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Natural organobromine in terrestrial ecosystems

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Abstract

Recent studies have shown that bromine undergoes biogeochemical cycling involving natural formation and degradation of organobromine compounds in marine systems. In the terrestrial environment, where background bromine levels tend to be low, the biogeochemistry of this element remains largely unexamined. We traced the path of bromine through plant growth, senescence, and decay of leaf litter on the forest floor. Using sensitive X-ray spectroscopic techniques, we show that all bromine in humified plant material, organic-rich surface soils, and isolated humic substances is bonded to carbon. Analysis of bromide-enriched plants suggests that bromide absorbed by the growing plants ultimately converts to organobromine when the plant litter decays. Application of isolated chloroperoxidase, a halogenating enzyme, to healthy plant material results in extensive bromination, with organobromine formed preferentially over organochlorine. The relative ease of bromide oxidation appears to promote biogeochemical transformations of Br from inorganic to organic forms, leading to its incorporation into soil organic matter through enzymatic processes related to plant litter decomposition. In combination with low concentration and susceptibility to leaching and plant uptake, natural bromination processes lead to the exhaustion of inorganic bromide in surface soils, making organic matter a reservoir of bromine in the terrestrial environment. This study provides the first detailed look into the terrestrial bromine cycle and lays the foundation for future studies of natural organobromine degradation, which may shed light on the fate of anthropogenic organobromine pollutants in the soil environment.

1. INTRODUCTION

Inorganic bromide (Br_{inorg}) has long been presumed unreactive in the soil environment and has often been used as a conservative tracer for hydrological studies (Levy and Chambers, 1987; Tanner and Sukias, 1995; Weaver et al., 2003). However, soil Br_{inorg} is subject to uptake by plants (Whitmer et al., 2000; Xu et al., 2004) as well as conversion to organobromine (Br_{org}) through natural mechanisms. Natural bromination may occur through the action of enzymes (Theiler et al., 1978; Jannun et al., 1981; Butler and Carter-Franklin, 2004), abiotic metal catalysis (Keppler et al., 2000; Schöler and Keppler, 2003), and photochemical reactions (Pelizzetti and Calza, 2002). Many of the known natural Br_{org} compounds are produced by sessile marine organisms (Schall et al., 1994; Gribble, 1999, 2000, 2010; Dembitsky, 2002; Maruya, 2003), for which they may play a role in chemical defense. In marine algae, for example, Br_{org} compounds have been associated with predator deterrence (La Barre et al., 2010) and may inhibit bacterial quorum sensing to prevent biofilm formation (Borchardt et al., 2001).

In marine sediments, total bromine (Br) concentrations can exceed 100 mg/kg, with natural Br_{org} constituting a significant proportion, in correlation with total organic carbon (Leri et al., 2010). In the terrestrial environment, Br tends to be less abundant. The few soil Br concentration [Br] measurements available exhibit a range of 5–40 mg/kg, with the lowest concentrations measured in sandy podzols and the highest in volcanic ash soils (Kabata-Pendias and Mukherjee, 2007). Positive correlations between total [Br] and organic matter content were found in soils decades ago (Yamada, 1968), though the study drew no conclusions about the molecular basis for the observed correlations. More recently,

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a detailed mass balance of a lacustrine system showed that lake sediments act as a sink for Br, implying the possibility of stable Br_{org} formation (Gilfedder et al., 2011).

The complex macromolecular structure of natural organic matter (NOM) and the low background [Br] in terrestrial samples make Br_{org} analysis challenging. The adsorbable organohalogen (AOX) sum parameter technique has provided much of the existing data on natural organohalogens in the terrestrial environment (Asplund et al., 1989, 1994; Nkusi and Müller, 1995; Müller et al., 1996; Verhagen et al., 1996; Putschew et al., 2003a). The AOX procedure relies on reaction of digested, acidified samples with activated carbon prior to combustion, followed by detection of hydrogen halides through coulometry or ion chromatography. Using the AOX technique, high levels of naturally produced Br_{org} have been measured in peat samples (Biester et al., 2004) and lake waters, in which it may be associated with photosynthetic organisms in the euphotic zone (Putschew et al., 2003b; Hütteroth et al., 2007).

In this study, the proportion of Br_{org} in natural samples was determined via X-ray absorption spectroscopy (XAS), an element-specific technique that requires physical sample preparation only. This minimizes the risk of unintended bromination of NOM in samples, which is particularly acute when samples are acidified during extraction or chemical digestion. To assemble a comprehensive description of terrestrial Br chemistry, we analyzed various field samples, including plant litter, soil, and isolated humic substances from different environments, employing several strategies to overcome low [Br]. For soil samples in which background Br levels fell below the bulk detection limit, we used X-ray spectromicroscopy to map the Br distribution, revealing localized areas of elevated [Br] at which it was possible to determine Br speciation. Bromide-enriched cattail plants provided a functional model of the translocation and chemical transformation of Br_{inorg} during plant growth and decay.

Natural bromination may occur through the action of halogenating enzymes such as chloroperoxidase (CPO), which is believed to facilitate oxidative degradation of plant material and produce organohalogens in the process (Ortiz-Bermúdez et al., 2003, 2007). To simulate enzymatic bromination during plant litter decay, we applied commercially isolated CPO to tree-harvested California redwood needles and assessed Br_{org} formation via XAS.

