Naturally halogenated organic matter in Atlantic rankers is concentrated in microbial rather than pyrogenic moieties

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Compiled January 16, 2019

Organohalogens in the environment are a booming research subject, due to their role in many ecosystem functions and ecotoxicology. A recent study showed that a series of colluvial soils in NW Spain, known as Atlantic rankers and having the idiosyncratic feature of high loads of aged pyrogenic soil organic matter (SOM) from anthropogenic palaeofires, contain large amounts of organically-bound natural bromine. Furthermore, it appeared that the Br/C ratio, indicative of biobromination status of the OM, increases steadily with age during the Holocene. We compared the organobromine record with OM composition as determined by pyrolysis-GC-MS. It appeared that negative excursions in the Br/C record coincide with peaks in pyrogenic matter content and positive excursions with peaks in the proportion of pyrolysis products from microbial constituents. Hence, in addition to age, SOM composition has a secondary controlling effect on organobromine concentrations. And the fact that the Br is associated with microbial SOM is strong evidence of a biogenic source (microbial halogenation), as abiotic surface binding would cause enrichment of Br in the pyrogenic SOM fraction, which has the largest specific surface area.

1. INTRODUCTION

Until recently organohalogen compounds were considered to be almost completely of anthropogenic origin, and interest has traditionally focused on the environmental problems caused by human-produced organohalogens such as dichloro-diphenyl-trichloroethane (DDT) and the dioxins, which deplete O_3 and are toxic to life on Earth. In addition to the synthetic organohalogens, a wide array of haloorganics are produced naturally, the vast majority of which are chlorinated and/or brominated biological compounds created in the oceans (Gribble, 2003). Nevertheless, biogenic organohalogens are also widespread in terrestrial soils (Asplund and Grimvall, 1991), where their sources, fate and interactions with soil constituents are black box phenomena.

In the last decade, the biogeochemical cycling of Cl species is beginning to unravel. Öberg and co-workers (2005) argued that the most important input (atmospheric deposition and chemical weathering) and output (leaching after dehalogenisation) fluxes of Cl are inorganic, while the main Cl pool is SOM. Myneni (2002) also showed that, as plant remains decompose, Cl is transformed from inorganic into organic forms; organo-chlorinated species being dominant in humic substances and phenolic-Cl compounds increasing with the degree of humification.

Although plants synthesise some organohalogens themselves, it is widely accepted that most chlorine is in situ incorporated into dead organic matter through enzymatically mediated electrophilic attack and carbon-Cl bond formation during biodegradation (Asplund et al., 1993; Hjelm et al., 1995; van Pee, 1996; van Pee and Unversucht, 2003; Öberg, 2003; Bastviken et al., 2007). Numerous organisms are capable of chlorination, the majority of which being fungi (Öberg et al., 1996) and bacteria (van Pee, 1996; Gribble, 2003). Decomposition of Cl-containing macromolecular OM may then release the sequestered organic Cl as inorganic Cl.

Whereas the basic principles of the biogeochemical cycling of the chloroorganics are reasonably understood, the production pathway, function and fate of bromoorganics is more enigmatical. The terrestrial cycling of Br may be expected to resemble that of Cl since (1) both elements enter terrestrial systems abiotically (and inorganically) through atmospheric deposition or rock weathering, (2) many chlorinating enzymes are also capable of bromination (Neidleman and Geigert, 1986) and (3) many authors reported a close relation between Br and Cl concentrations in soil (Yuita, 1983; Yuita et al., 1991). Leri and Myneni (2012) also suggested that the biogeochemical behaviour of Br may be similar to that of Cl. They found that Br in soil (humified plant material, O-horizons and humic substances) was covalently bound to organic carbon.

Another vexing hiatus in organohalogen research is the long-term fate of halogenated organic matter in soils (Öberg, 1998). The geographical distribution of natural organohalogens and the capacity of different soil types to store them are unknown. The lack of soil organohalogen inventory studies, especially in the deeper soil layers, hampers any calculation of natural soil organohalogens on Earth, which is needed for understanding the global cycling of halogens and for adequate organohalogen-related policy-making.

