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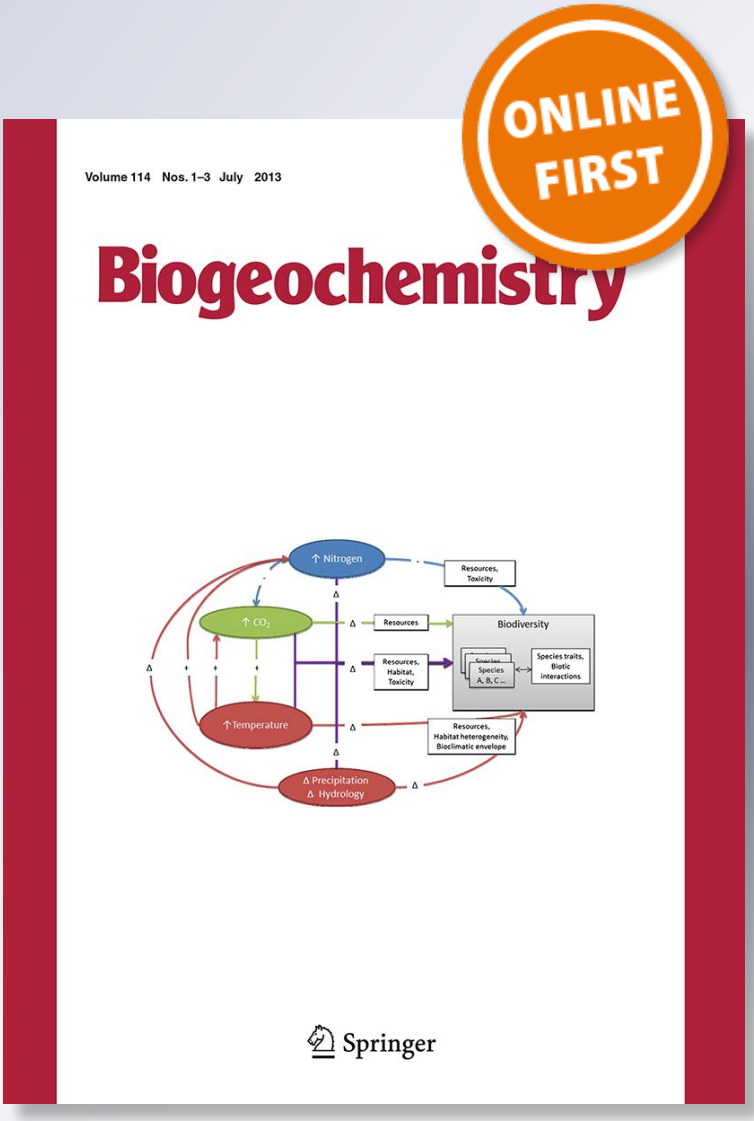


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# Biological chlorine cycling in the Arctic Coastal Plain

**Jaime E. Zlamal · Theodore K. Raab · Mark Little · Robert A. Edwards · David A. Lipson**

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**Abstract** This study explores biological chlorine cycling in coastal Arctic wet tundra soils. While many previous chlorine-cycling studies have focused on contaminated environments, it is now recognized that chlorine can cycle naturally between inorganic and organic forms in soils. However, these pathways have not previously been described for an Arctic ecosystem. We measured soil organic and inorganic Cl pools, characterized soils and plant tissues with chlorine K-edge X-ray absorption near-edge spectroscopy (Cl-XANES), measured dechlorination rates in laboratory incubations, and analyzed metagenomes and 16S rRNA genes along a chronosequence of revegetated drained lake basins. Concentrations of soil organic chlorinated compounds ($Cl_{org}$) were correlated with organic matter content, with a steeper slope in older soils. The concentration and chemical diversity of $Cl_{org}$ increased with soil development, with $Cl_{org}$ in younger soils more closely resembling that of vegetation, and older soils having more complex and variable Cl-XANES signatures. Plant $Cl_{org}$ concentrations were higher than previously published values, and can account for the rapid accumulation of $Cl_{org}$ in soils. The high rates of $Cl_{org}$ input from plants also implies that soil $Cl_{org}$ pools turn over many times during soil development. Metagenomic analyses revealed putative genes for synthesis (haloperoxidases, halogenases) and breakdown (reductive dehalogenases, halo-acid dehalogenases) of $Cl_{org}$, originating from diverse microbial genomes. Many genome sequences with close similarity to known organohalide respirers (e.g. *Dehalococcoides*) were identified, and laboratory incubations demonstrated microbial organohalide respiration in vitro. This study provides multiple lines of evidence for a complex and dynamic chlorine cycle in an Arctic tundra ecosystem.

**Keywords** Chlorine · Halogen · Organohalide respiration · XANES · Arctic · *Dehalococcoides*

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## Introduction

Biogeochemical cycling of naturally occurring chlorinated organic compounds ($Cl_{org}$) has received increased attention in recent scientific literature

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(Biester et al. 2006; Clarke et al. 2009; Leri and Myneni 2010; Öberg 2002; Svensson et al. 2007; Van den Hoof and Thiry 2012; Weigold et al. 2016). Chloride ($Cl^-$) has generally been considered inert in ecosystems, and is often used as a conservative tracer in hydrological studies (Leibundgut et al. 2009). Microbial chlorine (Cl) metabolism has been studied mainly in the context of contamination from chlorinated compounds used in industry (Asplund and Grimvall 1991; Hiraishi 2008; Holliger et al. 1997; Leys et al. 2013; Lohner and Spormann 2013; Öberg 2002), and radioactive $^{36}Cl$ released following decommissioning of nuclear reactors (Bastviken et al. 2013; Van den Hoof and Thiry 2012). Globally, soil Cl cycling also has significance for the destructive impact of methyl halides and other volatile halogenated organic compounds (VHOC) on the ozone layer (Keppler et al. 2000; Wetzel et al. 2015). Biogeochemical studies of Cl are rare in general (Öberg and Bastviken 2012). European forests are the best studied in terms of Cl cycling rates (Bastviken et al. 2009; Montelius et al. 2016; Öberg et al. 2005; Redon et al. 2011; Rohlenová et al. 2009; Weigold et al. 2016), though $Cl_{org}$ has also been quantified in grasslands and farmlands (Redon et al. 2013), and in the pore water of Chilean peat bogs (Biester et al. 2006). Evidence was found for biological chlorination of soil organic matter (SOM) in Canadian peat bogs (Silk et al. 1997). While several studies have measured VHOC fluxes (Rhew et al. 2007, 2008; Teh et al. 2009), there has been no previous comprehensive study of internal Cl cycling in Arctic soils.

Many organisms produce $Cl_{org}$, including fungi, lichen, bacteria, terrestrial and marine plants and invertebrates, and even higher animals such as frogs and mammals (Fielman et al. 1999; Gribble 2003; Peng et al. 2005). Halogenases and haloperoxidases catalyze the chlorination of organic compounds (Bengtson et al. 2013; Niedan et al. 2000; van Pée and Unversucht 2003). Some halogenated compounds produced by plants/algae are powerful insecticides, such as Telfairine produced by the red algae *Plocamium telfairiae*, and many bacterially-produced antibiotics (such as vancomycin) contain Cl (Gribble 1998). In addition to synthesis of antibiotics, bacteria may non-specifically halogenate organic compounds as a form of competitive antagonism or as a defense against reactive oxygen species (Bengtson et al. 2009, 2013). Abiotic processes such as volcanic eruptions and forest fires produce $Cl_{org}$, and ferrous iron [Fe(III)] can catalyze the abiotic formation of organohalogens from halides and organic matter (Comba et al. 2015; Keppler et al. 2000).

Some bacterial species, such as those in the *Dehalococcoides* genus, respire anaerobically using halogenated organic compounds as the terminal electron acceptor in organohalide respiration (also referred to as dehalorespiration, halorespiration, or chlororespiration) (Mohn and Tiedje 1992). This process results in the liberation of $Cl^-$ from $Cl_{org}$. A variety of dehalogenase enzymes exist and catalyze slightly different reactions depending on substrate specificity, utilizing one of several mechanisms to cleave the bond between the carbon and halogen atom (Bommer et al. 2014; Kurihara et al. 2000; Rupakula et al. 2013; Tang and Edwards 2013; Wagner et al. 2013). Organohalide respiring bacteria often have many different reductive dehalogenase (RDH) genes (up to 39) in their genome, probably corresponding to the complex suite of chlorinated substrates in their environment (Richardson 2013; Tang and Edwards 2013).

