

Supporting Information

Sequestration and re-mobilization of radioiodine (^{129}I) by soil organic matter and possible consequences of the remedial action at Savannah River Site

Chen Xu^a, Eric J Miller^a, Saijin Zhang^a, Hsiu-Ping Li^a, Yi-Fang Ho^a, Kathleen A Schwehr^a, Daniel I. Kaplan^b, Shigeyoshi Ootosaka^c, Kimberly A Roberts^b, Robin Brinkmeyer^a, Chris M. Yeager^b, Peter H. Santschi^a

^aLaboratory for Environmental and Oceanographic Research, Department of Marine Sciences, Texas A&M University, Building 3029, Galveston, Texas 77551

^b Savannah River National Laboratory, Aiken, SC 29808

^cResearch Group for Environmental Science, Japan Atomic Energy Agency, Tokai-Mura, Ibaraki 319-1195, Japan

17 pages containing 2 tables and 6 figures

Materials and methods

Study area and sampling protocols. The selected organic-rich soil sampling sites included site FSI18, in the path of the contaminated plume area, a background site (named “4Mile Creek”), close to the Four Mile Branch, where the contamination is regarded as minimal. A third organic-poor sandy soil was sampled to the north of the F-Area seepage basin (named “N Borrow”), where the surface soil had the same formation as that composed of the aquifer and regarded as “background” (Fig. S1). The soil samples were stored and shipped in zip lock bags under ice and immediately transferred to a refrigerator at 4 °C in the lab. Information on soil composition information is provided in Table S1.

^{125}I and $^{125}\text{IO}_3^-$ sources and validation of their application for tracking stable iodine behaviors. Carrier-free ^{125}I was purchased in the form of NaI from MP Biomedical, USA. $^{125}\text{IO}_3^-$ was prepared from ^{125}I by combustion and oxidation of Na ^{125}I . 0.5 mL of Milli-Q water and 5 μL of Na ^{125}I (45 μCi) was mixed with 100 mg V_2O_5 and combusted with a programmed procedure (see below). Oxygen flow was adjusted to 100 ml/min to ensure the well-oxidation of iodide. Still, the conversion was only 50% (i.e., $\text{I}:\text{IO}_3^-=1:1$). Thus a Strata X_A 33u polymeric strong anion exchange column (Phenomenex, USA, Cat #: 8B-S123-EBJ) was used to separate I^- from IO_3^- . The column was first conditioned with 2mL methanol and then 2mL Milli-Q water. 2mL of the solution after combustion and rinsing was loaded on the column and then eluted with 20 mM ammonium acetate. No I^- was detected in the eluate according to the speciation analysis. The purpose of using combustion method (SI) instead of other chemical conversion is to minimize the interference during soil organic matter (SOM) iodination experiment.

Though the uptake of the inorganic iodine by the different soils used in this study was calculated based on a mass balance (activity of total added inorganic iodine-125 minus that of

aqueous iodine-125), controls of only $^{127}\text{I}^-$ and $^{125}\text{I}^-$ (or $^{127}\text{IO}_3^-$ and $^{125}\text{IO}_3^-$) at two experimental concentrations (20 μM and 0.1 μM) shaken only with artificial freshwater (S2) for the same period of time and undergoing all processing steps showed that the sorption of inorganic iodine species onto the experimental devices or release out of the system (e.g., in the form of I_2) to be negligible, with recoveries very close to 100%. In addition, the incorporated iodine-127 into the solid, colloidal and dissolved phases were also measured by combustion followed by GC-MS analysis and quantified by the difference between the amendment and control groups. The results based on the radiotracer ($^{125}\text{I}^-$ or $^{125}\text{IO}_3^-$) agreed well with those of the combustion and GC-MS analysis (error within 6%), and therefore validate the experimental approach of using radiotracer $^{125}\text{I}^-$ or $^{125}\text{IO}_3^-$ as spikes to track the behavior of stable iodine in the soil and groundwater.