The soils of the study area in Campo Lameiro (NW Spain) are the outcome of thousands of years of erosion and sedimentation processes. These colluvial soils constitute a series of transported A-horizons, of which the age increases progressively with depth up to more than 10,000 years. These soils, referred to as Atlantic rankers, aluandic acidic soil or aluminic soils (Duchaufour, 1982; Macías et al., 1982; Delvaux et al., 2004; García-Rodeja et al., 2004; Kaal et al., 2008a), are characterised by a high SOM content (up to 15 % C, w:w) and organically bound Al (i.e. up to 2 % w:w pyrophosphate-extractable Al). A previous pyrolysis-GC-MS study of two of these soils showed that the NaOH-extractable SOM (ca. 80 % of total SOM) was a mixture of pyrogenic residues (black carbon), microbial carbohydrates and proteins, lipids and a negligible amount of intact plant-derived polysaccharides and lignin (Kaal et al., 2008b). A more recent study involving four of these soils (Martínez-Cortizas et al., 2016) showed that Br concentration largely depends on the amount of SOM stabilised as metal-SOM associations and actually recommend the use of Br/C as support in the modelling of soil age.

Here, we compare the long-term organohalogen records (Cl and Br) and molecular fingerprint records of the SOM, aiming to deepen our understanding of the long-term interactions between halogens and SOM. This is the first time such an attempt is made.

2. MATERIALS AND METHODS

The study area is located in Campo Lameiro (Pontevedra, Galicia, NW Spain) in the Rock Art Park (Parque Arqueolóxico de Campo Lameiro). For details please see Kaal et al. (2008b) or Kaal (2011), the latter of which is a PhD thesis on the Holocene (Mesolithic onwards) anthropogenic fire regime of the area.

Samples were collected from two soil monoliths sampled in the colluvial formations. The parent rock of the area is a migmatitic granite, upon which up to 3 meters of a colluvial sequence was deposited. Soil PRD-1 is 1.5 m thick representing more than 8500 yrs of erosion/sedimentation cycles. PRD-2 accumulated in the last ca. 6300 yrs, but is 2.4 m thick. Age estimations (cal yr BP) were based on radiocarbon dating and stone layers. Macroscopic charcoal (> 2 mm) was collected by wet sieving of soil samples and weighed. The fine earth fraction (< 2 mm) was analysed for total C by dry combustion and total Cl and Br were determined by XRF (Martínez Cortizas et al., 2016).

Soil OM was isolated from the fine earth fraction (< 2mm) by extraction using 0.1 M NaOH solution (twice for five minutes) followed by centrifugation (1000 g, 15 min). After collecting the supernatant, residues were washed with water (3–5 times) and centrifuged. Then, extracts were combined, acidified to pH 1–2 with HF/HCl solution, and manipulated through a 63 m mesh size sieve to remove fine roots and fine charcoal. Subsequently, the extracts were dialysed against distilled water and freeze-dried to obtain the purified SOM. Extraction yields for C, estimated from elemental analysis of the residues and the untreated fine earth fraction, were 82 ± 12 % and 82 ± 9 % for PRD-1 and PRD-2, respectively. More details can be found in Kaal et al. (2008b).

Curie-point (600 ºC) pyrolysis was performed using a Horizon Instruments pyrolyser. Pyrolysis products were transported to a Carlo Erba GC 8000 using He as the carrier gas. A 25 m long Chrompack fused silica column with an internal diameter of 0.25 m packed with a 0.40 m thick CP-Sil 51b film was used to separate the pyrolysis products. The GC oven parameters used were: initial temperature 40 ºC, heating rate 7 ºC min $^{-1}$, final temperature 320 ºC maintained for 20 minutes. The GC was coupled to a Fisons MD 800 operating at an electron impact energy of 70 eV. The mass-range was 45–650 m/z. Ninety-four pyrolysis products were quantified on their most abundant fragment ions (Kaal et al., 2008b).

