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Abiotic bromination of soil organic matter

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Abstract

Biogeochemical transformations of plant-derived soil organic matter (SOM) involve complex abiotic and microbially mediated reactions. One such reaction is halogenation, which occurs naturally in the soil environment and has been associated with enzymatic activity of decomposer organisms. Building on a recent finding that naturally produced organobromine is ubiquitous in SOM, we hypothesized that inorganic bromide could be subject to abiotic oxidations resulting in bromination of SOM. Through lab-based degradation treatments of plant material and soil humus, we have shown that abiotic bromination of particulate organic matter occurs in the presence of a range of inorganic oxidants, including hydrogen peroxide and assorted forms of ferric iron, producing both aliphatic and aromatic forms of organobromine. Bromination of oak and pine litter is limited primarily by bromide concentration. Fresh plant material is more susceptible to bromination than decayed litter and soil humus, due to a labile pool of mainly aliphatic compounds that break down during early stages of SOM formation. As the first evidence of abiotic bromination of particulate SOM, this study identifies a mechanistic source of the natural organobromine in humic substances and the soil organic horizon. Formation of organobromine through oxidative treatments of plant material also provides insights into the relative stability of aromatic and aliphatic components of SOM.

Graphical abstract

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1. Introduction

The geochemical behavior of bromine (Br) in soils has received little attention, in part because terrestrial concentrations tend to be low and in part because inorganic bromide (Br^-) is widely considered unreactive in the soil environment. The latter perception is rooted in the low sorption affinity of Br^- for anionic mineral surfaces,¹ a characteristic which, along with its solubility and general scarcity, makes Br^- useful as a tracer in soil hydrology.²⁻⁴ While Br^- may not be prone to adsorption, it does appear reactive towards terrestrial organic matter; recent studies have demonstrated fractionation of Br into organic and inorganic pools in lakes,⁵⁻⁷ peat bogs,⁸ and forest soil.⁹

This ubiquity of brominated SOM provided a molecular explanation for the indirect evidence of Br fractionation that had been accumulating since Yamada's 1968 measurements of extraordinarily high Br levels in humic-rich andosols.¹⁰ Globally, soil Br concentrations average around $5 \text{ mg} \cdot \text{kg}^{-1}$ ¹¹ but show a wide range, tending to increase with two distinct factors: 1) proximity to the sea shore; and 2) organic matter content.¹² As a result, total soil Br does not necessarily correlate with inorganic Br^- . In the organic fraction of highly leached forest soils, all detectable Br is bonded to carbon.⁹ In peats, organobromine content is proportional to total Br, which is not the case for Cl.¹³ These findings suggest that Br^- may function as a limiting reactant in soil bromination processes.

In principle, bromination of SOM could proceed through an assortment of mechanisms. There exist several biological pathways involving various brominating enzymes, including haloperoxidases and other halogenases.¹⁴⁻¹⁷ Vanadium-based bromoperoxidases, perhaps the best characterized of the brominating enzymes, catalyze the oxidation of inorganic Br^- by hydrogen peroxide (H_2O_2) to a " Br^+ -like" intermediate that goes on to brominate electron-rich organic substrates non-specifically.^{18, 19} Haloperoxidase-like activity has been detected in forest soil,²⁰ and application of the exogenous enzyme incorporates Br into plant litter,⁹ implicating haloperoxidase catalysis as one possible mechanistic explanation for the natural organobromine observed in SOM. Another pathway for enzymatic bromination involves methyl transferases, which are implicated in the production of volatile halocarbons like the methyl bromide emitted to the atmosphere by higher plants.²¹

Several abiotic bromination pathways have also been identified in natural systems. Volatile bromocarbons like methyl bromide (CH_3Br) form abiotically through alkylation of Br^- ions during the oxidation of SOM by ferric iron (Fe^{3+}).²² Combustion of biomass is another abiotic source of CH_3Br .²³ In aqueous systems, abiotic bromination may occur through photooxidation reactions. For example, phenol can be brominated under UV-Vis irradiation in the presence of oxidants like Fe^{3+} or nitrate (NO_3^-) through a suspected dibromide radical ($\text{Br}_2^{\cdot-}$) intermediate.²⁴ Formation of $\text{Br}_2^{\cdot-}$ in natural waters is thought to happen through radical mechanisms involving oxidation of Br^- by hydroxyl radicals and/or the triplet state of dissolved organic matter.²⁵ Irradiation of natural seawater spiked with phenol generates *para*-bromophenol, among other by-products;²⁶ goethite and Fe(II) may act as photosensitizers towards this bromination of phenol.²⁷ Salicylic acid can also be brominated through photochemical mechanisms in seawater.²⁸ In these studies, phenol and salicylic acid function as models for common aromatic moieties in natural organic matter. A recent study

has shown more generally that dissolved organic matter reacts with photochemically generated reactive Br species in seawater to produce organobromines.²⁹ Bromination of marine particulate organic matter has also been shown to occur through various peroxidative, photochemical, and Fenton-like mechanisms, creating both aliphatic and aromatic forms of organobromine.³⁰

The abiotic natural bromination reactions that have been documented thus far^{22–29} are associated with production of volatile and dissolved forms of organobromine. It remains unknown whether abiotic mechanisms play a role in the incorporation of Br into humus and other soil particulates. In this study, we performed laboratory-based reactions designed to model abiotic oxidations of decaying organic material by H₂O₂ and/or Fe³⁺ salts in the soil environment. Our models explore the effects of decay stage, H₂O₂ concentration gradients, light vs. dark reactions, and pH, among other environmental variables, on abiotic bromination of particulate SOM. Organobromine concentrations in treated particulates were measured using synchrotron-based X-ray absorption near-edge structure (XANES) spectroscopy, according to our rigorous quantification protocol.³¹ By differentiating aliphatic from aromatic forms of organobromine, we were able to assess the relative reactivity of different SOM components towards oxidative bromination.