In wet tundra soils of the Arctic Coastal Plain, continuous permafrost blocks drainage, and the dominant moss communities on the surface hold soil water (Brown 1967). The microbial communities in these waterlogged soils host a diverse range of anaerobic pathways (Lipson et al. 2013a, 2015). Our study site is located near Barrow, Alaska on the North Slope of the Arctic Coastal Plain. Much of the landscape is comprised of thermokarst lakes which drain and become revegetated slowly over the course of a roughly 5500 year cycle, with geomorphic stages defined as young (<50 y.b.p., years before present), medium (50–300 y.b.p.), old (300–2000 y.b.p.) and ancient (2000–5500 y.b.p) (Hinkel et al. 2003). As drained thermokarst lake basins (DTLB) age and develop, soil carbon accumulates in the surface organic layer, humic substances increase in complexity, and microtopographic features develop due to the formation of ice-wedge polygons (Bockheim et al. 2004; Grosse et al. 2013; Hinkel et al. 2005). The DTLB cycle in the Arctic Coastal Plain provides a convenient chronosequence to study ecosystem properties at different stages of soil development (Lipson et al. 2013b; Sturtevant and Oechel 2013; Zona et al. 2010). The goal of this study was to establish the internal biological cycling of Cl in soils of the Arctic Coastal Plain in northern Alaska, and to describe

variation in Cl cycling across this chronosequence. We quantify and chemically characterize soil Cl pools, we demonstrate the potential for organohalide respiration in laboratory incubations, and we present 16S rRNA and metagenomic sequences with high similarity to the genes and genomes of organisms that participate in Cl cycling pathways.

## Methods

### Site description

Soil and soil pore water samples for this study were collected from DTLB near Barrow, Alaska (centered around 71.24°N 156.48°W) on the North Slope of the Arctic Coastal Plain (see Fig. S1, Online Resource 1 for a map of the study area). Sampling occurred during summers of 2010–2013. The coastal wet tundra near Barrow is dominated by sedges, grasses and mosses (Walker et al. 2005), and the soils are characterized by a seasonally thawed active layer atop deep continuous permafrost, classified mostly as Aquorthels, Aquiturbels, Historthels and Histoturbels (Bockheim et al. 2004; Brown 1967). The depth of the active layer in these DTLB is around 30–40 cm (Lipson et al. 2013b; Shiklomanov et al. 2010). As DTLB age, organic layers develop over silty, organic rich lacustrine mineral layers (Bockheim et al. 2001). Average organic layer thickness for the four age classes are 7, 13, 20 and 35 cm for young, medium, old and ancient DTLB, respectively (Bockheim et al. 2004). Soil pH declines with age, ranging from an average of 6.12 for young DTLB to 4.96 for ancient DTLB (Fig. S2, Online Resource 1). Electrical conductivity also declines with age, with average values for young and medium DTLB (407–428 μS/cm) higher than old and ancient DTLB (180–181 μS/cm, Fig. S2). Other soil data for representative DTLB along this chronosequence have been published previously (Lipson et al. 2013b; Miller et al. 2015).

### Soil sampling

Soil samples were collected in June and August of 2011 along 30 m transects in replicate basins of all age classes. Samples of 3 cm diameter and 20–30 cm length were collected using coring drill bits and a handheld power drill. Deeper, 7.5 cm diameter cores were extracted to a depth of 40 cm using a Snow, Ice and Permafrost Research Establishment (SIPRE) corer (with niobium-steel drill bit) and gas-powered engine. Samples from July 2013 were collected from Y1, Ms, O1, and A0 basins (Fig. S1, Online Resource 1, and as described in Sturtevant and Oechel (2013)) using a long serrated knife. The entire thawed soil profile was collected up to the depth of the frozen layer (approximately 20 cm). Samples were immediately returned to the lab and frozen at −40 °C. Samples were shipped frozen overnight to labs in California by commercial courier.

### Soil solution $Cl^-$ measurements

Soil pore water samples were collected from a depth of 0–10 cm using installed soil water suction microlysimeters (Rhizon, Eijkelkamp) from late June through October 2010 (Lipson et al. 2013b). Seven to ten spatial replicate samples were collected from a representative basin of each age class. $Cl^-$ content was measured using a colorimetric microplate assay (Merchant 2009). Plates were read by a Spectra MAX 190 (Molecular Devices Corp.) at 480 nm and analyzed using SOFTmax PRO 4.0 software (Life Sciences Edition by Molecular Devices Corp).

### Quantification of Cl pools

Soil samples were analyzed for total halides (TX) using pyrohydrolysis and $Cl^-$ titration. The cores collected in June 2011 were used to study spatial variability among and within basins (three spatial replicates from three distinct basins for each of four age classes, 36 samples total). The SIPRE cores were used to analyze patterns by depth, using a single deep soil core from one basin of each age class (four depths × four classes = 16 samples). Depth profiles were created from SIPRE cores using the following approximate depth increments: 0–5, 10–15, 20–25, and 30–35 cm. Soils were prepared by drying overnight in a 65 °C drying oven and homogenizing using a clean mortar and pestle. Plant samples for total organic halide (TOX) analysis included ten replicates of the dominant sedge, *Carex aquatilis*, harvested from August 12 to September 1, 2007 and duplicate *Sphagnum* moss samples harvested from June 11 to July 18, 2006 from the BE experimental site (Fig. S1, Online Resource 1). *Sphagnum* species at this site

include *S. arcticum*, *S. tescorum*, *S. obtusum* and *S. orientale* (Zona et al. 2011). Between 10 mg and 60 mg of homogenized samples were weighed and combined with 100–150 mg tungsten powder (100 mesh, Santa Cruz Biotechnology) as a combustion accelerant before being placed into a clean, new, ceramic sample boat (COSA Xentaur; Yaphank, NY) and introduced by the Automatic Boat Controller to a Mitsubishi Chemical TOX-100 Cl analyzer set up for TOX analysis by pyrohydrolysis (Asplund et al. 1994) (see also Fig. S3, Online Resource 1). The gas (Matheson Inc.) profile was argon (carrier) and purified oxygen (for combustion). TOX-100 operating software (ver. 5.1.0.0) was used to analyze the resulting $Cl^-$ containing hydrolysate by coulometry. The method detection limit by coulometry for this equipment is <100 ppb Cl.

The procedure for TOX was based on Asplund et al. (1994), except, because our access to the TOX-100 instrument was limited, extractable inorganic Cl ($Cl_{in}$) was measured using a microplate method and $Cl_{org}$ was obtained by subtraction, rather than the original approach of directly measuring $Cl_{org}$ on leached subsamples by TOX, and obtaining $Cl_{in}$ by subtraction. $Cl^-$ measurements were made on subsamples of the same homogenized soil samples used for TX analyses. Each sample was shaken for two nights with a 1:10 or 1:20 ratio of dry soil to potassium nitrate solution acidified with nitric acid (0.2 M $KNO_3$, 0.02 M $HNO_3$) to extract $Cl^-$. These supernatants were analyzed using the colorimetric chloride assay described above (Merchant 2009). The $Cl^-$ content of the extraction solution ("extraction blank") was measured to correct for inadvertent addition of $Cl^-$, and none was detected. To increase sensitivity of the assay, the ratio of sample to working reagent was modified, as the original method was optimized to be linear over a much higher concentration of chloride (5 mM). Instead, 100 µL of sample was combined with 50 µL of working reagent, considerably lowering the background.

Soil Cl concentrations (per unit mass) were converted to a per meter square basis using bulk density calculated from SOM content using the relationship derived for these soils in Lipson (2013b). Soil Cl content measured in the upper ~25 cm was extrapolated to a nominal active layer depth of 30 cm. To calculate $Cl_{org}$ accumulation in the organic layer along the chronosequence we used published values of organic layer C (Bockheim et al. 2004), the relationship between $Cl_{org}$ and SOM derived in the present study, and a C content of 44% in SOM for these soils (Brown 1967). Changes in soil Cl pools and concentrations with soil age were tested using regression analysis with basin age coded non-parametrically (young = 1, medium = 2, old = 3 and ancient = 4), as the age categories are non-linear and the exact ages of the basins used in this study were not known. To estimate the annual input of $Cl_{org}$ from plant growth, we used published data to estimate net primary productivity (NPP) in DTLB of each age class. Gross primary productivity (GPP) was reported for young, medium and old DTLB's (Zona et al. 2010). To convert GPP to NPP, a factor of 0.636 was used based on a model comparison study for the region (Fisher et al. 2014). NPP was converted from C units to dry plant biomass assuming a plant C content of 40%. These values were multiplied by plant $Cl_{org}$ and $Cl_{in}$ concentrations from the present study. Relative contributions of vascular plants and mosses were derived from Zona (2011). $Cl^-$ inputs in precipitation were estimated from rain and snow melt data from Liljedahl (2011), and published $Cl^-$ content of snow (Jacobi et al. 2012) and rain (Kalff 1968).