3. RESULTS AND DISCUSSION

Both soils have a low C content in the surface layers deposited around 500–1000 cal yrs BP, a broad high range at 2000–4000 cal BP, after which C content declines steadily with depth (Figure 1a). The changes in C content are the result of changes in climate, vegetation pattern and landscape stability. Human disturbance of the area, particularly by the use of fire, is likely to have played a role in these changes. It is noted that the soils are not subject to 'cryptopodzolisation', i.e. visually unrecognisable vertical translocation of organic matter and adsorbed Fe and Al in the profile: the OM is immobile in these soils. Both exhibit peaks in macroscopic charcoal (> 2 mm) content around 1800 cal BP and 3300 cal BP (Fig. 1b), and PRD-1 also at ca. 6800 cal BP (PRD-2 has no record for that period).

Total Cl (r^2=0.59, $P<0.001$) and Br (r^2=0.69, $P<0.001$) closely follow C contents (Fig. 1c/d). In addition, the pyrophosphate-extractable C (C_p) and Al (Al_p) contents (not shown) are in strong correlation with Cl (Al_p: r^2=0.78, $P<0.001$; C_p: r^2=0.68, $P<0.001$) and Br contents (Al_p: r^2=0.91, $P<0.001$; C_p: r^2=0.79, $P<0.001$) (Kaal et al., 2008a). These results indicate that a large fraction of the halogens, especially Br, is intimately associated with the SOM.

As we calculate the molar Cl-to-C and Br-to-C ratios (Fig. 1e/f), it becomes clear that the amount of organically bound Br per unit of C increases with time (r^2=0.84, $P<0.001$), and that of Cl is also affected by age (r^2=0.39, $P<0.01$). The degree of chlorination of the SOM is not related to the concentration profile of any pyrolysis product (not shown). The remaining of this article is therefore concerned with the the degree of bromination.

By detrending the Br/C ratio from age, the deviations from that trend become apparent (Fig. 2a-d, white symbols). Minima of the detrended-Br/C curve (Br/C_{det}) coincide with peaks in macroscopic charcoal content (Fig. 2a). The inverse relation between the age-normalised degree of Br binding, Br/C_{det}, and the proportion of C in macroscopic charcoal shows that high levels of pyrogenic SOM coincide with low degree of bromination, probably due to the difficulties that the biobromination community experience in consuming pyrogenic OM on both a macroscale (impenetrable charcoal fragments) and at the molecular scale (inaccessible C in polycondensed aromatic domains that are barely consumable by soil microbes).For PRD-1 (n=28), the negative correlation between Br/C_{det} and macrocharcoal/C is significant at $P<0.05$ if the two bottom samples (>9000 cal BOP) are ignored and $P<0.001$ if four iron age samples are ignored as well. For PRD-2, the negative correlation is significant if all samples are included (n=42, $P<0.001$).

Pyrolysis-GC-MS showed that the extractable OM is composed of a mixture of pyrogenic SOM (producing benzenes, PAHs, benzonitrile, isoquinoline and probably some pyridine upon pyrolysis), intact primary OM (methoxyphenols and levoglucosan originating from lignin and polysaccharide, respectively), degraded microbial OM (pyrroles, furans, furfurals and some pyridine) and lipids (alkanes and alkenes). The negative effect of the presence of burning residues (macrocharcoal/C ratio) on the degree of bromination is supported by the variation

