are typically much more irregular in shape than the spherical particles  
17 detected in our samples by AFM <sup>[32]</sup> and the method that we used for AFM sample preparation has  
18 been optimised to minimise such artefacts. <sup>[42]</sup>

19 Our findings illustrate that AFM and FIFFF-UV are complimentary techniques, detecting different  
20 types of NP in groundwater samples. The reduction of the UV254 peak at 1.5 nm, and the removal of  
21 the UV575 peak at 3 nm in the FIFFF fractograms when the samples were aerated (Figures 4b and 4d)  
22 could be explained by co-aggregation or incorporation of organic macromolecules in Fe or Mn rich  
23 colloids (see Figures S7 and S8 for elemental compositions), such as those observed by AFM as a  
24 result of aeration (Figure 2). In addition, the application of phase contrast AFM could also help  
25 distinguish between hard and soft colloids. <sup>[43]</sup>

26 Diffusion coefficients are important for understanding the transport of natural organic matter in the  
27 subsurface where movement within porewaters is dominated by molecular diffusion. Number average

1 diffusion coefficients ( $D_N$ ) for organic NP calculated using the FIFFF-UV data (0-5 nm range) are  
2 shown in Table 1. These were found to be between  $3\text{-}3.3 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$  for suboxic samples and are  
3 10-15% higher for aerated samples. These values are consistent with values obtained using model  
4 humic and fulvic (Suwannee River) substances ( $2\text{-}3 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ) for comparable pH values using  
5 fluorescence correlation spectroscopy and FIFFF.<sup>[39]</sup> The aeration experiments (Figure 4b and 4d)  
6 show that there is contrasting reactivity between the two populations of organic NP, with the larger  
7 population (2-4 nm range) being rapidly removed while a proportion (ca. 30%) of the smaller sized  
8 population (0.5-2 nm range) remained in solution. This could be explained in terms of higher  
9 reactivity to oxidation of higher molecular weight DOM fraction compared to lower molecular weight  
10 fractions due to size-charge ratio effects and greater repulsion of fulvic like DOM compared to humic-  
11 like DOM.<sup>[44]</sup> Understanding the temporal variability of DOM is important for understanding trace  
12 metal binding and transport in these dynamic floodplain environments as the lower molecular weight  
13 fulvic acid fraction is known to exhibit a much higher negative charge density, at a given pH/ionic  
14 strength, compared to humic acid fractions.<sup>[45]</sup> The regular cycles of inundation may lead to rapid  
15 changes in the DOM characteristics as DOM is transported from the surface to depth within the  
16 alluvium and alluvial deposits.

17 **Morphology and surface chemistry.** A range of particles with differing sizes and morphology were  
18 observed from the TEM-micrographs of suboxic samples. Selected TEM micrographs from all four  
19 sites are shown in Figure 5 a-h illustrating the range of different NP the occurrence of few large (>100  
20 nm) colloids present in the suboxic groundwater. TEM images for the shallower groundwaters (Figure  
21 5b and 5f) show that these samples are dominated by a large number of compact NP in the 5-20 nm  
22 size range, with both electron dense and poor regions. These NP dominate over larger colloids in both  
23 AFM and TEM samples. The regions which were more transparent to electrons are presumably rich in  
24 organics, while those that are dark are rich in Fe. These contrast with the results for the deeper sites  
25 (figure 5d and 5h) which show a greater number of larger particles with more complex morphologies,  
26 corroborating the AFM results. Figure 5g illustrates the association of Fe rich (electron dense regions)  
27 and organic NP (electron poor regions), and the agglomeration of monomers to form macromolecules

1 with complex shapes and high polydispersity, which again corroborates the results for the AFM  
2 analysis (Table 1).

