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Microbial metabolism directly affects trace gases in (Sub) Polar snowpacks

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1. Title: Microbial metabolism directly affects trace gases in (Sub) Polar snowpacks

Running Title: Microbial metabolism in Polar snowpack

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2. Abstract

Concentrations of trace gases trapped in ice are considered to develop uniquely from direct snow/atmosphere interactions at the time of contact. This assumption relies upon limited or no biological, chemical or physical transformations occurring during transition from snow to firn to ice; a process that can take decades to complete. Here, we present the first evidence of environmental alteration due to *in situ* microbial metabolism of trace gases (methyl halides and dimethyl sulphide) in Polar snow. We collected evidence for ongoing microbial metabolism from an Arctic and an Antarctic location during different years. Methyl iodide production in the snowpack decreased significantly after exposure to enhanced UV radiation. Our results also show large variations in the production and consumption of other methyl halides, including methyl bromide and methyl chloride, used in climate interpretations. These results suggest that this long neglected microbial activity could constitute a potential source of error in climate history interpretations, by introducing a so far unappreciated source of bias in the quantification of atmospheric-derived trace gases trapped within the Polar ice caps.

3. Introduction

Snow is a highly porous environment, exchanging and entrapping air from the surrounding environment. As more snow is deposited onto the surface of the snowpack, older snow layers compress eventually into ice, encasing small samples of the atmosphere existing over and within the snow at the time of deposition. This simple mechanism of glacial formation was described in the 1990's (Bender *et al*, 1997), and has been presented as a justification to use greenhouse gases (CO_2, CH_4) entrapped in glacial ice as a proxy for atmospheric compositions (and hence, climate conditions) back in time. This same logic has been used to justify the quantification of shorter-lived, more reactive trace gases in ice cores including

methyl bromide (Saltzman *et al*, 2008) and methyl chloride (Saltzman *et al,* 2009, Verhult *et al*, 2013). However, these methods rest on the assumption that the snowpack is quasi-sterile metabolically, or at least, that microbial production/consumption of these trace gases is not significant.

Methyl halides, including methyl chloride, methyl bromide and methyl iodide are particularly interesting choices due to their roles in microbial metabolism and atmospheric chemistry. Methyl chloride and methyl bromide together are responsible for approximately 25% of the annual ozone loss (Butler, 2000). Methyl iodide affects local air quality and influences atmospheric degradation rates for longer lived compounds such as methane through its influence on hydroxyl radical concentrations (Tegtmeier *et al*, 2013). Methyl chloride and methyl bromide can be formed directly through chemical interactions in soil (Keppler et al, 2000), but are more commonly produced through active metabolism of eukaryotic organisms (fungi: Watling & Harper, 1998; Redeker *et al*, 2004; plants: Rhew *et al*, 2003; Redeker *et al*, 2004b; Saito & Yokouchi, 2006). To date, only prokaryotes (bacteria) have been observed to consume methyl chloride and methyl bromide (Borodina *et al*, 2005) and they are able to utilise these compounds as their sole energy substrate. Methyl iodide has been observed to be produced by bacteria (Amachi *et al*, 2001), fungi (Redeker *et al*, 2004) and plants (Redeker *et al*, 2004b) and is preferentially generated relative to the other methyl halides in most cases. Genetic sequences and enzymatic mechanisms for bacterial consumption of methyl chloride and methyl bromide have been identified (McAnnula *et al*, 2001), as well as a suite of homologues for methyl halide production in plants (Nagatoshi & Nakamura, 2007). There remains uncertainty regarding whether all primary mechanisms for monohalogenated metabolism have been identified (Redeker *et al*, 2004b; Manley, 2002).

Polar environments represent some of the most extreme environments on Earth, and the assumption of an effectively biologically inactive snowpack has been considered to be well

within reason. For example, Arctic average winter daytime temperatures range from -34° to 0° C, and available water, nutrients and sunlight are limited throughout the year (Przybylak, 2003). Antarctic conditions can be even more extreme (Carpenter *et al*, 2000). Furthermore, high UV levels occur commonly in Polar environments, and especially in the southern hemisphere spring (Sept-Nov) during the maximum extent of the ozone hole (Bargagli, 2005), further limiting the ability of microbial life to maintain significant levels of activity.

The limitations of these extreme conditions have recently been questioned. UV radiation appears to be significantly less harmful to sub-surface microbial communities since, while UV is easily transmitted once it has penetrated, penetration is limited by the surface snow which is a good scatterer (Gorton *et al*, 2003). Critically, lab-based results have shown that the temperatures experienced by polar snowpacks, even within the most remote and extreme locations, can support microbial metabolism (Price & Sowers, 2004).

Microbial presence is ubiquitous in the Polar Regions, and recent research into the Polar aerobiome points toward a dynamic Polar microbial community and the possibility of significant input of metabolically active bacteria onto the snowpack (Pearce *et al*, 2016), even to remote locations (Pearce *et al*, 2009; Herbold *et al*, 2014). To this end, research into the aerobiome and Polar environments have demonstrated that microorganisms in aerial fallout remain viable, as cultures from aerobiological samples can grow under favourable conditions (Sattler *et al*, 2001; Harding *et al*, 2011). Furthermore, the presence of microbes in remote, low nutrient, low water, very cold environments such as Polar glacial surfaces and their snowpacks is well established (Larose *et al*, 2013; Hodson *et al*, 2017).

However, the level to which microorganisms are metabolically active in the snow pack as its water content becomes scarce and temperatures drop remains contentious, as the only evidence to date remains correlative or circumstantial (Carpenter *et al*, 2000; Price & Sowers,

2004; Michaud *et al*, 2014). Research has shown that microorganisms can be incredibly persistent, even deep within high plateau polar ice, remaining culturable even after hundreds of thousands of years (See Price & Sowers, 2004 references). Lab-based evidence suggests that microbes are at least capable of metabolic maintenance activities, even at very low temperatures (Price & Sowers, 2004) but what potential thresholds exist that determine active versus maintenance metabolism in polar snowpack, are unknown (Price, 2006).