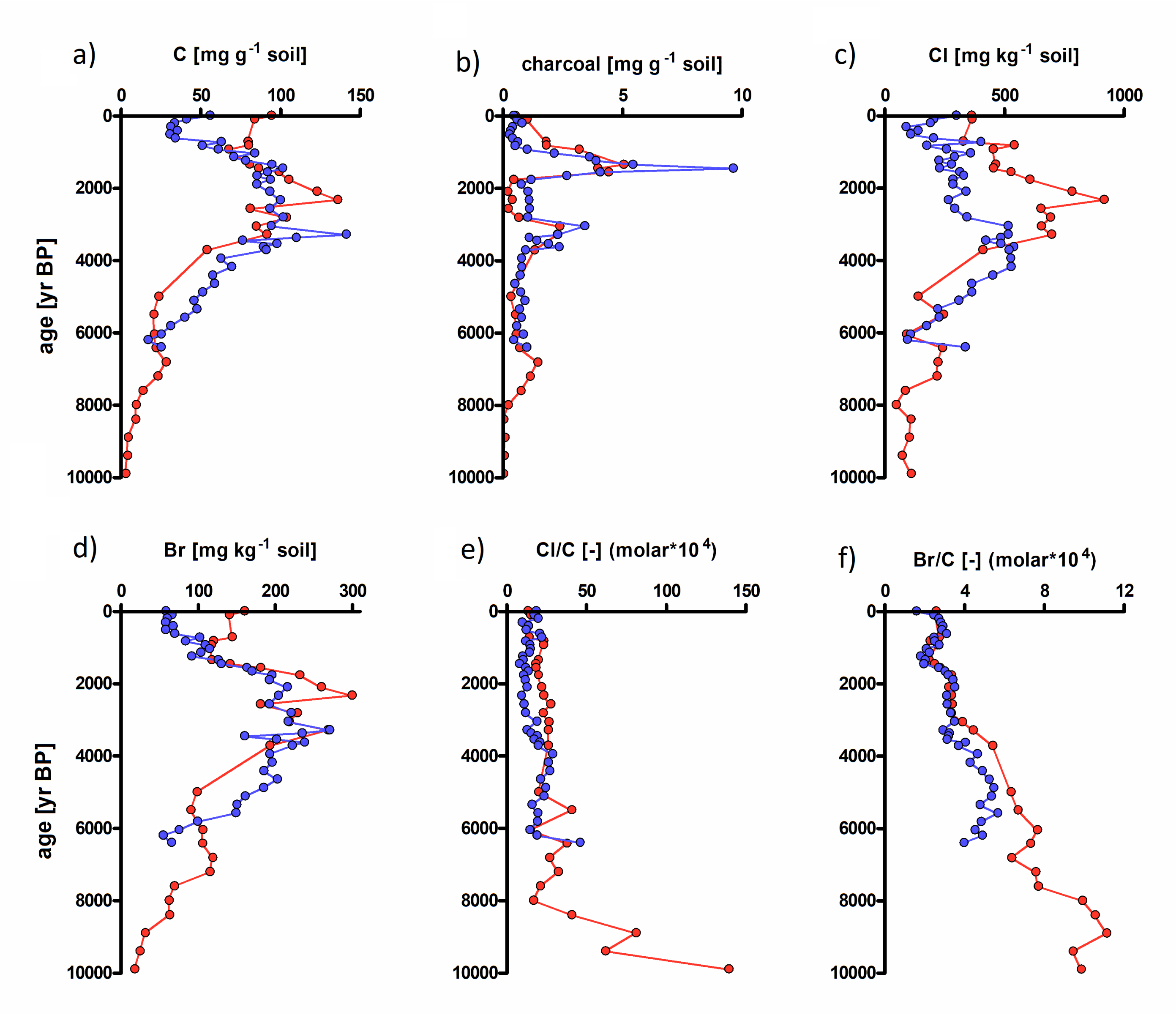

Fig. 1. Results from elemental analysis (C, Cl, Br, Cl/C and Br/C) and macroscopic charcoal content, plotted against age for the PRD-1 (red symbols and line) and PRD-2 (blue symbols and line) sequences.