2. Materials and Methods

2.1. Field sample details

Plant material was collected from the Brendan Byrne State Forest in New Jersey, USA (DMS 39° 53' 27.66" N 74° 34' 46.63" W), an area of the Pine Barrens lying ~15 km from the Atlantic coast. This forest features a mix of coniferous and deciduous vegetation above sandy, acidic soil (pH 3–5). Fresh, healthy pine needles and oak leaves were harvested from pitch pine (*Pinus rigida*) and white oak (*Quercus alba*) trees in late summer. Mixed, weathered plant litter was grab-sampled from the forest floor below the tree canopy, along with samples of dark-colored, fine-grained humus from the top of the soil organic horizon. Plant material (both fresh and decayed) was air-dried for one week in the laboratory, then pulverized in a mechanical mill. Humus was passed through a 5-mm sieve. The resulting powders were stored at –20°C until treatment or analysis.

2.2. Abiotic bromination treatments

Treatments were carried out on pulverized material (1.0 g of pine powder, oak powder, litter powder, or humus) in thick suspensions buffered at pH 3.0 (phosphate) or 5.0 (acetate) with various combinations of the following reagents: potassium bromide (KBr; 40 mM), hydrogen peroxide (H₂O₂; 2.0 mM except where otherwise specified), ferric nitrate nonahydrate (Fe(NO₃)₃ • 9H₂O; 20 mM), ammonium ferric citrate (C₆H₈O₇ • xFe³⁺ • yNH₃; 20 mM), ferric sulfate (Fe₂(SO₄)₃; 20 mM), and ferrous sulfate (FeSO₄; 100 µM). Specified concentrations refer to final solution volume, which was 10.0 mL for all treatments. The mixture of 1.0 g particulates and 10.0 mL aqueous solution created a thick heterogeneous suspension intended to resemble conditions of moist plant litter decomposing on the forest floor. The levels of added Br[–] are similar to those used in a previous lab-based study showing production of halomethanes from halide-treated soil.²² These millimolar Br[–] levels

are high relative to typical concentrations in natural environments. The intention of using large excesses of Br^- was to promote bromination on relatively short experimental timescales.

Reagents were combined with plant material in 50-mL centrifuge tubes, vortexed, and placed on a shaker for 24, 48, or 96 hours of reaction time at room temperature. For the “dark” reactions, centrifuge tubes were wrapped in aluminum foil. Following the treatments, solids were rinsed using six successive rinse/vortex/centrifuge/decant cycles with 50-mL portions of deionized water to remove unreacted Br^- . Thorough rinsing is crucial to prevent excess Br^- from overrunning the Br XANES signal. Centrifugation was performed at 10°C and 3000 rpm for 20 min per cycle. Rinsed solids were spread on watch glasses to dry overnight at 35°C before being compressed into 13-mm pellets in a hydraulic laboratory press at 15,000 lbs.

2.3. Br *K*-edge X-ray absorption near-edge structure (XANES) spectroscopy

2.3.1. Beamline and data collection specifics—Br *K*-edge XANES spectra were collected at the National Synchrotron Light Source (Upton, NY, USA) at beamline X23A2, an unfocused bend magnet beamline with a Si(311) fixed-exit monochromator of a Golovchenko-Cowan design.³² Sample pellets were mounted on halogen-free XRF tape and exposed at a 45° angle to the incoming X-ray beam, with a spot size of 4.5 mm (H) × 0.5 mm (V) centered on the pellet. XANES scans were measured from 13,374 to 13,674 eV using a 0.25 eV step size around the Br *K*-edge (13,474 eV) and 0.5–5.0 eV step sizes above and below the edge, with 1.0-sec dwell times. Spectra were collected in fluorescence mode using a four-element Si-drift detector with the algorithm of Woicik *et al.*³³ used to correct detector deadtime. Between four and twelve XANES scans were collected per sample, depending on spectral noise.

Samples were measured using two internal standards: an energy calibration transmission standard mounted behind the sample and a [Br] quantification standard mounted alongside the samples. The transmission standard consisted of bromophenol blue, which has a discrete absorption maximum well suited to energy alignment. The quantification standards were pellets containing specific concentrations of KBr homogenized via dissolution in a matrix of sodium polyacrylate. Energy alignment and quantification were achieved as described below.

2.3.2. Analysis of XANES data for Br concentration and speciation—XANES spectra were processed using the ATHENA program in the Demeter software package.³⁴ Individual XANES scans were aligned using the absorption maximum of the bromophenol blue transmission spectrum, which was calibrated to 13,473.4 eV. After energy alignment, XANES scans were averaged. Each averaged spectrum was background-subtracted and normalized with polynomials fit through the pre-edge (13,412 eV to 13,442 eV) and post-edge (13,552 eV to 13,652 eV) regions. The “edge step” of each XANES spectrum was recorded as the difference at 13,472.0 eV between these two regression lines.

The edge step of a Br XANES spectrum is linearly related to the absolute concentration of Br in the sample.³¹ A series of KBr pellet standards were used to produce a calibration line

from 0–500 mg•kg⁻¹ total Br concentration. Beamline X23A2 featured a moveable sample stage on which several pellets were mounted at once. Once the calibration line was established, an internal KBr standard was included adjacent to sample pellets on each mount, and the edge step of this quantification standard was used to position the detector for subsequent measurements of all pellets on the mount. Edge steps of the sample spectra could therefore be converted into total Br concentrations using the calibration line. This could be achieved directly provided the pellets were of uniform mass (335 ± 5 mg). If sample pellets were underweight, a thickness correction factor was applied as we have described in detail previously.³¹ Uncertainties in the edge step measurement were computed as 1σ error.

