UC Berkeley
UC Berkeley Previously Published Works

Title
Chloroform (CHCl3) Emissions From Coastal Antarctic Tundra

Permalink
https://escholarship.org/uc/item/1s17x0dd

Journal
Geophysical Research Letters, 48(18)

ISSN
0094-8276

Authors
Zhang, Wanying
Jiao, Yi
Zhu, Renbin
et al.

Publication Date
2021-09-28

DOI
10.1029/2021gl093811

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Chloroform ($CHCl_3$) emissions from coastal Antarctic tundra

Wanying Zhang [1, †], Yi Jiao [2, †], Renbin Zhu [1,3,*], Robert C. Rhew [2,4,*], Bowen Sun [1] and Haitao Dai [1]

1. Institute of Polar Environment and Anhui Province Key Laboratory of Polar Environment and Global Change, School of Earth and Space Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China

2. Department of Geography, University of California, Berkeley, California 94720, United States

3. Frontiers Science Center for Planetary Exploration and Emerging Technologies, University of Science and Technology of China, Hefei, Anhui, 230026, China

4. Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720, United States

*** Corresponding authors:** Renbin Zhu (zhurb@ustc.edu.cn) or Robert C. Rhew (rrhew@berkeley.edu).

† These two authors contributed equally to this work.

Key points:

1. Antarctic tundra is a natural source of $CHCl_3$, emitting up to 0.1 Gg each year into the atmosphere.
2. Penguins input organic matter and marine halides into soil through excrement, which facilitate microbial-mediated $CHCl_3$ production.
3. The strength of $CHCl_3$ source will vary in response to changes in penguin population/colony size, and the extent of Antarctic warming.

Abstract

In this study, the first *in situ* static-chamber measurements were conducted at coastal Antarctica tundra for $CHCl_3$ fluxes, which showed that $CHCl_3$ was naturally emitted from the Antarctic tundra at 35 ± 27 nmol m^{-2} d^{-1}, comparable to other reported important natural sources. Significantly enhanced $CHCl_3$ emission rates (66 ± 20 nmol m^{-2} d^{-1}) were observed from ornithogenic soil on the island populated with penguins, which was rich in organic matter and halides coming from penguin excrements. It is estimated that Antarctic tundra emits up to 0.1 Gg $CHCl_3$ per year, which is an important source for regional atmospheric $CHCl_3$. Laboratory-based incubations suggested that organic carbon and chlorine inputs by penguins may stimulate O_2 dependent microbial-mediated $CHCl_3$ emission from the Antarctic tundra, and all tundra soils showed the maximum $CHCl_3$ emission at 4 °C. The strength of this $CHCl_3$ source is also expected to change in response to Antarctic warming.

Plain language summary

Chloroform ($CHCl_3$) is the second largest natural carrier of atmospheric chlorine, which can catalyze stratospheric ozone depletion. Natural sources of $CHCl_3$ are believed to predominate over anthropogenic sources, accounting for 50-90% of global $CHCl_3$

emissions. Among the natural sources, soils are the second largest source, after the ocean. This study conducted the first *in situ* static-chamber measurements and lab-based incubations on $CHCl_3$ emissions from Antarctic tundra, and found that it was an important regional source, emitting up to 0.1 Gg $CHCl_3$ each year (< 1% of natural terrestrial sources) into the atmosphere. Penguin activites deposited large amounts of excrement in colony tundra and enhanced organic matter and chlorine content in the soil, which promoted the production of $CHCl_3$ mediated by microbial activities. Temperature-controlled incubations indicated that tundra soils showed the maximum $CHCl_3$ emission at 4 °C, and temperature increase and freeze-thaw cycles might influence annual and seasonal $CHCl_3$ emissions from Antarctic tundra. This study suggested that the strength of $CHCl_3$ source will vary in response to changes in penguin population/colony size, and the extent of Antarctic warming.

Introduction

Chlorine-containing halocarbons, such as chloromethane (CH_3Cl), chloroform ($CHCl_3$), and chlorofluorocarbons (CFCs), can photo-dissociate in the stratosphere, releasing reactive chlorine radicals that catalyze ozone depletion. Among the naturally produced atmospheric chlorocarbons, $CHCl_3$ is the second largest natural carrier of chlorine after CH_3Cl. With an average tropospheric lifetime of 149 days (Engel et al., 2018), it is categorized as a very short-lived substance (VSLS). Hence, the ozone-depleting capacity of $CHCl_3$ was thought to be minor and was not regulated by the Montreal Protocol. However, observations over the past decade showed its atmospheric molar fractions have been steadily increasing (Engel et al., 2018; Fang et al., 2019). Numerical model simulations also indicated the recovery of stratospheric ozone may be significantly delayed if atmospheric concentrations of VSLSs, including $CHCl_3$, continue to grow (Fang et al., 2019).

Natural sources of $CHCl_3$ are believed to predominate over anthropogenic sources, accounting for 50-90% of global $CHCl_3$ emissions (McCulloch, 2003; Worton et al., 2006). The natural emissions of $CHCl_3$ are mainly from surface ocean (~360 Gg yr^{-1}) and terrestrial soils (over 200 Gg yr^{-1}). Rhew et al (2008a) first measured $CHCl_3$ fluxes from the Arctic tundra, and an extrapolation of these fluxes suggested that tundra globally could contribute to 1–2% of atmospheric $CHCl_3$. Tundra in Greenland and Northern Scandinavia also showed large emissions of $CHCl_3$ (Johnsen et al., 2016; Macdonald et al., 2020). Ice-free tundra in the Antarctica is as large as 7.2×10^{10} m^2, but no $CHCl_3$ flux measurement has ever been conducted due to the remote location and adverse weather conditions (Lee et al., 2017; Terauds & Lee, 2016).

Different from other terrestrial ecosystems, Antarctic tundra is often colonized by large numbers of sea animals, such as penguins (Croxall et al., 2002; Sun et al., 2000). Penguin

activities strongly alter local biogeochemical environments through depositing guano, promoting the formation of ornithogenic tundra soil, which is fertile in organic matter and marine elements (Michel et al., 2006; Schaefer et al., 2008). The activities of microorganisms are often higher in these ornithogenic soils, compared to normal tundra soil (Tscherko et al., 2003; Zdanowski et al., 2005). Significantly higher emission rates of greenhouse gases and other trace gases were observed from ornithogenic soil in penguin colonies (Zhu et al., 2006, 2008, 2009). Therefore, given the abundant organic carbon, chloride contents and microorganism populations in Antarctic colony tundra soil, the unique biogeochemical environment may facilitate the chlorination of organic matter to produce $CHCl_3$.

In this study, *in situ* static-chamber measurements of $CHCl_3$ fluxes were conducted in Antarctic tundra during the 36th Chinese Antarctic Research Expedition (CHINARE-36). Soil samples were also collected from the tundra on Fildes Peninsula and Ardley Island, and then incubated in the laboratory (1) under simulated natural conditions to obtain $CHCl_3$ fluxes, and to compare with the *in situ* results; (2) in the range of -12 °C to 12 °C to quantify $CHCl_3$ emissions under simulated seasonal cycles and projected Antarctic warming; and (3) under different manipulated conditions (such as N_2-anoxic and thermal treatments) to explore the $CHCl_3$ formation pathway in Antarctic tundra.