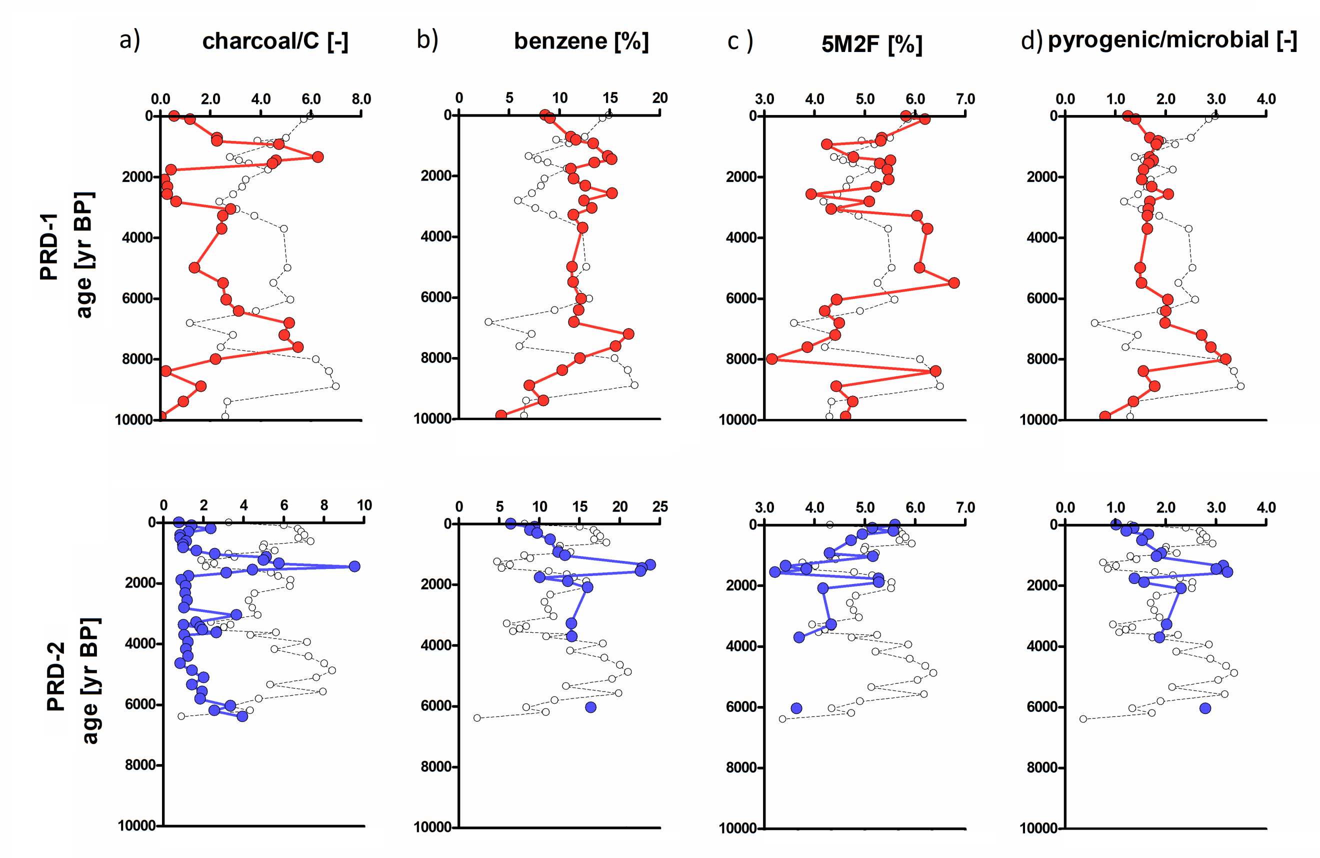

Fig. 2. Selected parameters from the PRD-1 (upper row) and PRD-2 (lower row) sequences. The white line in all figures is the age-detrended ratio of Br to C (molar basis) (Br/C_{det}). Charcoal/C is the ratio between macroscopic charcoal (>2 mm) content and total C content of the samples. Benzene is the most abundant pyrolysate of pyrogenic SOM from palaeofires, whereas 5-methyl-2-furaldehyde (5M2F) is a product of microbial SOM. The ratio of all products of pyrogenic and microbial SOM is also depicted.