3 In the TEM micrographs, NP of a few nm in size were observed in the suboxic samples (Figure 5), but  
4 were not present in aerated samples (Figure S4, supplementary material). This is also consistent with  
5 the observations made using AFM. The presence of high dissolved and nanoparticulate Fe suggests  
6 that DOC from the landfill has mobilised Fe oxides by reductive dissolution and produced in-situ  
7 colloidal and nanoparticulate Fe oxides.<sup>[30]</sup> Fibrillar material was also observed in association with the  
8 colloidal sized material in sample 28c (Figure 5e), presumably indicating a source of biopolymeric  
9 organic matter in the shallow groundwater. The fact that the larger colloids (>50 nm) were observed  
10 in the suboxic samples by TEM but not by AFM can be explained by the fact that the probability of  
11 finding them with AFM was much lower, since AFM-images were acquired on random locations of  
12 the mica substrate, while TEM-micrographs were acquired on particles selected after searching large  
13 areas of the TEM-grids. In addition, the electron dense particles show up clearly on the TEM images  
14 while less dense (e.g. organic) are less obvious. The formation of NP by the oxidation of iron and  
15 manganese by the electron beam cannot be ruled out. However, such perturbations are assumed to be  
16 minimal, since no changes of the samples could be observed during TEM-analysis.

17 SEM micrographs taken for suboxic samples from all four sites are given in Figures S5-S8. These  
18 showed colloids with similar shapes as the larger electron dense colloids on the TEM-micrographs.  
19 EDX (energy dispersive X-ray spectroscopy) enabled the determination of major element composition  
20 at selected locations. The EDX results show two different types of colloids that dominated these  
21 samples: i) larger colloids rich in Ca and O (as calcite, given these samples are calcite saturated) with  
22 minor amounts of Fe, Mn, S, Al and Si, ii) Fe, Mn and oxygen rich colloids (Fe/Mn-oxyhydroxides)  
23 associated with more minor quantities of Ca and P.

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4 permission to access the field site. This manuscript is published with the permission of the BGS  
5 director.

## 6 **Supporting Information Available**

7 Text, tables and figures describing the study site, groundwater chemistry, FIFFF-fluorescence results,  
8 TEM/SEM micrographs of aerated samples and summary EDX results. This information is available  
9 free of charge via the Internet at <http://pubs.acs.org/>.

10

## 11 **Tables:**

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1 **Table 1. Summary table of number and weight average particle heights by AFM, polydispersity**  
 2 **index, number average hydrodynamic diameter and diffusion coefficients for organic NP by**  
 3 **FIFFF-UV**

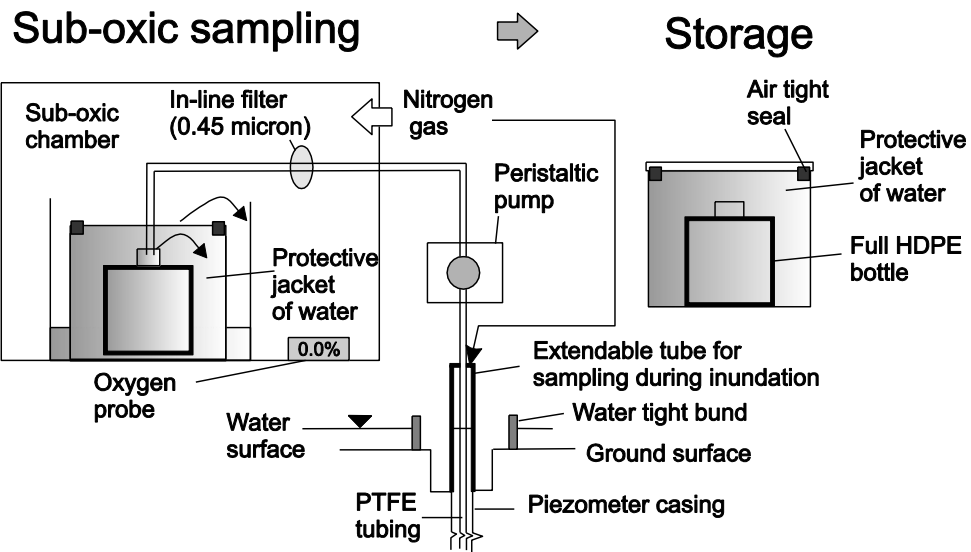
Site	<i>P</i>	$N_{(z)}$ (nm)	$S_{(z)}$ (nm)	$d_H$ (nm)	$d_H^*$ (nm)	$D_N$ ( $10^{-10} \text{ m}^2 \text{ s}^{-1}$ )	$D_N^*$ ( $10^{-10} \text{ m}^2 \text{ s}^{-1}$ )
26c	1.2	11.7	13.5	2.0	1.4	3.04E-10	3.51E-10
26d	1.4	11.3	15.7	1.8	1.3	3.2E-10	3.45E-10
28c	1.2	10.0	11.7	N/A	N/A	N/A	N/A
28d	2.1	9.2	19.5	1.6	N/A	3.29E-10	N/A