Materials and methods

Study sites

The study area is located on Fildes Peninsula (61°51′S-62°15′S, 57°30′W-59°00′W) and Ardley Island (62°13′S, 58°56′W) in West Antarctica (Figure 1). The annual average temperature is about -2.5 °C, in a range of -26.6~11.7 °C, and the annual average precipitation (mainly in snowfall) is about 630 mm (Zhu et al., 2013). Fildes Peninsula (Figure 1b) is the largest ice-free area in King George Island, the middle of the peninsula

is bedrock-exposed hills, and a well-developed tundra ecosystem is located along the coastline, with the lichens and mosses as the main vegetation types (Borchhardt et al., 2017). The western coast of the peninsula is inhabited by a variety of seals (Wang et al., 2019). The eastern side of Ardley Island is an important habitat for Adélie, gentoo and chinstrap penguins with a combined population over 10,000 (Zhu et al., 2013) (Figure 1c). The adjacent penguin-lingering tundra areas are covered by cushions of mosses, lichens, and algae (Borchhardt et al., 2017), and penguins occasionally wander here. The western side of the island, which is in the prevailing wind direction, is a lowland tundra marsh as well as an abandoned former penguin colony (Yang et al., 2019). Each year, large amounts of excrement are deposited on Ardley Island and leached into tundra soil by snowmelt from surrounding hills, and seawater is also brought inland by penguins. In general, sea animal colony tundra soils have higher gravimetric moisture and soil organic matter levels in comparison to normal tundra soils (soil properties are given in Table S1).

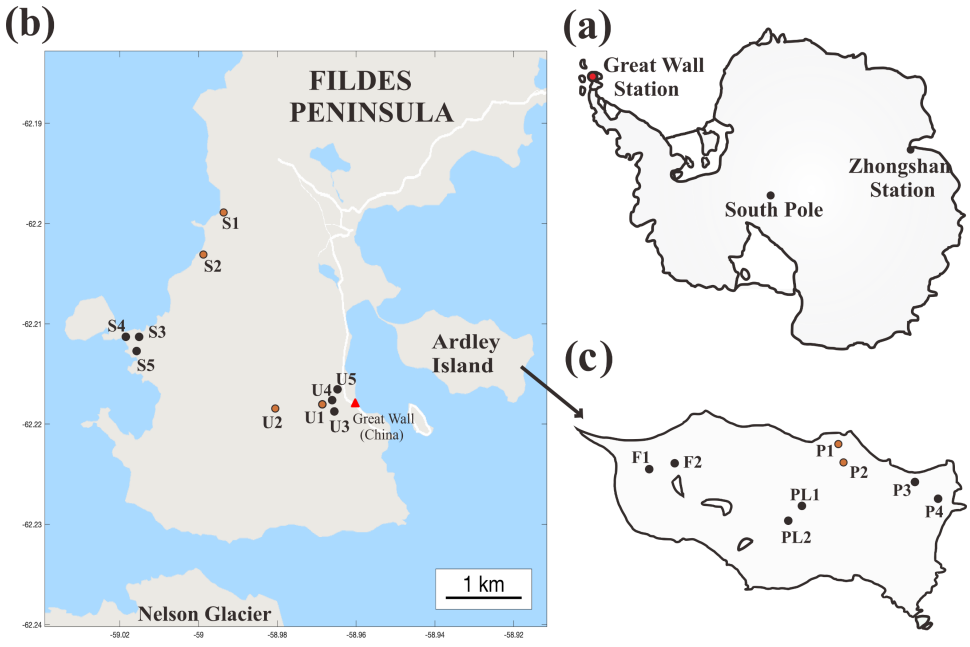

Figure 1. Maps of the study area and sampling sites. (a) Antarctic continent and the location of the Great Wall Antarctic Research Station (red dot); (b) Sampling sites on the Fildes Peninsula, including the seal colony tundra SCS (S1-5) and normal upland tundra UTS (U1-5); (c) Sampling sites on the Ardley Island, including the western abandoned former penguin colony FPS (F1, F2), the eastern active penguin colony tundra PCS (P1-4), and the adjacent penguin-lingering

tundra PLS (PL1, PL2). The orange and black dots represent *in situ* flux measurement sites and soil sample collection sites, respectively.

In situ $CHCl_3$ flux measurements

During the 36th Chinese National Antarctic Research Expedition, *in situ* static-chamber measurements (Figure S1) for $CHCl_3$ fluxes were conducted from seal colony tundra (S1, S2; Histic Cryosols), penguin colony tundra (P1, P2; Ornithogenic Cryosols) and normal upland tundra (U1, U2; soil combinations of Turbic Cryosols and Hyperskeletic Leptosols) (Figure 1, orange dots). Air samples within the chamber were drawn into pre-evacuated air flasks (1-litre; Entech, California, United States) at 1 min, 16 min and 31 min through a stainless-steel tube extended into the chamber center point. During sampling, the chamber was vented through a tube to allow for pressure equilibrium and to avoid over-sampling the soil pore air (additional information on sampling procedure is given in Text S1). Each location had two replicate static-chamber measurements.

$CHCl_3$ within the flask samples were cryotrapped and analyzed by a gas chromatograph-mass spectrometer (GC/MS, Agilent 7890B/5973N, Agilent Technologies, California, United States). The samples were calibrated against a commercial standard mixture of TO-15 compounds (Linde PLC, New Jersey, United States). The $CHCl_3$ molar fraction was reported in units of parts per trillion (p.p.t; the same hereafter). Based on daily standard measurements, the instrumental precision (1σ) for $CHCl_3$ analysis was less than 4%. Details on the cryotrapping procedure, capillary column selection and GC oven temperature setting are given in Supporting Information (Text S2).

Laboratory soil incubation experiment

Soil samples (top 5-10 cm) were collected from Ardley Island at penguin colony (P1-P4), penguin-lingering area (PL1, PL2), and abandoned former penguin colony (F1, F2). Soils were also collected on Fildes Peninsula from normal upland tundra (U1-U5) and seal

146 colony (S1-S5) (Figure 1). Each sample (25 grams) had at least two replicates ($n \geq 2$)
147 incubated as described below.

