Impact of bromide exposure on natural organochlorine loss from coastal wetland soils in the Winyah Bay, South Carolina†

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Naturally formed halogenated organic compounds are common in terrestrial and marine environments and play an important role in the halogen cycle. Among these halogenated compounds, chlorinated organic compounds are the most common halogenated species in all soils and freshwater sediments. This study evaluated how a previously observed phenomenon of bromination of organic matter in coastal soils due to salt-water intrusion impacts the stability and fate of natural organochlorine (org-Cl) in coastal wetland soils. The reacted solid and liquid samples were analyzed using X-ray spectroscopy (in cm and at micron scales for solids) and ion chromatography. We find that introduction of Br/C0 species and their subsequent reactions with organic carbon are associated with an average of 39% loss of org-Cl species from leaf litter and soil. The losses are more prominent in org-Cl hotspots of leaf litter, and both aliphatic and aromatic organochlorine compounds are lost from all samples at high Br/C0 concentrations. The combination of solid and aqueous phase analysis suggests that org-Cl loss is most likely largely associated with volatilization of org-Cl. Release of labile org-Cl compounds has detrimental environmental implications for both ecosystem toxicity, and stratospheric ozone. The reactions similar to those observed here can also have implications for the reactions of xenobiotic chlorinated compounds in soils.

Environmental significance

The fate of naturally occurring organochlorine (org-Cl) compounds has become increasingly important as we gain more insight into their role in contributing to detrimental environmental impacts. Org-Cl compounds are persistent organic pollutants which can bioaccumulate and contribute to ecosystem and human toxicity. Further, org-Cl compounds emitted to the atmosphere undergo photolysis, producing chlorine radicals which catalytically destroy ozone. In this study we show that bromination of organic carbon as salt-water intrudes into coastal wetlands results in ubiquitous loss of org-Cl, most likely to the gaseous phase, allowing for further perpetuation of ozone depletion and subsequent sea level rise. Our research underscores the importance of halogen reactions in coastal ecosystems on global cycling of org-Cl and ultimately on climate change.

1. Introduction

Natural organochlorine (org-Cl) compounds occur ubiquitously in soils and sediments, where they form and accumulate as a result of organic matter decomposition. Many studies have reported that these compounds occur in the concentration range of a few hundred to a few thousand mg kg\(^{-1}\) (ppm) depending on the biogeochemistry of the local environment. In each of these studies, org-Cl compounds were reported from diverse ecosystems, including soils, marine and estuarine sediments, peat bogs, and coniferous forests.

The mechanisms of natural org-Cl compound formation have been examined, and these studies have shown that chloride (Cl\(^{-}\)) in soils and leaf litter can be converted to org-Cl through a variety of pathways. These mechanisms may be biotic, where haloperoxidase enzymes produced by a variety of microbial and fungal communities present in soil are used, or abiotic, where iron-catalyzed oxidation of organic matter or halides are key reactants. Once produced, these natural org-Cl compounds accumulate in soils and sediments over time because of the increased stability from strong C–Cl bonds.

The biological toxicity and environmental impact (other than their role in the halogen cycle) of these natural org-Cl compounds are unknown. However, many known anthropogenic org-Cl compounds, such as polychlorinated biphenyls, for example, are highly toxic because of their high stability and lipophilicity and are known to bioaccumulate in humans and cause detrimental health effects, including hormone and...
endocrine system disruption and acute reproductive cancers. In addition to org-Cl toxicity, small org-Cl compounds, such as chloroform (CHCl₃), methyl chloride (CH₃Cl), and a variety of other very short-lived substances (generally a few months lifetime in the atmosphere), can volati-
tilize from terrestrial systems, enter the atmosphere, and undergo photolysis producing Cl radicals which react with and destroy stratospheric ozone. For these reasons, transport, reactivity, and fate of naturally occurring org-Cl species and the biogeochemical variables that control their behavior are of particular interest and concern.

This study on natural org-Cl associated with leaf litter and soil showed a surprising result and indicated that these compounds are unstable in the presence of elevated levels of common nucloephiles, such as bromide (Br⁻). Due to recent trends in global warming and associated sea-level rise in coastal systems, freshwater wetlands and their soil organic carbon are exposed to elevated levels of Br⁻, along with changes in other natural conditions, such as pH and elevated concentration of other ions present in seawater. While each of these variables may influence the stability of natural org-Cl compounds present in coastal wetlands of the Winyah Bay (SC), we have previously observed that rise in sea level in this region has resulted in rapid, pervasive, and preferential bromination of organic matter as freshwater wetlands are converted to salt-affected wetlands. Because Br⁻ appears to be the key reactant in these coastal conditions, for this study, we explored the exposure of Br⁻ on the natural org-Cl present in freshwater wetlands as they undergo salination as a first step in understanding the complex reactions that may occur in these ecosystems and impact overall Cl biogeochemistry and cycling. The findings from this study have implications for the stability and fate of both natural and anthropogenic haloge-
nated organic compounds.