4  $N_{(z)}$  is the number average particle height by AFM,  $S_{(z)}$  is the weight average particle height by AFM.  $D_N$  is the number average diffusion  
 5 coefficient by FIFFF-UV (245 nm for the 0-10 nm range),  $d_H$  is the number average hydrodynamic diameter by FIFFF-UV (245 nm for the  
 6 0-10 nm range), \* indicates values for aerated samples

7  
 8  
 9  
 10  
 11

1 **Figures:**

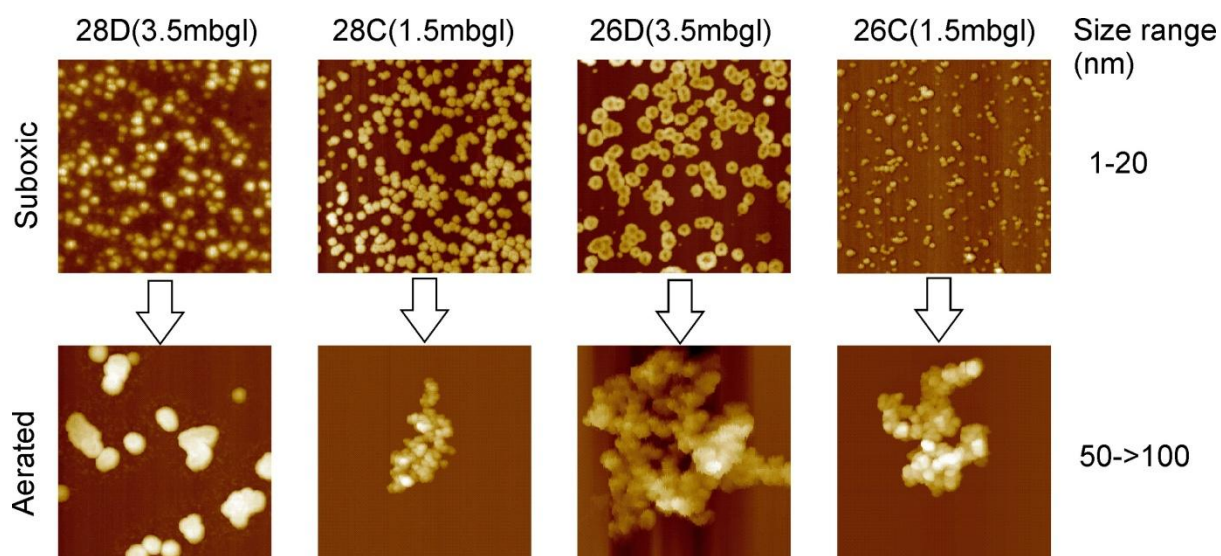
2



3

4 Figure 1. Schematic of suboxic groundwater sampling during inundation and storage methodology.

5

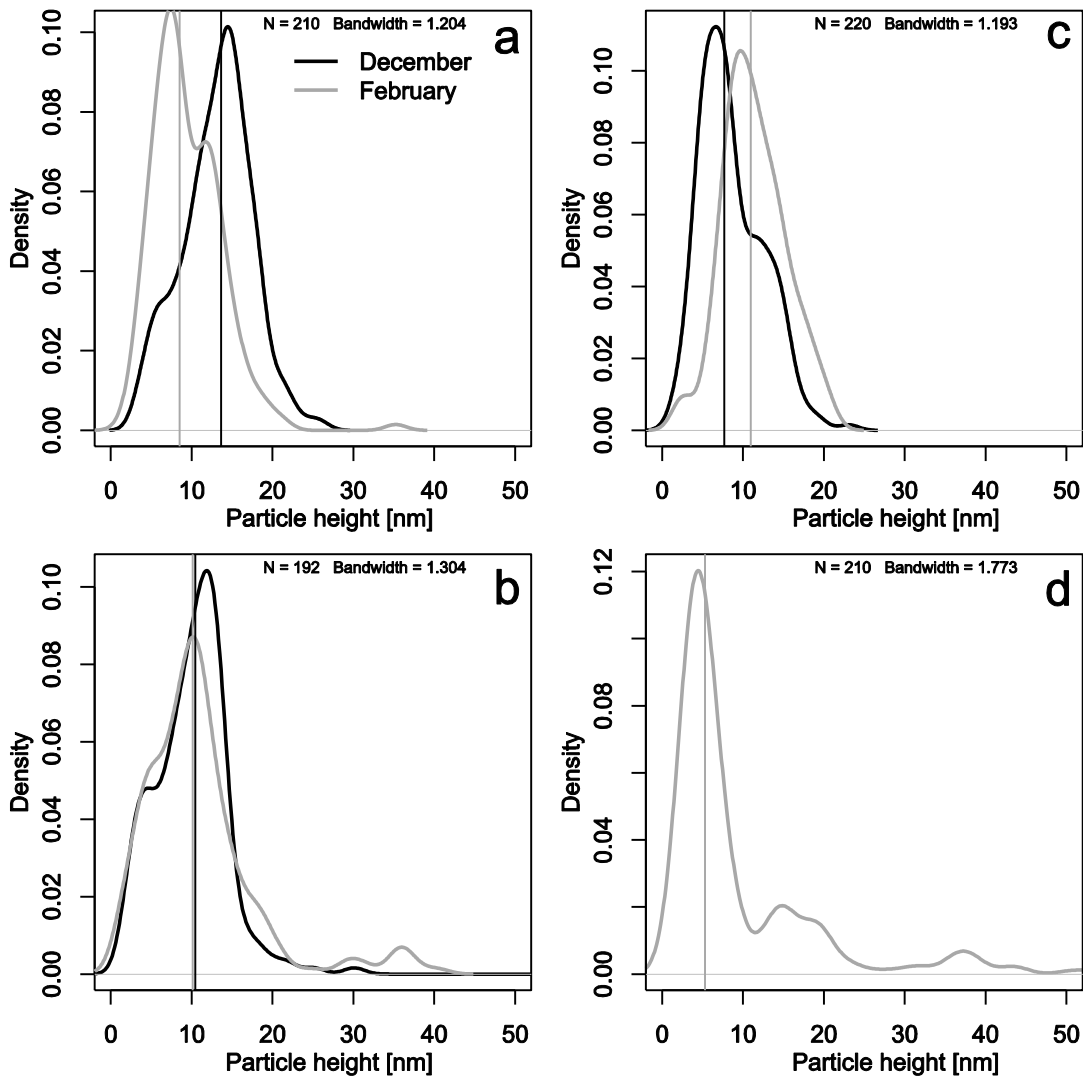


1

2 Figure 2. Typical AFM scans ( $1 \times 1 \mu\text{m}$ ) for all four samples before and after aeration experiments.