It is clear that microorganisms have played a major role in the Earth's current and past climate (Falkowski *et al*, 2008), and affect Polar biogeochemical cycles (Larose *et al*, 2013; Hodson *et al*, 2017). Therefore, identifying whether microorganisms remain active in the Polar snow pack, and hence which type of metabolic activity and ecological role they play, is important.

Exploring Polar snowpack environments for microbial metabolism is challenging, in particular due to the complex nature of the unconsolidated snow and a range of interfering signals from physical, chemical and biological sources. Snowpack tends to be a high exposure environment, with substantial wind-driven mixing of boundary layer air with sub-surface snow pore space air (Massman & Frank, 2005; Redeker *et al*, 2015). Concurrently, snow is readily transparent to a range of UV-Visible light, which is known to drive substantial photochemical reactions, including methyl halide production (Swanson *et al*, 2007). The quasi-liquid layer on the surface of snow particles incorporates complex chemical reactions and provides limited habitat for microbial life (Price, 2006) while seasonality drives snow pack thinning and expansion (Bender *et al*, 1997), and longer timeframes lead to compression, consolidation and removal from atmospheric influence (Bender *et al*, 1997). Nearby and sub-snowpack soils can also influence snowpack air chemistry through diffusion/advection from local biological sources/sinks with access to more favourable environments (Swanson *et al*, 2005; Helmig *et al*, 2009).

To explore whether it is possible to directly detect signals of ongoing metabolism from microbial constituents in Polar snowpack we have developed and deployed a trace gas sampling system that minimizes interfering signals from physical, chemical and alternative biological sources. This sampling system uses methyl halides (and other parts-per-trillion-by-volume, pptv, concentration metabolites) as chemical probes, to maximize the potential of observing substantial change in metabolite concentrations over short time scales (<2 hours). We tested the sampling system in optimal temperature and biological loading conditions at Signy Island, Antarctica during the Antarctic Spring of 2012 and the system was re-deployed in Svalbard during the Arctic Summer of 2015.

Here, we provide the first direct *in situ* evidence of continuous microbial metabolism of methyl halides in Polar snowpack. Our results show active methyl iodine production and some level of methyl bromide consumption. Thus, we show how microbial activity can alter the concentration of trace gases trapped within the snowpack, which could potentially constitute a source of error in climate history interpretations based on firn and ice core data.

4. Methods

Study Sites

Methyl halide and dimethyl sulphide fluxes were measured in two locations, one Arctic and one Antarctic. The Antarctic site was located at Signy Island (60.718 S, 45.632 W) on the Gourlay Snowfield, where measurements were taken between December 3rd and December 21st, 2012. The Arctic site was Larsbreen glacier, near the settlement of Longyearbyen, Svalbard (78.223 N, 15.627 E), where measurements were taken between June 29th and July 19th, 2015 (Figure 1). Thus, the sampling dates correspond with the Antarctic Spring and the Arctic Summer. All sampling sites presented relatively thick snowpacks (0.8 – 1.5 m) over glacial ice, and they were at least 100 m from the glacier edge. Sites were selected to be far

enough from soils to avoid soil biological effects from trace gases diffusing through the snowpack (Swanson *et al*, 2005; Helmig *et al*, 2009; Redeker *et al*, 2015).

Environmental conditions at Signy were similar to those in Svalbard, with temperatures in ambient air ranging from -3.0 to +15.8° C (Signy) and from +1.9 to 8.2° C (Svalbard). Snowpack temperatures lay at the melting point at Signy and from -2.8 to 0° C on Svalbard. Ambient temperatures in Signy were strongly affected by daytime sunlight, with highest temperatures occurring at mid-day and coldest temperatures during the limited night. Therefore, localised re-freezing at the surface of the snow occurred at Signy. Wind speeds were between +1.5 to +8.2 m/s at Signy while Svalbard experienced winds ranging from 0.0 to +6.8 m/s (6.8 m/s is equivalent to ~15 miles per hour) during sampling periods.

Site preparation

We installed three paired sample chambers in Signy Island and four pairs in Svalbard. Each pair was composed of one less-impacted, control chamber ("living snowpack") and one irradiated with UV light. Chamber placements of this nature will influence the local snowpack environment through heat retention and wind blocking. Efforts were made to reduce these impacts, particularly through limited placement periods prior to sampling. The chambers were either placed directly into the snow (Signy), or pinned to the snowpack using 50 cm stainless steel pegs (Svalbard). The PVC chamber bases were 30 cm inner diameter and 6 cm height. The distance between each pair of chambers was approximately 10 m (Figure 2). Trace gas measurements were taken 2 to 4 days after the chamber bases were installed.

Snow in the enhanced UV exposure chamber was irradiated using UV sterilization lamps (UV Light Technology) with 2 parallel UV bulbs (17 W Phillips F17T8 bulbs UV-C), placed vertically in the snowpack (UV lamp length = 61 cm), in line with the snow beneath the

irradiated chamber. Thus, the irradiated snowpack was directly exposed to high intensity UV-C light (Figure 2). The UV lamps were placed so that there would be no direct effect on the living control chamber. Although subject to surface scattering, UV transmission within snowpack is enhanced by minimal absorption, travelling well over 1 m with high transmission efficiencies (Wiscombe & Warren, 1980).

Each pair of chambers was covered by an opaque 3x3 m black plastic tarp, leaving 1 m from the chamber to the edge of the tarp, to avoid sunlight-driven photochemical reactions (Swanson *et al*, 2007). In addition, the distance between the chambers and tarp edge reduced the impact of wind-driven horizontal transport and mixing of atmospheric air with pore spaces in the snowpack (Bender *et al* 1997; Redeker *et al*, 2015).