To determine Br speciation in the samples, normalized XANES spectra were fit via linear combination (LC) with spectra of Br-containing model compounds, several examples of which are shown in Fig. 1A. The vertical lines on the chart intersect the characteristic absorption maxima of aliphatic organobromine (represented by *tert*-butylbromide and 1-bromoadamantane, Fig. 1A, a–b) and aromatic organobromine (represented by *para*-bromophenol and –dibromobenzene, Fig. 1A, c–d). These sharp, low-energy features correspond to transitions of the 1s electron to σ* and π* orbitals associated with the C-Br bond. The typical absorption maximum, or “white line,” for an aromatic organobromine appears almost a full eV higher in energy than that of an aliphatic compound, due to differences in bond energy imparted by partial π character of the aromatic C-Br bond. When there is no covalent bond present, XANES spectral features appear broader and at higher energies, as exemplified by the spectrum of aqueous KBr (Fig. 1A, e). The LC fitting routine provides quantitative estimates of the relative proportions of inorganic Br⁻ and aromatic/aliphatic organobromine in sample XANES spectra with 10–15% error. Spectral fitting was carried out in ATHENA on the first derivative of the near-edge region of each spectrum, from 13,465 to 13,500 eV.

3. Results and Discussion

3.1. Background Br in untreated plant material and soil humus

Tree-harvested white oak leaves and pitch pine needles have very little Br—undetectable in the case of the former and at the detection limit (~1 mg•kg⁻¹) in the latter, with a weak aromatic organobromine signal. Compared with fresh plant materials, soil humus is enriched in Br, with 5 mg•kg⁻¹ aromatic organobromine revealed in a noisy XANES spectrum (Fig. 1B, a). These low concentrations are consistent with the low background Br in the highly leached soil environment of the Pine Barrens.

3.2. Rationale for treatments of plant material and soil humus

Treatments of SOM particulates with Br⁻ and H₂O₂ at acidic pH were designed to create hypobromous acid (HOBr) or a similar reactive Br species through a peroxidative reaction:

Oxidized forms of Br, including HOBr, hypobromite (OBr⁻), and elemental bromine (Br₂), are highly reactive towards olefins and phenolic molecules,³⁵ and plant material contains

myriad potential substrates for electrophilic bromination, including unsaturated lipids (fatty acids, waxes, terpenoids), aromatic amino acids, polyphenolic molecules, and lignin. Hydrogen peroxide is ubiquitous in the soil environment. Substantial quantities of H_2O_2 are introduced to soil from wet and dry deposition, and decomposition of H_2O_2 to the hydroxyl radical may have far-reaching effects on the oxidative chemistry of soils.³⁶

Some treatments incorporated oxidants other than H_2O_2 , including various forms of ferric iron, which could function as a potential metal catalyst for Eq. 1 or be involved in an alternative bromination mechanism, such as the previously demonstrated oxidation of SOM with concomitant emission of CH_3Br .²² We also investigated the effects of several other variables on bromination, including pH, H_2O_2 concentration, treatment time, and substrate characteristics (oak vs. pine and fresh vs. decayed plant material).

The objectives of this study are twofold: 1) certain model experiments are intended to elucidate abiotic bromination pathways that are likely to occur in real soil environments; and 2) models using powerful oxidants not likely to occur in nature are useful for providing insight into the comparative reactivity of different SOM components to bromination.

3.3. Br speciation in treated plant material and soil humus

Following treatment with aqueous Br^- and H_2O_2 for 48 h at pH 3.0, oak, pine, and humus particulates display strong organobromine signals in their XANES spectra (Fig. 1B, b–d). The absorption maxima of the spectra differs by substrate—while treated humus shows a white line position corresponding to aromatic organobromine (Fig. 1B, b), the white line of treated pine needles falls slightly below this energy (Fig. 1B, c), and that of treated oak leaves falls closer to the characteristic energy of aliphatic organobromine (Fig. 1B, d). LC fitting of the spectra confirmed higher proportions of aliphatic organobromine in treated oak leaves, with approximately 3:2 aliphatic:aromatic compared with 1:4 aliphatic:aromatic for treated pine needles and 100% aromatic in treated humus. Br speciation will be presented quantitatively for various treatments in the sections that follow.

3.4. Br enrichment in treated humus

Br enrichment of treated particulates was revealed by their absolute fluorescence intensity, as exemplified in the unnormalized spectra of treated humus in Fig. 2. Beyond the oscillations of the near-edge region that reveal Br speciation, the post-edge rise in spectral intensity is linearly proportional to Br concentration in the sample. Br concentrations are computed from the edge step value with high precision following our previously described quantification procedure.³¹ Total [Br] values are then combined with LC fitting results to give absolute concentrations of aliphatic/aromatic and inorganic Br species in the sample.

Treatment of soil humus with H_2O_2 and aqueous Br^- at pH 3.0 for 48 h resulted in dramatic Br enrichment to $64 \text{ mg}\cdot\text{kg}^{-1}$ (from $5 \text{ mg}\cdot\text{kg}^{-1}$ in untreated humus), all in the form of aromatic organobromine (Fig. 2). Combination of H_2O_2 and Br^- with ferric citrate or nitrate produced less organobromine, $36 \text{ mg}\cdot\text{kg}^{-1}$ and $52 \text{ mg}\cdot\text{kg}^{-1}$, respectively, with ferric nitrate producing $6 \text{ mg}\cdot\text{kg}^{-1}$ aliphatic organobromine in addition to $46 \text{ mg}\cdot\text{kg}^{-1}$ aromatic. However, each of the ferric oxidants combined with Br^- in the absence of H_2O_2 increased the organobromine content compared with the control, with the ferric citrate treatment

producing 14 mg·kg⁻¹ aromatic organobromine and ferric nitrate 13 mg·kg⁻¹ aromatic and 5 mg·kg⁻¹ aliphatic. Application of aqueous Br⁻ to humus in the absence of oxidants did not significantly increase organobromine concentrations compared with the untreated control (Fig. 2).