3 Aeration carried out by bubbling with compressed air for 10 minutes.

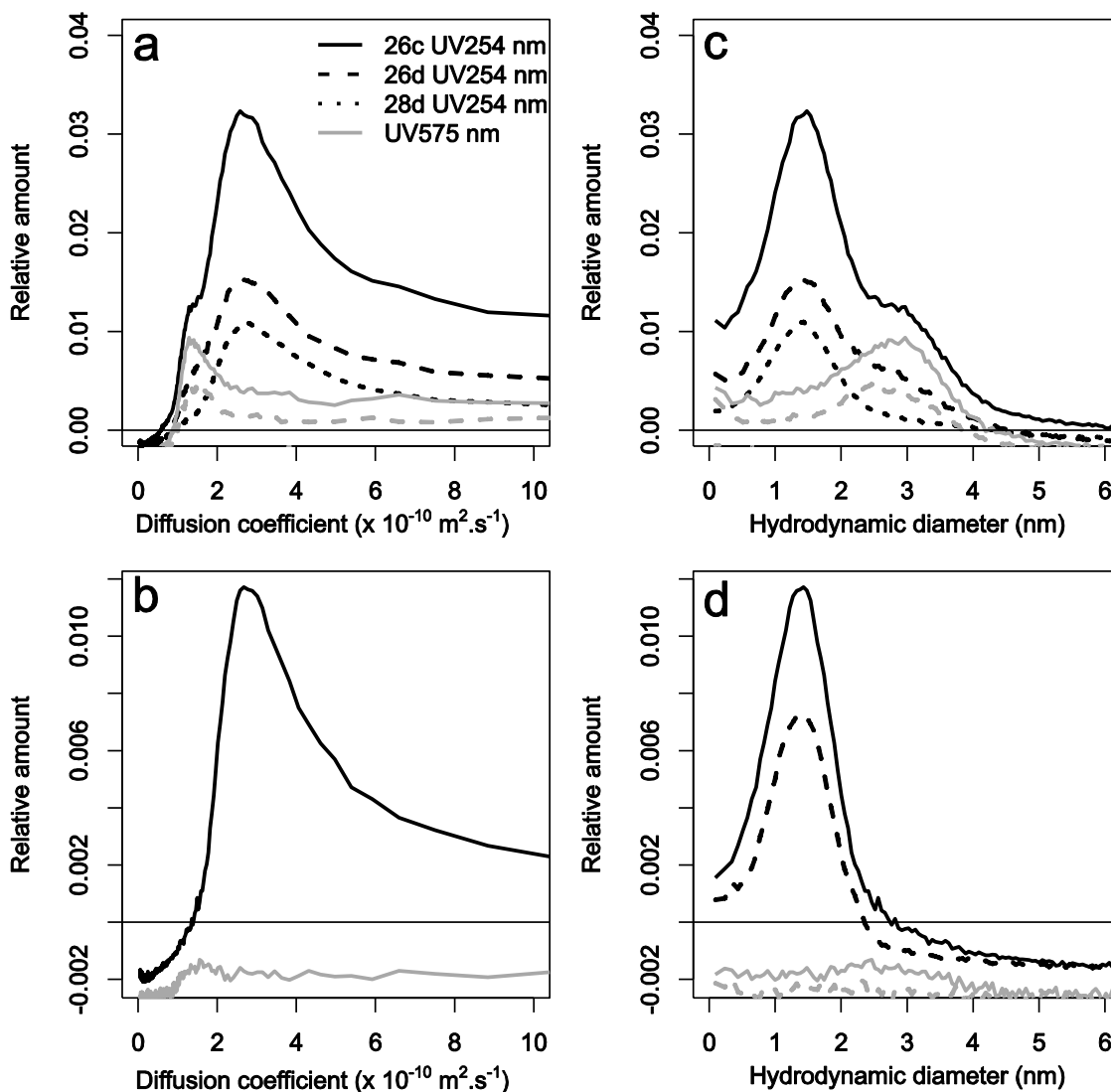
4



1

2 Figure 3. Kernel density estimates for suboxic AFM results for all four sites: (a) 26c, (b) 26d, (c) 28c  
 3 and (d) 28d. Results from December 2010 sampling round shown in black, results from February  
 4 round are shown in grey. Results comprise >190 individual measurements (N) for each sample, AFM  
 5 carried out in a N<sub>2</sub> atmosphere following absorption to mica under suboxic conditions. Vertical lines  
 6 show median values for the distributions. Kernel density estimates <sup>[46]</sup> are similar to histograms but  