148 **(i) Soil incubation under simulating summertime temperature**. Each soil sample
149 (U3-U5; S3-S5; F1-F2; PL1-PL2; P3-P4) was enclosed in glass Mason jars, and then
150 incubated overnight at 4 ℃ using a water/ethylene glycol bath (VWR Model 1180S,
151 Pennsylvania, United States) to simulate Antarctic mean summer temperature. A stainless-
152 steel lid to the glass jar was connected to a pre-evacuated stainless-steel tube ($V \approx 25$ mL),
153 which was further connected to the cryo-trapping system (Khan et al., 2012) of a gas
154 chromatograph/mass spectrometer (GC/MS; Agilent 6890N/5973, Agilent Technologies,
155 California, United States). The headspace air was drawn, cryofocused, and injected into
156 the GC/MS at 1 min, 31 min and 61 min after sealing. Weekly calibration curves were
157 constructed with a natural whole air standard collected and calibrated at the Scripps
158 Institution of Oceanography, University of California at San Diego (SIO-98 calibration
159 scale). The same standard was run before and after each batch of incubations to correct for
160 the daily drift of the mass spectrometer signal.

161 **(ii) Incubation under anoxic condition and post thermal sterilization.** Two sets of
162 incubation experiments were carried out at 4 ℃ under anoxic conditions and post thermal
163 sterilization, respectively, with the soil samples (U3-U5; S3-S5; F1-F2; PL1-PL2; P3-P4)
164 to explore the potential effects of oxygen levels and soil microorganisms. The anoxic
165 condition was induced by flushing and filling the jar with ultra-pure nitrogen gas (≥
166 99.999%, Praxair, Connecticut, United States); thermal sterilization was conducted by
167 autoclaving the soil samples at 150 ℃ for 2 hours. The moisture was brought back by
168 adding the same amount of distilled water as mass loss. The soil samples were incubated
169 for $CHCl_3$ flux following the same procedure above.

(iii) Temperature-gradient incubation. Soil samples (U3-U5; S3-S5; P3-P4) were also incubated along a temperature gradient between -4 and 12 ℃ at 4 ℃ intervals, in order to construct the relationship between $CHCl_3$ fluxes and soil temperature, and to explore projected $CHCl_3$ emissions under Antarctic warming.

(iv) Freeze-thaw cycles (FTCs) incubation. To simulate Antarctic seasonal temperature cycles, soil samples (U1-U2; S1-S2, S4; P1-P2, P4) were incubated over three consecutive FTCs. Within each FTC, soil sample was frozen at -12 °C (mean wintertime temperature) for 8 hours, and then 20 ml of headspace air was extracted and analyzed every 2 hours for four times. The sample was then thawed at 4 °C (mean summertime temperature) for 8 hours, after which the headspace air was analyzed following the same procedure. After each sampling, the jar was flushed with the standard whole air to conserve constant pressure within the jar.

Flux calculation

For $CHCl_3$ productions, most of the flux measurements showed significant linear relationship ($R^2 > 0.70$, $p < 0.05$) between $CHCl_3$ concentrations in chamber/jar headspace and enclosure time. Therefore, net positive fluxes were calculated using the linear least squares fit between $CHCl_3$ molar fraction and time, multiplied by the number moles of air in the static-chamber or glass jar, and then normalized to the surface area of soil (Jiao et al., 2018).

$$F = \frac{1}{\mathrm{A}} \frac{dc}{dt} N_a \quad \cdots\cdots\cdots \quad (\mathrm{Eq.}\,1)$$

Where F represents the $CHCl_3$ flux from the soil [nmol m^{-2} d^{-1}]; A represents soil surface area; and N_a is the number moles of air in the chamber or jar. For soil incubations, $CHCl_3$ emission rates are normalized to the dry mass of soil, and reported in the unit of

pmol g^{-1} d^{-1}. For linear regressions with low coefficients of determination (r^2<0.70), the curve fits were not considered robust and the fluxes were treated as zero.

$CHCl_3$ degradation in soil was assumed to follow first-order kinetics to the ambient $CHCl_3$ concentration (Khan et al., 2011).

$$\frac{d[c]}{dt} = -k[c] \quad \cdots\cdots \quad (\text{Eq. } 2)$$

Therefore, when uptake of $CHCl_3$ was observed, a linear least squares fit was applied to the natural log of headspace $CHCl_3$ concentration (ln[c]) over incubation time to get the first-order constant, k, which was then multiplied by background $CHCl_3$ concentration in Southern Hemisphere (Prinn et al., 2018) to obtain *in situ* uptake rates.

Analysis of soil properties

Soil pH, gravimetric moisture content (Mc), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and chlorine (Cl) contents were also analyzed. The methodology is given in Supporting Information (Text S3).

Results and discussion

$CHCl_3$ Emission from Antarctic Tundra

In situ static-chamber measurements showed that normal upland tundra (UTS), seal colony tundra (SCS), and active penguin colony tundra (PCS) were $CHCl_3$ sources, with emission rates of 16 ± 6 nmol m^{-2} d^{-1}, 22 ± 11 nmol m^{-2} d^{-1}, and 66 ± 20 nmol m^{-2} d^{-1}, respectively (Figure 2a). Laboratory-based incubations showed that all Antarctic tundra soils emitted $CHCl_3$, with mean emission rates ranging from 0.4 to 7.5 pmol g^{-1} d^{-1} (Figure 2b), further supporting the *in situ* finding that Antarctic tundra was a $CHCl_3$ source.

This study provides the first $CHCl_3$ flux measurements from the Antarctic tundra. The average $CHCl_3$ flux (35 ± 27 nmol m^{-2} d^{-1}) of different types of Antarctic tundra was within

the range of those from the Alaskan Arctic tundra (45 nmol m^{-2} d^{-1}) (Rhew et al., 2008a), and Arctic glacial forefield tundra (74 ± 33 nmol m^{-2} d^{-1}) (Macdonald et al., 2020); the flux from penguin colony tundra (66 ± 20 nmol m^{-2} d^{-1}), where the highest emission rates were observed in this study, was higher than or close to those from Arctic tundra.

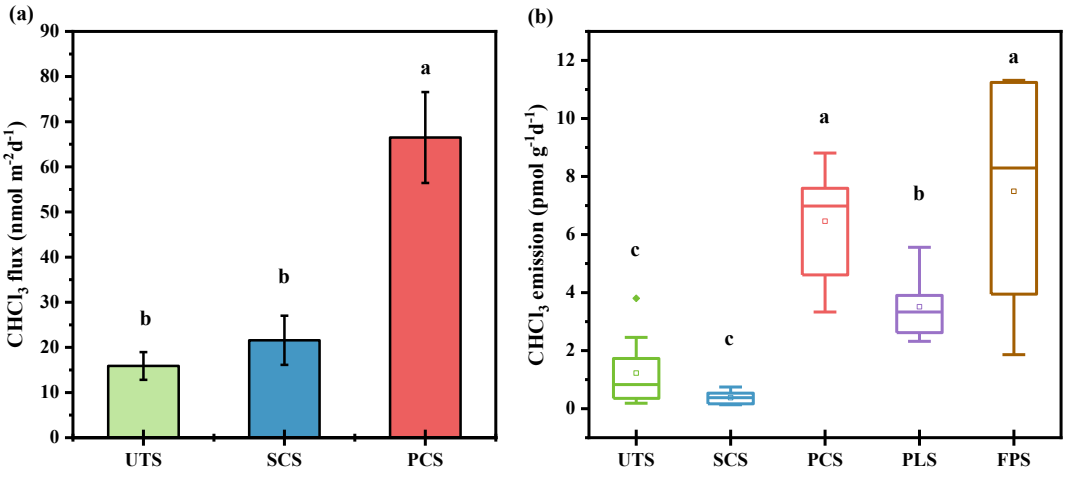

Figure 2. (a) Bar plots of *in situ* measurements on chloroform ($CHCl_3$) fluxes from normal upland tundra (UTS, n = 4), seal colony tundra (SCS, n = 4), and active penguin colony tundra (PCS, n = 4) in coastal Antarctica. (b) Box plots of laboratory-based measurements on chloroform ($CHCl_3$) emission rates from the soils of normal upland tundra (UTS, n = 8), seal colony tundra (SCS, n = 8), active penguin colony tundra (PCS, n = 7), penguin-lingering tundra (PLS, n = 6) and abandoned former penguin colony tundra (FPS, n = 6). The different lowercase letters (a, b, or c) indicate significant differences between the means (LSD, $p < 0.05$).