Trace gas sampling

After ≥2 days under tarpaulin-induced blackout conditions, the section of the tarpaulin directly over the chamber base was removed and each PVC chamber base was immediately covered with an opaque, blacked-out polycarbonate chamber top for headspace sampling. Trace gas samples were taken at 0 (immediately after placement), 60, and 120 min post-chamber top placement. Trace gas sampling canisters were connected to the lid of the polycarbonate chamber top (total chamber volume = ~28 L) with a ¼" sulfinert-coated stainless steel sampling line (Restek, Bellefonte, PA) that incorporated a 15 cm long Ascarite trap. Gas samples were drawn via pressure differential into previously evacuated 0.5-L electropolished stainless steel canisters (LabCommerce Inc, San Jose, CA) (Figure 2). Chamber base, top and Ascarite traps (for carbon dioxide and partial water removal) have previously been used for similar experiments and shown to be inert for the gases measured here (Redeker & Cicerone, 2004; Redeker *et al*, 2007; Redeker & Kalin, 2012).

After the first round of trace gas sampling the central sections of the blackout tarps were re-installed and the irradiated chambers were exposed to high intensity UV light for one hour. After UV-C light exposure the chambers were left for 30 minutes then resampled (Signy) or a further 24 hours before re-sampling (Svalbard). Post-exposure time allowed reactive (Signy) and both reactive and moderately reactive (Svalbard) photochemically-derived products to dissipate.

Snowpack and air temperature were measured for each trace gas flux chamber placement, as was local wind speed. General weather conditions in the days before and during sampling were also recorded.

Trace gas flux analysis

Canisters were shipped directly post-sampling to the University of York for analysis. Trace gas concentrations were analysed on a HP 5972 GC/MSD fitted with a PoraPlot Q column (25m, 0.32 ID, 5µm thickness; Restek, Bellefonte, PA), similar to methods used in previous studies (Redeker & Cicerone, 2004; Redeker & Kalin, 2012). This instrument has been equipped with low concentration halocarbon and dimethyl sulphide (DMS) gas standards and calibration tests indicate detection limits of ~0.2 pptv for methyl iodide, <1.0 pptv for methyl bromide, <10pptv for dimethyl sulphide and <50 pptv for methyl chloride. Method reproducibility is better than 8% for standard injections (Redeker & Cicerone, 2004; Redeker & Kalin, 2012).

Fluxes from snowpack are calculated based on the difference in headspace concentration over time,

$$Flux_{MeX} = \Delta[MeX]/ \Delta t$$

where Δ[MeX] represents the change in headspace MeX concentration over the time period sampled, Δt. The chambers used in this study are designed to allow pressure equilibration between the interior and exterior as samples are removed. Pressure equilibration is necessary to avoid oversampling snowpack pore spaces (Xu *et al*, 2006). However, as a consequence, our reported fluxes slightly underestimate due to a ~3% dilution of chamber air over the course of the experiment. Living snow samples, either pre-treatment or post-treatment were not significantly different and were therefore combined in the comparative analyses between irradiated treatments and non-irradiated treatments.

Microbial sampling and analysis

Signy

Snowpack was collected after the second set of trace gas flux samples (post-irradiation) from within chamber footprints. At least 2 L of snow was collected, transported directly to lab facilities located in Signy Research Station, located in Factory Cove, Borge Bay, and analysed on site.

At the research station, we filtered 2 L of melted snow per site through a 47 mm diameter 0.2 µm filter (Millipore, GTTP04700). DNA was recovered from the filter using a RapidWater DNA Isolation kit (MoBio, 14810-50-NF), eluted in 100 µL of water and stored at -20°C. Subsequently, 5 µL of purified DNA was subjected to 35 rounds of PCR in a 25 µL reaction volume, with an annealing temperature of 50°C using GoTaq Colourless MasterMix (Promega, M7142) and primer pairs 8f (5'-CAG ACT TTG ATY MTG GCT CAG-3') and 1492r (5'- RGY TAC CTT GTT ACG ACT T-3'), or ARCH349f (5'- GYG CAS CAG KCG MGA AW-3') and ARCH806R (5'- GGA CTA CVS GGG TAT CTA AT-3') (Takai & Horikoshi, 2000) at a final concentration of 10 µM. Successful PCR reaction was confirmed

by electrophoresis using 1.2% Flashgel (Lonza), 4 µL of the completed PCR reaction and 1 µL 5x Flashgel loading dye (Lonza).

Svalbard

Surface snow was collected in Twirl'em® sterile sampling bags with sterile gloves after the second set of trace gas flux samples (post-irradiation) and from within the chamber footprints. Samples were taken to The University Centre in Svalbard (UNIS) to be analysed within the following 24 hours. Samples were stored in the interim at 6° C.

150 g of snow from each site was filtered thru a 0.2 µm Whatman® hydrophilic polycarbonate membrane. 10 µl of filtrate from the first paired set of living control and irradiated samples were inoculated on 3 different solid media: Bacto Agar, Polygalacturonate (PGA) and Reasoner's 2A agar (R2A); and grown at room temperature (21° C) and at 6° C. Two replicates were made for each media at each temperature. Observations were made 10 days after inoculation. 50 µl filtrate from the remaining paired sets was placed on 0.2 µm Whatman® hydrophilic polycarbonate membranes with 10 µl of 1 mM 5-cyano-2, 3-ditotyl tetrazolium chloride (CTC- a fluorescent stain that binds to DNA of actively respiring cells) for 10 minutes. Excess stain was removed with 500 µl PBS and the filter was air-dried for 5 minutes before it was mounted on a glass slide. Viable, CTC-binding cells were counted (in 12 randomly selected, separate visual fields) using a Nikon ECLIPSE E200 microscope with an E2-FM epi-fluorescence attachment. In filters where limited cells were observed, the process was repeated with another 50 µl of sample as described but with the addition of 10 µl of 1 g/ml 4'-6 diamino-2 phenylindole (DAPI) solution instead of CTC. DAPI binds to both alive and dead cells and this step was performed as a positive control to quantify the number of dead cells present.