The results presented here demonstrate that natural Br_{org} is ubiquitous in the terrestrial environment and implicate haloperoxidase-like enzymes in bromination reactions on the forest floor. This first glimpse into the reactivity of Br_{inorg} in soil systems suggests that Br undergoes dynamic biogeochemical cycling, which could further diminish its effectiveness as a hydrological tracer.

2. MATERIALS AND METHODS

2.1. Sample details

2.1.1. Soil and litter layer sampling

Soil and leaf litter samples were collected from the Brendan Byrne State Forest in the Pine Barrens, NJ, USA. The soil in this forest is sandy below the organic (*O*-)horizon, acidic (pH 3.5–5.5), and well drained (Boyd, 1991). The decaying plant material on the soil surface contains leaf litter from numerous oak, pine, and maple tree species. The leaf litter occurs in two or three distinct layers, depending on the season, with the top layer containing material from the most recent litter deposition and the bottom layer the most degraded litter. After about three years on the forest floor, litter has decayed to the extent that individual leaf species are no longer recognizable.

2.1.2. Isolated humic substances

Isolated humic and fulvic acids of fluvial, soil, peat, and lignitic origin were obtained from the International Humic Substance Society (St. Paul, MN). Fulvic acid isolated from Lake Fryxell, Antarctica, was obtained from G. Aiken (US Geological Survey) and Y. Chin (Department of Geological Sciences, The Ohio State University).

2.1.3. Degradative study of Br_{inorg}-fed plant material

Br_{inorg}-fed cattail (*Typha latifolia*) plants were cultivated in a greenhouse as described previously (Xu et al., 2004). Detached Br_{inorg}-fed cattail leaves were placed in trays in an experimental station in the Brendan Byrne State Forest for controlled degradation experiments. Experimental trays consisted of polypropylene plastic and contained a layer of coarse, rigid polypropylene mesh on which the plant material rested. Fine-gauge polypropylene mesh fitted over the tops of the trays prevented the loss of experimental substrates and the entry of extraneous solid material. This mesh was secured over the trays with nylon string and was easily removable for sampling purposes. Tygon tubing (attached to the trays and carboy lids with nylon hose fittings, plastic screws, and epoxy) channeled leachate from the trays to carboys. Trays were situated in a clearing to minimize inputs from the forest canopy. "Above-ground" trays were suspended five feet above the soil surface, enabling a semi-abiotic study of leaf degradation. Trays positioned "on-ground" had holes drilled into the sides to allow for microbial exchange with the surrounding litter. See Supplementary material for schematics and images of the field station apparatus.

2.2. Laboratory-based models of natural bromination reactions

California redwood (*Sequoia sempervirens*) needles collected from healthy trees in Menlo Park, CA, were treated with CPO enzyme under different conditions and analyzed for Br speciation. CPO (isolated from the fungus *Caldariomyces fumago*; Sigma, St. Louis, MO) was diluted with pH 3.5 phosphate buffer (prepared from H_3PO_4 and NaH_2PO_4 in deionized water). Intact redwood needles (0.5 g) were treated with ~140 units of CPO and dilute (0.3%) H_2O_2, with and without addition of exogenous KBr (5 mM). Needles were also treated with H_2O_2 in the absence of CPO and KBr. Treatments were performed in microcentrifuge tubes with the minimal amount of solution necessary to coat the plant material. Solids were analyzed *in situ* after 24 h and 4.5 days of reaction time, respectively. The samples were also analyzed for Cl speciation after 24 h by a previously described procedure (Reina et al., 2004).

2.3. Br 1*s* XANES spectroscopy

Br 1*s* XANES spectroscopy was performed at the Stanford Synchrotron Radiation Laboratory (SSRL) on beamlines 4–3 and 2–3 using a Si (220) double crystal monochromator with slit openings set to $1 \times 15\ mm^2$ upstream and downstream. Samples were mounted on Br-free polypropylene sample holders between layers of X-ray-clean polyfilm secured at the edges with Kapton tape. Sample fluorescence was measured over an energy range of 13,415–13,550 eV using a 13-element Ge detector. Spectra were acquired using a 0.6 eV step size around the Br *K*-edge and 0.9–2.5 eV step sizes above and below the edge. Energy calibration was performed using an internal KBr (s) standard, with the inflection point of its spectrum set at 13,474.0 eV (the Br *K*-edge).

X-ray data were processed using SixPACK version 0.43 (Newville, 2001; Webb, 2005) and WinXAS version 2.0 (Ressler, 1998). SixPACK was used for energy calibration and averaging of scans. Averaged fluorescence scans were imported into WinXAS for background correction, normalization, and spectral fitting. A smooth background was obtained by fitting a first-order polynomial to the pre-edge region, and another first-order polynomial fit to the post-edge region normalized the edge jump to 1.0 at 13,540 eV. This normalization allows for comparative analysis of spectral features in the near-edge region, where absorption intensity is dependent on the bonding environment of Br. Br speciation was computed by least-squares fitting sample spectra iteratively with a library of ten representative Br-containing model compounds. In general, the linear combinations produced high-quality fits with low residuals and proved consistent in distinguishing inorganic from organic forms of Br (see Supplementary Fig. S1 for fitting details). Least-squares fitting gave less consistent results in distinguishing between aliphatic and aromatic forms of Br_{org}, leading to an uncertainty of $\pm 15\%$ depending on the standards used. Therefore, unless Br concentrations in the samples were sufficient to give very clean spectra, as for the humic substances, we sought only to quantify proportions of inorganic vs. organic Br by least-squares fitting.