2. Methods

2.1 Selection of field site and sample collection

The Winyah Bay, South Carolina is a coastal region in south-
eastern United States adjacent to the Atlantic Ocean which has been well monitored and has experienced significant sea-level rise in the past few decades. The Winyah Bay area has a humid, subtropical mid-latitude climate with equally spread precipitation throughout the year including mid-latitude cyclones through the winter months and convective thunder-
storms during the summer months. Annual temperatures vary between an average high of 25 °C to low of 16 °C. The coastal region contains wetlands occurring across a salinity gradient from salt marsh, at highest salinity, to salt-affected wetland, and to freshwater wetland, lowest salinity. For this study, leaf litter and soil samples were collected only from the freshwater wetlands, which have not been exposed to seawater. The salinity in the freshwater wetland is 0.25 (±0.06) ppt (parts per thou-
sand) throughout the year. The freshwater wetland is forested with dominate tree species of bald cypress (Taxodium dis-
tichum), water tupelo (Nyssa aquatic), and swamp tupelo (Nyssa sylvatica var. biflora). Samples were collected once in three sections from a small area: leaf litter, 0–10 cm, and 10–20 cm. The samples were transferred to Ziploc bags, sealed, and shipped on ice to Princeton, New Jersey, where they were stored in a cold room in order to sustain the natural conditions prior to incubations with Br⁻.

2.2 Soil incubations with Br⁻

Soil and leaf litter incubations were conducted in aerobic conditions (open to exchange with atmospheric oxygen) intended to mimic aerobic and fluctuating hydrological conditions and the transition of freshwater wetlands to salt-affected wetlands. This is in contrast to the salt marsh conditions, which are considered to be relatively anoxic due to their being consistently submerged with seawater. Additionally, the salt-
affected wetland, oxic conditions were previously shown to have the highest rate of bromination, likely due to increased organic carbon content and exposure to oxygen. Leaf litter and soils in their natural state were reacted with Br⁻ (prepared as a solution from KBr(s)) of varying concentrations (0.1, 0.5, 1.0, and 3.0 mM). The concentration of Br⁻ in seawater is ~0.8 mM, and the selected Br⁻ concentrations represent different amounts of either dilution with freshwater or concentration of salinity. Concentrations of Br⁻ above those found in seawater, and explored in this study, can potentially occur in conditions such as significant evaporation of seawater from an isolated area inundated with seawater or presence of specific types of vegetation that concentrate salt into their root systems. Further, this range of Br⁻ concentrations creates a spectrum in which to explore the extent of bromination in end-case scenarios. Samples without any externally added Br⁻ (0.0 mM) are ‘controls’, and these samples went through wet–dry cycles with additions of just deionized Milli-Q water. For each incubation, 5 grams of soil were reacted with 7.5 mL of Br⁻ solution at room temperature over the course of 12 days by 6 additions of 1.25 mL of solution every other day. This Br⁻ addition scheme was intended to create a wetting and drying cycle similar to natural tidal patterns observed in the coastal wetland system. The incubations were open to the atmosphere throughout the 12 day period to allow for natural oxygen exchange. Following the 12 day incubation, samples were thoroughly mixed to reduce heterogeneity and washed with deionized Milli-Q water in order to remove excess unreacted salt and prepare them for speciation analysis. A flow chart of sample phases and the methods used to analyze them, described in the following paragraphs, is shown in Fig. S1.†