The average $CHCl_3$ fluxes from Antarctic tundra were an order of magnitude smaller than those from several mid- and low- latitudes ecosystems, such as temperate peatland (258 ± 288 nmol m^{-2} d^{-1}) (Khan et al., 2012), pasture (241 ± 221 nmol m^{-2} d^{-1}) (Cox et al., 2004), subtropical degraded forested wetland (209 ± 183 nmol m^{-2} d^{-1}) (Jiao et al., 2018; Wang et al., 2016), and tropical rice paddies (201 nmol m^{-2} d^{-1}) (Khalil et al., 1998). These results from such diverse ecosystems (Table S2) suggest that soils globally are important and widespread sources for atmospheric $CHCl_3$, even in coastal Antarctica.

Exploring $CHCl_3$ production mechanism

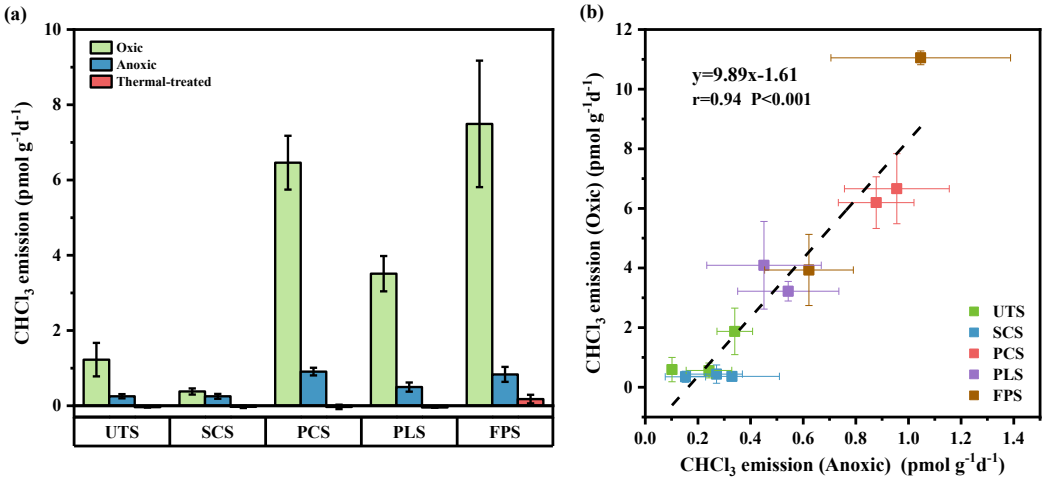

Figure 3. (a) Bar plots of chloroform ($CHCl_3$) emission rates from normal upland tundra (UTS), seal colony tundra (SCS), active penguin colony tundra (PCS), penguin-lingering tundra (PLS) and former penguin colony (FPS) based upon laboratory incubations under simulated natural conditions, N_2-anoxic conditions and post thermal treatment. (b) Linear correlation between chloroform ($CHCl_3$) emission rates from coastal Antarctic tundra soils under ambient oxic conditions and N_2-anoxic conditions.

In this study, soil $CHCl_3$ emission rates significantly declined to near zero after thermal sterilization (Figure 3a). A number of studies have shown that $CHCl_3$ formation within soil may be primarily of biotic origins (Bastviken et al., 2009; Breider & Hunkeler, 2014a; Macdonald et al., 2020; Ruecker et al., 2014, 2015). Chloroperoxidases (CPOs), commonly found in soil fungi, such as *Caldariomyces fumago* and *Peniophora pseudopini*, are capable to produce hypochlorous acid (HOCl), which can chlorinate soil organic matter to produce intermediate organohalogens (such as trichloroacetyl-containing compounds), and subsequently cleave to yield $CHCl_3$ (Albers et al., 2017; Breider & Hunkeler, 2014a, 2014b; Wever & Barnett, 2017). The enzymatic activities of various CPOs are all suppressed at high temperatures. For example, the activity of iron-CPO in *Caldariomyces fumago* is reduced to almost 0% after a 15-minute incubation at 60 °C (Pickard & Hashimoto, 1988). Thus, the dramatic reduction of emissions post thermal sterilization suggests that the observed $CHCl_3$ production in Antarctica tundra soils was predominantly by enzymatic or microbial activities. However, on the other hand, thermal sterilization may have altered the

chemical composition of soil organic matter to some extent, such as by decomposing or volatilizing labile carbon compounds, and inhibited $CHCl_3$ production potential of abiotic process which is usually catalyzed by Fe^{3+} (Huber et al., 2009).

The optimal pH for chlorinating activities of CPOs was around 3-4 (Asplund et al., 1993; van Schijndel et al., 1994), lower than the soil pH of Antarctic tundra (Table S1). Consistent with this, an inverse proportional relationship was observed between $CHCl_3$ fluxes and soil pH across different types of Antarctic tundra ($r = -0.72$, $p = 0.008$, Figure S2, S3).

$CHCl_3$ emission rates from Antarctic tundra soils under anoxic conditions (0.5 ± 0.4 pmol g^{-1} d^{-1}) declined to about 14.3 % of that under oxic conditions (3.5 ± 3.4 pmol g^{-1} d^{-1}), but still conserved a strong linear correlation ($r = 0.94$, $p < 0.001$) with those under ambient conditions (Figure 3), suggesting that the production of $CHCl_3$ can occur both aerobically and anaerobically, although the aerobic production mechanism dominates. Most of the CPOs are heme-dependent, or prosthetic groups of transition metals are in oxidized states, such as iron (III) or vanadium (V) (Breider & Hunkeler, 2014a; Wever & Barnett, 2017). An oxic environment is essential to keep these transition metals in oxidation states for soil $CHCl_3$ production. Laboratory incubations of soil samples from a coniferous forest confirmed that O_2 is required in the dominant CPO-mediated chlorination process (Bastviken et al., 2009). The anaerobic environment may have reduced the oxidation states of transition metals, and subsequently suppressed the enzymatic pathway of $CHCl_3$ formation in Antarctic tundra soils.