Time series soil resuspension experiment. A series of 40 mL soil slurries were gently shaken with an orbital shaker at ~160 rpm in 50 mL centrifuge tubes containing some headspace. Samples were then allowed to sit without caps for half an hour twice a day to exchange air with the atmosphere to avoid anoxic conditions. At each time point (the 4th, 10th, 17th, 24th and 31th day) two duplicate tubes were taken and processed for each treatment. Particles were allowed to settle by low-speed (~3200 g) centrifugation and the supernatant was immediately filtered by using sterile disposable vacuum filter/storage systems (Corning, USA). The filtrate (< 0.45 μm) was ultrafiltered through an Amicon Ultra-15 centrifugal device with a cut-off of 3 kDa. The soil pellet was exhaustively washed several times with 0.1 M KCl to measure exchangeable I^- or IO_3^- that was reversibly bound onto the solid phase, until no more activity was observed in the aqueous phase (<0.45 μm).

Iodine speciation analysis. Determination of iodide and iodate followed Zhang et al. (S3). Basically, iodide was oxidized by 2-iodosobenzoate to iodine, which then subsequently iodinated

N,N-dimethylaniline to become 4-iodo-dimethylaniline. The resulting derivative was extracted by cyclohexane and detected either by GC-MS or liquid scintillation counter if ^{125}I has been added. Iodate was converted to iodide first by sodium meta-bisulfite, followed the same reaction as iodide and calculated as the difference between total inorganic iodine and iodide. The presence of high organic matter “smeared” the detection of inorganic iodine by interacting with the reagents, thus sample amended with known I^- (or IO_3^-) was also measured and compared to the sample without the addition of standard, to calculate the recovery.

The determination of total iodine (^{127}I and ^{129}I) in aqueous solution followed the procedure of Zhang et al. (S3), with slight modification. 2mL of sample was mixed with 150 mg of vanadium pentoxide (V_2O_5), which acted as a catalyst for rapid combustion, and were placed in a porcelain boat. ^{125}I was added to the sample- V_2O_5 mixture as a tracer for the pyrolysis recovery. The boat with the sample and catalyst was gently pushed into the center of a quartz combustion tube and subjected to combustion on a tube furnace (Lindberg/Blue M Mini-Mite, Thermo Scientific). The temperature of the furnace was programmed as follows: the temperature was increased to 200 °C within one minute and then held constant for three minutes. After that, the temperature was increased to 900°C over ten minutes and left there for an additional 10 minutes. Oxygen was used as carrier gas at a constant flow rate of 113mL/min during the whole program. The iodine released by pyrolysis was collected into a pyrex glass tube containing 1 mL of Milli-Q water. After the pyrolysis program was finished, carrier gas was switched from oxygen to nitrogen at a flow rate of 134 mL/min and allowed to purge the receiving water for 30 minutes. Total iodine was measured by Finnigan Trace GC and Polaris Q EI-MS from Thermo, after being derivatized to iodinated N, N-dimethylaniline. Quantification of ^{127}I and ^{129}I was based on the different masses of their respective derivatives in the mass spectrum, which were used as the “screening

filter” to distinguish their respective responses in the gas chromatograms. Organo-iodine was calculated as the difference between total iodine and total inorganic iodine.

Aromaticity. Aromaticity, as defined as the peak area in the 110-160 ppm chemical shift band of quantitative ^{13}C -NMR spectra, was determined according to its relationship with UV absorbance (S4). Briefly, freeze-dried samples were re-dissolved in a phosphate buffer and passed through a 0.45 μm syringe filter. Total organic carbon of the filtrate was determined using a Shimadzu TOC-5000 analyzer. UV absorbance (280nm) was determined using a Turner spectrometer. Absorptivity (L/mole of OC/cm) of eight IHSS humic substances was determined by the same way and plotted against their reported aromaticity, which was obtained from IHSS website and determined by ^{13}C -NMR. The resulting calibration equation ($y=0.063x-3.679$, x and y denote absorptivity and aromaticity, respectively) was used to estimate aromaticity from sample molar absorptivity.