2.3 Cl & Br speciation in soils & leaf litter

Speciation of Cl was conducted using X-ray absorption spec-
troscopy (XAS) at the Cl absorption edge, and this was con-
ducted at two different spatial scales: centimeter scale (2.5 × 0.2 cm²) to evaluate the bulk, or average, Cl speciation in samples, and at the micron (1–15 μm²) scale to evaluate the possible heterogeneity in Cl speciation in individual particles. The samples were analyzed in their natural state without further preparation, in situ, by sealing a thin layer of the sample (about ½ cm thick) between X-ray clean polyfilm and Kapton tape before placing in
a He-filled X-ray chamber for spectral collection. The He-environment was used in the sample chamber to both improve the detection of Cl fluorescence signal and to reduce the probability of forming reactive oxygen species throughout X-ray exposure to the sample during the approximately 20 min X-ray scan. All wet, soil and leaf litter samples were analyzed for bulk Cl speciation on beamline 4-3 at the Stanford Synchrotron Radiation Light Source (SSRL). The Cl K-edge XANES spectra were collected using a Vortex fluorescence detector with an energy step size of 0.08 eV close to the Cl edge. The beam size was 1 × 0.1 cm² and the sampled area was 1.414 × 0.1 cm². Chlorophenol red was used as a standard to calibrate the Cl absorption edge, with the Cl 1s absorption maximum of the C–Cl aromatic bond set at 2821.1 eV. Spectra were normalized using Demeter Athena²⁰ by fitting a first-order polynomial to the pre-edge region and normalizing the post-edge region to 1.0 by fitting a first- or second-order polynomial depending on the spectral shape. The Cl XANES spectra of structurally known model inorganic and organic compounds were used to fit sample spectra using a linear combination fitting (LCF) procedure in order to determine Cl valence state and coordination environment in each sample. These standard spectra are: chlorophenol red (org-Clₗₑᵣᵈ), chlorodecane (org-Clₜᵣᵢᵢ), glycine H–Cl (H-bonded inorg-Cl), and NaCl[ₐₖₕ] (hydrated inorg-Cl) (Fig. 1A). Fitting was done using these four standards, as they are indicative of typical Cl bonding and are easily differentiated due to their energy shift depending on Cl speciation. This analysis followed previous studies utilizing Cl K-edge XANES to determine Cl speciation in soil and leaf litter samples.²⁴,⁴¹ Some soil samples were also analyzed on beamline 14-3 at SSRL (using a de-focused beam) and an energy step size of 0.15 eV close to the Cl edge. In addition to Cl, cm-scale XANES were performed at the Br K-edge as part of a previously published study with the same sample set up, and results showing production of org-Br species after reaction are presented in that study.¹⁵

The relative abundances of Cl in wet samples was determined using the intensity of edge-step, ~40–50 eV above the absorption edge of the background-corrected and unnormalized XANES spectra.

2.3.2 Speciation at the micron scale. High-resolution elemental maps of weathered bald-cypress leaf litter were made using spectromicroscopic techniques in order to assess the heterogeneity in Cl speciation at the micron-scale. Leaf samples were washed with deionized Milli-Q water to remove excess salt adsorbed to the surface and mounted on plastic sample holders using XRF Tape TF-500 which is both halogen and sulfur free (Fig. S2†) backing and imaged at different energies (redox maps) close to the Cl K-edge corresponding to the energy shifts.

![Normalized Cl XANES spectra of standards (A) and wetland samples (B–D). (B) Leaf litter, (C) 0–10 cm soil depth, and (D) 10–20 cm soil depth. Spectra in panels (B–D) correspond to samples reacted with increasing Br⁻ concentration from top to bottom, and the topmost spectrum of 0.0 mM Br⁻ indicates control (the Br spectrum of this sample represents native Br in the sample). Arrows in panel (A) indicate edge energies for Cl in different bonding environments, and these energies were used for Cl-speciation maps presented in Fig. 3.](image-url)
determined by Cl standards. Org-Cl (chlorophenol red) was run as a standard to determine the peak position of org-Cl\textsubscript{atorn} from which the org-Cl\textsubscript{all} and inorg-Cl edge energies could be determined based on known energy shifts of standards discussed above. These energies were 2820.5 eV (org-Cl\textsubscript{all}), 2821.1 eV (org-Cl\textsubscript{atorn}), and 2823.1 eV (inorg-Cl), and are shown with arrows in Fig. 1A. A higher energy map was also collected at 2900 eV to observe total Cl abundance. After initial imaging, the mounted leaves were reacted with 1 mL of 0.1 mM Br\textsuperscript{-} solution [as a thin-film of solution on the surface of the leaf].\textsuperscript{16} Excess solution was siphoned off after approximately 2–3 hours, and the samples were allowed to air dry for an additional 3–4 hours. The leaves were washed with deionized Milli-Q water to remove excess soluble salts from the leaf surface and re-imaged at the exact same locations and energies to examine speciation variations after the reaction. Micro-XANES (\(\mu\)-XANES) spectra for Cl were collected at Cl hotspots (high Cl fluorescence intensity) both before and after the reaction in the same locations.

In addition to Cl, Br speciation maps were simultaneously collected at the same locations and with the same parameters as with Cl maps in order to observe Br speciation in leaf litter and correlation with Cl. Br redox maps were collected at 13 473.0 eV (org-Br), 13 476.0 eV (inorg-Br), and 13 500 eV (high energy). Only one energy was selected for org-Br because the intense X-ray absorption features for org-Br\textsubscript{all} and org-Br\textsubscript{atorn} are too close to differentiate from one another (Fig. S6A\textsuperscript{+}). A set of Br \(\mu\)-XANES was also collected from Br hotspots in the same locations before and after reaction. Aqueous potassium bromide (KBr) was used as a standard to calibrate the Br absorption edge, with the Br 1s absorption maximum set at 13 476.0 eV.