Effects of penguin activities on tundra $CHCl_3$ emission

In situ $CHCl_3$ fluxes showed that the average fluxes from penguin colony tundra were more than 3-4 times higher than those from seal colony tundra and normal upland tundra (Figure 2a). For lab-based incubations, the highest emission rate was observed from the

285 abandoned former penguin colony (FPS, mean 7.5 ± 4.1 pmol g^{-1} d^{-1}) and the active
286 penguin colony (PCS, mean 6.5 ± 1.9 pmol g^{-1} d^{-1}), followed by the adjacent penguin-
287 lingering tundra (PLS, mean 3.5 ± 1.2 pmol g^{-1} d^{-1}). These values were about 3-6 times
288 higher than normal upland tundra (UTS) (1.2 ± 1.3 pmol g^{-1} d^{-1}). These results indicated
289 that penguin activities may have increased the $CHCl_3$ emission from tundra soils.

290 Microorganisms, which are responsible for $CHCl_3$ production, are often unevenly
291 distributed and can lead to differences in $CHCl_3$ forming activity (Albers et al., 2011).
292 Ornithogenic soil on Ardley Island affected by penguin activities was more abundant in
293 organic carbon (TOC), nitrogen (TN) and phosphorus (TP) than other tundra soils (Table
294 S1), which were major factors influencing microbial populations in Antarctic soil
295 (Tscherko et al., 2003; Wang et al., 2019; Zhu et al., 2015). Fungi abundance analysis of
296 the Antarctic tundra soil samples showed that the majority of the microorganisms was in
297 the phylum of *Ascomycota* and *Basidiomycota*, to which most of CPOs-related fungi
298 belonged (Durán et al., 2019). Therefore, the higher $CHCl_3$ emission rates from penguin-
299 related tundra may be attributed to the colonization of CPO-related microorganisms in this
300 organic matter rich soil.

301 $CHCl_3$ fluxes across all the types of tundra soils also showed significant correlations (p
302 < 0.05) with TOC, TP, Cl, and Mc (Figure S2, S3). Compared to seal colony soil and
303 normal upland tundra soil, ornithogenic soil within the penguin colony and its adjacent
304 tundra were much richer in organic carbon (Table S1), which can provide organic
305 substrates for CPOs-catalyzed $CHCl_3$ production. The high frequency transit of penguins
306 between the sea and tundra brings seawater inland, and the sea breeze also blew in abundant
307 salt aerosols onto Ardley Island (especially the western side), which increased soil chloride
308 and moisture. All these factors induced by penguin activities and wind patterns potentially
309 led to the enhanced $CHCl_3$ emissions from tundra populated with penguins.

Temperature effects on $CHCl_3$ emission

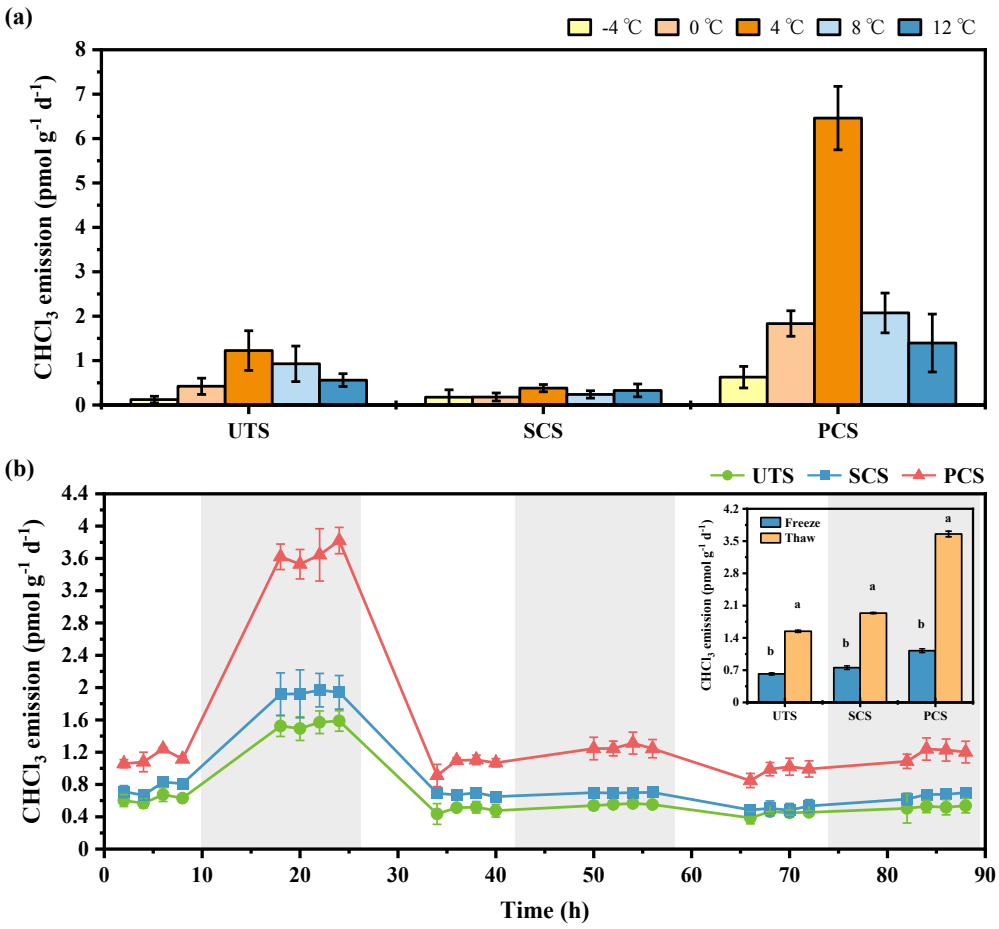

Figure 4. Chloroform ($CHCl_3$) emission rates based on laboratory soil incubations **(a)** at different temperatures; and **(b)** during three consecutive freeze-thaw cycles (FTCs) between -12 °C and 4 °C. The inserted plot showed the comparison of $CHCl_3$ emission rates in the first FTC. The incubated samples were collected from normal upland tundra (UTS), seal colony tundra (SCS), and active penguin colony tundra (PCS).

The temperature-gradient incubation experiments showed that Antarctic tundra soils were a source for $CHCl_3$ across the entire temperature range of -4 ~ 12 °C (Figure 4a). All tundra soils showed maximum production rates of $CHCl_3$ at 4 °C (close to local mean air temperature in summer); mean $CHCl_3$ emission rates decreased to close to zero at -4 °C and increased significantly when temperature rose above zero. Compared to 4 °C, $CHCl_3$ emission rates from tundra soils decreased at 8 °C and 12 °C, but they were still higher than those at -4 °C. The Antarctic region is highly sensitive to climate change (Bockheim et al., 2013; Schofield et al., 2010), and Western Antarctica, especially the Antarctic

Peninsula, has been experiencing the highest temperature increase (Bromwich et al., 2013; Vaughan et al., 2003). With additional warming, more days above freezing can be expected, which would influence the annual pattern of $CHCl_3$ emissions from Antarctic tundra.