## X-ray absorption near-edge spectroscopy (XANES)

K-edge Cl XANES was performed at energies of 2800–2860 eV to elucidate chemical forms of Cl. The symmetry of some $Cl^-$ salts is evident in distinct pre-edge features, and the position of the pre-edge peak can be related to the degree of covalency in a metal-Cl bond. XANES spectra are extremely sensitive to the immediate neighborhood of Cl in terms of oxidation state, local symmetry of the absorber, and bond lengths (Leri et al. 2006, 2007). XANES spectra were collected at Beamline 9-BM-C at the Advanced Photon Source (Argonne National Laboratory; Lemont, IL) in partial fluorescence mode in an experimental arrangement essentially as described in (Bolin 2010) and consisted of a Si(111)-monochromator, with focusing achieved using a Rhodium-coated toroidal mirror. Harmonics were rejected through a flat, Rhodium-coated mirror; this provided a maximum energy resolution of 0.1–0.2 eV at 2.8 keV. Qualitative exploration of chlorinated compounds was achieved by linear combination fitting of normalized soil/plant spectra to a standard library collected under the identical beamline

conditions as the soil spectra (Manceau et al. 2012). Standards dispersed in boron nitride mulls ($BN_3$ to minimize Cl over-absorption) served as Cl-peak energy standards by which we compared whole soils. Vulcan Carbon XC72 (Cabot Corporation) was used for immobilizing organic solvents.

ATHENA software was used to analyze and compare the spectra (Ravel and Newville 2005). All sample spectra were normalized after pre-edge and post-edge corrections. The relative contributions of known Cl-containing compounds to each spectrum was analyzed using linear combination fitting (LCF) (Manceau et al. 2012). Standards were chosen to span the canonical oxidation states of Cl, and those used to fit spectra in this study included NaCl, KCl, $CaCl_2$, monochlorodimedone, sucralose, polyvinylchloride, trichloroethylene, chrome azurol-S, and chloroacetic acid. LCF analysis produced weights attributed to each standard, and these weights were used in a Principal Component Analysis (PCA) to qualitatively compare the overall similarity of each sample.

Cl XANES was performed on subsamples from the soil monoliths collected in July 2013. Soils were cut into horizons with a band saw and dried fully in a 65 °C drying oven. Vegetation samples included the grass, *Arctophila fulva* (from the ancient basin, A0), the sedge, *Carex aquatilis* (from the medium basin, Ms), and *Sphagnum* moss (from the medium basin, BE). Humic acid extracts from a young and an ancient basin were prepared as described previously (Lipson et al. 2013b). Briefly, humic substances from 5 to 10 g of wet, frozen soil were extracted overnight in $N_2$-bubbled 0.1 M sodium hydroxide (NaOH)/0.1 M sodium pyrophosphate ($Na_4P_2O_7$), precipitated by acidification with hydrochloric acid (HCl) to pH 1, rinsed with 0.001 M HCl, and finally redissolved in $N_2$-bubbled 50 mM sodium bicarbonate ($NaHCO_3$). To prepare these extracts for XANES, they were precipitated in 0.1 M sulfuric acid ($H_2SO_4$) and rinsed with 0.1 μM $H_2SO_4$ before being dried at 65 °C. Dried samples were ground to a fine paste with a clean, ethanol-rinsed mortar and pestle before mounting onto carbon tape. Polyethylene glycol was used as a binder for some soil samples.

## Organohalide respiration in laboratory incubations

To measure potential rates of organohalide respiration, soil slurries were incubated in the laboratory under a variety of conditions. About 1 g of soil from monoliths collected from a medium aged basin in July 2013 was added to 25 mL of sterile, oxygen free, 10 mM pH 5.5 sodium acetate buffer containing 900 μM tetrachloroethylene (PCE), 1.8 mM dichloroethylene (DCE), and 0.1 mg/L thiamine in sterile 50 mL Wheaton crimp top glass vials fitted with butyl rubber septa. Septa were secured with metal clamps, and the headspace was flushed with $N_2$ gas. Treatments for this experiment included a matrix of the following: with and without vitamin $B_{12}$ (cobalamin, 0.5 mg/L), with and without carbon dioxide gas (sufficient to replace $N_2$ headspace), and with and without hydrogen gas as an electron donor (10 cc/vial). The five different non-sterilized treatments were: (1) $+B_{12}$, $+H_2$, $+CO_2$; (2) $+H_2$, $+CO_2$; (3) $+B_{12}$, $+H_2$; (4) $+B_{12}$, $+CO_2$; (5) $+CO_2$. A subset of vials was autoclaved as a control for abiotic Cl liberation. This treatment received vitamin $B_{12}$, hydrogen and carbon dioxide gases to allow direct comparison with the most favorable conditions for reductive dechlorination. Vials were incubated at 10 °C and liquid samples were extracted periodically with a syringe, briefly centrifuged to remove suspended soil particles, and assayed for $Cl^-$ as described above. Concentrations were normalized to μmole $Cl^-$ per gram of wet soil.

## Sequencing and bioinformatics

Eight metagenomes were created using two depths from each of four age classes; each metagenome library included combined DNA from three spatial replicate samples taken from different locations along a 30 m transect to make each metagenome more representative of spatial variability. Shallow (5–6 cm depth) and deep (15–16 cm depth) soils were analyzed from the following basins: Y1, Ms, O1, and A0.

Samples were thawed and processed ($\sim$1 g) using MO BIO PowerSoil® DNA isolation kit (MO BIO Cat# 12888-100). DNA was quantified using Quant-iT pico green dsDNA assay kit (Life Technologies, Cat# P11496). Environmental DNA samples (500 ng) were sheared using a Covaris focused-ultrasonicator M220 with a target fragment size of 500 bp. Shotgun libraries were prepared using Roche rapid library Lib-L, Multiple-Prep (MV) preparation methods and multiplex identifier (MID) adaptors. Libraries were sequenced on a Roche 454 Life Sciences GS Junior platform at San Diego State University.

MID's, reads with mean quality scores less than 20, reads less than 60 bp, duplicates, and reads with more than 1% ambiguous bases were removed. Read ends with quality scores less than 20 were trimmed from left and right using PRINSEQ v0.20.4 (Schmieder and Edwards 2011). Processed sequencing reads were uploaded to MG-RAST (Meyer et al. 2008) and are publicly available (Project ID 7998, MG-RAST ID numbers 4554152.3-4554159.3).

We re-analyzed our previously published metagenomes from a medium aged basin as detailed by Lipson et al. (2013a). These four metagenomes are from four depths (0–10, 10–20, 20–30, and 30–40 cm); each metagenome explored pooled soil DNA from four spatially replicated soil cores (GenBank SRA accession number SRP020650). The four previously published metagenomes had larger coverage than the eight described in the current study; however, all twelve were generated using the same sequencing platform and analyzed using MG-RAST with hit comparisons to SEED and GenBank databases. To search more sensitively for reductive dehalogenase (RDH) genes, we used a Hidden Markov Model (HMM) analysis using HMMER v3 (Eddy 2011). The RDH gene family is well-curated (Hug et al. 2013), and has a defined protein family (TIGR02486). We searched all open reading frames in the metagenomes using hmmer3. Resulting sequences were manually inspected using tblastn searches to exclude probable false matches (some genes identified in the HMM search appeared to be epoxyqueuosine reductases, a related gene family (Payne et al. 2015). We also used a local BLAST search to identify *cmu* genes using BioEdit (because no authentic matches were found using MG-RAST). Predicted proteins from our metagenomes were downloaded from MG-RAST and used to construct a local protein database. Published *cmuABC* genes from *Methylobacterium extorquens* were queried against this database and the top hits were then investigated by searching against the public database.