in the proportion of pyrolysis products from pyrogenic SOM (using benzene as an example, Fig. 2b), indicating that pyrogenic OM is relatively scarcely brominated (PRD-1, $P<0.001$ if two oldest samples >9000 cal BP are excluded; PRD-2, $n=16$, $P<0.05$ and $P<0.01$ if surface sample is omitted). Note that the negative relation between fire markers and the Br/C_{det} curve suggests that the majority of the bromine does not originate from forest fire (Gribble, 2003).

Because the dominant pyrolysis products of plant-derived polysaccharides are very scarce, and a large amount of microbial OM was observed in a micromorphological survey (Kaal and van Mourik, 2008), 5-methyl-2-furaldehyde is most likely a pyrolysis product of microbial SOM (Kaal et al., 2008b). The synchronous biobromination during biodegradation of the OM explains why the degree of bromination and this furfural are positively related (Fig. 2c). For PRD-1 this positive correlation is significant if again the bottom samples are omitted ($P<0.01$), and for PRD-2 if, again, the surface sample is ignored ($P<0.05$). Finally, 2-methylphenol also showed a positive relation with Br/C_{det} ratio (not shown).

Figure 2d shows the ratio of pyrogenic SOM markers (benzene, benzonitrile, isoquinoline, PAHs) to biodegradation markers (furaldehydes, pyrroles, etc.). The negative relation between Br/C_{det} and this ratio suggests that the resistance of pyrogenic OM against bromination and the biogenic pathway of organobromine compounds can explain a large part of the deviations of the Br/C ratio from its increase with time (not significant in PRD-1, significant in PRD-2 at $P<0.05$ and $P<0.01$ if surface sample is excluded). A micromorphological study showed that the non-pyrogenic OM mainly exists in charcoal fragments and organomineral microaggregates (Kaal and van Mourik, 2008). The results presented here suggest that the organobromines are likely concentrated in microaggregates and, thus, their breakdown is likely very slow due to e.g. occlusion (Six et al., 2002) in combination with sorptive preservation by the abundant short-range ordered Al that is adsorbed to the SOM.

4. CONCLUSIONS

These results show that the degree of SOM bromination in the studied soils is not only affected by age, but also by the composition of the SOM, in particular the balance between microbial and pyrogenic SOM constituents. The organobromine could probably accumulate on the long-term due to the stabilisation of microbial SOM in Al-rich microaggregates. All evidence points towards a biogenic origin of the bromoorganics. There were no relationships between the degree of chlorination and the pyrolysis fingerprints of the SOM.

5. REFERENCES

Asplund, G., Grimvall, A., 1991. Organohalogens in nature, more widespread than previously assumed. Environmental Science and Technology 25, 1346–1350.

Asplund, G., Christiansen, J.V., Grimvall, A., 1993. A chloroperoxidase-like catalyst in soil: detection and characterization of some properties. Soil Biology and Biochemistry 25, 41-46.

Bastviken, D., Thomsen, F., Svensson, T., Karlsson, S., Sanden, P., Shaw, G., Matucha, M., Öberg, G., 2007. Chloride retention in forest soil by microbial uptake and by natural chlorination of organic matter. Geochimica et Cosmochimica Acta 71, 3182-3192.

Delvaux, B., Strebl, F., Maes, E., Herbillon, A. J., Brahy, V. and Gerzabek, M., 2004. An Andosol-Cambisol toposequence on granite in the Austrian Bohemian Massif. Catena 56, 31–43.

Duchaufour, P., 1982. Pedology: Pedogenesis and Classification. George Allen Unwin, London. 448 pp.

García-Rodeja, E., Nóvoa-Muñoz, J.C., Pontevedra-Pombal, X., Martínez-Cortizas, A., Buurman, P., 2004. Aluminium fractionation of European volcanic soils by selective dissolution techniques. Catena 56, 155–183.