These results demonstrate that the brominating effects of H₂O₂ and the ferric salts are not additive. On the contrary, ferric oxidants act as brominating agents on their own but impede bromination in the presence of H₂O₂. Combination of H₂O₂ with ferric salts may have caused catalytic H₂O₂ decomposition³⁷ or created such strongly oxidizing conditions that organobromine was broken down as well as produced. The latter possibility is supported by the production of aliphatic organobromine in the ferric nitrate treatments, as these aliphatics could represent breakdown products from oxidation of brominated aromatics.

All concentrations reported subsequently were obtained from unnormalized XANES spectra similar to those of the humus treatments in the Fig. 2 example.

3.5. Bromination of various substrates

To compare the susceptibility of assorted SOM precursors to abiotic bromination, treatments at pH 3.0 were carried out on fresh plant material, partially decayed plant litter from the forest floor, and highly degraded soil humus (Fig. 3). For this comparison, “oak” and “pine” refer to green, tree-harvested plant material. “Mixed litter” consists of brown, decayed oak leaves and pine needles of various species after approximately nine months of weathering on the forest floor, an intermediate decay stage compared with the dark-colored, oxidized “humus” material.

Results for treated oak and pine substrates contrast sharply with those of soil humus, with more organobromine produced in fresh material than in humus. Even in the absence of added oxidants, application of 40 mM Br⁻ caused substantial production of both aromatic and aliphatic forms of organobromine in oak and pine substrates compared with untreated controls (Fig. 3). Aliphatic organobromine production was greater in oak than pine material. This general pattern continues in treatments incorporating H₂O₂, ferric nitrate, or both in addition to Br⁻, with some interesting distinctions among the oxidants. Combination of H₂O₂ with Br⁻ produced no more organobromine in fresh oak than Br⁻ alone and only slightly more in fresh pine (Fig. 3). This held true for treatments with varying H₂O₂ concentrations, from 0.1 to 2.0 mM, and in the dark as much as the light (Fig. S1 in Supporting Information). By contrast, addition of ferric nitrate with Br⁻ dramatically increased organobromine production in oak and pine, in both aromatic and aliphatic fractions (Fig. 3). Using both ferric nitrate and H₂O₂ with Br⁻ brominated oak and pine somewhat less than ferric nitrate and Br⁻ alone.

While application of Br⁻ by itself did not enrich humus in organobromine compared with the untreated control, combination of Br⁻ and H₂O₂ increased the aromatic organobromine content of the humus by an order of magnitude (Fig. 3). Addition of ferric nitrate, H₂O₂, and Br⁻ to humus produced less organobromine than H₂O₂ and Br⁻ alone and created a small aliphatic fraction in addition to aromatic organobromine. Even less bromination was observed with the application of Br⁻ and ferric nitrate to humus.

The results of these treatments illuminate several phenomena. The straightforward incorporation of Br into fresh oak and pine material suggests a limiting reagent role for Br⁻ in the bromination of fresh organic material in acidic soils. In contrast with the natural soil environment, the addition of large excesses of Br⁻ switched the limiting reagent to reactive organic moieties in plant material, resulting in extensive bromination. The superfluity of added H₂O₂ in the bromination of fresh plant material does not preclude Eq. 1 as a bromination pathway, because mechanical pulverization of the plant material could cause endogenous H₂O₂ release.³⁸ Bromination is just as effective in the dark, implying the reaction is not photochemical. More bromination in fresh vs. oxidized substrates is indicative of more labile organics in fresh plant material. In all treatments, oak material has higher organobromine concentrations than pine, and most of the additional organobromine is aliphatic. For each treatment, results for mixed plant litter fall intermediate between those for fresh oak/pine and humus, with the caveat that the mixed litter treatments were carried out for 24 h compared with 48 h for the other materials (Fig. 3). Partially decayed litter has more brominatable aliphatics than humus, though not as many as fresh oak leaves and pine needles.

3.6. Effects of pH on abiotic bromination

If Eq. 1 represents the dominant reaction at work in our models, lower pH should enhance bromination. To assess the effects of pH, we treated oak and pine materials at pH values of 3.0 and 5.0, bracketing a typical range for forest soil. In pine material, bromination occurred at both pH values, but each treatment produced less than half of the organobromine at pH 5.0 than at pH 3.0 (Fig. 4A). This held true for treatments incorporating H₂O₂ and ferric nitrate oxidants as well as those applying Br⁻ by itself. Oak material also showed less bromination at higher pH, with particular diminishment in the aliphatic organobromine fraction (Fig. 4B).