5. Results

Trace gas fluxes from snowpack

All compounds studied behaved in ways consistent with biological influence, however there were substantive differences in behaviour between sites, compounds and UV treatment (Table 1; Figure 3).

Methyl iodide

Methyl iodide showed consistent, significant differences in fluxes originating from enhanced UV exposure versus living snowpack (t-test; $p<0.05$; Fig 3). At both Signy Island and at Svalbard living snow generated methyl iodide at low rates (31±17 and 9±8 ng m^{-2} d^{-1} at Signy and Svalbard respectively, Fig 3), despite methyl iodide's highly reactive nature (methyl iodide has a very strong methylating capacity) (Baowei *et al*, 2006). Once irradiated, the snowpack at both locations consumed methyl iodide (-290±270 and -30±24 ng m^{-2} d^{-1} at Signy and Svalbard respectively, Fig 3). Fluxes of methyl iodide were consistently, significantly different from zero flux between snowpack and ambient air (t-test, $p<0.05$ for both living controls at Signy and Svalbard, as well as snow with enhanced UV radiation at Svalbard). There were no significant correlations between methyl iodide fluxes and snowpack temperature, chamber temperature or local wind speeds.

Methyl bromide and methyl chloride

Methyl bromide and methyl chloride fluxes varied substantially across the sampling sites chosen at Signy and Svalbard (Table 1). Despite this large variability in chamber-to-chamber behaviour, methyl bromide was consistently consumed by the snowpack at both Signy and Svalbard, for both living and irradiated conditions (-74±47 and -19±20 ng m^{-2} d^{-1} in living controls at Signy and Svalbard respectively, as well as -130±50 and -6±20 ng m^{-2} d^{-1} in irradiated chambers at Signy and Svalbard). Fluxes were significantly different from zero for living controls at Signy (t-test, $p<0.1$) and Svalbard (t-test, $p<0.05$), and for enhanced UV

radiation snowpack at Signy (t-test, $p<0.05$) (Table 1, Fig. 3). No statistical difference in methyl bromide behaviour was observed between irradiated and living snowpack. Similarly, the majority of living (14 out of 21) and sterilized (5 out of 7) chamber locations at Svalbard and Signy removed methyl chloride from chamber headspace (Table 1, Fig. 3) although average fluxes were not significantly different from zero. While not significant, there is a trend towards greater methyl chloride removal from irradiated chambers. There were no significant correlations between methyl bromide and methyl chloride fluxes and snowpack temperature, chamber temperature or local wind speeds.

Dimethyl sulphide

At Signy Island dimethyl sulphide fluxes were not significantly different from zero (0±100 and 70±130 ng m^{-2} d^{-1} in living controls and irradiated chambers respectively). At Svalbard however, consumption within the snowpack was observed (-80±150 and -130±60 ng m^{-2} d^{-1} in living controls and irradiated chambers respectively; t-test, $p<0.05$; Fig. 3). UV irradiated snowpack did not behave significantly differently from living snowpack during this study period. There were no significant correlations between dimethyl sulphide fluxes and snowpack temperature, chamber temperature or local wind speeds.

Microbial analyses

Inoculated microbial cultures from Svalbard showed that viable cells were present in living control snowpack samples, and that a variable number of viable cells persisted in irradiated snowpack after UV exposure. These results were supported by CTC fluorescent staining, which detected the presence of viable cells within all sites after UV exposure (Table 2). Although viable cells were present after irradiation, CTC stain counts show that their number was significantly lower in irradiated sites than in living controls (ANOVA: $F = 47.16$; $d.f. = 1.66$; $p\text{-value} < 0.001$).

DNA recovered from two experimental sites at Signy were examined by PCR to determine whether a measurable effect could be detected in snowpack microbial communities treated with UV. Results were consistent with the inoculated microbial cultures, in that they show reduction (but not complete restriction) in UV exposed microbial populations. However, domain-specific effects were also observed. Archaea-specific probes demonstrated significant reduction, up to complete removal (2 out of 5 samples), after UV treatment, but differences between treated and untreated samples were not detected when using universal bacterial 16S primers (n = 5).

6. Discussion

Our data represents the first unequivocal and *in situ* measurement of ongoing microbial metabolism in Polar snowpack. Our observed fluxes from living snowpack are consistent with microbial metabolisms previously observed in terrestrial and marine environments, including methyl iodide production (Amachi *et al*, 2001), and methyl chloride and methyl bromide consumption (McAnnula *et al*, 2001). Likewise, the snowpack response to irradiation broadly conforms to the reduction of a microbial signal combined with an enhanced chemical signal, with reduced methyl bromide consumption and little or no methyl iodide production. Probable chemical uptake of methyl iodide and dimethyl sulphide is observed post irradiation while methyl chloride and methyl bromide responses to irradiation are variable.

Based on our observed results, fluxes of trace gases from the snowpack are derived from a complex mixture of physical, chemical and biological processes. Methyl iodide fluxes in living, non-irradiated samples are determined primarily by biological production processes, masking chemical removal rates. Fluxes of methyl bromide appear to combine chemical

substitution reactions with biological consumption to generate greater removal rates in snowpack than either individually.

Our sampling methodology minimized the effects of sunlight since methyl chloride, methyl bromide and methyl iodide are known to be photochemically generated in snowpack (Swanson *et al*, 2007). As a consequence of this we observe, in the living snowpack, methyl iodide production while methyl bromide is uniformly consumed. These processes are consistent with the known metabolisms of marine and terrestrial microorganisms but are inconsistent with a photochemical signal in which both methyl iodide and methyl bromide would be expected to be produced. Furthermore, if photochemistry was the driving mechanism for trace gas fluxes, we would expect to see significant increases in production of all methyl halides, and especially methyl chloride, post irradiation (Swanson *et al*, 2007). In the irradiated samples however methyl chloride removal rates appear to be enhanced while methyl iodide is removed, in contrast to living control samples. Methyl bromide fluxes also contradict a photochemically dominated process. We might expect significant enhancement of methyl bromide production after UV irradiation but instead we see site-specific, variable reduction in uptake, as we might expect if the bacterial population responsible for consumption was both heterogeneously distributed and variably sensitive to irradiation.