One set of Cl maps was collected on beamline 14-3 at SSRL under a He environment using a vortex detector with a 15-micron step size. Br maps were also collected at beamline 5-ID (SRX) at the National Synchrotron Radiation Lightsource II with 1-micron step size. All other sets of leaf litter Cl and Br maps were collected on GSECARS beamline 13-IDE at the Advanced Photon Source (APS) under a He atmosphere. The maps and \(\mu\)-XANES spectra were collected using a vortex ME4 silicon drift diode array detector. Maps were collected with a 10-micron step size, and \(\mu\)-XANES for both Cl and Br were collected with a 0.2 eV step size around the Cl or Br edge, respectively.

All images were first normalized to the incident photon counts, and analyzed using Sam’s Microprobe Analysis Kit (SMAK)\textsuperscript{12} and Larch: Data Analysis Tools for X-Ray Spectroscopy.\textsuperscript{17} The Larch software was also used to determine total Cl and Br fluorescence intensity (counts per second, cps) of leaf litter hotspots, intermediate spots, and background spots. The \(\mu\)-XANES spectra for both Cl and Br were analyzed using Athena as previously described for the XANES spectral analysis of bulk soil and leaf litter samples.

2.4 Estimating Cl abundances in solid samples using X-ray fluorescence (XRF)

The concentration of total Cl (inorg-Cl + org-Cl) in incubated and dried soil samples was measured using XRF analysis. Each sample, including controls, was washed thoroughly with 25 mL of deionized Milli-Q water to remove excess soluble halides. Following washing, each sample was dried overnight in an oven at 50 °C and then ground to a fine powder using a diamond mortar and pestle. From each sample 3–5.9 mm pellets were made using a hydraulic press by applying 5 tons of pressure for 2 minutes. Each pellet was placed between a polycarbonate disk and X-ray clean polyfilm and examined using a Rigaku Supermini200 wavelength dispersive XRF Spectrometer under vacuum. An RX25 monochromator crystal and a scintillation counter were used to detect Cl. For each of the three sample depths (leaf litter, 0–10 cm, and 10–20 cm), a set of Cl standards was created by standard addition of KCl solution in increasing concentrations (0–200 ppm) to create a standard curve. These standard curves were used to estimate the concentration of Cl in each sample. In addition to Cl, XRF was performed to analyze total Br concentration as part of a previously published study with the same sample set up, and results showing increased total Br after reaction are presented in that study.\textsuperscript{15}

2.5 Aqueous phase Cl\textsuperscript{-} analysis with ion chromatography (IC) and XANES

All reacted soil and leaf litter samples were washed with 25 mL of deionized Milli-Q water to remove any water-soluble Cl\textsuperscript{-} and other Cl species that may have formed during the course of the reaction. The collected solution was filtered using a 0.2 μm syringe filter to remove all suspended solids. All sample filtrates were analyzed for Cl\textsuperscript{-} concentration on a Dionex ion chromatography system equipped with an IC25 Ion Chromatograph, AS40 autosampler, an LC25 chromatography oven and an EG40 eluent generator. Anions were separated using a Dionex IonPac AS15 (3 × 150 mm) analytical column connected to a Dionex IonPac AG15 (3 × 50 mm) guard column. A set of Cl\textsuperscript{-} standards with varying Cl concentrations was run and used to calculate the Cl concentrations of samples. The output from the IC analysis determined concentration of Cl\textsuperscript{-} in the aqueous phase on a mg L\textsuperscript{-1} basis. This value was then converted to total amount of mg Cl\textsuperscript{-} kg\textsuperscript{-1} soil lost from each sample using the total amount of water (25 mL) with which each sample was washed and the total amount of reacted soil per sample (0.005 kg). The collected aqueous portions from leaf litter and soil reactions were further analyzed by XANES in order to determine Cl speciation in aqueous and particulate organic matter phases. Because the Cl concentration is too low in the water extracts for Cl XANES analysis, the filters (used for IC) were dried onto a halogen-free tape surface as a thin film to study with XANES spectroscopy. Unfiltered sample solutions where filtered through P5 grade filter paper, and the particulate organic matter was collected on the filter paper and allowed to dry. Both the thin-film filtrates and particulate organic matter were analyzed for Cl speciation on beamline 13-IDE at the APS by Cl K-edge XANES as previously described.