Extraction of HA, FA and WEC. Extraction of HA and WEC was described elsewhere (S5), but briefly mentioned here. Five HA fractions were obtained by repeatedly extracting the same soil five consecutive times following a standard procedure recommended by IHSS (S6). The resulting HAs were named HA1, HA2,..., HA5. Two successive glycerol-extracted HA fractions and one alkaline extracted fraction were obtained from the soil residue after five times of HA extraction, by twice extracting the residue with 50% glycerol followed by removal of major cations (e.g., Fe) through complexation with 0.5 M citric acid, and extraction one additional time with 0.1 M NaOH (S7). The three HA fractions were named as HA6, HA7 and HA8. Thus the first five HAs and FAs probe the SOM that are loosely bound to the soil mineral and thus can be more easily released under alkaline/acid extraction conditions. Glycerol has the ability of penetrating into the interlayers of the phyllosilicates and thus releasing any HS still trapped in

the interlayers. SRS soils commonly have high Fe-oxide contents. For example, Hixon et al. (S8) used Mossbauer spectroscopy to determine that the sediment collected in an adjacent aquifer at the F-Area contained 16,000 ppm Fe, consisting of 34% hematite, 59% goethite, and 7% Fe in clay structures. Citric acid was used to complex iron or other major cations, which are involved in the formation of HS-metal-clay complexes, and make the humic acid accessible to the extraction solvent (NaOH).

To collect water extractable colloid (WEC), the moist soil was dispersed in 20 L artificial freshwater to reach a final concentration of 5 g-dry soil/ L and shaken very gently at 150 rpm on an orbital shaker for three days (S9). In order to investigate the yield of WEC based on the dry soil weight and make the radioiodine data comparable among bulk soil, HA, FA and WEC, the ratio of weight of oven-dried soil to that of original damp soil was calculated in a preliminary experiment. Later, the soil resuspension was centrifuged for 30 minutes at $3200 \times g$, and the supernatant was successively filtered through 10 μm and 0.45 μm cartridge filters. Filtration was followed by cross-flow ultrafiltration, utilizing a spiral-wound 1kDa SOC 1812 cartridge (Separation Engineering, Inc.), until 1L retentate remained. The retentate was diafiltered against 20 L Milli-Q until it was concentrated to 1 L. Then, the cartridge was rinsed with 250 mL Milli-Q water with the pump on for 30 min, and then the pump was turned off. The cartridge was allowed to soak for four hours or more. The cartridge was consequently rinsed with another 250 mL of Milli-Q water, and the procedure was repeated twice or more until the rinse water became clear. The retentate and rinse solution were then subsequently combined and concentrated utilizing an Amicon 8400 stirred-cell ultrafiltration unit with a 1 kDa regenerated cellulose filter at 275 kPa. The retentate was freeze-dried for further radioiodine analysis and chemical characterization (S9).

Molecular weight distribution. The fractions of HA, FA, and WEC were dissolved in 0.05M Tris HCl /0.02M KCl buffer at a concentration of 1mg/mL (*S10*). Samples were then allowed to equilibrate overnight before being passed through a 0.2 μ m syringe filter. The molecular weight distribution for the fractions was then determined using size exclusion chromatography (SEC) on a Waters High Performance Liquid Chromatographic (HPLC) system, using Waters EmpowerTM 2 software to operate, acquire, and integrate all chromatograms. The Waters system components used in this analysis are a 600S gradient controller, a 626 non-metallic pump, a 200 μ L sample loop, a 717-plus autosampler, a 2417 refractive index (RI) detector. The RI detector was set at a temperature of 30°C and a sensitivity of 4. The mobile phase was a solution of 0.05M Tris HCl/ 0.02M KCl, maintained at a flow rate of 0.5 mL/min through Tosoh Biosciences guard and analytical columns G3000 PWx1, 6 mm x 4 cm and 7.8 mm x 30 cm, respectively, particle size 7 μ m. The injection volume was 150 μ L. Polystyrene sulfonate molecular weight standards of 1.6, 4.3, 8, and 35 kDa, from Polysciences, Inc., were used to calibrate the samples. These standard solutions were prepared in the same manner and were run in series with the samples. The logarithm of the molecular weight (MW) of standards were plotted against their respective retention times and the obtained equation ($y = -0.3183x + 5.9554$, $R^2 = 0.9985$, x, y denote retention time and log (MW)) was used to calculate the sample's MW based on its retention time.