Pyrosequencing was performed on 16S rRNA gene amplicons from old and ancient basin soils collected in June 2011 (Lipson et al. 2015). Four depths were studied from high/dry and low/wet topographical features (rims and centers of low-centered ice wedge polygons). Sample processing, sequencing, and core amplicon data analysis were performed by the Earth Microbiome Project (www.earthmicrobiome.org), and all amplicon and meta-data made public through the data portal (www.microbio.me/emp) (Gilbert et al. 2010). EMP protocols are available at http://www.earthmicrobiome.org/emp-standard-protocols/. All amplicon and metadata is available through the European Nucleotide Archive (ENA) of the European Bioinformatics Institute (EBI) (www.ebi.ac.uk/ena, study ID: ERP010098 and PRJEB9043).

## Results

### Seasonal changes in soil pore water $Cl^-$

Soil pore water $Cl^-$ concentrations were measured in a single representative DTLB of each age class over the summer of 2010 (Y0, M0, O0 and A0). $Cl^-$ concentrations in the medium aged basin were higher than in the other three basins (Fig. 1). Linear regression analysis shows the medium and ancient basin $Cl^-$ concentrations increased significantly over the season (Medium: slope = 14.7 μM/day, $R^2 = 0.168$, $P = 0.008$; Ancient: slope = 5.2 μM/day, $R^2 = 0.181$, $P = 0.006$), while $Cl^-$ in the young and old basins did not (Young: slope = −0.67 μM/day, $R^2 = 0.003$, $P = 0.75$; Old: slope = −0.47 μM/day, $R^2 = 0.002$, $P = 0.79$). The high slope in the medium basin was driven by the initial increase early in the season, after which the concentrations remained relatively constant.

### Quantification of soil and plant Cl pools

The concentrations of total Cl, $Cl_{org}$ and $Cl_{in}$ were measured in soils of all age classes (Fig. 2). This analysis included three replicates within each basin,

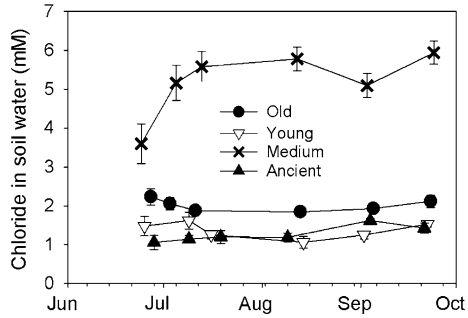


**Fig. 1** Soil pore water chloride ($Cl^-$) concentrations over the 2010 growing season

three basins per age class, four age classes, 36 samples total. Two samples from one of the medium-aged basins (Ms) had extremely high Cl concentrations and were identified as statistical outliers by their extreme Cook's D values (Fig. 2a, b, c). Omitting these two outliers, there were significant increases across the age gradient in total soil Cl (Fig. 2a, $P = 0.007$), $Cl_{org}$ (Fig. 2b, $P = 0.001$), but not $Cl_{in}$ (Fig. 2c, $P = 0.358$). The ratio of $Cl_{org}$ to total Cl also increased across the age gradient (Fig. 2d, $P = 0.029$, the two Ms samples were not outliers in this analysis). The increase of $Cl_{org}$ with soil development is shown clearly in the relationship between SOM and $Cl_{org}$ (Fig. 2e; this analysis includes the data from Fig. 2a–d, as well as a depth profile through the entire active layer from one basin of each age class). An analysis of covariance (ANCOVA) was used to test whether the relationship between $Cl_{org}$ and SOM varies by basin age. $Cl_{org}$ increased with SOM content ($P < 0.001$), but $Cl_{org}$ in old and ancient basins increased more steeply with SOM than young and medium basin soils (Fig. 2e, Basin × SOM interaction, $P = 0.013$). The choice of combining the youngest two basins and comparing them to the two oldest basins was justified by the corrected Akaike Information criterion ($AIC_C$), which compares models by balancing predictive power against complexity. In the regression with separate slopes for all four basins the $AIC_C$ was 580.80. Collapsing the basins into two categories lead to a significant improvement in $AIC_C$ (572.48, a decrease of 2 or more is commonly considered significant, Burnham and Anderson 2003). This analysis indicates higher Cl contents in soil organic matter of old and ancient basins than young and medium aged basins.

Total soil Cl and $Cl_{org}$ tended to decline with depth (Fig. S4A–B, Online Resource 1), though these patterns were statistically weak ($P = 0.037$ and

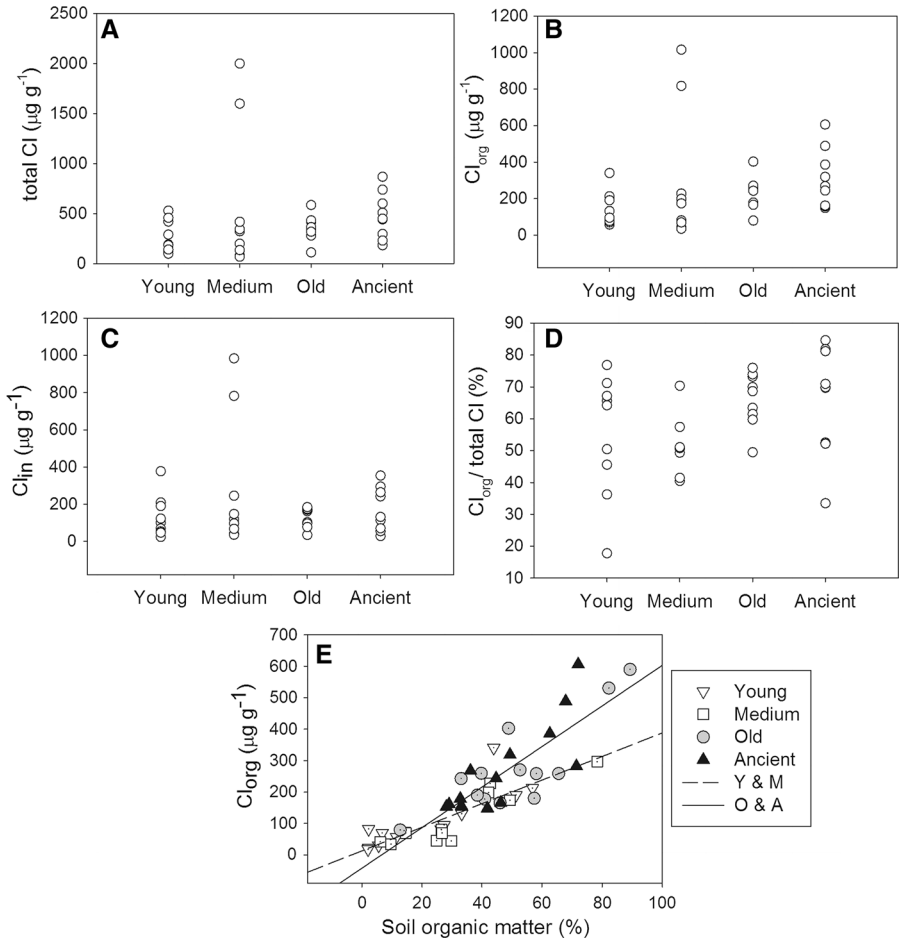


**Fig. 2** Analysis of soil Cl concentrations (μg $g^{-1}$ dry mass) by basin age: **a** Total Cl, **b** $Cl_{org}$, **c** $Cl_{in}$, **d** $Cl_{org}$ as percent of total Cl. **e** The relationship between $Cl_{org}$ and soil organic matter by basin age. Regression lines are shown for young and medium (YM, $y = 3.75X + 13.0$, $r = 0.851$) versus old and ancient (OA, $y = 6.45X - 42.1$, $r = 0.818$) soils

0.075, respectively). Some of this variability was due to the smaller change with depth in the young basin profile. $Cl_{org}$ as a percent of total Cl showed a non-significant ($P = 0.166$), positive trend with soil depth (Fig. S4C, Online Resource 1).