3. Results and discussion

3.1 In situ Cl speciation of bulk soil and leaf litter

The in situ, wet XANES analyses of the bulk leaf litter and soil samples not exposed to Br\textsuperscript{-} show a sharp and strong peak
corresponding to org-Cl, while the Br\textsuperscript{-}/C\textsubscript{0} reacted samples clearly show a loss in org-Cl signal at higher concentrations of added Br\textsuperscript{-} (Fig. 1). The org-Cl XANES spectra of examined standards show a sharp, lower energy peak when compared with inorg-Cl XANES (Fig. 1A). In leaf litter and both examined soil depths, the Cl-XANES spectra of samples lose the spectral features at low energy, and the spectra shift to higher energy progressively with increasing Br\textsuperscript{-}/C\textsubscript{0} concentration. The changes are pronounced at highest concentrations of Br\textsuperscript{-} added, which indicates a change in speciation from org-Cl to largely inorg-Cl coordination. Unnormalized edge step intensity in the XANES spectra, which is an indicator of relative total Cl abundance (inorg-Cl + org-Cl), shows a greater than 50% loss in Cl signal in leaf litter (70 (±3)%) and the 10–20 cm soil depth (63 (±3)%) (Fig. 2). The 0–10 cm depth (38 (±3)%) also shows a similar trend in intensity loss, but not as pronounced as the others. The average loss in total Cl fluorescence intensity across all three depths is 57 (±3)%. The percent error was calculated as a function of variability in intensity changes between each sample in order to include variability that may arise from variation in composition and preparation of samples. While samples for this analysis were mounted as consistently as possible, absolute concentrations are difficult to estimate with high accuracy in this wet \textit{in situ} analysis because of differences in sample packing, density and water content, and thus these represent relative concentrations and relative error. The LCF analysis of XANES spectra, which provides speciation information, shows both org-Cl\textsubscript{ali} and org-Cl\textsubscript{arom} species are present initially in all samples, and both forms are lost at the highest concentration of added Br\textsuperscript{-} concentration (3.0 mM) (Tables 1 and S1). At lower concentrations of added Br\textsuperscript{-} in some samples, the org-Cl spectral character actually increases, for

![Fig. 2](image_url) 

**Fig. 2**  \textit{In situ} estimation of Cl abundances in soils reacted with aqueous Br\textsuperscript{-}. The Cl abundance in these wet samples was estimated directly using the unnormalized XANES spectral intensity of the edge step.

<table>
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<tr>
<th>Depth</th>
<th>Br\textsuperscript{-} added (mM)</th>
<th>% Org-Cl\textsubscript{ali}</th>
<th>% Org-Cl\textsubscript{arom}</th>
<th>% Inorg-Cl (H-bonded)</th>
<th>% Inorg-Cl (hydrated Cl\textsuperscript{-})</th>
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<td>57.7</td>
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<tr>
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example from 0 to 1.0 mM Br\(^{-}\). This is most likely a result of sample heterogeneity, and while the org-Cl spectral character may increase, the total Cl content decreases simultaneously as is observed by the unnormalized edge-step intensity. The LCF overall shows an average of 39% loss in org-Cl spectral character, corresponding to loss in total org-Cl compounds (Cl\(_{ali}\) + Cl\(_{arom}\)). Based on in situ XANES analysis of wet samples, addition of Br\(^{-}\) results in overall loss in total Cl abundance, but more significantly, a substantial loss of org-Cl compounds present in soil or leaf litter.

3.2 Spectromicroscopy of Cl and Br in leaf litter

Because the bulk, wet, in situ XANES analysis of leaf litter showed the most dramatic loss in org-Cl upon exposure to Br\(^{-}\), these samples were analyzed further at the micron-scale in order to assess the heterogeneity in Cl speciation and reactivity throughout the sample. Heterogeneity in both location and speciation of Cl hotspots may be dependent on a variety of variables, but are likely mainly dependent on mechanisms of chlorination, such as biotic versus abiotic. For example, those produced through enzymatic processes might be found in proximity to fungal structures in soil or leaf litter, and their speciation may generally differ from those formed through abiotic mechanisms.\(^{24}\) Maps of degraded leaf litter imaged before and after reaction with dilute Br\(^{-}\) solution (0.1 mM; \(\sim\)10% of Br\(^{-}\) in seawater) show a loss of Cl fluorescence intensity in all parts of the leaf (Fig. 3). Cl containing regions in the maps were separated into 3 categories based on Cl relative fluorescence intensity (counts per second, cps) in the org-Cl\(_{ali}\) map (Fig. 3A): hotspots (>10 000 cps), intermediate spots (5000–10 000 cps), and background spots <5000 cps (Fig. 4). The same intensity scales were used for Org-Cl\(_{arom}\) and inorg-Cl map analysis, however fluorescence intensity ranges were shifted up by 5000 cps and 10 000 cps, respectively. This adjustment accounts for increased total Cl fluorescence intensity with increasing map energy, which results from incorporation of all fluorescence from Cl species below that map’s energy. Hotspots and background spots are an average of 10 measured spots, and intermediate spots are an average of 5 measured spots in each map.