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Table S1 Soil characterization

Parameters	FSI18	N Borrow	4Mile Creek
pH	5.63 ± 0.1	5.2 ± 0.1	4.1 ± 0.0
OM, %	24.1	<0.01	7.9
Sand/silt/clay (wt%)	92.1/7.0/0.9	N/A	85.5/11.7/2.8
¹²⁷ I (μg- ¹²⁷ I /g-soil)	4.26	N/A	2.59
¹²⁹ I (μg- ¹²⁹ I /g-soil)	0.32	N/A	BD
FeII/Fe(III), %/%	N/A	1.9 / 98.1	N/A
Fe(III), Hematite, Goethite, %	N/A	8, 28, 63	N/A
Reduction Capacity, meq/kg	N/A	2.88 ± 0.82	N/A
Mineralogy (<53μm)	N/A	Kaolinite, goethite, hematite, gibbsite, muscovite, rutile (no quartz; very slight 14Å)	Kaolinite, goethite, quartz, hematite, muscovite/ 10 Å
DCB Al, <53μm (ppm)	N/A	961.9 ± 78	N/A
DCB Fe, <53μm (ppm)	N/A	5346.3 ± 563	2.24
DCB Mn, <53μm (ppm)	N/A	8.8 ± 1	N/A

Table S2 Composition of solution for interaction between inorganic iodine (I^- or IO_3^-) and soil organic matter (SOM)

pH	0.2 ml	1.475 mL	0.3 mL buffer	0.025 mL
3	1mg/L HA/FA/WEC in 20 mM Tris-HCl)	Artificial freshwater	0.05 M phosphate buffer, pH 2.46	20 μL 0.1 μM $^{127}\text{I}^-$ (or
4			0.05 M acetate buffer, pH 3.8	$^{127}\text{IO}_3^-$) + 5 μL 0.36
5			0.05 M acetate buffer, pH 4.75	mCi/L $^{127}\text{I}^-$ (or $^{127}\text{IO}_3^-$)
6			0.05 M acetate buffer, pH 4.75 and trace amount of NaOH	
7			0.05 M phosphate buffer, pH 6.5	
8			0.05 M Tris-HCl buffer, pH 7.95	
9			0.05 M Tris-HCl buffer, pH 7.95 and trace amount of NaOH to the desired pH	

Figures:

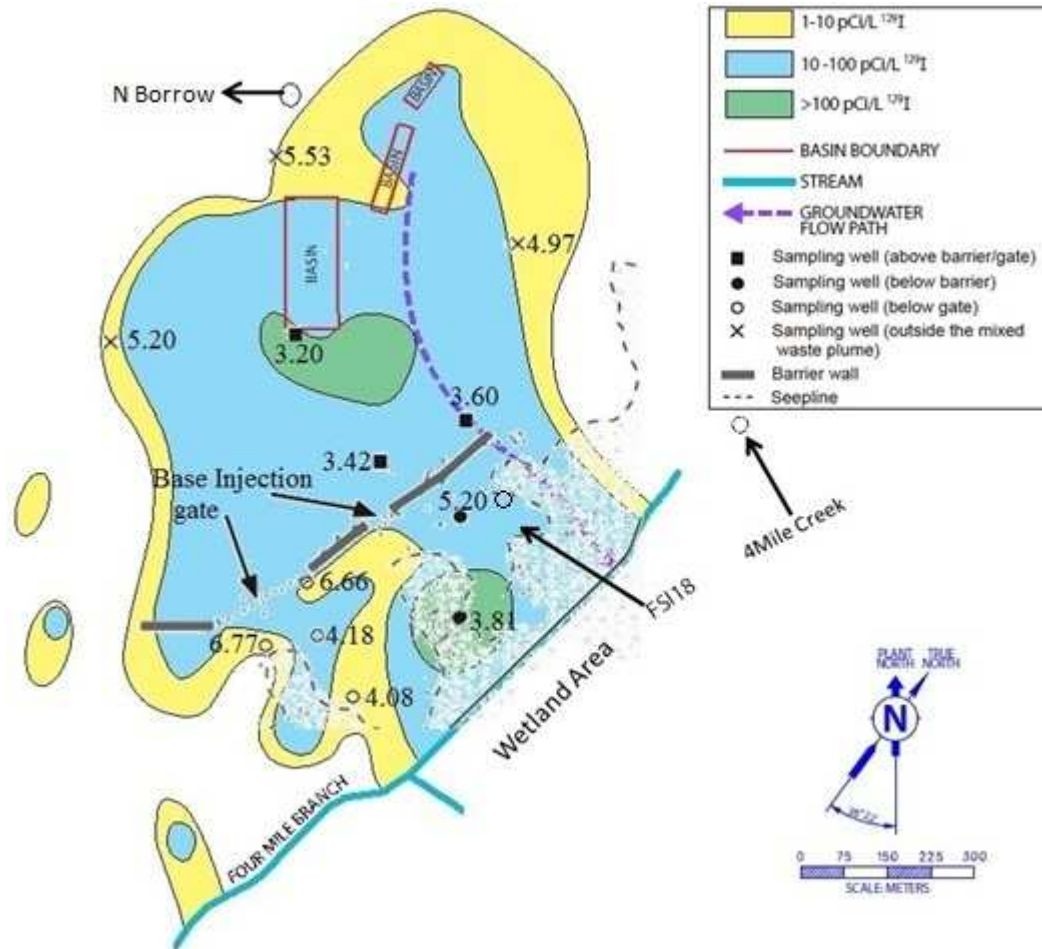


Fig. S1 Locations of sampling sites at F-Area of Savannah River Site. Numbers on this map denote the pH values of the local groundwater and adopted from Otosaka et al. (S11). FSI18 was located along the seep line where the grassland was abundant in organic matter (24% OC) but high amount of radioiodine was found in the local groundwater. It is a few hundred meters upward the wetland area.

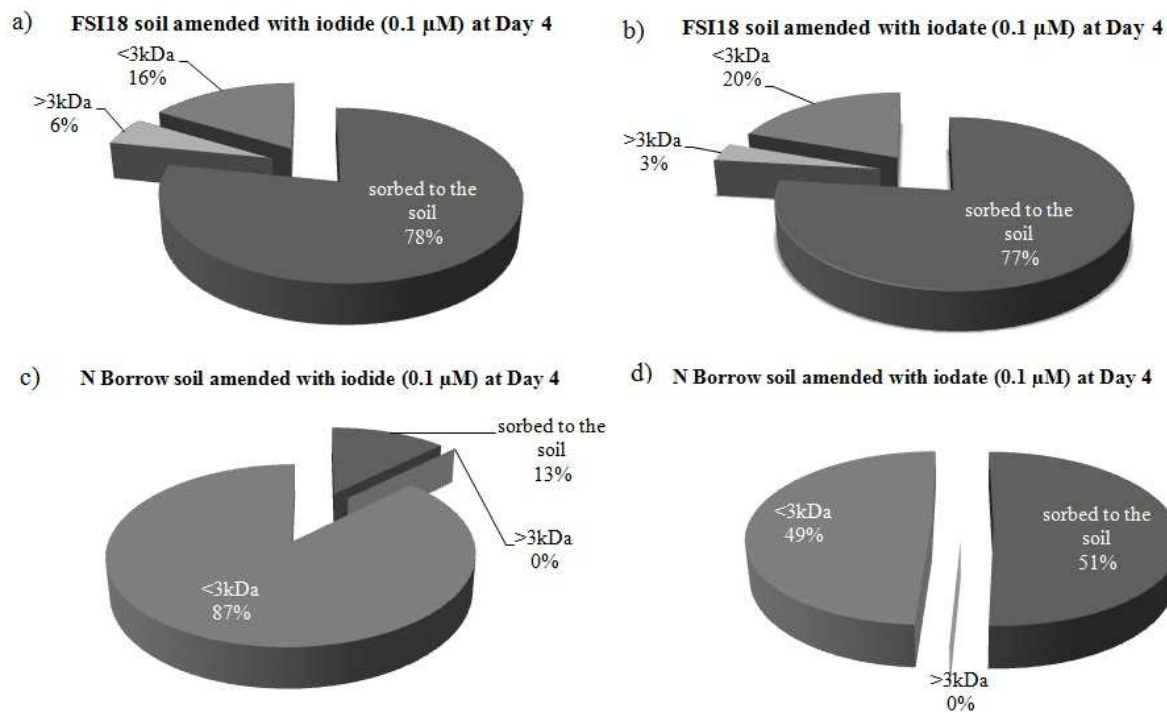


Fig.S2 Partitioning of (a) iodide or (b) iodate at ambient concentration (0.1 μM) into the three phases (particulate, colloidal and truly dissolved fractions) of organic-rich soil (FSI18) during a four-day resuspension, and partitioning of (c) iodide or (d) iodate at ambient concentration (0.1 μM) into the three phases of organic-poor soil (N Borrow) during a four-day resuspension.