Methyl halides are chemically removed in aqueous systems through substitution reactions following the precedence of hydroxyl>chloride>bromide>iodide ions (Elliot & Rowland, 1993). In these reactions we would expect methyl iodide to be removed most rapidly since available hydroxyl, chloride and bromide ions in the quasi-liquid layer substitute efficiently to transform methyl iodide into methanol, methyl chloride and methyl bromide respectively. These chemical reactions cannot be the determining factor for snowpack methyl iodide flux, since living, non-irradiated sample fluxes were uniformly positive. The substitution reaction may be an important component of the processes by which methyl iodide is removed post

irradiation, however the predicted reaction rates for methyl iodide substitution reactions are lower than the observed snowpack removal rates.

Observed loss rates of methyl bromide in chambers were 12.5% over 2 hours in Signy samples, and 10% over 2 hours in Svalbard. These equate to daily removal rates of >70%. If we take seawater substitution reaction rates (King & Saltzman, 1997) as an extreme example (temperature in snowpack is lower, and ionic concentration is higher in seawater) it is clear that the observed degradation rates in snowpack are significantly higher than expected through chemical reactions alone. For instance, we would expect approximately 10% of the starting concentration of methyl bromide within the chamber to react over the course of a day through substitution with hydroxyl and chloride ions and reactions with other available organics (King & Saltzman, 1997). The room temperature, filtered/autoclaved seawater chemical reaction rate measured in King and Saltzman (1997) is much smaller than the observed reaction rate in Signy and Svalbard snowpack and the chemical reaction rate is expected to diminish by a factor of four for each 10° C temperature drop.

The observed signal for methyl bromide is also greater than expected for microbial consumption rates alone. Methyl bromide and methyl chloride are consumed by bacteria in soils (Borodina *et al*, 2005; Redeker & Kalin, 2012). Fungal production may play a role in net fluxes from terrestrial surfaces (Watling & Harper, 1998; Redeker *et al*, 2004). The impact of archaea on methyl halide cycling is not yet established and they may play a role in either methyl halide production or consumption within soils and snowpacks. In temperate forest soils, with an estimated 0.1 billion microbial cells per cubic centimetre (Raynaud & Nunan, 2014), methyl bromide is reported to be consumed at a rate of 5 μg m^{-2} day^{-1} (Redeker & Kalin, 2012). If we assume that the density of microbial cells in snowpack is ~50,000 cc^{-1} (Hell *et al*, 2013), then we would expect the microbial consumption rate for methyl bromide in snowpack to be roughly equal to 2.5 ng m^{-2} day^{-1}, assuming all else to be

equal. Observed rates of reaction within living control snowpack are roughly equivalent to these estimates in Svalbard samples but exceed this estimate by an order of magnitude in Signy snowpack.

When biological processes are impaired through irradiation the removal rate of methyl iodide is significantly more rapid than that of methyl bromide, nearly 60% methyl iodide is removed from the chamber headspace over 2 hours. This is equivalent to nearly complete (99.8%) daily removal of methyl iodide from the surface snowpack. In non-irradiated snow pack we see instead a significant enhancement of methyl iodide in the chamber headspace that cannot be explained through (photo)chemical reactions. Biological explanations, however, remain plausible. Cultures of marine microbes capable of producing methyl iodide do so at rates between 2 and 900 fmol 10^{10} $cells^{-1}$ day^{-1} (Amachi *et al*, 2001). If we take the snowpack beneath a square meter footprint to the depth of 0.5 meters (which equates to 500 litres of snowpack) this would provide 2.5 x 10^{10} microbial cells. From this we might expect 0.7 to 25 ng m^{-2} day^{-1} of methyl iodide production, which is broadly similar to the fluxes observed in Signy and Svalbard snowpack (Table 1). If irradiated samples represent chemical removal for living control treatments, then microbial productivity would need to double in order to generate the fluxes observed (Table 1).

While methyl bromide and methyl iodide fluxes were broadly consistent across both sampling sites, methyl chloride and dimethyl sulphide fluxes were variable. There exist a number of sources of variability within the sites selected; including snowpack and methodology, site location relative to larger land masses, distance from the coast and height above sea level, wind effects, annual UV intensity at ground level, as well as within-community individual species' resistance to UV radiation.

Signy Island is a small island (~19km^2) which is part of a small island chain in the Southern Ocean, itself only 90km long, and is found approximately 1000km distant from the tips of both South America and the Antarctic peninsula. Svalbard (~61,000km^2) is located centrally within the Greenland Sea, and is between 1000 and 1500km distant from Greenland, Iceland, Norway, Sweden, Finland and Russia. Therefore, based upon location, the microbial community found at Signy Island is more likely to be representative of oceanic microbes due to the presence of the Antarctic circumpolar current whereas Svalbard snow and ice communities are likely to have a larger terrestrial microbial component (Burrows *et al*, 2009).

Signy's sampling location, the Gourlay snowfield, is ~0.5km from the coast and 100m above sea level while the sampling site at Svalbard, Larsbreen glacier, is ~7km from the coast and 600m above sea level. Hodson *et al* (2017) show how such differences in distance from the coast can result in marked differences in snowpack microbial community composition and resultant biogeochemical conditions. Orientation and placement of the glacier within the local geological context will also play a role in modifying the snow, dust and sea salt deposition by local winds. The resultant heterogeneity and variability in snowpack microorganism communities is therefore a likely explanatory variable for the differences observed between Signy and Svalbard, as well as the intra-site variability between replicates.