These results implicate a pH-dependent pathway like peroxidative bromination (Eq. 1) in abiotic organobromine production and further imply that such processes will be of greater importance in more acidic soils. Ferric nitrate promotes bromination of oak and pine at both pH 3.0 and 5.0 (Fig. 4). Iron-bearing soil minerals could potentially serve as Lewis acid catalysts to facilitate abiotic Br⁻ oxidation, as they do in certain brominations devised by synthetic chemists. Inspired by the action of vanadium bromoperoxidases, chemists have sought biomimetic transition metal catalysts for mild peroxidative bromination,^{39–43} mainly to circumvent the need for the corrosive and toxic Br₂ reagent in organic syntheses. However, there has been some controversy over the efficacy of various transition metal catalysts in biomimetic oxybromination reactions, with Rothenberg and Clark finding that reactions ostensibly catalyzed by molybdenum and vanadium were dependent rather on stoichiometric quantities of acid,⁴⁴ in accordance with Eq. 1. The acidity of the reaction medium thus appears to be a crucial variable in peroxidative bromination, with the need for activation of peroxide either by Brønsted or Lewis acids. This translates into pH values below 3 or else substantial amounts of vanadate or a similar Lewis acid to make this reaction efficient enough for organic syntheses in aqueous solution.⁴⁵ Our reactions were buffered to minimize pH changes from added reagents. The role of ferric nitrate in enhancing bromination in our treatments will be explored further in the subsequent section.

Since peroxidative bromination proceeds with good yields in aqueous solution at pH 2–3,⁴⁵ then it likely occurs with reduced efficiency in soils. Soil pH values below 5 are common, particularly in certain highly leached and/or organic-rich environments. An important natural system for abiotic bromination is likely to be peat lands, which cover approximately 2–3% of total land area and are one of the largest terrestrial reservoirs of halogens.⁴⁶ Peats bogs are acidic (sphagnum or “typical” bogs have pH < 5), and peats are highly enriched in Br.¹³ The dominant mechanisms of Br incorporation into peats have not yet been established, but the abiotic reactions demonstrated here are probable contributors.

3.7. Effects of ferric salts on abiotic bromination

The most effective treatments in brominating oak and pine material involve ferric nitrate (Figs. 3–4). The maximum organobromine produced in any experiment was 550 mg·kg⁻¹, in oak material treated with ferric nitrate and Br⁻ for two days. Interestingly, similar treatment of oak for four days produced only 360 mg·kg⁻¹ organobromine (Fig. 5), suggesting degradation upon extended exposure to ferric nitrate. Most of the loss comes from the aliphatic organobromine fraction, which decreases from 385 mg·kg⁻¹ after two days to 255 mg·kg⁻¹ after four days. The lability of the aliphatic fraction in oaks is also supported by the result that ferric nitrate yields substantially more aliphatic organobromine in the absence of H₂O₂ (385 vs. 250 mg·kg⁻¹—see Fig. 3). As posited in section 3.4, organobromines may break down under the highly oxidizing conditions created by combining ferric nitrate and H₂O₂.

Likewise, more aliphatic organobromine is produced in pine material by ferric nitrate and Br⁻ than by ferric nitrate, Br⁻, and H₂O₂; however, the difference is less dramatic than for oak, in which the concentrations of aliphatic organobromine produced are much higher (Fig. 3). The reaction time trend differs for pine; four days of treatment with ferric nitrate and Br⁻ resulted in slightly more aliphatic organobromine than two days, although still far less than in oak (Fig. 5).

Unlike some of the milder treatments, the ferric nitrate experiments do not directly model reaction conditions in the natural environment. A strong oxidant like ferric nitrate is apt to carry out rapid oxidation of labile organics in plant material, much like potassium permanganate, the reagent that operationally (and controversially) defines labile soil carbon.⁴⁷ Application of ferric nitrate to fresh oak and pine had dramatic effects on their appearance, darkening the material so that it took on the deep brown color of naturally oxidized soil humus. Like the production of aliphatic organobromine, the darkening effect was more pronounced in oak (Fig. S2) than in pine (Fig. S3) material.

Although ferric nitrate contains two powerful oxidants, Fe³⁺ and NO₃⁻, neither has sufficient potential to oxidize aqueous Br⁻ directly to Br₂ or HOBr. To clarify the roles of Fe³⁺ and NO₃⁻, we varied the counteranion in a series of oak and pine treatments with ferric salts, finding that treatments with ferric citrate or sulfate did not produce nearly as much organobromine as those with ferric nitrate (Fig. 5). This implicates NO₃⁻ rather than Fe³⁺ as the key oxidant leading to bromination in the ferric nitrate treatments. Moreover, the large production of aliphatic organobromine in oak and pine is specific to the ferric nitrate treatments.

These data suggest that the bromination mechanism at work in the ferric nitrate treatments involves oxidation of SOM rather than the oxidation of Br^- as in Eq. 1. Such a pathway would be consistent with the findings of Keppler *et al.*, who demonstrated production of volatile bromocarbons resulting from oxidation of natural organics by Fe^{3+} and concomitant methylation of Br^- .²² Accordingly, in our experiments, NO_3^- , and to a lesser extent Fe^{3+} , could oxidize electron-rich organic substrates in plant material that proceed to alkylate Br^- . Such a pathway could account for the particularly large increases in brominated aliphatics upon treatment of oak and pine with ferric nitrate. Alternatively, Fe^{3+} may promote aromatic ring cleavage to produce low molecular weight bromoalkanes.⁴⁸

Although ferric nitrate dramatically enhances bromination of fresh plant material, it does not increase organobromine levels in humus (Fig. 3), presumably because the labile carbon has already been consumed through natural oxidative processes. Thus, the substrates susceptible to bromination through the treatment of fresh plant material with ferric nitrate represent labile forms of carbon that break down during initial decay stages on the forest floor.

3.8. Effects of Fenton-like conditions on abiotic bromination

The well-known Fenton reaction involves the Fe^{2+} -catalyzed disproportionation of H_2O_2 to produce hydroxyl radicals ($\text{OH}\cdot$), which may proceed to react with halides, forming reactive halogen species.⁴⁸ The combination of Fe^{2+} with H_2O_2 in seawater has been shown to induce dramatic bromination of marine organic matter.³⁰ To investigate whether Fenton-like conditions would lead to abiotic bromination of SOM, we carried out experiments on plant material and humus with Br^- , H_2O_2 , and Fe^{2+} (in the form of ferrous sulfate) at pH 3.0. For oak and humus, the addition of Fe^{2+} made no significant difference in organobromine yield (Fig. 6). For pine, Fe^{2+} increased the production of aromatic organobromine, although the effect is within the margin of error (Fig. 6).