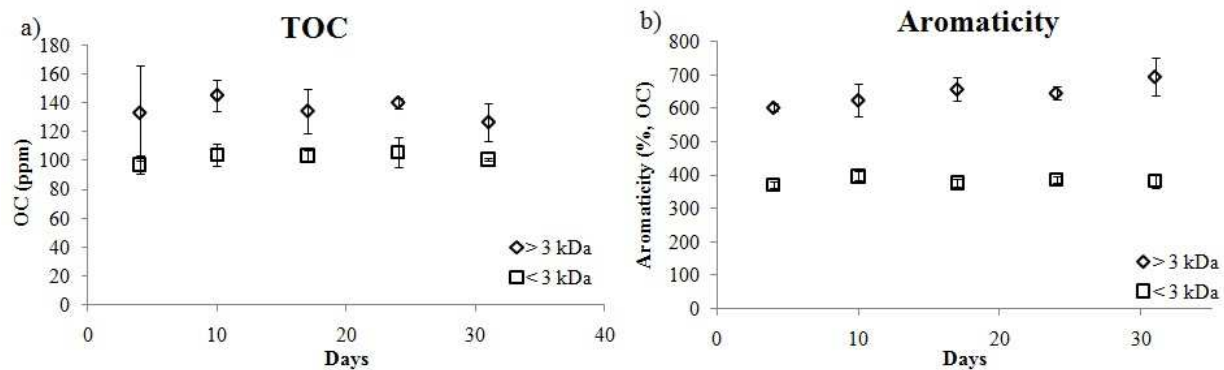


Fig. S3 Time-elapsing release of colloidal organic carbon (COC) and dissolved organic carbon (DOC) from the organic-rich soil and change of aromaticity. No significant difference in the organic matter characterization was found out among the control, I^- amendment and IO_3^- amendment groups, thus data were averaged from six samples for each time point (duplicates for each group).

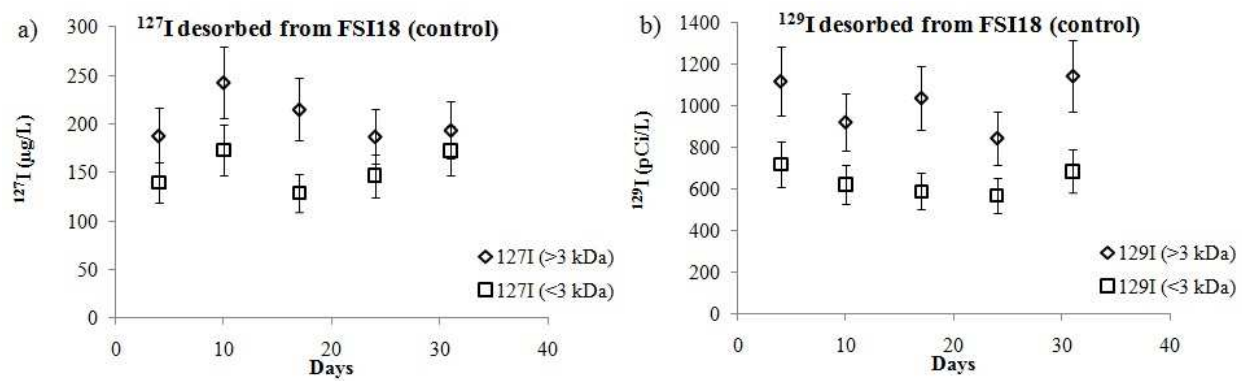


Fig. S4 Temporal desorption of a) ^{127}I and b) ^{129}I from an organic-rich soil (FSI18) of F-Area (soil resuspended in artificial freshwater).