2.4. X-ray microanalysis and Br 1*s* micro-XANES spectroscopy

The micron-scale distribution and speciation of Br was established *in situ* in heterogeneous natural samples using synchrotron-based X-ray spectromicroscopic techniques involving: (1) micron-scale X-ray fluorescence (micro-XRF) imaging of elemental distributions and (2) establishment of Br speciation through micro-XANES spectroscopy using a focused beam (Bertsch and Hunter, 2001). These X-ray spectromicroscopic studies were performed on beamline 10.3.2 (Marcus et al., 2004) at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory (Berkeley, CA, USA).

Samples were mounted between layers of X-ray-clean polyfilm on metal alloy-framed sample holders designed to fit the movable stage in the X-ray beam path. The X-ray beam struck the samples at a 45° angle and did not come into contact with the sample holder or the Kapton tape used to secure the sample. The 7-element Ge detector was oriented at 90° from the X-ray beam in the plane of the sample. Micro-XRF elemental distribution maps were acquired at 16 μm^2 resolution by training the micro-focused X-ray beam on the sample while the stage moved in the horizontal and vertical directions. The X-ray beam was set to the maximum size available at the focus of the beamline, 16 (horizontal) × 7 (vertical) μm^2. Fluorescence counts were collected exciting at 14 keV. Micro-XRF maps were processed using the XY-mapping software associated with beamline 10.3.2.

Areas of interest on the micro-XRF maps were probed via Br 1*s* micro-XANES spectroscopy, using the energy range, step sizes, and calibration standard specified in Section 2.3. Dwell times at each point were set to at least 3 s; the monochromator was allowed to settle for 0.5 s between steps. EXAFS Editor, a data analysis utility associated with beamline 10.3.2, was used for preliminary inspection, deadtime correction, and averaging of Br 1*s* micro-XANES scans. Averaged scans were imported into WinXAS for energy calibration, background subtraction, and normalization, as described in Section 2.3.

3. RESULTS AND DISCUSSION

3.1. Br 1*s* XANES spectroscopy and micro-XRF imaging

XANES spectroscopy is sensitive to the bonding state of Br, allowing distinctions to be drawn between Br_{inorg} and Br_{org} species in natural samples without chemical digestion or extraction. The increase in X-ray absorption around 13,474 eV (the Br *K*-edge) and the spectral features in the near-edge region correspond to transitions of Br-1*s* electrons to empty atomic and molecular orbitals. The energy of the Br transition shifts to higher values with an increase in Br oxidation state; sodium bromate (Fig. 1d) exhibits an absorption maximum at 13,478.1, whereas aqueous KBr (Fig. 1c) absorbs at 13,477.3 eV. By contrast, Br_{org} compounds give rise to sharp lower-energy peaks around 13,473 eV, as in 4-bromophenol (Fig. 1a) and 1-bromoeicosane (Fig. 1b). These discrete features arise from the $1s \rightarrow \pi^*$ or σ^* transitions associated with the C–Br bonds. The precise energies of these peaks depend on C–Br bond length: Br atoms bonded to aromatic carbon have absorption maxima occurring 0.6 eV higher than those of aliphatic Br_{org} (Fig. 1, top right inset). Spectral variations depending on the oxidation state and coordination environment of Br allow the relative contributions of Br_{inorg} and Br_{org} to total Br in natural samples to be estimated via least-squares fitting with model spectra (Supplementary Fig. S1).

Samples with high total [Br] produce clean bulk (mm-scale) Br 1*s* XANES spectra in which aliphatic and aromatic Br_{org} can be resolved. For samples with low Br content, the spectra tend to be too noisy to make this distinction, allowing for estimation of the proportions of Br_{org} and Br_{inorg} only. Several samples display no bulk Br 1*s* XANES signal, an indication of very low [Br] (estimated detection limit: total [Br] ~10 ppm). For samples in which [Br] is too dilute to detect at the bulk level, μm-scale XRF image mapping