These Fenton-like conditions did not meaningfully change the yields of organobromine, suggesting that hydroxyl radicals do not promote bromination. Furthermore, performing treatments (Fenton-like or otherwise) in the dark did not diminish organobromine yields (Fig. 6), discounting a photochemical pathway. The results of these experiments imply that peroxidative bromination is more likely than a radical-based pathway.

3.9. Insights into relative stabilities of SOM components

In addition to illuminating environmentally feasible pathways of abiotic bromination, our experiments have identified aliphatic and aromatic fractions of varying reactivity in SOM and its plant-derived precursors. The chemical composition and reactivity of various SOM fractions, which are crucial to understanding soil carbon turnover, have not been adequately characterized.^{49, 50} One of the most striking results of our experiments is the existence of readily brominatable aliphatics in fresh oak and pine material, a fraction that is no longer present in humus. In the natural oxidative transformation of plant litter to soil humus, these aliphatic compounds must be broken down or chemically altered in a way that diminishes their susceptibility to bromination.

Brominatable aliphatics in oak and pine litter probably make up part of the so-called “labile soil carbon” pool. The quickest SOM fraction to turn over, labile soil carbon is chemically

heterogeneous and poorly defined, consisting of carbohydrates, polysaccharides, proteins, amino acids, waxes, fatty acids, and other unspecified compounds.⁵¹ Unsaturated moieties in this pool would be highly susceptible to bromination through a mechanism like peroxidative bromination (Eq. 1). The labile fraction decomposes over a period between days and few years,⁴⁹ comparable to the timescale over which plant litter is converted to soil humus. Our treatments produced substantially more aliphatic organobromine in oak leaves than in pine needles, potentially signifying that deciduous leaves contribute more labile aliphatics to SOM than do coniferous needles.

Our treatments were also effective in producing aromatic organobromine, in fresh oak and pine as well as humus. Bromination of aromatics likely involves oxidation of Br^- to HOBr (Eq. 1) or a similarly reactive species that proceeds to brominate phenolic and anisolic moieties. Such activated benzene rings are common in SOM, from plant polyphenolics to lignin, the recalcitrant plant polymer. The brominated aromatic fraction, which is more stable than the aliphatic fraction on the timescale of our experiments, could represent a reservoir of less decomposable organic matter—part of the stable or inert fractions of SOM, with longer turnover on the order of decades to centuries.⁴⁹

This and other recent mechanistic explorations^{24, 28–30} have begun to illuminate the geochemical dynamics of Br under various environmental conditions. Our results demonstrate that peroxidative bromination can occur abiotically in the soil environment, particularly under acidic conditions. The incorporation of Br^- into fresh plant material seems to be limited primarily by Br^- concentration. This suggests that in natural environments where total Br concentrations are low and organic matter concentrations are high (e.g. the soil organic horizon), inorganic Br^- is actively scavenged, effectively operating as a limiting reactant in abiotic oxidations. Thus, our study has revealed a natural, abiotic source of particulate organobromine in the terrestrial environment. Furthermore, by assessing the susceptibility of different substrates to bromination, we have identified a labile aliphatic fraction of plant material that does not persist in humus as well as a more stable, recalcitrant aromatic fraction—another small step in the chemical characterization of particulate SOM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Gilley JE, Finkner SC, Doran JW, Kottwitz ER. Adsorption of bromide tracers onto sediment. *Applied Engineering in Agriculture*. 1990; 6(1):35–38.
2. Nobles MM, Wilding LP, Lin HS. Flow pathways of bromide and Brilliant Blue FCF tracers in caliche soils. *Journal of Hydrology*. 2010; 393(1–2):114–122.