Local winds, as determined through local topography, bring aerosols for deposition but also influence trace gas fluxes through purging the sub-surface of volatile metabolites and producing quasi-advective flow in sub-surface snowpack pore spaces (Redeker *et al*, 2015). We reduced the influence of wind by placing a 3m x 3m tarp over the chamber flux measurement site but horizontal transport of material within the snowpack, driven by wind, may have influenced our results and may be the source of some of the chamber-to-chamber variability in the observed fluxes.

Local biology effects are also probable. Signy Island, and the Gourlay snowfield, are more accessible to regionally important animal populations (seals and penguins in particular) and they may have provided nutrients through faecal and urine deposits that enhance the activity and modify the community of microorganisms within the snowpack (Hodson, 2006). Further biological complications arise from the dispersed and spatially variable nature of the biological community within the snowpacks, as observed in maritime Antarctic snow covers by Fogg (1968) and Hodson *et al* (2017). Such variability, at spatial scales from centimetres to kilometres, is well-known in other ecosystems. Microbial communities in terrestrial ecosystems demonstrate substantial variability over all spatial scales, from centimetres to kilometres (Raynaud & Nunan, 2014), leading to similar variations in microbial metabolisms and metabolic outcomes that are detectable over similar spatial scales (Hartman & Richardson, 2013).

Antarctic ecosystems are exposed to greater UV radiation throughout the year, particularly during the Antarctic spring during the period of maximum stratospheric ozone depletion. The variable levels of resistance in archaea, algae and bacteria to irradiation, as observed in this study and others (de Bakker *et al*, 2001; Jacobs *et al*, 2005), will likely lead to significant variation in observed fluxes from irradiated snowpacks in Signy versus Svalbard, and differences in snowpack temperature and local surface winds from chamber to chamber are likely to enhance these differences (Hell *et al*, 2013; Larose *et al*, 2013).

Using low concentration metabolites and taking precautions against wind and photochemistry allows the unravelling of these small, variable biological signals from chemical and physical processes with far greater sensitivity than is possible with other parameters such as CO_2. We calculate that, in an isolated environment, it would take ~50 to 100 years for the consumption and production of methyl halides to cause a 1 ppm deviation in carbon dioxide concentration within snowpack pore space. This is well below the detection limits for most analytical

measurements for carbon dioxide (Landwehr *et al*, 2014). This estimate, however, assumes that all biologically-produced trace gases that are not consumed within the snowpack are transferred into the glacial ice, and can be subsequently detected. Other potential metabolites are available in ice and volatile forms within snowpack however (Price, 2000), and it is as yet unclear how rapid the overall microbial metabolism in snowpack may be. These results highlight the need of further studies to assess whether the gases produced by this found biological activity are vertically transferred to the ice as the firn transforms into glacial ice.

The compounds described here have complex, often catalytic, chemistry with important impacts on climate. Methyl chloride and methyl bromide trap solar energy more efficiently than carbon dioxide, so biological removal and transformation of these compounds trades a more effective greenhouse gas (MeX) for a less effective greenhouse gas (CO_2). However, methyl chloride and methyl bromide are both catalytically involved in ozone chemistry, so reduction of these compounds in the lower atmosphere will lead to greater concentrations of ozone, which itself is an effective greenhouse gas at these elevations. Production of methyl iodide generates a short-lived, effective greenhouse gas which reacts rapidly to generate iodide radicals which catalytically destroy ozone (more efficiently than chlorine or bromine radicals), and which chemical products lead to aerosol nucleation. Both of these indirect effects from methyl iodide release act to cool the planet (Table 3). Dimethyl sulphide is widely recognized as the primary naturally produced organosulfur compound responsible for non-sea salt sulfate aerosols, so removal of this through biological processes in snowpack will act to warm the planet by reflecting less incoming sunlight.

Total impacts for any given compound are difficult to predict due to the often conflicting nature of direct versus indirect radiative impacts (Table 3). Furthermore, a significant amount of methyl halide consumption in snowpack will reduce the photochemically produced methyl chloride and methyl bromide before it is mixed with overlying air, in a manner similar to the

reduction of methane efflux by methylotrophs in soils. Sub-snowpack soils will generate significant amounts of methyl halides and these are also likely to be consumed *in situ* before they can escape, especially in short-term coverage sites (winter snowpack). Snowpack in direct contact with soil may act to consume methyl iodide as well (Swanson *et al*, 2005), inverting the effects observed in soil-free snowpack. With these concerns noted, if we take the estimated global area coverage of snow (~10% of the global surface area on average) and apply our average living snowpack fluxes we find that approximately 1% of the annual methyl bromide budget sink can be explained through snow-atmosphere biological processes. Similarly, methyl chloride sinks are one half of 1%, and the production of methyl iodide globally is enhanced to a similar degree. We propose that diminished snowpack may be, in a small degree, responsible for slightly delaying the recovery of the ozone layer through a reduction in methyl halide sinks.

Beyond climate and air quality impacts the demonstrated potential for microbes to metabolise in this challenging environment has significant implications for xenobiology (expanding the realms in which we might expect life to persist and reproduce), industry (through exploitation of low nutrient, cold-tolerant metabolisms) and biogeochemistry (the developing fields of Aerobiology and Cryosphere biology). In particular, however it requires a reconsideration of the use of firn air to quantify pre-industrial levels of methyl halides (Aydin *et al*, 2004) and dimethyl sulfide metabolism by-products (methane sulfonate: Saltzman *et al*, 2006; carbonyl sulfide: Aydin *et al*, 2016). These measurements have not considered the impact of photochemistry (Swanson *et al*, 2007) or biology (this study) on these long term storage concentrations and until these impacts have been quantified and discounted the reported values should be considered the net overall result of all possible biological, chemical and physical effects.

7. Data, code and materials

The datasets supporting this article have been uploaded as part of the supplementary material.

8. Competing interests

I/We have no competing interests.