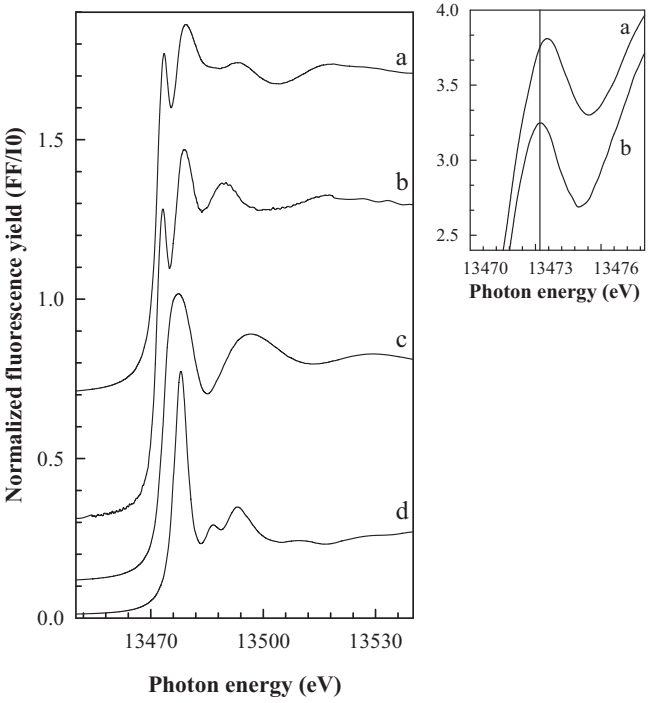

Fig. 1. Normalized Br 1*s* X-ray absorption near-edge structure (XANES) spectra of model compounds: (a) 4-Bromophenol. (b) 1-Bromoeicosane. (c) KBr (aq); amplitude × 0.7. (d) Sodium bromate; amplitude × 0.25. Top right inset: spectral amplitudes enhanced 3×.

techniques enable identification of localized areas of concentrated Br, and micro-XANES spectroscopy reveals the Br speciation at these Br "hot spots".

In isolated humic substances and Br-enriched plant material, total [Br] is generally sufficient to ensure clean and informative bulk Br 1*s* XANES spectra. For decayed plant litter and soil samples with low total [Br], spectromicroscopy provides an effective means to probe Br distribution and speciation.

3.2. Speciation of Br in isolated humic materials

Isolated humic substances from soil, peat, lignitic, and aquatic sources display intense Br 1*s* XANES features corresponding to C–Br bonds, with no detectable Br_{inorg} (Fig. 2a–h). Most of the signals are sufficiently clean to distinguish between aliphatic and aromatic Br_{org} based on least-squares fitting with Br-containing model compound spectra (Supplementary Fig. S1). The humic substances examined display aromatic Br_{org} speciation (Fig. 2a–d and f–g), with the exception of fulvic acid from the sediments of a dry Antarctic valley, in which aliphatic Br_{org} constitutes approximately 25% of the Br signal (Fig. 2e; Supplementary Fig. S1A). The Br chemistry of these isolated humic and fulvic acids is analogous to their reported Cl speciation (Myneni, 2002), implying that the molecular structure of natural organohalogens in humic substances may depend on the chemical ecology of the forest ecosystem and the proportion of lignitic vs. aliphatic inputs to the NOM pool.

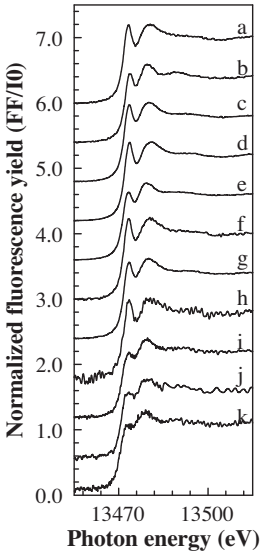

Fig. 2. Normalized Br 1*s* bulk XANES spectra of humic substances (a–h) and decayed plant material (i–k). Humic substances isolated from soil, river water, peat, and lignite; HA = humic acid, FA = fulvic acid: (a) Suwannee River FA. (b) Suwannee River HA. (c) soil FA. (d) Soil HA. (e) Lake Fryxell FA. (f) peat HA. (g) peat FA. (h) Leonardite HA. Decayed plant material from the floor of the Brendan Byrne State Forest (NJ). (i) Middle litter layer (estimated decay time approximately 1.5 years). (j) Bottom litter layer (estimated decay time approximately 2.5 years). (k) Soil *O*-horizon. Spectral noise in h–k reflects low total [Br].

3.3. Speciation of Br in soil profiles

Recently fallen oak leaves and pine needles from the top mulch layer in the Brendan Byrne State Forest have no detectable Br 1*s* XANES signal. However, degraded leaf litter from previous years' depositions and samples from the soil organic (*O*-)horizon display Br_{org} speciation at the exclusion of Br_{inorg} (Fig. 2i–k). Unlike in the isolated humic substances, relatively low total [Br] in these unextracted samples renders the spectra too noisy to distinguish aliphatic from aromatic Br_{org}. However, the appearance of Br_{org} in untreated NOM samples substantiates that the Br_{org} observed in the isolated humic materials is not merely a product of chemical isolation procedures. The Br signal intensity in plant litter increases with decay time, suggesting that Br becomes incorporated into NOM during decay, through conversion of Br_{inorg} and/or adsorption of Br_{org} from atmospheric deposition or throughfall sources. The increase in Br_{org} concentration with decay time may also be a sign of refractory Br_{org} that remains in the plant litter as other components are degraded, emerging in the Br 1*s* XANES signal only at more advanced decay stages. Such phenomena have been documented as part of the Cl cycle in forest ecosystems (Leri and Myneni, 2010). Like their anthropogenic counterparts (Sørmo et al., 2006), natural Br_{org} compounds may exhibit enhanced resistance to biodegradation, thus constituting a relatively stable form of carbon in soils.