To place our measured soil Cl concentrations in a landscape context, we converted the data in Fig. 2 to an area basis (Fig. 3). $Cl_{org}$ in the active layer increases with basin age (Fig. 3a, $P = 0.014$). Much of this is accounted for by an increase in $Cl_{org}$ associated with the organic layer (calculated from published soil C data and the $Cl_{org}$ vs. SOM relationship in Fig. 2e) as the organic layer develops over time (Bockheim et al. 2004). To estimate the potential contribution of plant $Cl_{org}$ to the soil pool we multiplied our measured concentrations for plant leaf tissue from a medium-aged DTLB (Table 1) by published values of plant productivity and calculated the time it would take for this input to account for the observed increase in the profile (or in the case of young DTLB's, the appearance of $Cl_{org}$ in the organic layer). These estimates are within or below the estimated ages of each age category, and so plants could account for the initial accumulation of soil $Cl_{org}$. These rates imply that the growing soil $Cl_{org}$ pool turns over many times during development (~9–25 times for an ancient basin with respect to plant $Cl_{org}$ inputs, and even more if other atmospheric and microbial inputs are considered). It is also clear from Fig. 3a that $Cl_{org}$ in pre-existing lacustrine-derived organic matter makes up a substantial fraction of the total pool. In contrast to $Cl_{org}$, $Cl^-$ does not accumulate in soil profiles over time, and turns over on the order of 10 years with respect to $Cl^-$ in precipitation and runoff (Fig. 3b). Much of the inorganic Cl that is deposited by precipitation passes through plants before release as $Cl^-$ into the soil. Based on our measurements of plant total Cl concentrations (Table 1) and previously published productivity data, 63% of the $Cl^-$ in precipitation could be absorbed by growing vegetation (0.99 g Cl $m^{-2}$ $year^{-1}$), with 0.84 g Cl $m^{-2}$ $year^{-1}$ of this remaining in inorganic form (Fig. 3b).

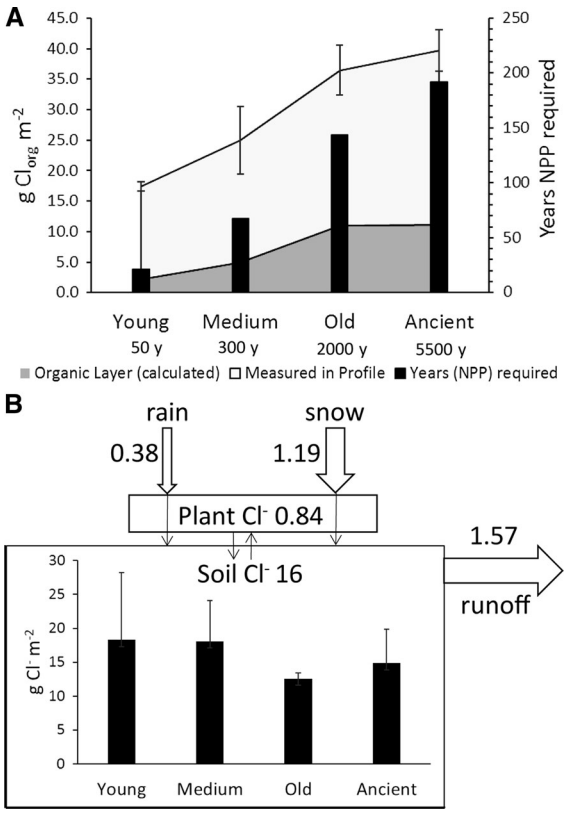


**Fig. 3** Input and accumulation of $Cl_{org}$ and $Cl^-$ in soils. **a** $Cl_{org}$ measured in the active layer of basins along the chronosequence (maximum age for each category shown), $Cl_{org}$ content of the organic layer, and the number of years it would take for the $Cl_{org}$ in the soil profile to accumulate given only $Cl_{org}$ inputs from plants. **b** $Cl^-$ measured in the active layer across the chronosequence (and the average for all basins), $Cl^-$ associated with annual plant production, inputs of $Cl^-$ from precipitation, and $Cl^-$ lost in runoff, assuming steady state conditions

**Table 1** Total ($Cl_{tot}$), organic ($Cl_{org}$) and inorganic ($Cl_{in}$) chlorine concentrations in plant biomass (mg per kg dry mass, standard errors shown in parentheses)

| Plant type | $Cl_{tot}$ | $Cl_{org}$ | $Cl_{in}$ |
|---|---|---|---|
| Sedge | 4420 (324) | 243 (17) | 4241 (407) |
| Moss | 541 (3) | 256 (74) | 285 (72) |

Sedges samples were *Carex aquatilis*, moss samples were *Sphagnum* spp.

## Cl XANES

Cl-XANES was performed to reveal chemical structure information of chlorinated compounds in bulk soils, soil humic acids, and plant tissue. The energy of the edge (or "white line") feature of XANES spectra reflects the oxidation state of the Cl in the sample, and can be used to determine whether the compound signature is more organic or inorganic (Leri et al. 2006, 2007; Leri and Myneni 2010). The edge occurs at lower energies for

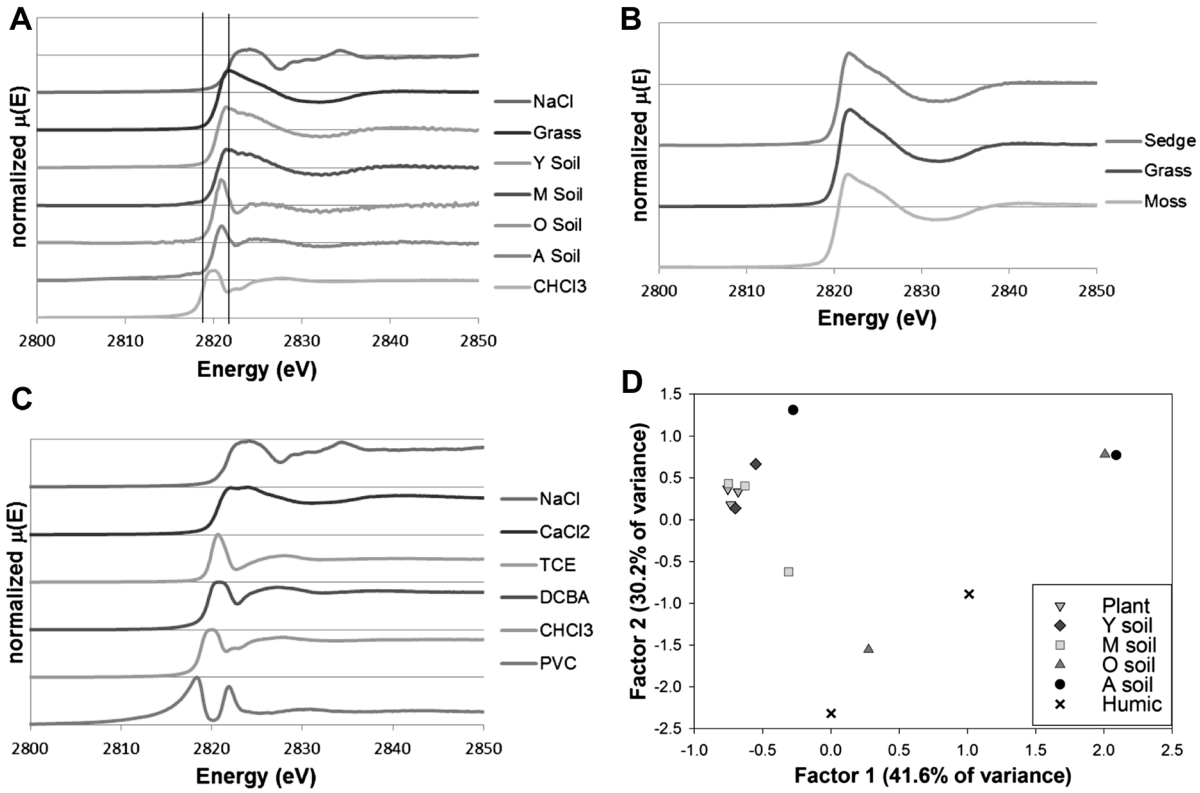


**Fig. 4** Cl-XANES of soils, plant and standards. **a** Spectra of representative soil samples from young (Y), medium (M), old (O) and ancient (A) basins, and grass tissue, bracketed between organic (chloroform, $CHCl_3$) and inorganic Cl (NaCl) standards. For reference, the locations of the K-edge are shown for $CHCl_3$ and NaCl with vertical lines. The spectra are stacked arbitrarily along the Y axis for purposes of comparison. **b** Spectra of three plant samples, a sedge, a grass and a moss. **c** Spectra of several organic (polyvinyl chloride (PVC), $CHCl_3$, dichlorobenzoic acid (DCBA), trichloroethylene (TCE)) and inorganic Cl standards. **d** Principle component analysis of curve-fitting results for spectra of soils, humic acid and plant tissues, using all relevant standards

organic chlorinated compounds than inorganic salts (shown by vertical lines in Fig. 4a). Spectral edges of the young and medium soils and the plant samples occurred at higher energy than old and ancient soils, indicating higher proportions of inorganic Cl in younger soils and vegetation, and higher contributions of organic Cl in older soils. The pre- and post-edge features give information on the chemical bonding environment of the Cl atoms. Qualitatively, the spectra of the medium and young soils were similar to spectra of vegetation, while old and ancient soils were not (Fig. 4a, b). The spectra of soils, plants, and soil humic acid extracts were analyzed with linear combination fitting (LCF), using a variety of organic and inorganic Cl standards (examples are shown in Fig. 4c). While our Cl-XANES libraries are too incomplete to provide a comprehensive, quantitative description of the types of Cl-containing molecules in these samples, LCF with our available standards allows us to compare the Cl chemistry among our samples. For each sample, LCF yielded the percent contribution of each standard, and these were reduced to two dimensions using principle components analysis (PCA, Fig. 4d). Young and medium soil samples tended to cluster with vegetation samples (moss, sedge, and grass), demonstrating the similarity in Cl signature among these samples. Old and ancient soil spectra were dispersed on the graph, indicating diversification in soil Cl signatures as soils develop. Likewise, the spectra from humic acids (relatively complex and old compounds) extracted from both young and ancient aged basins were distinct from the spectra of both soils and vegetation, indicating the diversification of Cl chemistry during the humification process.