9. Authors contributions

KR participated in the design of the study, carried out components of the field work in Svalbard, analysed trace gas samples, performed data analysis, and drafted the manuscript; JPJC participated in the design of the study, performed all field and microbial work at Signy Island, and aided in manuscript preparation; AA collected field samples and culturing data from Svalbard; AH aided deployment of the field campaign in Signy and Svalbard and helped draft the manuscript; DP participated in the design of the study, aided deployment of the field campaign in Signy/Svalbard, aided in microbial culture analyses in Svalbard, participated in data analysis and reviewed the manuscript. All authors gave final approval for publication.

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12. References

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Table 1: Net fluxes of methyl halides and dimethyl sulphide from snowpack (in ng m^{-2} day^{-1} ± stderr). Samples taken from chambers before irradiation treatments and "non-irradiated" post-irradiation treatments were combined, as they showed no statistical difference in behaviour. Listed replicate numbers (in brackets) may not equal the maximum replicates possible for "live" (9 in Signy, 12 in Svalbard) and irradiated (3 in Signy, 4 in Svalbard) snowpack. When the trace gas of interest was not quantifiable (below detection limits), they were not included in the replicate count. Negative fluxes indicate net biological or chemical consumption within the snowpack whereas positive fluxes indicate the dominance of production (biological) processes.

Signy Island					
		Methyl iodide	Methyl bromide	Methyl chloride	Dimethyl sulphide
	"Non-irradiated" control measurements	+31 ± 17 (n = 5)	-74 ± 47 (n = 7)	-380 ± 470 (n = 9)	0 ± 100 (n = 4)
	90% CI	+3 → +59	-150 → +3	-1200 → +390	-160 → +160
	UV Irradiated chambers	-290 ± 270 (n = 2)	-130 ± 50 (n = 3)	-1000 ± 1100 (n = 3)	70 ± 130 (n = 3)
	90% CI	-730 → +150	-210 → -48	-2800 → +810	-140 → +280
Svalbard					
		Methyl iodide	Methyl bromide	Methyl chloride	Dimethyl sulphide
	"Non-irradiated" control measurements	+9 ± 8 (n = 5)	-19 ± 20 (n = 12)	20 ± 600 (n = 12)	-80 ± 150 (n = 12)
	90% CI	-4 → +22	-52 → +14	-970 → +1000	-330 → +170
	UV Irradiated chambers	-30 ± 24 (n = 2)	-6 ± 20 (n = 4)	-280 ± 160 (n = 4)	-130 ± 30 (n = 4)
	90% CI	-69 → +9	-39 → +27	-540 → -20	-180 → -80

Table 2: CTC-staining-based viable cell counts from inoculated microbial cultures. Samples were obtained from Svalbard snowpack directly beneath paired control and irradiated chambers. Numbers indicate viable cells per 50 µl snowpack filtrate ± one standard error.

	Control chamber	Irradiated chamber
1st paired chambers	6.2 ± 0.9 (n = 12)	1.2 ± 0.4 (n = 12)
2nd paired chambers	28.8 ± 4.6 (n = 12)	10.0 ± 1.3 (n = 12)
3rd paired chambers	15.1 ± 1.3 (n = 8)	13.8 ± 1.2 (n = 8)

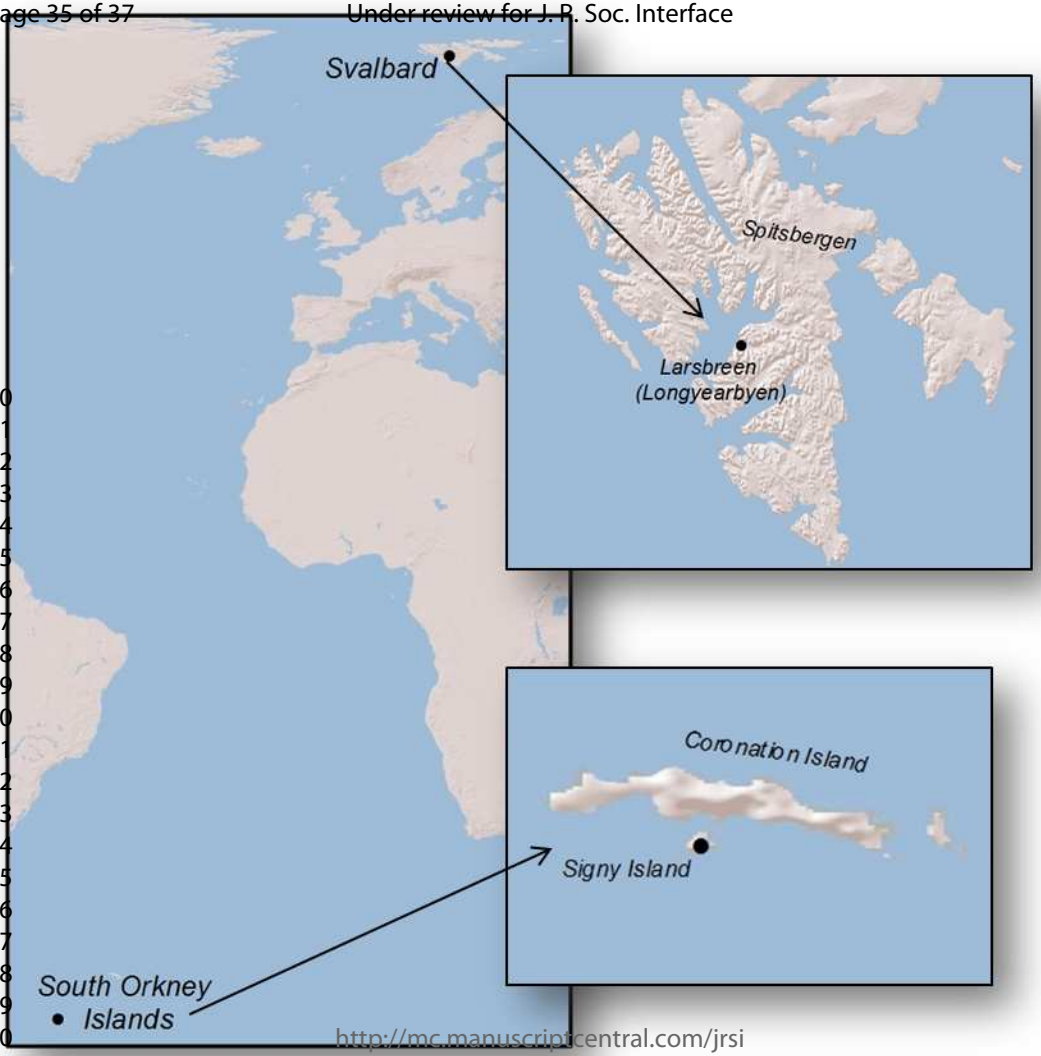
Table 3: Snowpack activity, global direct and indirect effects of each trace gas measured within this study.