3. Ma R, Zheng C, Zachara JM, Tonkin M. Utility of bromide and heat tracers for aquifer characterization affected by highly transient flow conditions. *Water Resour Res.* 2012; 48(8):W08523.
4. Mollerup M, Abrahamsen P, Petersen CT, Hansen S. Comparison of simulated water, nitrate, and bromide transport using a Hooghoudt-based and a dynamic drainage model. *Water Resour Res.* 2014; 50(2):1080–1094.
5. Putschew A, Mania M, Jekel M. Occurrence and source of brominated organic compounds in surface waters. *Chemosphere.* 2003; 52(2):399–407. [PubMed: 12738262]
6. Gilfedder BS, Petri M, Wessels M, Biester H. Bromine species fluxes from Lake Constance's catchment, and a preliminary lake mass balance. *Geochimica et Cosmochimica Acta.* 2011; 75(12): 3385–3401.
7. Hüttersoth A, Putschew A, Jekel M. Natural production of organic bromine compounds in Berlin lakes. *Environ Sci Technol.* 2007; 41(10):3607–3612. [PubMed: 17547185]
8. Biester H, Keppler F, Putschew A, Martinez-Cortizas A, Petri M. Halogen Retention, Organohalogenes, and the Role of Organic Matter Decomposition on Halogen Enrichment in Two Chilean Peat Bogs. *Environ Sci Technol.* 2004; 38(7):1984–1991. [PubMed: 15112797]
9. Leri AC, Myneni SCB. Natural organobromine in terrestrial ecosystems. *Geochimica et Cosmochimica Acta.* 2012; 77(0):1–10.
10. Yamada Y. Occurrence of Bromine in Plants and Soil. *Talanta.* 1968; 15(11):1135–1141. [PubMed: 18960416]
11. Yuita K, Nobusawa Y, Shibuya M, Aso S. Iodine, bromine and chlorine contents in soils and plants of Japan. *Soil Sci Plant Nutr.* 1982; 28(3):315–336.
12. Flury M, Papritz A. Bromide in the Natural Environment: Occurrence and Toxicity. *J Environ Qual.* 1993; 22(4):747–758.
13. Putschew A, Keppler F, Jekel M. Differentiation of the halogen content of peat samples using ion chromatography after combustion (TX/TOX-IC). *Analytical and Bioanalytical Chemistry.* 2003; 375(6):781–785. [PubMed: 12664178]
14. Butler A, Sandy M. Mechanistic considerations of halogenating enzymes. *Nature.* 2009; 460(7257):848–854. [PubMed: 19675645]
15. Winter JM, Moore BS. Exploring the Chemistry and Biology of Vanadium-dependent Haloperoxidases. *Journal of Biological Chemistry.* 2009; 284(28):18577–18581. [PubMed: 19363038]
16. Blasiak LC, Drennan CL. Structural Perspective on Enzymatic Halogenation. *Accounts of Chemical Research.* 2009; 42(1):147–155. [PubMed: 18774824]
17. van Pee KH. Enzymatic chlorination and bromination. *Methods Enzymol.* 2012; 516:237–57. [PubMed: 23034232]
18. Everett RR, Butler A. Bromide-assisted hydrogen peroxide disproportionation catalyzed by vanadium bromoperoxidase: absence of direct catalase activity and implications for the catalytic mechanism. *Inorg Chem.* 1989; 28(3):393–395.
19. de Boer E, Wever R. The reaction mechanism of the novel vanadium-bromoperoxidase. A steady-state kinetic analysis. *The Journal of biological chemistry.* 1988; 263(25):12326–32. [PubMed: 3410844]
20. Laternus F, Mehrtens G, Grøn C. Haloperoxidase-like activity in spruce forest soil – a source of volatile halogenated organic compounds? *Chemosphere.* 1995; 31:3709–3719.
21. Gan J, Yates SR, Ohr Hd, Sims JJ. Production of methyl bromide by terrestrial higher plants. *Geophysical Research Letters.* 1998; 25:3595–3598.
22. Keppler F, Eiden R, Niedan V, Pracht J, Schöler HF. Halocarbons produced by natural oxidation processes during degradation of organic matter. *Nature.* 2000; 403:298–301. [PubMed: 10659846]
23. Mano S, Andreae MO. Emission of Methyl-Bromide from Biomass Burning. *Science.* 1994; 263(5151):1255–1257. [PubMed: 17817427]
24. Vione D, Maurino V, Man SC, Khanra S, Arsene C, Olariu RI, Minero C. Formation of Organobrominated Compounds in the Presence of Bromide under Simulated Atmospheric Aerosol Conditions. *ChemSusChem.* 2008; 1(3):197–204. [PubMed: 18605206]

25. De Laurentiis E, Minella M, Maurino V, Minero C, Mailhot G, Sarakha M, Brigante M, Vione D. Assessing the occurrence of the dibromide radical (Br_2^-) in natural waters: Measures of triplet-sensitised formation, reactivity, and modelling. *Science of The Total Environment*. 2012; 439(0): 299–306. [PubMed: 23085471]
26. Calza P, Massolino C, Pelizzetti E, Minero C. Solar driven production of toxic halogenated and nitroaromatic compounds in natural seawater. *Science of the Total Environment*. 2008; 398(1–3): 196–202. [PubMed: 18452974]
27. Calza P, Massolino C, Pelizzetti E, Minero C. Role of iron species in the photo-transformation of phenol in artificial and natural seawater. *Science of the Total Environment*. 2012; 426(0):281–288. [PubMed: 22503675]
28. Tamtam F, Chiron S. New insight into photo-bromination processes in saline surface waters: The case of salicylic acid. *Science of the Total Environment*. 2012; (0):435–436. 345–350. [PubMed: 23026150]
29. Méndez-Díaz JD, Shimabuku KK, Ma J, Enumah ZO, Pignatello JJ, Mitch WA, Dodd MC. Sunlight-Driven Photochemical Halogenation of Dissolved Organic Matter in Seawater: A Natural Abiotic Source of Organobromine and Organoiodine. *Environ Sci Technol*. 2014
30. Leri AC, Mayer LM, Thornton KR, Ravel B. Bromination of marine particulate organic matter through oxidative mechanisms. *Geochimica et Cosmochimica Acta*. 2014; 142(0):53–63.
31. Leri AC, Ravel B. Sample thickness and quantitative concentration measurements in Br K-edge XANES spectroscopy of organic materials. *J Synchrotron Radiat*. 2014; 21(3):623–626. [PubMed: 24763653]
32. Golovchenko JA, Levesque RA, Cowan PL. X-ray monochromator system for use with synchrotron radiation sources. *Review of Scientific Instruments*. 1981; 52(4):509–516.
33. Woicik JC, Ravel B, Fischer DA, Newburgh WJ. Performance of a four-element Si drift detector for X-ray absorption fine-structure spectroscopy: resolution, maximum count rate, and dead-time correction with incorporation into the ATHENA data analysis software. *J Synchrotron Radiat*. 2010; 17(3):409–413. [PubMed: 20400841]
34. Ravel B, Newville M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *J Synchrotron Radiat*. 2005; 12(4):537–541. [PubMed: 15968136]
35. Heeb MB, Criquet J, Zimmermann-Steffens SG, von Gunten U. Oxidative treatment of bromide-containing waters: formation of bromine and its reactions with inorganic and organic compounds—a critical review. *Water Res*. 2014; 48:15–42. [PubMed: 24184020]
36. Petigara BR, Blough NV, Mignerey AC. Mechanisms of hydrogen peroxide decomposition in soils. *Environ Sci Technol*. 2002; 36(4):639–645. [PubMed: 11878378]
37. Lin SS, Gurol MD. Catalytic Decomposition of Hydrogen Peroxide on Iron Oxide: Kinetics, Mechanism, and Implications. *Environ Sci Technol*. 1998; 32(10):1417–1423.
38. Olson PD, Varner JE. Hydrogen-Peroxide and Lignification. *Plant J*. 1993; 4(5):887–892.
39. Sels B, Vos DD, Buntinx M, Pierard F, Kirsch-De Mesmaeker A, Jacobs P. Layered double hydroxides exchanged with tungstate as biomimetic catalysts for mild oxidative bromination. *Nature*. 1999; 400(6747):855–857.
40. Conte V, Di Furia F, Moro S. Mimicking the vanadium bromoperoxidases reactions: Mild and selective bromination of arenes and alkenes in a two-phase system. *Tetrahedron Letters*. 1994; 35(40):7429–7432.
41. Meister GE, Butler A. MOLYBDENUM(VI)-MEDIATED AND TUNGSTEN(VI)-MEDIATED BIOMIMETIC CHEMISTRY OF VANADIUM BROMOPEROXIDASE. *Inorg Chem*. 1994; 33(15):3269–3275.
42. Hamstra BJ, Colpas GJ, Pecoraro VL. Reactivity of Dioxovanadium(V) Complexes with Hydrogen Peroxide: Implications for Vanadium Haloperoxidase. *Inorg Chem*. 1998; 37(5):949–955.
43. Podgorsek A, Zupan M, Iskra J. Oxidative halogenation with “green” oxidants: oxygen and hydrogen peroxide. *Angewandte Chemie (International ed. in English)*. 2009; 48(45):8424–50. [PubMed: 19827067]
44. Rothenberg G, Clark JH. On oxyhalogenation, acids, and non-mimics of bromoperoxidase enzymes. *Green Chemistry*. 2000; 2(5):248–251.