### Organohalide respiration in laboratory incubations

The potential for organohalide respiration in Arctic Coastal Plain soils was verified by a laboratory experiment measuring the release of $Cl^-$ in anaerobic slurries of autoclave-sterilized and non-autoclaved soils, supplied with a mix of organic chlorinated compounds (PCE and DCE) that are dechlorinated by various species (Futagami et al. 2008). Non-sterilized samples received various combinations of vitamin $B_{12}$ (cobalamin, a cofactor required by *Dehalococcoides*), $H_2$ (an energy source) and $CO_2$ (required for autotrophic growth). However, no differences were observed among non-sterilized treatments, and so these are combined in Fig. 4. In non-sterilized treatments, $Cl^-$ increased linearly over the course of the incubation (slope = 0.0012 µmol Cl $g^{-1}$ soil $h^{-1}$, $R^2 = 0.464$), signifying cleavage of carbon-Cl bonds via organohalide respiration (Fig. 5). Conversely, autoclaving the soils appeared to liberate $Cl^-$ initially, after which there was no significant increase in chloride (the first measurement occurred prior to autoclaving the vials; all other measurements were taken after this step). A Mann–Whitney U test, performed on the slopes of individual flasks from this experiment, confirmed that the slopes were significantly different between autoclaved and non-autoclaved treatments ($P = 0.002$).

### Metagenomics and 16S rRNA gene analysis

We used shotgun metagenomes and surveys of 16S rRNA genes from our sites to assess the genetic potential for Cl cycling. One of the two metagenomics data sets and the 16S rRNA data set were previously published (Lipson et al. 2013a, 2015), and the reader is referred to these publications for a more thorough description of the microbial communities in the Arctic Coastal Plain near Barrow, AK. The previously published metagenome represented a single, medium-aged basin, while the 16S rRNA study included only old and ancient basins, and so additional metagenomes were constructed from basins of all age categories (Table 2). A general taxonomic description of these new metagenomes is given in Table S1 (Online Resource 1). Metagenomic data revealed the presence of Cl cycling genes in different depths of Arctic soils of all age classes (Table 2). Matches to genes annotated as haloperoxidases, (primarily non-heme, vanadium-dependent chloroperoxidases) were found in all but one soil surveyed (a relatively small metagenome with only 6407 sequences), and included matches to a diverse range of microbes, including some strict anaerobes (Table S2, Online Resource 1). Haloperoxidases catalyze the oxidation of halides by hydrogen peroxide and are involved in the synthesis of a variety of halogenated compounds (Winter and Moore 2009). Sequence matches to halogenases, which catalyze the halogenation of organic compounds, were found in young and medium aged basins, primarily at the shallower depths of the soil profile. Haloacid dehalogenases, responsible for liberating halogens from 2-halo carboxylic acids (Goldman et al. 1968), were found throughout the landscape everywhere except the shallow ancient soil (a small metagenome with only 4802 sequences). Reductive dehalogenase (RDH) genes, responsible for transferring electrons to $Cl_{org}$ in the final step of organohalide respiration (Holliger et al. 1999), were found in young and medium soils. Our initial BLAST search revealed only 8 sequence matches to RDH genes. The HMM search for RDH genes resulted in the 21 matches shown in Table 2. These Cl cycling genes originated from an unexpectedly diverse set of genera of Bacteria and Archaea (Tables S2, S3, S4, and S5, Online Resource 1). For example, the 21 sequence matches to RDH genes represented ten genera from seven different phyla (Table S5, Online Resource 1). The metagenomes from all depths and age categories also included numerous matches to the genomes of Cl-cycling bacteria, such as the obligate organohalide respiring genus *Dehalococcoides* (Taş et al. 2010), the

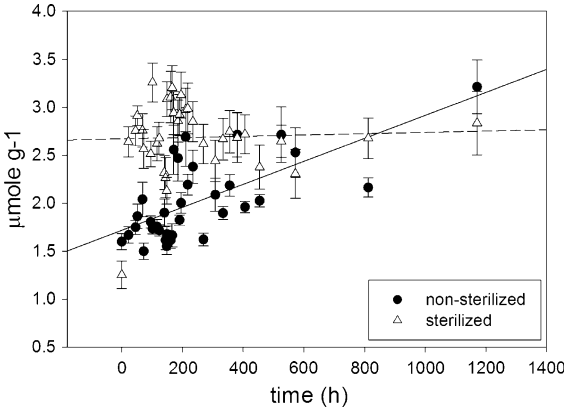


**Fig. 5** Chloride concentration (µmole $g^{-1}$ soil) over time in autoclaved sterilized and non-sterilized soils provided with dichloroethylene (DCE) and tetrachloroethylene (PCE). Regressions lines for the two treatments are shown

**Table 2** Numbers of sequences matching chlorine cycling genes and organisms in metagenomes

| Metagenome | HPO | H | HADH | RDH | *Anaeromyxobacter* | *Dechloromonas* | *Dehalococcoides* | Total post-QC sequences |
|---|---|---|---|---|---|---|---|---|
| Young 5–6 cm | 6 | 1 | 7 | 0 | 234 | 154 | 89 | 30,320 |
| Young 15–16 cm | 7 | 0 | 2 | 1 | 85 | 23 | 36 | 9250 |
| Medium 5–6 cm | 4 | 1 | 6 | 1 | 210 | 67 | 135 | 31,309 |
| Medium 15–16 cm | 3 | 0 | 1 | 0 | 66 | 20 | 26 | 6886 |
| Old 5–6 cm | 6 | 0 | 2 | 0 | 143 | 41 | 42 | 15,558 |
| Old 15–16 cm | 0 | 0 | 1 | 0 | 64 | 24 | 30 | 6407 |
| Ancient 5–6 cm | 7 | 0 | 0 | 0 | 41 | 9 | 27 | 4802 |
| Ancient 15–16 cm | 4 | 0 | 2 | 0 | 69 | 32 | 20 | 8765 |
| Medium 0–10 cm | 49 | 5 | 26 | 6 | 1528 | 410 | 296 | 128,370 |
| Medium 10–20 cm | 59 | 2 | 33 | 1 | 1310 | 438 | 376 | 159,070 |
| Medium 20–30 cm | 33 | 3 | 21 | 9 | 727 | 217 | 301 | 90,764 |
| Medium 30–40 cm | 29 | 0 | 20 | 3 | 721 | 210 | 321 | 79,096 |
| Total | 207 | 12 | 121 | 21 | 1645 | 5198 | 1699 | 570,597 |

*HPO* haloperoxidase, *H* halogenase, *HADH* haloacid dehalogenase, *RDH* reductive dehalogenase

dechlorinating genus *Anaeromyxobacter* and the perchlorate-reducing genus *Dechloromonas* (Table 2). The relative abundance of these three Cl-cycling genera was fairly constant across the basins and depths, though Chi squared tests revealed some significant trends: *Dechloromonas* was slightly over-represented in the young soil ($P < 0.001$); *Anaeromyxobacter* ($P < 0.001$) and *Dechloromonas* ($P = 0.004$) were enriched in shallower samples of the 0–40 cm study, while *Dehalococcoides* ($P < 0.001$) was more abundant at depth (Table S6, Online Resource 1). The metagenomes contained genomic matches to the methyl halide degrading bacterial species, *Methylobacterium chloromethanicum* and *Hyphomicrobium dentrificans*, though no authentic *cmuABC* genes were found (McDonald et al. 2002). Ten putative matches to *cmu* genes were initially identified, but these proved to be corrinoid methyltransferase proteins in strict anaerobes, more likely associated with acetogenesis than methyl halide degradation.