Gribble, G.W., 2003. The diversity of naturally produced organohalogens. Chemosphere 52, 289–297.

Hjelm, O., Johansson, M., Öberg, G., 1995. Organically bound halogens in coniferous forest soil. Chemosphere 30, 2353–2364.

Kaal, J., 2011. Identification, molecular characterisation and significance of fire residues in colluvial soils from Campo Lameiro (NW Spain). PhD Thesis. Available online at: http://digital.csic.es/handle/10261/35227.

Kaal, J., van Mourik, J.M., 2008. Micromorphological evidence of black carbon in colluvial soils from NW Spain. European Journal of Soil Science 59, 1133-1140.

Kaal, J., Costa Casais, M., Ferro Vázquez, C., Pontevedra Pombal, X., Martínez Cortizas, A., 2008a. Soil formation of "Atlantic Rankers" from NW Spain - A high resolution aluminium and iron fractionation study. Pedosphere 18, 441 - 453.

Kaal, J., Martínez Cortizas, A., Nierop, K.G.J., Buurman, P., 2008b. A detailed pyrolysis-GC/MS analysis of a black carbon-rich acidic colluvial soil (Atlantic ranker) from NW Spain. Applied Geochemistry 23, 2395-2405.

Léri, A.C., Myneni, S.C.B., 2012. Natural organobromine in terrestrial ecosystems. Geochimica et Cosmochimica Acta 77, 1-10.

Macías, F., Calvo de Anta, R., García-Paz, C., García-Rodeja, E., Silva, B., 1982. The parent material: Influence on the formation and properties of soils from Galicia. Anales de Edafología y Agrobiología 41, 1747-1768.

Martínez Cortizas, A., Ferro Vázquez, C., Kaal, J., Biester, H., Costa Casais, M., Taboada, T., Rodríguez Lado, L., 2016. Bromine accumulation in acidic black colluvial soils. Geochimica et Cosmochimica Acta. 174, pp. 143 - 155.

Myneni, S.C., 2002. Formation of stable chlorinated hydrocarbons in weathering > plant material. Science 295, 1039-1041.

Neidleman, S.L., Geigert, J., 1986. Biohalogenation: Principles, Basic Roles, and Applications. Ellis Horwood, Chichester, England.

Öberg, G., 1998. Chloride and organic chlorine in soil. Acta Hydrochimica et Hydrobiolobiologica 26, 137-144.

Öberg, G., 2003. The biogeochemistry of chlorine in soil. In: The Handbook of Environmental Chemistry Vol. 3, part P. The Natural Production of Organohalogen Compounds (ed Gribble, G.), Springer-Verlag, pp 43-62.

Öberg, G., Nordlund, E., Berg, B., 1996. In situ formation of organically bound halogens during decomposition of Norway spruce litter – Effects of fertilization. Canadian Journal of Forest Research 26, 1040– 1048.

Öberg, G., Holm, M., Parikka, M., Sandén, P., Svensson, T., 2005. The role of organic-matter-bound chlorine in the chlorine cycle: A case study of the Stubbetorp catchment, Sweden. Biogeochemistry 75, 173-201

Six, J., Conant, R.T., Paul, E., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155-176. DOI: 10.1023/A:1016125726789

Van Pée, K.-H., 1996. Biosynthesis of halogenated metabolites by bacteria. Annual Reviews in Microbiology 50, 375-399.

Van Pée, K.H., Unversucht, S., 2003. Biological dehalogenation and halogenation reactions. Chemosphere 52, 299–312.

Yuita, K., 1983. Iodine, bromine and chlorine contents in soils and plants of Japan. Soil Science and Plant Nutrition 29, 403-428, DOI: 10.1080/00380768.1983.10434645.

Yuita, K., Tanaka, T., Abe, C., Aso, S., 1991. Dynamics of iodine, bromine, and chlorine in soil. Soil Science and Plant Nutrition 37, 61-73