As elucidated in Section 3.1, spectromicroscopy provides additional insight into Br distribution and speciation for plant litter samples with low [Br]. The spatial distribution of Br in decayed oak leaves appears largely homogeneous (Fig. 3A), with occasional "hot spots" of elevated Br fluorescence (Fig. 3B). Br 1*s* micro-XANES spectra collected at these spots display a strong Br_{org} pre-edge feature at the energy associated with aromatic C–Br (Fig. 3C(b)). By contrast, Br micro-speciation in areas of diffuse, low Br fluorescence includes a combination of Br_{org} and Br_{inorg} (Fig. 3C(c–d)). The distinct Br_{org} signals observed in the bulk Br 1*s* XANES measurements of such samples (Fig. 3C(a)) appear therefore to be attributable to the cumulative effect of sparsely distributed but highly concentrated Br_{org} hot spots.

Below the organic-rich mulch layers on the soil surface, total [Br] again falls below the bulk Br 1*s* XANES detection limit, but X-ray spectromicroscopy reveals changing Br

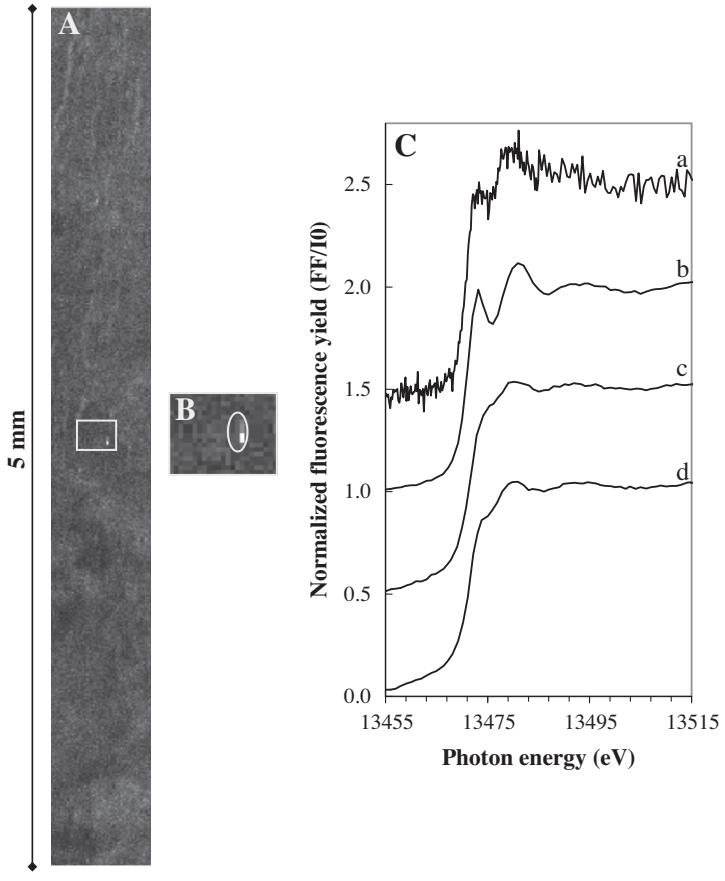

Fig. 3. Br distribution and micro-speciation in decaying plant material from the bottom litter layer in the Brendan Byrne State Forest (NJ). (A) Br Kα micro-XRF map ($5.0 \times 0.6\ mm^2$) of highly degraded litter (estimated decay time approximately 2.5 years). Lighter shade corresponds to greater fluorescence intensity, i.e., greater [Br]. Central Br "hot spot" is highlighted in box. (B) Close-up of Br hot spot. (C) Normalized Br 1*s* XANES spectra: (a) Bulk spectrum of plant material imaged in A. (b) micro-XANES spectrum corresponding to the circled Br hot spot in B. (c–d) Micro-XANES spectra corresponding to diffuse areas of low [Br] in A.

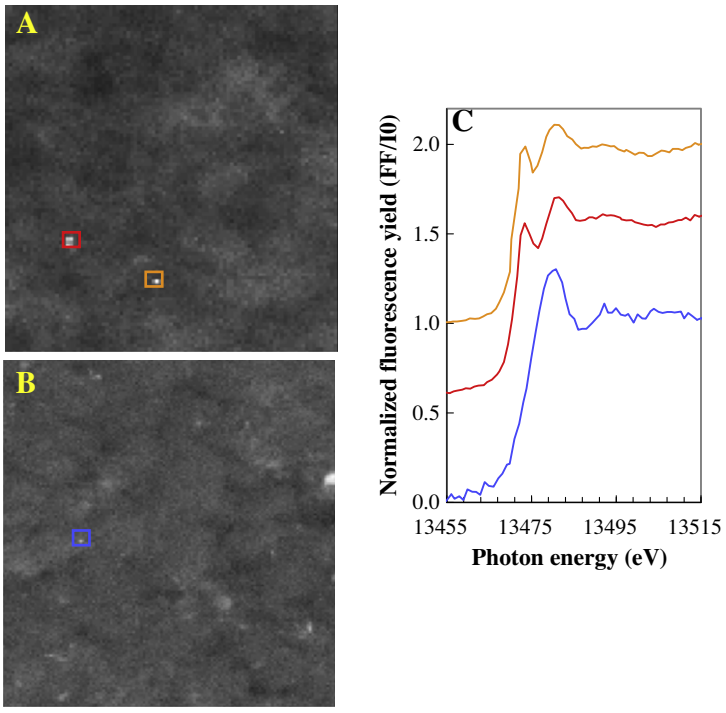

Fig. 4. Br distribution and micro-speciation in soil from the Brendan Byrne State Forest (NJ) at different depths. (A–B) Br K_α micro-XRF maps (2.0×2.0 mm^2) of soil at ~1 cm (A) and ~7 cm (B) below the *O*-horizon. Lighter shade corresponds to greater fluorescence intensity, i.e., greater [Br]. (C) Normalized Br 1*s* micro-XANES spectra: colors of boxes in A and B match colors of associated micro-XANES spectra in C. The Br signal in diffuse areas of low [Br] proved too weak to permit micro-XANES acquisition in soil samples.