The 16S rRNA gene survey showed higher relative abundance of *Dehalococcoides* in lower topography, with a peak at the intermediate depth of 15 cm below the surface (Fig. 6). The proportion of *Dehalococcoides* sequences varied with topography and depth ($P = 0.012$ and $P = 0.011$, respectively, $R^2 = 0.485$, ANOVA on log-transformed relative abundance data). 16S rRNA genes of bacterial and archaeal genera

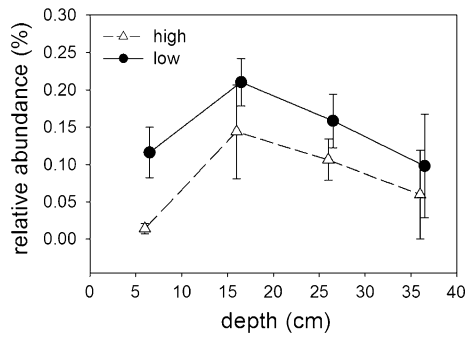


**Fig. 6** Relative abundance of *Dehalococcoides* 16S rRNA sequences by depth along the soil profile in areas of high and low topography (ice-wedge polygon rims and centers, respectively)

known for their capacity to dechlorinate halogenated organic compounds occur in these soils (the number of sequences detected, out of 2027,920 total, is shown in parentheses): *Delftia* (9) (Zhang et al. 2010), *Desulfobacterium* (52) (Egli et al. 1987), *Desulfomonile* (16) (Louie and Mohn 1999), *Desulfovibrio* (36) (Sun et al. 2000), *Methanobacterium* (10,649) (Egli et al. 1987), and *Methanosarcina* (2135) (Holliger et al. 1992). Notably, four genera found throughout these soils are capable of both dechlorination and dissimilatory iron reduction, another critical component of Arctic soil metabolism: *Anaeromyxobacter* (199) (He and Sanford 2002), *Desulfitobacterium* (1) (Nonaka et al. 2006), *Desulfuromonas* (67) (Löffler et al. 2000), and

*Geobacter* (33,602) (Sung et al. 2006). Additionally, 59 sequences matching the perchlorate-reducer containing genus *Dechloromonas* were found throughout the samples (Achenbach et al. 2001).

## Discussion

Substantial concentrations of $Cl_{org}$ and the presence of Cl cycling genes in soils of all basin ages indicate widespread Cl cycling activity in this ecosystem, driven by diverse microbial communities. The positive relationship between $Cl_{org}$ and SOM, its increased slope in older basins, and the increase in $Cl_{org}$ along the chronosequence indicate that $Cl_{org}$ accumulates as soils age and SOM develops. These results are similar to studies of agricultural and forest soils in Sweden and France, where $Cl_{org}$ (Redon et al. 2011; Svensson et al. 2007) and chlorination rates (Gustavsson et al. 2012) were positively correlated with SOM. The Arctic soil $Cl_{org}$ values found in the present study range from 18 to 1016 ppm, with an average of 245 ppm. This average concentration is somewhat higher than previously published values, for example, 87 ppm in a coniferous forest soil in southeast Sweden (Svensson et al. 2007), 45–100 ppm for arable, grassland and forest soils of France (Redon et al. 2013), and 133 ppm for a spruce forest soil in northwest Denmark (Öberg and Grøn 1998). The relatively high $Cl_{org}$ concentrations in these Arctic peat soils are presumably due to higher levels of SOM, as well as the natural enrichment of halogens in peat soils (Biester et al. 2006; Keppler and Biester 2003; Silk et al. 1997). The average concentrations of $Cl_{org}$ in plant tissues found in the current study (243 ppm for sedge tissue, 256 for moss) were generally higher than previously published $Cl_{org}$ values for plant tissues and litter, which tend to not exceed 100 ppm (Öberg 1998; Öberg and Grøn 1998; Öberg et al. 1996). Plant $Cl_{org}$ concentrations in our study are several fold higher than reported values for grass (10 ppm) and moss (60 ppm) in southeast Sweden (Flodin et al. 1997). This might be explained by the relatively low salinity of the Baltic sea compared to the Arctic ocean, although the total plant Cl concentrations in the two studies are similar (3200 and 700 ppm for grass and moss in Sweden, 4420 and 541 ppm for sedge and moss in this study). The $Cl_{org}$ fraction of the total soil Cl pool (mean of 61.4% in our study) was similar to the average value for Swedish boreal forest (68.5%) (Svensson et al. 2007), though somewhat lower than the average for French forest soils (85%) (Redon et al. 2011). The relative rates of chlorination and dechlorination vary in space and time, but the presence of these two opposing processes in soils may tend to balance soil Cl pools between $Cl_{org}$ and $Cl_{in}$. This idea is consistent with our observation that the soil $Cl_{org}$ pool must turn over many times during soil development, presumably cycling between organic and inorganic forms due to microbial activity. There is opportunity for loss of soil Cl as $Cl^-$ during snow melt. During this period, early in the summer, some fraction of the soil $Cl^-$ pool might diffuse upward into the overlying water and exit as runoff. As discussed below, Cl losses through VHOC fluxes are probably quite small compared to the soil $Cl_{org}$ pool.

The relative rates of $Cl_{org}$ accumulation in soils and production by plants show that plants contribute an important fraction of the $Cl_{org}$ that accumulates in soils over time. The similarity of XANES spectra of soils from young and medium-aged basins to those of vegetation further shows that mosses and graminoids contribute to the Cl pools in the early developmental stages of these soils. A development of soil $Cl_{org}$ with time is indicated by the shift in spectra toward a more organic signature seen as soils age, and by the increased variability in spectra of older soils and humic substances (which represent an older, more complex fraction of the SOM). The TX data support this interpretation, as the $Cl_{org}$/total Cl fraction increases with basin age and SOM is enriched in $Cl_{org}$ in old and ancient basins. Furthermore, the relatively rapid turnover rate of the soil $Cl_{org}$ pool with respect to plant inputs implies that soil Cl pools cycle between inorganic and organic forms many times during development of the older basins, allowing ample opportunity for chemical transformations of soil $Cl_{org}$. It follows that, while vegetation is a key input of soil $Cl_{org}$, this pool transforms into more complex structures with increased functional group diversity as a result of microbial activity over the course of soil development, as others have proposed (Fahimi et al. 2003; Leri and Myneni 2010; Myneni 2002). The Cl cycle we describe for this Arctic peat soil contrasts with the Cl cycle in Scandinavian forests, where the concentration of $Cl_{org}$ per unit soil organic matter increases sharply from the O to A horizon, indicating that $Cl_{org}$ is primarily generated in

the soil (Hjelm et al. 1995; Öberg 1998). This pattern was not observed in the present study. The relative contribution of plants and microbes to initial $Cl_{org}$ generation may differ between these ecosystems, but both exhibit rapid cycling between inorganic and organic forms of Cl in the soil due to microbial chlorination and dechlorination reactions.

The soil landscape around Barrow generally becomes less anoxic with development of ice wedge polygons that create microtopographic relief, resulting in more high, dry, oxic areas (Lipson et al. 2013b). Our metagenomic evidence indicates a prevalence of reductive dehalogenation pathways, which would be restricted to anoxic microenvironments. Supporting this idea, genomic sequences from the obligate organohalide respiring genus *Dehalococcoides* are underrepresented near the surface and highest at depth. Likewise, 16S rRNA genes of this genus are more abundant in lower, wetter areas than in high, dry areas, and are generally less abundant near the surface (Fig. 5). Conversely, the abundance of haloperoxidase genes indicates that formation of $Cl_{org}$ would be favored in oxic conditions where $H_2O_2$ is present, possibly as an adaptation to oxygen stress (Bengtson et al. 2013). Therefore, it is possible that an increase in aerobic conditions as soils age may contribute to the increased $Cl_{org}$ content of SOM in older soils. Young and medium basin Cl metabolism may be dominated by reductive dehalogenation, slowing the accumulation of $Cl_{org}$, while synthesis pathways (e.g., haloperoxidases, which require peroxide) may dominate in the more aerated soils of older basins. Alternatively, the rate of $Cl_{org}$ generation may be slightly faster than the rate of degradation at all stages of soil development; simply leading to accumulation of $Cl_{org}$ in older soils. Soil development over the thaw lake cycle in coastal tundra contrasts with longer-term chronosequences elsewhere in Alaska. For example, moist nonacidic tundra exists in some recently glaciated sites of the foothills of the Brooks Range, but older sites are often taken over by *Sphagnum* mosses, producing moist acidic tundra with increased plant productivity, microbial activity and more reducing conditions (Hobbie et al. 2002; Walker et al. 1998). Older acidic soils with more plant production and reducing conditions would probably host a more active Cl cycle than in the nonacid soils.