 7 represent a continuous, smooth, distribution giving a more realistic representation of the particle  
 8 height distributions, removing the dependence on the end points of bins and minimising bin width  
 9 effects. A Gaussian kernel was used and the smoothing bandwidth was optimised and scaled to be the  
 10 standard deviation of the smoothing kernel.

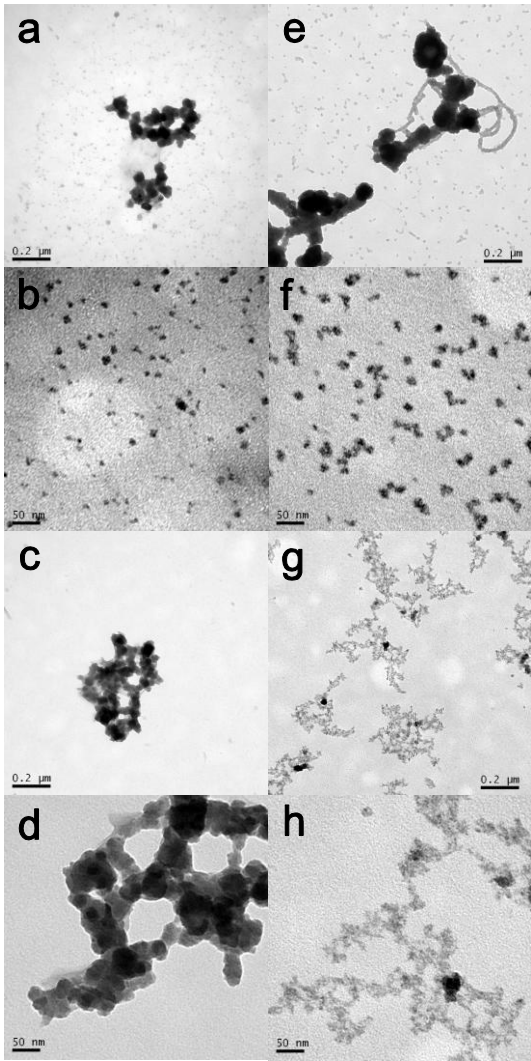
11



1

2 Figure 4. FIFFF fractograms: (a) diffusion coefficient for suboxic samples, (b) diffusion coefficient  
 3 for aerated samples, (c) hydrodynamic diameter for suboxic samples, (d) hydrodynamic diameter for  
 4 aerated samples. Plots in black show result for UV 254 signal, plots in grey show results for UV 575  
 5 nm. FIFFF setup: channel flow of  $0.5 \text{ mL min}^{-1}$  and a focus flow of  $2.5 \text{ mL min}^{-1}$  for 30 min, elution  
 6 and fractionation carried out with a crossflow of  $3 \text{ mL min}^{-1}$  and detector flow of  $0.5 \text{ mL min}^{-1}$ .  
 7 Suboxic analysis carried out after degassing buffer solution with  $\text{N}_2$ .

8



1

2 Figure 5. Typical TEM micrographs for suboxic samples: (a) 26c, (b) 26c, (c) 26d, (d) 26d, (e) 28c,

3 (f) 28c, (g) 28d, (h) 28d.

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