chemistry with soil column depth. Localized spots of Br intensity appear in micro-XRF maps of *O*-horizon soil from 1 cm below the surface (Fig. 4A). As in the plant litter samples, these spots yield Br 1*s* micro-XANES spectra characteristic of aromatic Br_{org} (Fig. 4C). Deeper down the soil profile, the rare spot with sufficient Br signal to permit micro-XANES acquisition (Fig. 4B) yields spectra with distinctly Br_{inorg} features (Fig. 4C). The Br signal in regions of low Br fluorescence proved insufficient for micro-XANES acquisition in any soil samples from below the mulch layers.

The change in Br micro-speciation with depth suggests that Br_{org} may be debrominated as NOM is degraded in the soil column. Anaerobic microenvironments at deeper depths may allow for reductive debromination of Br_{org} by bacteria, with consequent release of Br_{inorg} (Müller et al., 1996; Ahn et al., 2003). In marine environments, increasing Br_{inorg} concentrations in sediment porewaters with depth imply that the anion is regenerated as a result of the decomposition of OM during diagenesis (Martin et al., 1993; Mahn and Gieskes, 2001; Leri et al., 2010), and it seems reasonable that a similar phenomenon could occur in terrestrial systems.

3.4. Bromination of plant material

Numerous terrestrial and aquatic plants actively take up Br_{inorg} (Schnabel et al., 1995; Bowman et al., 1997; Whitmer et al., 2000; Xu et al., 2004), and plants are known to contain higher Br levels when grown in Br-rich soils (Kabata-Pendias and Mukherjee, 2007). Upon entry into plant vascular systems, Br_{inorg} is subject to translocation within the plant tissues as well as chemical transformation. Notably, methyl bromide (MeBr) is produced enzymatically from Br_{inorg} in plants inhabiting salt marshes (Rhew et al., 2000) and terrestrial environments (Gan et al., 1998). MeBr evolves from plant tissue into the atmosphere, thus implicating certain plants as contributors to stratospheric ozone depletion.

Since the uptake of Br_{inorg} increases the total [Br] in plant tissues, we used Br_{inorg}-amended *T. latifolia* (cattail) plants to trace the fate of absorbed Br from growth through senescence and decay. Cattails were cultivated in Br_{inorg}-amended greenhouse pots as described previously (Xu et al., 2004). Br 1*s* XANES measurements demonstrate the accumulation of Br_{inorg} in healthy cattail leaves, stems, and roots, substantiating the upward translocation of Br_{inorg} in the plant tissue (Fig. 5A). The absolute Br intensity of cattail leaf spectra (Fig. 5A(a)) is 2.5–4 times greater than that in the roots and stems (Fig. 5A(b–c)), suggesting that the leaves have the highest Br_{inorg} concentrations, consistent with previous observations (Kabata-Pendias and Mukherjee, 2007). The spectral intensity is not an absolute measurement, given the variable density and thickness of the materials analyzed; however, since the leaves are less dense than the roots and stems, the relative concentration of Br in the leaves compared with stems and roots is likely to be even higher than suggested. Senescent cattail leaves (Fig. 5A(d)) display lower total Br levels than healthy leaves (Fig. 5A(a)) but similar Br_{inorg} speciation. Br_{org} is not