In general, microbial chlorination and dechlorination reactions probably occur simultaneously in most areas of the landscape, albeit at different depths in the soil profile. The sharp increase in $Cl^-$ observed early in the summer in the medium basin, M0, (Fig. 1) could have resulted from net dechlorination of $Cl_{org}$. This increase is not likely due to concentration of salts by evaporation, as the other basins were subjected to the same conditions and did not produce similar trends. These four basins shared the same seasonal patterns in water table depth (Lipson et al. 2013b). Also, net primary productivity and evapotranspiration (ET) peak later in the growing season, from mid-July to early-August (Zona et al. 2010), so the $Cl^-$ increase early in the season is unlikely to result from ET. Furthermore, if any basin were to show more rapid ET it would be the young basins that have the highest plant productivity (Zona et al. 2010). On the other hand, this medium basin had much higher $Cl^-$ levels than the others, and may have had unique hydrology. For example, M0 is closer to Elson Lagoon than the others (Fig. S1, Online Resource 1), and could be more subject to coastal flooding (Brown et al. 2003; Reimnitz and Maurer 1979). If $Cl^-$ were concentrated at a certain depth, then thawing of this layer could also give rise to the pattern seen in Fig. 1. Similarly, the outliers in the soil Cl analysis (Fig. 2a–c) both came from a single medium-aged basin (Ms, Fig. S1, Online Resource 1) with a relatively low elevation (3 m compared to mean of 6.5 m for all basins in this study). Storm surges of greater than 3 m have been reported for this region (Lynch et al. 2008).

$Cl^-$ liberation in the laboratory incubation confirmed the organohalide respiring capability of these Arctic soils in vitro. Interestingly, the process of autoclaving the experimental control soils in the laboratory incubation resulted in a large release of chloride (Fig. 5). Assuming bacterial populations around $10^9$ cells/g soil, an estimated cell volume of 1 $\mu m^3$, and the reported range of intracellular $Cl^-$ concentrations for bacteria (typically hundreds of mM (Fagerbakke et al. 1999)), it is plausible that the $\sim$1 μmol $Cl^-$/g soil increase after autoclaving largely resulted from cytoplasmic $Cl^-$, though it is also possible that autoclaving liberated $Cl^-$ from dead OM (such as plant cell walls). $Cl^-$ concentrations in the incubation experiment were variable over time; the fluctuations observed in non-sterilized samples may point to the dynamic nature of multiple, opposing Cl metabolic pathways occurring during the incubation. The lack of response to vitamin $B_{12}$, $H_2$, or $CO_2$

indicates these factors were not limiting in these incubations, or that our technique was not sensitive enough to detect these effects. It is also unknown whether the $Cl^-$ liberated during the incubation originated from naturally occurring $Cl_{org}$ or the PCE/DCE cocktail. Cl tracing experiments using isotopic labels would allow for more sensitive measurements of Cl cycling (Bastviken et al. 2009; Montelius et al. 2016).

Previous studies in this Arctic ecosystem showed that the soil microbial community and its metagenome are dominated by anaerobic respiratory pathways (Lipson et al. 2013a, 2015). Fe(III) and humic substances are important electron acceptors for anaerobic respiration in soils of the Arctic Coastal Plain (Lipson et al. 2010, 2013b), and can compete with other anaerobic processes such as methanogenesis (Miller et al. 2015). Organohalide respiration occurs simultaneously with Fe(III) reduction in some environments (Azizian et al. 2010) though inhibitory effects of Fe(III) on reductive dechlorination have been reported (Aulenta et al. 2007; Paul and Smolders 2014). Though the redox potential of $Cl_{org}$ is difficult to gauge due to its complex and varied chemical composition (Gribble 2003; Montelius et al. 2016), the coexistence of organohalide respiration with Fe(III) reduction in these soils suggests their reduction potentials may overlap (Shani et al. 2013). This is consistent with thermodynamic and empirical evidence that $H_2$ thresholds are similar for Fe reduction and organohalide respiration, both orders of magnitude lower than the threshold for methanogenesis (Löffler et al. 1999; Lovley et al. 1994). This implies that, like Fe and humic reduction, organohalide respiration has the potential to inhibit methane production in this ecosystem by reducing $H_2$ to very low levels. Furthermore, Fe(III) and humic substances can react with $Cl^-$ to create $Cl_{org}$ (Fahimi et al. 2003; Keppler et al. 2000), potentially increasing the opportunity for Cl cycling in these soils. The Cl, Fe, and C cycles may be highly intertwined in this ecosystem. Cl inputs from the Arctic Ocean to this coastal tundra ecosystem are presumably higher than inland sites (Frontasyeva and Steinnes 2004; Simpson et al. 2005). However, there are over 400,000 km of permafrost-affected coastline in the northern hemisphere, representing 34% of the world's coasts (Lantuit et al. 2012), and so even if Cl cycling only plays a major biogeochemical role in coastal tundra, this would still represent a globally important phenomenon.

This is the first study to describe internal Cl cycling in an Arctic ecosystem. However, fluxes of VHOC have been previously measured in this ecosystem (Rhew et al. 2007, 2008; Teh et al. 2009). Fluxes of methyl halides and chloroform are very small compared to the overall soil Cl pools and our estimates of Cl cycling rates. For example, with respect to $Cl_{org}$ content from the current study, gross rates of methyl chloride uptake in this ecosystem [793 nmoles $m^{-2}$ - $days^{-1}$, Teh (2009)] represent a residence time of about 24,000 years. For comparison, the potential dechlorination rates found in our laboratory incubations, or those reported by Montelius et al. (2016), were both four orders of magnitude higher than the methyl chloride uptake rate reported by Teh et al. (2009). The sources and sinks of methyl halides seem more related to methane cycling microbes than those that cycle Cl into and out of organic matter: methyl halide fluxes were correlated with methane fluxes, and dry sites were larger sinks than wet sites (Rhew et al. 2007; Teh et al. 2009). Thus, methyl halides are probably degraded aerobically (McDonald et al. 2002), rather than by reductive dechlorination, and so the landscape patterns for methyl halides are different from the overall patterns of chlorination and dechlorination we propose based on soil redox conditions.

Many studies of organohalide respiration have focused on model organisms such as *Dehalococcoides* or *Desulfitobacterium* in contaminated sites. Our results from this Arctic environment with no major anthropogenic sources of $Cl_{org}$ illustrate a naturally occurring Cl cycle involving a diverse biological community. While the range of microorganisms potentially participating in the Cl cycle is vast, the limited annotation of genomes restricts the ability to use genetic insight alone to determine the full diversity of Cl cycling organisms in the environment. Metagenomic analysis relies on the annotation of sequenced genomes, and many gene functions have not been verified. For example, we found close sequence matches to genes from strict anaerobes, such as *Methanosarcina acetivorans* and *Geobacter uraniireducens*, that are annotated as chloroperoxidases. While peroxidases are found in anaerobic prokaryotes (Passardi et al. 2007), chlorinated products have not (to our knowledge) been described in anaerobes (van Pée et al.

2006). The genes annotated as chloroperoxidases in the genomes of strict anaerobes might instead be acid phosphatases or members of the large alpha/beta hydrolase family (Xu and Wang 2016). On the other hand, we are doubtlessly missing many other Cl-cycling genes that have not yet been described and annotated in sequenced genomes. Despite this uncertainty, observing putative Cl cycling genes in 11 phyla ranging from Cyanobacteria to Archaea was one of the more surprising aspects of the present research.

This is one of the most thorough studies to date on soil Cl cycling, combining chemical, genetic, field-based, and laboratory-based results; furthermore, it is the first to demonstrate the internal cycling of Cl in an Arctic ecosystem. Significant amounts of $Cl_{org}$ enter these soils through the vegetation, and this pool accumulates, turns over and diversifies due to microbial activity as soils age. Diverse Cl-cycling microbes are ubiquitous across this landscape. The Cl cycle has the potential to interact with the biogeochemical cycling of Fe and C in this ecosystem. Even if such an active Cl cycle is mainly restricted to coastal tundra, this study still represents an important and previously unrecognized aspect of Arctic biogeochemistry, and provides further evidence that natural Cl cycling in ecosystems is a widespread phenomenon.

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