Freeze-thaw cycles (FTCs) incubations showed that $CHCl_3$ emission rates were 3.3 times higher during thaw than during freeze under the first FTC for PCS, while for UTS and SCS, the emission rates were about 2.5 times higher (Table S3). As permafrost thaws, the increase of soil moisture and temperature can enhanced soil microbial activity and organic material release for microorganisms to access (Ludwig et al., 2006; Schimel & Clein, 1996). However, $CHCl_3$ emission rates did not significantly differ between the freeze and thaw incubations in the two following FTCs, with rates remaining low, similar to those during the first freeze period, but $CHCl_3$ emission rates from PCS were consistently higher than those from SCS and UTS (Figure 4b, Table S3). The lack of significant $CHCl_3$ emission rates in the two following FTCs is not necessarily predicted. It might be attributed to the depletion of substrates in the soil, such as chloride ions, iron (III), etc. More likely, the inhibited $CHCl_3$ emission rates in the following cycles may be attributed to a rapid FTC-induced reduction of microbial populations. A single rapid FTC can kill up to half of the soil microorganisms, leading to either reduced decomposition rates or to immobilization of nutrients tied up in the microbial biomass (Larsen et al., 2002; Schimel & Clein, 1996). Hence, the seasonal pattern of $CHCl_3$ emission needs to be considered when quantifying the annual fluxes.

Regional Assessment

Given the spatial and temporal variabilities of $CHCl_3$ fluxes and the limited number of measurements, the attempt to extrapolate to the continental scale would introduce significant uncertainties. More *in situ* studies are needed to capture diurnal, seasonal, and spatial variations of $CHCl_3$ emissions. However, since no prior fluxes have been measured

from Antarctic environment, a simple quantitative upscaling of these first-reported $CHCl_3$ fluxes is still worthwhile in assessing its influence on regional atmospheric compositions and on global $CHCl_3$ inventory.

Assuming the $CHCl_3$ fluxes measured from this study are representative of the 7.2×10^{10} m^2 ice-free tundra in Antarctic continent (Lee et al., 2017; Terauds & Lee, 2016), among which 30% is colonized or influenced by penguins or other sea animals (Zhu et al., 2008), during the austral summertime (assuming an active period of 100 days), and $CHCl_3$ flux in winter (the other 265 days) accounts for one-third of that based upon the FTCs experiments, it is estimated that Antarctic tundra emits 0.08 ± 0.02 Gg of $CHCl_3$ per year naturally, which constitutes a small fraction (< 1%) of global $CHCl_3$ terrestrial sources. The size of this $CHCl_3$ source is minor compared to other sources, largely due to the relatively small area of ice-free tundra in the Antarctica. Therefore, it may not exert a significant impact on global atmospheric $CHCl_3$ concentrations, such as mid-/low-latitudes and the other hemisphere. However, it may have a relatively greater influence on local $CHCl_3$ concentrations, especially given the relatively small portion of the troposphere in the Antarctic region. On the other hand, the rapid warming of the Antarctic continent is expanding the area of ice-free tundra and influencing the size of penguin populations and colonies (Barbraud et al., 2020; Lee et al., 2017; Lyver et al., 2014). Hence, the relative contribution to atmospheric $CHCl_3$ from tundra ecosystems will vary in response to these changes in the future.

Acknowledgments

This work was funded by National Natural Science Foundation of China (No. 41776190; 41976220), and National Key Research and Development Program of China (No. 2020YFA0608501) and the Strategic Priority Research Program of Chinese Academy of Sciences (No. XDB40010200). GC/MS analysis of soil incubations was conducted at

the University of California, Berkeley; GS/MS analysis of flask samples was conducted at Fujian Agriculture and Forestry University under the help of Prof. Zhigang Yi; fieldwork was conducted with assistance from Chinese National Antarctic Research Expedition team.

The data that supports the discussion and conclusion is all presented in manuscript and Supporting Information. A copy of the data is archived on Zenodo and available to download at (http://doi.org/).

References

Albers, C. N., Jacobsen, O. S., Flores, É. M. M., Pereira, J. S. F., & Laier, T. (2011). Spatial variation in natural formation of chloroform in the soils of four coniferous forests. *Biogeochemistry*, *103*(1–3), 317–334. https://doi.org/10.1007/s10533-010-9467-9

Albers, C. N., Jacobsen, O. S., Flores, E. M. M., & Johnsen, A. R. (2017). Arctic and subarctic natural soils emit chloroform and brominated analogues by alkaline hydrolysis of trihaloacetyl compounds. *Environmental Science & Technology*, *51*(11), 6131–6138. https://doi.org/10.1021/acs.est.7b00144

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*(1), 41–46. https://doi.org/10.1016/0038-0717(93)90239-8

Barbraud, C., Delord, K., Bost, C. A., Chaigne, A., Marteau, C., & Weimerskirch, H. (2020). Population trends of penguins in the French Southern Territories. *Polar Biology*, *43*(7), 835–850. https://doi.org/10.1007/s00300-020-02691-6

Bastviken, D., Svensson, T., Karlsson, S., Sandén, P., & Öberg, G. (2009). Temperature sensitivity indicates that chlorination of organic matter in forest soil is primarily biotic. *Environmental Science & Technology*, *43*(10), 3569–3573. https://doi.org/10.1021/es8035779

401 Bockheim, J., Vieira, G., Ramos, M., López-Martínez, J., Serrano, E., Guglielmin, M., et
402 al. (2013). Climate warming and permafrost dynamics in the Antarctic Peninsula
403 region. *Global and Planetary Change*, *100*, 215–223.
404 https://doi.org/10.1016/j.gloplacha.2012.10.018
405 Borchhardt, N., Schiefelbein, U., Abarca, N., Boy, J., Mikhailyuk, T., Sipman, H. J. M.,
406 & Karsten, U. (2017). Diversity of algae and lichens in biological soil crusts of
407 Ardley and King George islands, Antarctica. *Antarctic Science*, *29*(3), 229–237.
408 https://doi.org/10.1017/S0954102016000638
409 Breider, F., & Hunkeler, D. (2014a). Investigating chloroperoxidase-catalyzed formation
410 of chloroform from humic substances using stable chlorine isotope analysis.
411 *Environmental Science & Technology*, *48*(3), 1592–1600.
412 https://doi.org/10.1021/es403879e
413 Breider, F., & Hunkeler, D. (2014b). Mechanistic insights into the formation of
414 chloroform from natural organic matter using stable carbon isotope analysis.
415 *Geochimica et Cosmochimica Acta*, *125*, 85–95.
416 https://doi.org/10.1016/j.gca.2013.09.028
417 Bromwich, D. H., Nicolas, J. P., Monaghan, A. J., Lazzara, M. A., Keller, L. M.,
418 Weidner, G. A., & Wilson, A. B. (2013). Central West Antarctica among the most
419 rapidly warming regions on Earth. *Nature Geoscience*, *6*(2), 139–145.
420 https://doi.org/10.1038/ngeo1671
421 Cox, M. L., Fraser, P. J., Sturrock, G. A., Siems, S. T., & Porter, L. W. (2004). Terrestrial
422 sources and sinks of halomethanes near Cape Grim, Tasmania. *Atmospheric*
423 *Environment*, *38*(23), 3839–3852. https://doi.org/10.1016/j.atmosenv.2004.03.050