The Cl-hotspots show a dramatic decrease in Cl fluorescence intensity (average loss 57%) after reaction with Br\(^{-}\) for all Cl speciation types (inorg-Cl + org-Cl) (Fig. 4). Intermediate spots show a small decrease in fluorescence intensity, while background spots show little to no change. The Cl-hotspots show the greatest loss in total Cl and org-Cl fluorescence intensity after reaction, suggesting that org-Cl loss is variable with respect to heterogeneity of leaf litter. Further, pervasive loss of Cl fluorescence intensity in both org-Cl\(_{ali}\) and org-Cl\(_{arom}\) maps suggests that org-Cl loss is not necessarily dependent on local coordination of org-Cl. Rather, both org-Cl\(_{ali}\) and org-Cl\(_{arom}\) can be lost as a result of bromination of org-Cl containing organic matter.

The Cl hotspots were selected from the org-Cl maps (Fig. 3A) and were analyzed further in greater detail for Cl-speciation variations by collecting \(\mu\)-XANES spectra at these spots before and after reaction with Br\(^{-}\). Before reaction, the Cl \(\mu\)-XANES spectra were less noisy and showed sharp, low energy org-Cl peaks (with the exception of spot 4, which has more inorganic-Cl) (Fig. S3A†). The peak intensities decreased in many of the spectra after reaction (nearly lost in some spots, such as in spots 3 and 5; Fig. S3B†). In addition, spectra collected after Br\(^{-}\) reaction are noisy, indicating very low Cl signal or low Cl abundance. Analysis of the unnormalized edge-step for the \(\mu\)-XANES spectra from different spots before and after reaction showed 87% (±3%; average) loss of Cl fluorescence intensity (Fig. S3C†). This indicates a dramatic loss in
relative total Cl abundance in the leaf litter (inorg-Cl + org-Cl), and this is greater than the loss reported for the bulk analysis of leaf litter, discussed above. The LCF of these spectra (Table S2†) shows consistently high loss in org-Clali species, whose fraction drop to nearly zero in some of the μ-XANES spectra, and a significant loss in org-Clarom character after reaction. This further indicates that org-Cl loss occurs regardless of org-Cl bonding environment in leaf litter or soil. Overall, μ-XANES LCF shows a combined loss of 47% of org-Cl character corresponding to loss in total org-Cl compounds (Clali + Clarom) in Br⁻ reacted leaf litter. This is consistent with observed loss in org-Cl from the leaf litter bulk analysis reported above.

The leaf litter samples, described above, were also analyzed at the Br K-edge before and after reaction in order to assess Br speciation at the micron-scale and its relationship with Cl species. Br speciation maps show an increase in Br fluorescence intensity at both the org-Br and inorg-Br edge energies (Fig. S4†). As with the Cl maps, Br abundance in Br maps was also analyzed by tracking changes of Br fluorescence intensity before and after reaction in Br hotspots (>50 000 cps), intermediate spots (10 000–50 000 cps), and background spots (<10 000 cps). Hotspots show an overall increase in fluorescence intensity after reaction for all Br forms (average increase 65%) (Fig. S5†), which is relatively close to the observed loss in Cl fluorescence intensity (57%). The locations (x,y coordinates) of Cl and Br hotspots can be compared qualitatively in order to assess if org-Cl loss occurs exactly where bromination takes place. Maps showing changes in fluorescence intensity for both org-Cl and org-Br are shown in Fig. 5. In these maps, spots of highest org-Cl fluorescence intensity were determined by subtraction of org-Cl cps after reaction from org-Cl cps before reaction. Similarly, spots of highest org-Br fluorescence intensity were determined by subtraction of org-Br cps before reaction from org-Br cps after reaction. While there is some overlap in the location of org-Cl and org-Br hotspots, the majority of org-Br spots do not appear to correlate with instances of high org-Cl intensity before reaction. This simply suggests that incorporation of Br species does not directly replace Cl in molecules. Because org-Cl loss appears to occur heterogeneously with respect to the location of bromination, it is possible that bromination of organic material destabilizes or degrades macromolecules containing org-Cl, creating smaller species of org-Cl that can more easily partition to other phases, such as aqueous or gaseous. Analysis of Br hotspots by μ-XANES (Fig. S6†) shows both inorg-Br and org-Br species to be present before and after reaction with Br⁻. The inorg-Br fraction appears to increase slightly relative to org-Br species after reaction, which is likely due to adsorption of Br⁻ to the leaf surface during the short reaction time (Table S3†).