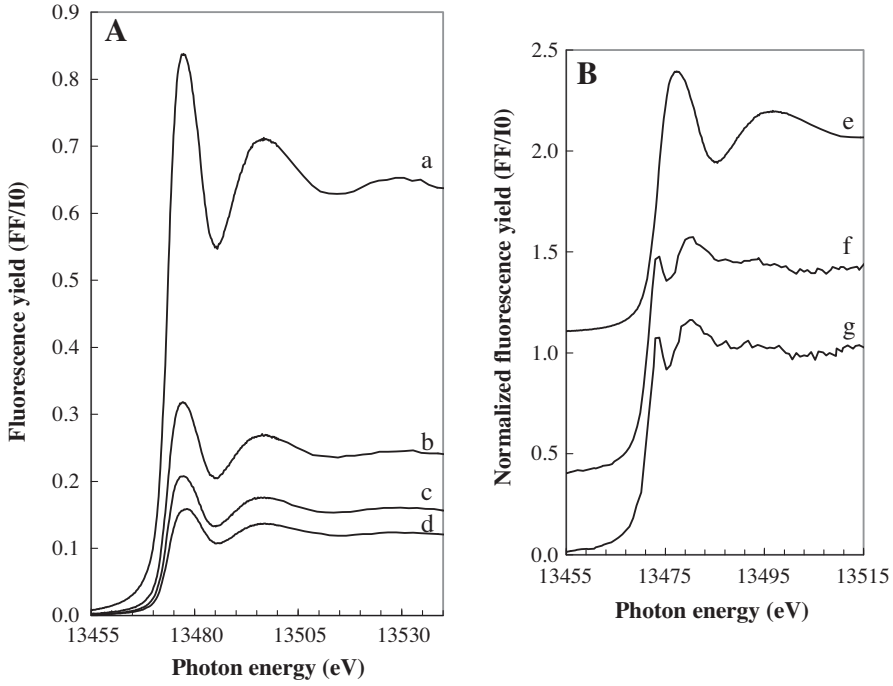

Fig. 5. Accumulated Br in healthy *T. latifolia* plant materials and change in Br speciation as a result of weathering. (A) Unnormalized Br 1*s* bulk XANES spectra (to highlight absolute Br fluorescence intensities, which are roughly proportional to [Br]): (a) Healthy leaf, growing ~7 cm above sediment surface. (b) Root from ~1.5 cm below surface. (c) Stem from ~2.5 cm above surface. (d) Senescent leaf from ~3 cm above surface. (B) Normalized Br 1*s* bulk XANES spectra: (e) Healthy *T. latifolia* leaf. (f) *T. latifolia* leaf after approximately 5 months' weathering in above-ground field set-up. (g) *T. latifolia* leaf after approximately 5 months' weathering among litter on the forest floor.

detectable in healthy or senescent cattail materials; if present, its signal in the spectra is likely overwhelmed by that of Br_{inorg}.

Because the occurrence of Br_{org} in plant material seems linked to decay processes, Br_{inorg}-fed cattail leaves were harvested from the greenhouse and placed into the field station apparatus described in Section 2.1.3. After several months of weathering in the field, the Br speciation in detached Br_{inorg}-amended cattail leaves had changed to Br_{org} (Fig. 5B). This transformation in Br speciation occurred regardless of whether the leaves were allowed to weather in "above-ground" trays, having minimal contact with the soil microbial milieu (Fig. 5B(f)), or placed in more direct contact with the forest floor litter in the "on-ground" trays (Fig. 5B(g)). This suggests that the appearance of Br_{org} in decaying cattail leaves does not necessarily depend on microbial mediation (although the "above-ground" set-up was not strictly abiotic). Thus, the change in Br speciation may be attributable to *de novo* production of Br_{org} during decay and/or to leaching of soluble Br_{inorg} revealing more refractory Br_{org} that had been present all along in the plant tissue.

3.5. Haloperoxidase-catalyzed bromination of plant material

Natural Br_{org} may form through the action of halogenating enzymes, of which five major classes have been identified (Blasiak and Drennan, 2009; Butler and Sandy, 2009). The iron-heme- and vanadium-based haloperoxidases (HPOs) are perhaps the most thoroughly characterized halogenating enzymes. HPOs catalyze the two-electron oxidation of halides by hydrogen peroxide to hypohalous acid (HOX)-like intermediates that react with electron-rich organic substrates to produce organohalogen compounds. HPO-like activity has been reported in forest soils (Asplund et al., 1993; Laturnus et al., 1995). Individual HPOs are named after the most electronegative halogen they can oxidize; thus, Br_{inorg} should be susceptible to oxidation by various HPOs, including chloroperoxidases (CPO) in addition to bromoperoxidases (BPO). The reactive halogen species produced by these enzymes are powerful oxidants that may facilitate the degradation of lignin and other recalcitrant components of decaying plant material by fungi, producing halogenated organic matter as a by-product (Ortiz-Bermúdez et al., 2003, 2007). The possible link between biodegradation of lignin and natural organohalogen production represents an important area of future study.

California redwood needles are rich in phenolic groups that can function as substrates of electrophilic aromatic bromination. We previously showed CPO catalysis to stimulate rapid conversion of the Cl_{inorg} in healthy redwood needles to stable Cl_{org} (Reina et al., 2004). Similarly, treatment with isolated CPO causes the Br_{inorg} naturally present in redwood needles to convert to Br_{org}, with longer reaction times resulting in greater conversion (Fig. 6A(a, d and f)). Addition of exogenous Br_{inorg} to the system results in significant bromination of the plant material, as evidenced by the clean Br_{org} signals in the Br 1*s* XANES spectra (Fig. 6A(b–c)). Even in the absence of added CPO, exogenous H_2O_2 stimulates