Croxall, J. P., Trathan, P. N., & Murphy, E. J. (2002). Environmental Change and Antarctic Seabird Populations. *Science*, *297*(5586), 1510–1514. https://doi.org/10.1126/science.1071987

Durán, P., Barra, P. J., Jorquera, M. A., Viscardi, S., Fernandez, C., Paz, C., et al. (2019). Occurrence of soil fungi in Antarctic pristine environments. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00028

Engel, A., Rigby, M., Burkholder, J. B., Fernandez, R. P., Froidevaus, L., Hall, B. D., et al. (2018). *Update on ozone-depleting substances (ODSs) and other gases of interest to the Montreal Protocol* (Chapter 1 in Scientific Assessment of Ozone Depletion: 2018, Global Ozone Research and Monitoring Project No. Report No. 58). Geneva, Switzerland: World Meteorological Organization. Retrieved from https://www.esrl.noaa.gov/csd/assessments/ozone/2018/citations.html

Fang, X., Park, S., Saito, T., Tunnicliffe, R., Ganesan, A. L., Rigby, M., et al. (2019). Rapid increase in ozone-depleting chloroform emissions from China. *Nature Geoscience*, *12*(2), 89. https://doi.org/10.1038/s41561-018-0278-2

Jiao, Y., Ruecker, A., Deventer, M. J., Chow, A. T., & Rhew, R. C. (2018). Halocarbon emissions from a degraded forested wetland in coastal South Carolina impacted by sea level rise. *ACS Earth and Space Chemistry*, *2*(10), 955–967. https://doi.org/10.1021/acsearthspacechem.8b00044

Johnsen, A. R., Jacobsen, O. S., Gudmundsson, L., & Albers, C. N. (2016). Chloroform emissions from arctic and subarctic ecosystems in Greenland and Northern Scandinavia. *Biogeochemistry*, *130*(1–2), 53–65. https://doi.org/10.1007/s10533-016-0241-5

Huber, S. G., Kotte, K., Schöler, H. F., & Williams, J. (2009). Natural abiotic formation of trihalomethanes in soil: results from laboratory studies and field samples.

Environmental Science & Technology, 43(13), 4934-4939. https://doi.org/10.1021/es8032605

Khalil, M. A. K., Rasmussen, R. A., Shearer, M. J., Chen, Z.-L., Yao, H., & Yang, J. (1998). Emissions of methane, nitrous oxide, and other trace gases from rice fields in China. *Journal of Geophysical Research: Atmospheres*, *103*(D19), 25241–25250. https://doi.org/10.1029/98JD01114

Khan, M. A. H., Rhew, R. C., Whelan, M. E., Zhou, K., & Deverel, S. J. (2011). Methyl halide and chloroform emissions from a subsiding Sacramento–San Joaquin Delta island converted to rice fields. *Atmospheric Environment*, *45*(4), 977–985. https://doi.org/10.1016/j.atmosenv.2010.10.053

Khan, M. A. H., Whelan, M. E., & Rhew, R. C. (2012). Effects of temperature and soil moisture on methyl halide and chloroform fluxes from drained peatland pasture soils. *Journal of Environmental Monitoring*, *14*(1), 241–249. https://doi.org/10.1039/C1EM10639B

Larsen, K. S., Jonasson, S., & Michelsen, A. (2002). Repeated freeze–thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology*, *21*(3), 187–195. https://doi.org/10.1016/S0929-1393(02)00093-8

Lee, J. R., Raymond, B., Bracegirdle, T. J., Chadès, I., Fuller, R. A., Shaw, J. D., & Terauds, A. (2017). Climate change drives expansion of Antarctic ice-free habitat. *Nature*, *547*(7661), 49–54. https://doi.org/10.1038/nature22996

Ludwig, B., Teepe, R., Lopes de Gerenyu, V., & Flessa, H. (2006). CO_2 and N_2O emissions from gleyic soils in the Russian tundra and a German forest during freeze–thaw periods—a microcosm study. *Soil Biology and Biochemistry*, *38*(12), 3516–3519. https://doi.org/10.1016/j.soilbio.2006.06.006

Lyver, P. O., Barron, M., Barton, K. J., Ainley, D. G., Pollard, A., Gordon, S., et al. (2014). Trends in the breeding population of Adélie penguins in the Ross Sea, 1981–2012: a coincidence of climate and resource extraction effects. *PLOS ONE*, *9*(3), e91188. https://doi.org/10.1371/journal.pone.0091188

Macdonald, M. L., Wadham, J. L., Young, D., Lunder, C. R., Hermansen, O., Lamarche-Gagnon, G., & O'Doherty, S. (2020). Consumption of CH_3Cl, CH_3Br, and CH_3I and emission of $CHCl_3$, $CHBr_3$, and CH_2Br_2 from the forefield of a retreating Arctic glacier. *Atmospheric Chemistry and Physics*, *20*(12), 7243–7258. https://doi.org/10.5194/acp-20-7243-2020

McCulloch, A. (2003). Chloroform in the environment: occurrence, sources, sinks and effects. *Chemosphere*, *50*(10), 1291–1308. https://doi.org/10.1016/S0045-6535(02)00697-5

Michel, R. F. M., Schaefer, C. E. G. R., Dias, L. E., Simas, F. N. B., Benites, V. de M., & Mendonça, E. de S. (2006). Ornithogenic gelisols (cryosols) from maritime Antarctica. *Soil Science Society of America Journal*, *70*(4), 1370–1376. https://doi.org/10.2136/sssaj2005.0178

Pickard, M. A., & Hashimoto, A. (1988). Stability and carbohydrate composition of chloroperoxidase from Caldariomyces fumago grown in a fructose–salts medium. *Canadian Journal of Microbiology*, *34*(8), 998–1002. https://doi.org/10.1139/m88-175

Prinn, R. G., Weiss, R. F., Arduini, J., Arnold, T., DeWitt, H. L., Fraser, P. J., et al. (2018). History of chemically and radiatively important atmospheric gases from the Advanced Global Atmospheric Gases Experiment (AGAGE). *Earth System Science Data*, *10*(2), 985–1018. https://doi.org/10.5194/essd-10-985-2018

Rhew, R. C., Teh, Y. A., Abel, T., Atwood, A., & Mazéas, O. (2008a). Chloroform emissions from the Alaskan Arctic tundra. *Geophysical Research Letters*, *35*(21). https://doi.org/10.1029/2008GL035762