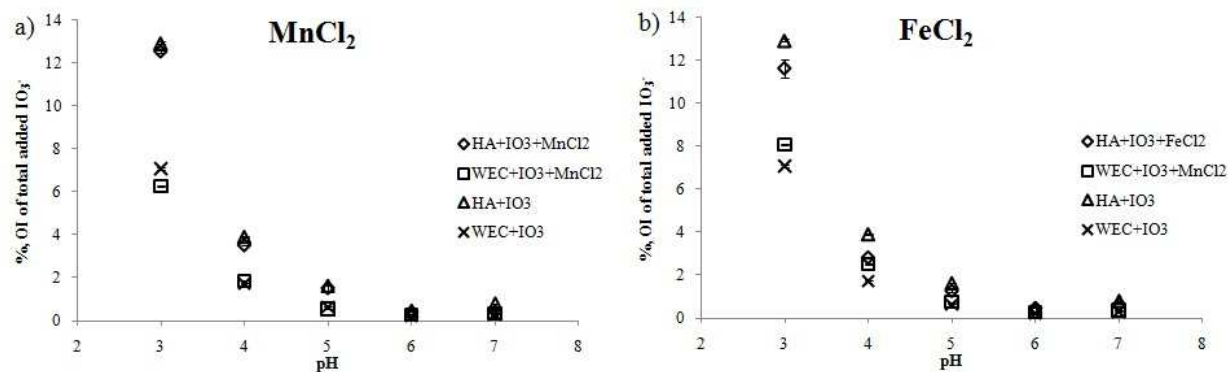


Fig. S5 a) pH-dependent iodate incorporation into HA or WEC at the presence of MnCl_2 (HA or WEC, 100 mg/L; MnCl_2 , 10^{-5}M ; reaction time, 24 hours); d) pH-dependent iodate incorporation into HA or WEC at the presence of FeCl_2 (HA or WEC, 100 mg/L; FeCl_2 , 10^{-5}M ; reaction time, 24 hours). All samples were performed in duplicates with the error < 1%.

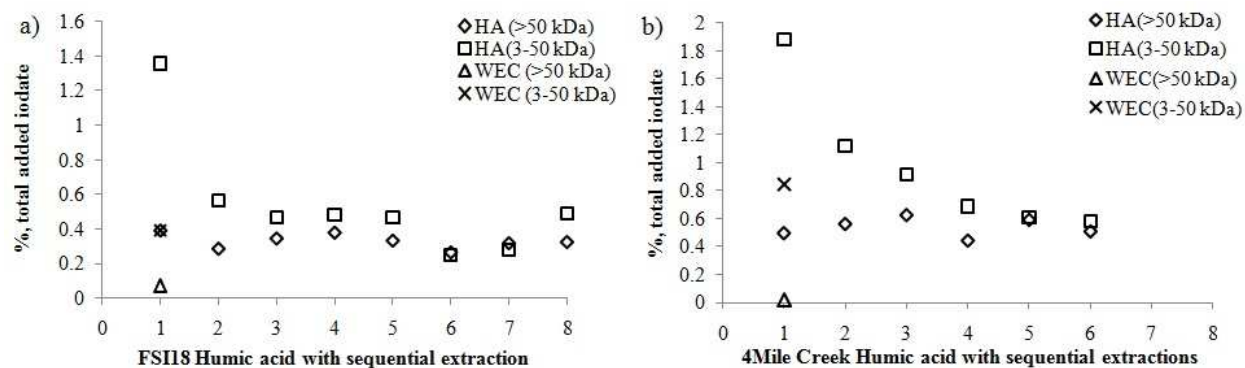


Fig. S6 Abiotic incorporation of iodate at pH 5 into two-size-fractionated humic acids (HAs) obtained by a sequential extraction method and water extractable colloid (WEC) of a) FSI18 soil; b) 4Mile Creek soil (NOM, 100 mg/L; $^{127}\text{IO}_3^-$, 0.1 μM ; $^{125}\text{IO}_3^-$, 0.9 $\mu\text{Ci/L}$; reaction time, 72 hours; 1, 2, 3, 4, ..., 8 represent the order of sequential extraction)