3.3 Ex situ analysis of Cl abundances in soil and leaf litter

To complement the in situ synchrotron X-ray analysis, total Cl abundances (inorg-Cl + org-Cl) in reacted soil and leaf litter samples were also estimated utilizing XRF (Fig. 6A). However, samples for this measurement must be in a dry state. In both the leaf litter and soil samples from the 10–20 cm depth, Cl loss occurred on a similar scale of org-Cl loss observed in the wet in situ bulk CI-XANES analysis (39%), but to a smaller extent than when compared with the total Cl loss observed in the wet in situ bulk analysis (57%). The 0–10 cm depth samples show relatively
unchanging Cl concentration with increasing added Br\(^-\), which is consistent with the variability observed for this soil depth from the XANES analysis. While the XRF analysis shows some agreement with the \textit{in situ} XANES analysis of bulk leaf litter and soil incubations in general, the XRF data is highly variable with a calculated error of 20.5% total Cl.

The observed inconsistency and high analytical variability of XRF when compared to \textit{in situ} XANES analysis may be due to artefacts associated with sample drying for XRF analysis. Recent studies assessing the transport and precipitation of salts in soils have shown that salts become increasingly concentrated in soil pore waters and are transported to the soil surface and precipitate on the surface as evaporation and drying occurs.\(^\text{25,26}\) While the soils for XRF analysis were washed prior to drying, differences in precipitation of trapped salts on the sample surface during drying would lead to inaccurate and variable estimation of total Cl for XRF analysis. The X-ray penetration depth at the Cl K-edge is on the scale of 1 micron, while the full sample is about \(\frac{1}{4}\) of a centimeter thick (the Br K-edge, for comparison, has a penetration depth of a few hundred microns). For this reason, the dry XRF analysis is significantly affected by surface artefacts, rather than the bulk composition of the sample. Further this surface transport and precipitations would vary based on the inherent structural differences of porous media in soil (e.g.
porosity, amount of organic matter and amorphous materials that can retain hydrated ions). However, this surface precipitation is not an issue for the in situ XANES analysis of wet samples, as salts are less likely to precipitate on the surface without drying, and strongly indicates that org-Cl is consistently lost from the solid phase. Sample drying and analysis by XRF can lead to incorrect estimates of light elements because of shallow X-ray penetration, and researchers should be cautious with the interpretation of XRF results of dry porous materials.

3.4 Aqueous phase analysis of Cl species

Aqueous phase analysis of Cl\textsuperscript{−}, conducted using ion chromatography (IC), indicates that Cl\textsuperscript{−} concentration did not change with Br\textsuperscript{−} exposure (Fig. 6B). In other words, the org-Cl lost from bulk soil and leaf litter incubations was not dehalogenated, broken down, and released as Cl\textsuperscript{−} to the aqueous phase. The average amount of Cl\textsuperscript{−} that is predicted to be in the aqueous phase of each sample, assuming all the lost org-Cl species were completely broken down to Cl\textsuperscript{−}, was determined in order to compare with the amount of Cl\textsuperscript{−} measured in the aqueous phase of each sample. The percentages of total Cl abundance loss (inorg-Cl + org-Cl) observed from XANES analysis (70% for leaf litter, 38% for 0–10 cm, and 63% for 10–20 cm) of bulk, reacted leaf litter and soil samples were applied to the Cl concentration determined in the unreacted leaf and soil samples. This value is the potential Cl\textsuperscript{−} concentration in the aqueous phase if all lost Cl was released as Cl\textsuperscript{−} (dashed lines, Fig. 6B). In all samples, org-Cl lost as Cl\textsuperscript{−} is small. For all three depths in this study, the released Cl\textsuperscript{−} is between 8.2–20.9 mg Cl\textsuperscript{−} kg\textsuperscript{−1} soil. For both the leaf litter and 10–20 cm depth, this value is between 4–8% of the predicted total Cl loss. For 0–10 cm depth, the Cl\textsuperscript{−} concentration in the filtrate only accounts for between 25–35% of predicted total Cl loss. These results suggest a very small percentage of Cl species present in leaf litter or soil are completely dehalogenated and lost as aqueous Cl\textsuperscript{−} species.