Rhew, R. C., Miller, B. R., & Weiss, R. F. (2008b). Chloroform, carbon tetrachloride and methyl chloroform fluxes in southern California ecosystems. *Atmospheric Environment*, *42*(30), 7135–7140. https://doi.org/10.1016/j.atmosenv.2008.05.038

Ruecker, A., Weigold, P., Behrens, S., Jochmann, M., Laaks, J., & Kappler, A. (2014). Predominance of biotic over abiotic formation of halogenated hydrocarbons in hypersaline sediments in Western Australia. *Environmental Science & Technology*, *48*(16), 9170–9178. https://doi.org/10.1021/es501810g

Ruecker, A., Weigold, P., Behrens, S., Jochmann, M., Barajas, X. L. O., & Kappler, A. (2015). Halogenated hydrocarbon formation in a moderately acidic salt lake in Western Australia – role of abiotic and biotic processes. *Environmental Chemistry*, *12*(4), 406–414. https://doi.org/10.1071/EN14202

Schaefer, C. E. G. R., Simas, F. N. B., Gilkes, R. J., Mathison, C., da Costa, L. M., & Albuquerque, M. A. (2008). Micromorphology and microchemistry of selected Cryosols from maritime Antarctica. *Geoderma*, *144*(1), 104–115. https://doi.org/10.1016/j.geoderma.2007.10.018

Schimel, J. P., & Clein, J. S. (1996). Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology and Biochemistry*, *28*(8), 1061–1066. https://doi.org/10.1016/0038-0717(96)00083-1

Schofield, O., Ducklow, H. W., Martinson, D. G., Meredith, M. P., Moline, M. A., & Fraser, W. R. (2010). How do polar marine ecosystems respond to rapid climate change? *Science*, *328*(5985), 1520–1523. https://doi.org/10.1126/science.1185779

521 Sun, L., Xie, Z., & Zhao, J. (2000). A 3,000-year record of penguin populations. *Nature*,
522 *407*(6806), 858. https://doi.org/10.1038/35038163
523 Terauds, A., & Lee, J. R. (2016). Antarctic biogeography revisited: updating the
524 Antarctic conservation biogeographic regions. *Diversity and Distributions*, *22*(8),
525 836–840. https://doi.org/10.1111/ddi.12453
526 Tscherko, D., Bölter, M., Beyer, L., Chen, J., Elster, J., Kandeler, E., et al. (2003).
527 Biomass and enzyme activity of two soil transects at King George Island,
528 maritime Antarctica. *Arctic, Antarctic, and Alpine Research*, *35*(1), 34–47.
529 https://doi.org/10.1657/1523-0430(2003)035[0034:BAEAOT]2.0.CO;2
530 Van Schijndel, J. W., Barnett, P., Roelse, J., Vollenbroek, E. G., & Wever, R. (1994).
531 The stability and steady-state kinetics of vanadium chloroperoxidase from the
532 fungus Curvularia inaequalis. *European Journal of Biochemistry*, *225*(1), 151–
533 157. https://doi.org/10.1111/j.1432-1033.1994.00151.x
534 Vaughan, D. G., Marshall, G. J., Connolley, W. M., Parkinson, C., Mulvaney, R.,
535 Hodgson, D. A., et al. (2003). Recent Rapid Regional Climate Warming on the
536 Antarctic Peninsula. *Climatic Change*, *60*(3), 243–274.
537 https://doi.org/10.1023/A:1026021217991
538 Wang, J.-J., Jiao, Y., Rhew, R. C., & Chow, A. T. (2016). Haloform formation in coastal
539 wetlands along a salinity gradient at South Carolina, United States. *Environmental*
540 *Chemistry*, *13*(4), 745–756. https://doi.org/10.1071/EN15145
541 Wang, Q., Zhu, R., Zheng, Y., Bao, T., & Hou, L. (2019). Effects of sea animal
542 colonization on the coupling between dynamics and activity of soil ammonia-
543 oxidizing bacteria and archaea in maritime Antarctica. *Biogeosciences*, *16*(20),
544 4113–4128. https://doi.org/10.5194/bg-16-4113-2019

545 Wever, R., & Barnett, P. (2017). Vanadium chloroperoxidases: the missing link in the
546 formation of chlorinated compounds and chloroform in the terrestrial
547 environment? *Chemistry – An Asian Journal*, *12*(16), 1997–2007.
548 https://doi.org/10.1002/asia.201700420
549 Worton, D. R., Sturges, W. T., Schwander, J., Mulvaney, R., Barnola, J.-M., &
550 Chappellaz, J. (2006). 20th century trends and budget implications of chloroform
551 and related tri-and dihalomethanes inferred from firn air. *Atmospheric Chemistry*
552 *and Physics*, *6*(10), 2847–2863. https://doi.org/10.5194/acp-6-2847-2006
553 Yang, L., Gao, Y., Sun, L., Xie, Z., Yang, W., Chu, Z., et al. (2019). Enhanced westerlies
554 drove penguin movement at 1000 yr BP on Ardley Island, west Antarctic
555 Peninsula. *Quaternary Science Reviews*, *214*, 44–53.
556 https://doi.org/10.1016/j.quascirev.2019.04.026
557 Zdanowski, M. K., Zmuda, M. J., & Zwolska, I. (2005). Bacterial role in the
558 decomposition of marine-derived material (penguin guano) in the terrestrial
559 maritime Antarctic. *Soil Biology and Biochemistry*, *37*(3), 581–595.
560 https://doi.org/10.1016/j.soilbio.2004.08.020
561 Zhu, R., Kong, D., Sun, L., Geng, J., Wang, X., & Glindemann, D. (2006). Tropospheric
562 phosphine and its sources in coastal Antarctica. *Environmental Science &*
563 *Technology*, *40*(24), 7656–7661. https://doi.org/10.1021/es061601e
564 Zhu, R., Liu, Y., Xu, H., Ma, J., Zhao, S., & Sun, L. (2008). Nitrous oxide emissions
565 from sea animal colonies in the maritime Antarctic. *Geophysical Research*
566 *Letters*, *35*(9). https://doi.org/10.1029/2007GL032541
567 Zhu, R., Liu, Y., Ma, E., Sun, J., Xu, H., & Sun, L. (2009). Greenhouse gas emissions
568 from penguin guanos and ornithogenic soils in coastal Antarctica: Effects of

freezing–thawing cycles. *Atmospheric Environment*, *43*(14), 2336–2347. https://doi.org/10.1016/j.atmosenv.2009.01.027

Zhu, R., Liu, Y., Xu, H., Ma, D., & Jiang, S. (2013). Marine animals significantly increase tundra N_2O and CH_4 emissions in maritime Antarctica. *Journal of Geophysical Research: Biogeosciences*, *118*(4), 1773–1792. https://doi.org/10.1002/2013JG002398

Zhu, R., Shi, Y., Ma, D., Wang, C., Xu, H., & Chu, H. (2015). Bacterial diversity is strongly associated with historical penguin activity in an Antarctic lake sediment profile. *Scientific Reports*, *5*(1), 17231. https://doi.org/10.1038/srep17231