45. Wischang D, Brücher O, Hartung J. Bromoperoxidases and functional enzyme mimics as catalysts for oxidative bromination—A sustainable synthetic approach. *Coordination Chemistry Reviews*. 2011; 255(19–20):2204–2217.
46. Biester H, Selimovi D, Hemmerich S, Petri M. Halogens in pore water of peat bogs – the role of peat decomposition and dissolved organic matter. *Biogeosciences*. 2006; 3(1):53–64.
47. Tirol-Padre A, Ladha JK. Assessing the Reliability of Permanganate-Oxidizable Carbon as an Index of Soil Labile Carbon. *Soil Sci Am J*. 2004; 68(3):969–978.
48. Comba P, Kerscher M, Krause T, Schöler HF. Iron-catalysed oxidation and halogenation of organic matter in nature. *Environ Chem*. 2015; 12(4):381–395.
49. Strosser E. Methods for determination of labile soil organic matter: An overview. *Journal of Agrobiology*. 2010; 27(2):49–60.
50. Nichols KA, Wright SF. Carbon and nitrogen in operationally defined soil organic matter pools. *Biol Fertil Soils*. 2006; 43(2):215–220.
51. Poirier N, Sohi SP, Gaunt JL, Mahieu N, Randall EW, Powlson DS, Evershed RP. The Chemical Composition of Measurable Soil Organic Matter Pools. *Organic Geochemistry*. 2005; 36(8):1174–1189.

Figure 1.

Normalized Br *K*-edge X-ray absorption near-edge structure (XANES) spectra. **A)** Br-containing model compounds: **a–b**, aliphatic organobromine standards; **c–d**, aromatic organobromine standards; **e**, inorganic bromide standard. **B)** Particulate SOM samples: **a**, untreated soil humus; **b–d**, particulates treated with 40 mM Br⁻ and 2.0 mM H₂O₂ for 48 h at pH 3.0. Vertical lines intersect the characteristic absorption energies of aliphatic and aromatic organobromine.

Figure 2.

Unnormalized Br *K*-edge XANES spectra of soil humus after various treatments, all for 48 h at pH 3.0. The post-edge fluorescence intensities (the rise of the “edge step”) are linearly proportional to total Br concentrations [Leri & Ravel, 2014]. The near-edge features around 13,473 eV reveal Br speciation in the samples and can be resolved into inorganic, aliphatic, and aromatic proportions via linear combination fitting of normalized spectra. The information from XANES spectra can thus be transformed into the data in the table at right. All concentrations reported in subsequent figures were obtained similarly from Br *K*-edge XANES spectra.

Figure 3.

Aliphatic and aromatic organobromine concentrations in fresh oak leaves and pine needles, mixed plant litter, and humus from the soil organic horizon, before and after oxidative treatments. Treatments were carried out in phosphate buffer (pH 3.0) with 40 mM KBr, 2 mM H₂O₂, and 20 mM Fe(NO₃)₃. Oak leaves, pine needles, and soil humus were treated for 48 h; mixed plant litter was treated for 24 h.

Figure 4.

Effect of pH on bromination of fresh: **A**) oak leaf; and **B**) pine needle material. Treatments were carried out in phosphate (pH 3.0) or acetate (pH 5.0) buffer for 48 h with 40 mM KBr, 2 mM H₂O₂, and 20 mM Fe(NO₃)₃.

Figure 5.
Effect of different ferric salts on bromination of fresh oak and pine material, with treatment times as indicated.

Figure 6.

Effect of Fenton-like reaction conditions on bromination of fresh oak, fresh pine, and humus material. Indicated treatments were performed in the dark.