While the columns used in IC are sensitive to inorganic Cl\textsuperscript{−}, Cl-XANES spectroscopy was utilized to determine the possible presence of org-Cl in filtrates and in particulate organic matter isolated from the aqueous phase after reaction with Br\textsuperscript{−}. The Cl XANES of filtrates indicate that Cl is mostly in the inorg-Cl form (Fig. S7A and Table S4†). Speciation analysis of aqueous phase particulate organic matter shows increased org-Cl character when compared to filtrates (Fig. S7B and Table S4†). The particulate organic matter shows 20–50% org-Cl character in the XANES spectra, while the aqueous filtrates did not show any org-Cl character in all samples except for one. However, the low fluorescence intensity of the particulate organic matter spectra indicates that, while some org-Cl is released into aqueous phase particulate organic matter, this portion is small compared with the total org-Cl loss observed from the bulk reacted samples. These combined data predict that Cl species may be volatilized and released to the gaseous phase.

4. Conclusions

In summary, our studies show that org-Cl, the primary form of Cl in leaf litter and soils, is lost upon exposure to Br\textsuperscript{−} containing aqueous solutions, and especially at higher concentrations of Br\textsuperscript{−}, and this loss varies with the amount of Br\textsuperscript{−} exposure, and the nature of the sample. In addition, bromination of organic matter is concomitant with org-Cl loss, however, microscopy studies do not suggest that they co-occur in the same regions and that both org-Cl\textsubscript{ali} and org-Cl\textsubscript{alom} speciations can be lost. Among all the examined samples, leaf litter showed the highest org-Cl loss, suggesting differences in org-Cl speciation in soils may be playing an important role in the reactions. The org-Cl species present in leaf litter and soils may not be water soluble (as noticed from little loss of org-Cl species when samples are washed/‘reacted’ with DI water), and this is likely because these molecules are incorporated into larger insoluble macromolecules of natural organic matter. However, additions of Br\textsuperscript{−} may be responsible for promoting the breakdown of the Cl containing macromolecules, allowing them to be released from the solid phase. The reaction mechanisms involving the breakdown of natural organohalogen are unknown at this stage; however, these are expected to be more complex when compared with smaller anthropogenic org-Cl compounds.\textsuperscript{27} Some studies of org-Cl compounds in the natural environment have indicated that dechlorination and dehalogenation reactions may occur readily, however they are mechanistically diverse and dependent on local environmental conditions.\textsuperscript{28} Many of these studies focus on biotic pathways in the natural environment, while abiotic pathways are not well understood. Based on the results presented in this study, we suggest that bromination of organic matter may influence subsequent degradation of organic carbon and org-Cl containing organic matter present in leaf litter and wetland soils. The absence of decay products, inorganic Cl\textsuperscript{−} and org-Cl, in the aqueous phase suggest that Cl containing molecules are volatilized from the Br\textsuperscript{−} reacted soils during the reactions. While the mechanisms for this process are unclear at this stage in the research, it is possible that they follow natural biodegradation pathways associated with production of volatile org-Cl compounds, and that reactivity of Br\textsuperscript{−} with soil organic carbon induces similar degradation mechanisms and end results. Based on observed org-Cl loss, the abundances of gaseous chlorinated compounds are expected to be in the range of parts per trillion. These measurements are difficult to accomplish with typical gas chromatography instrumentation set ups due to the expected high volatility and very low concentration, and will thus be explored with specialized equipment in continuing work.

The results from this study point to the loss of natural org-Cl compounds, possibly as volatile org-Cl molecules, in freshwater wetlands as soil organic carbon undergoes bromination, an emerging phenomenon in coastal wetlands impacted by sea level rise. While there are a variety of variables associated with sea-level rise that may influence overall fate of org-Cl compounds, this study focused specifically on understanding the role of bromination on organic carbon and natural org-Cl compounds as a first step in uncovering the complex and ongoing halogen chemistry occurring in coastal ecosystems. Org-Cl compounds emitted into the atmosphere can photolyze, creating chlorine radicals which catalytically destroy stratospheric ozone. A number of studies have shown coastal
wetlands and salt-marshes to be a major source of natural halocarbon emissions due to seawater intrusion, and loss of org-Cl compounds and possible biogeochemical reactions in these ecosystems may be similar to those observed in this study. As seawater intrusion into freshwater ecosystems occurs more frequently in a changing climate, it is possible that volatile org-Cl emissions will be further observed in areas not previously accounted for in org-Cl biogeochemical cycling. In addition, the results of this study are of particular interest to the fate of manmade org-Cl compounds, as they are considered to be persistent organic pollutants which can bioaccumulate and contribute to ecosystem, animal, and human toxicity. Ultimately, these reactions play an important role in the global cycling of org-Cl and their downstream influence on ecosystem health and climate change.

Conflicts of interest

There are no conflicts to declare.

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