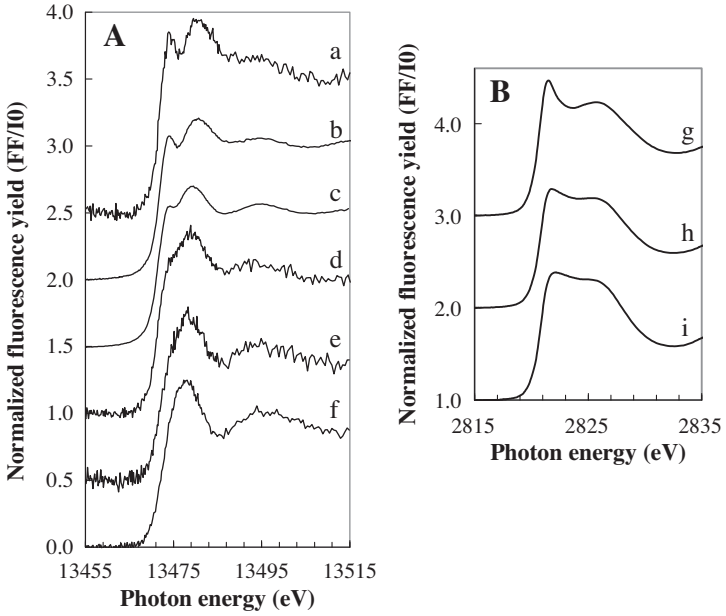

Fig. 6. Br and Cl speciation results for California redwood needles treated with chloroperoxidase (CPO) enzyme under different conditions. (A) Normalized Br 1*s* bulk XANES spectra: (a) Needles + CPO + H_2O_2 (4.5 days). (b) Needles + KBr + CPO + H_2O_2 (4.5 days). (c) Needles + KBr + CPO + H_2O_2 (24 h). (d) Needles + CPO + H_2O_2 (24 h). (e) Needles + H_2O_2 (24 h). (f) Untreated needles. (B) Normalized Cl 1*s* bulk XANES spectra: (g) Needles + CPO + H_2O_2 (24 h). (h), Needles + KBr + CPO + H_2O_2 (24 h). (i), Needles + H_2O_2 (24 h).

production of small amounts of Br_{org} in redwood needles (Fig. 6A(e)). This may indicate that redwood needles (or the microorganisms associated with them) contain HPO-like enzymes, or that the Br_{inorg} in the needles can be oxidized abiotically. In a model reaction system, phenol was readily brominated in the presence of $Fe(NO_3)_3$, KBr, and H_2O_2 at pH 2.1 (Supplementary Fig. S2), suggesting that abiotic bromination of phenolic moieties in plant material could be feasible under acidic soil conditions.

In principle, Br_{inorg} should be more susceptible than the more electronegative Cl_{inorg} to oxidation by CPO and electrophilic substitution on an organic substrate. Redwood needles treated with CPO and H_2O_2 display significant conversion of Cl_{inorg} to Cl_{org} after 24 h, as evidenced by the appearance of a sharp Cl_{org} feature around 2,821 eV (Fig. 6B(g)). When KBr, as a source of Br_{inorg}, is added to the system under identical conditions, chlorination occurs to a much smaller degree, with the spectrum retaining the broad features characteristic of Cl_{inorg} (Fig. 6B(h)) (For a more detailed explanation of Cl 1*s* XANES features, please refer to Myneni, 2002.) Exogenous Br_{inorg} seems therefore to inhibit CPO-catalyzed chlorination of redwood needles in favor of bromination (Fig. 6A(c)).

Haloperoxidative bromination thus represents a viable source of Br_{org} in decaying plant material. However, the relative contributions of various enzymatic and abiotic bromination processes to the terrestrial Br_{org} pool remain poorly understood. It seems feasible that abiotic oxidation of Br_{inorg} by H_2O_2 could occur to some degree at soil pH, possibly through metal catalysis. Non-enzymatic bromination may also occur as a result of metal-catalyzed oxidative breakdown of soil NOM with concomitant alkylation of Br_{inorg} (Keppler et al., 2000).

4. SUMMARY AND IMPLICATIONS FOR THE TERRESTRIAL BR CYCLE

Together with recent findings in marine and estuarine sediments (Leri et al., 2010), this study illuminates the ubiquitous appearance of Br_{org} throughout diverse sedimentary environments. The spectral data in Fig. 2 unambiguously show that all Br in isolated humic substances, decaying plant material, and the organic fraction of soils is covalently bonded to carbon. The appearance of Br_{org} in NOM is linked with plant litter decay, and its formation may be catalyzed by HPO or HPO-like enzymes in the soil environment. The addition of Br_{inorg} to a system of redwood needles, CPO, and H_2O_2 impedes Cl_{org} formation while resulting in extensive conversion of Br_{inorg} to Br_{org}, highlighting the relative ease of enzymatic bromination.

The susceptibility of Br_{inorg} to oxidation, in combination with its solubility and low background concentration, suggests that this halide effectively functions as a limiting reactant in oxidative halogenation processes during plant litter decay. This theory is supported by the observation that Br_{org} constitutes the sole form of Br in the organic fraction of soil and in humified plant material. These findings indicate that Br_{inorg} may be significantly more reactive than previously assumed in the soil environment, a characteristic that, along with uptake by plants, could compromise its effectiveness as a hydrological tracer. The reactivity of Br_{inorg} tracers towards natural oxidation in the soil environment should be established to determine whether they would be labile towards NOM on a pertinent timescale.

The Br_{org} in humified plant material and soil organic matter may function as a reservoir of Br that may ultimately be released as Br_{inorg} upon degradation of NOM,

accounting for our detection of Br_{inorg} in the soil column below the *O*-horizon. In marine environments, reductively debrominating bacteria have been identified (Steward et al., 1995; Ahn et al., 2003), and microorganisms with similar capabilities may exist in terrestrial systems as well. Further investigation of the degradation of naturally produced Br_{org} compounds will ultimately shed light on the fate of PBDEs and other anthropogenic organohalogen pollutants in the soil environment.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2011.11.012.

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