	Dark processes	Net direct impact	Radiative impact of DI	Indirect effects (IE)	Radiative impact of IE (and type)
Methyl chloride	Biological and Chemical removal	MeCl → CO2	Cooling (long-wave)	-Enhanced local low atmosphere ozone concentration -Enhanced stratospheric ozone	Warming (long-wave) Warming (long wave)
Methyl bromide	Biological and Chemical removal	MeBr → CO2	Cooling (long-wave)	-Enhanced local low atmosphere ozone concentration -Enhanced stratospheric ozone	Warming (long-wave) Warming (long wave)
Methyl iodide	Biological production Chemical removal	DOC/POC → MeI	Warming (long wave)	-Reduction of local low atmosphere ozone concentration -Enhanced local aerosol concentration	Cooling (long-wave) Cooling (short-wave)
Dimethyl sulphide	Biological removal	DMS → CO2	Cooling (long-wave)	-Reduced local aerosol loading	Warming (short-wave)

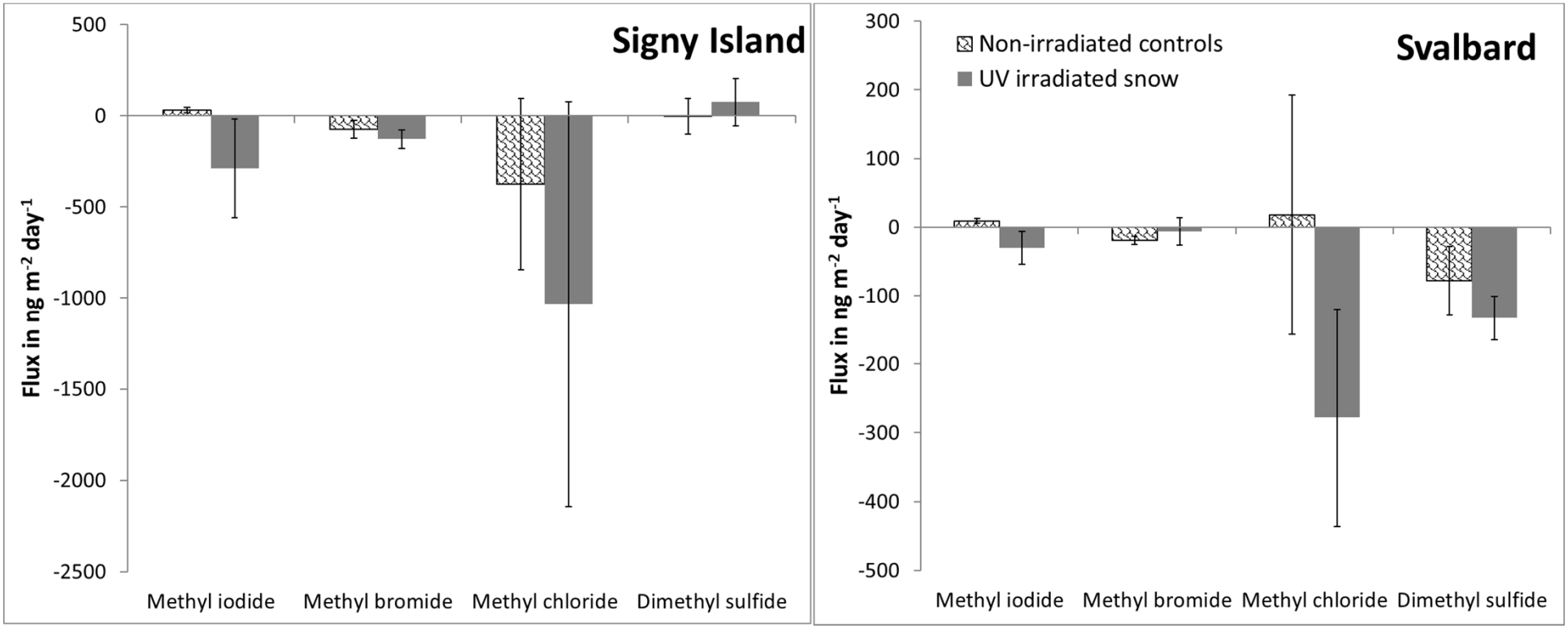
Figure 1: Site locations for Polar snowpack measurements. The Antarctic site was located at Signy Island (60.718 S, 45.632 W) on the Gourlay Snowfield and the Arctic site was Larsbreen glacier, near the settlement of Longyearbyen, Svalbard (78.223 N, 15.627 E).

Figure 2: Snowpack trace gas-sampling methodology. Chamber installation (A); prior to tarpaulin cover the chamber base is visible at top, while the UV lamp is positioned vertically within the snow, 50cm from the chamber center. Trace gas sampling in process (B); both irradiated and non-irradiated chambers are visible, with tarpaulin cover outlined by wooden poles. Opaque chamber tops have been placed on top of the chamber bases shown in (A), with electropolished stainless steel canisters attached to Ascarite traps, in turn connected to glass-coated stainless steel lines connected to the chamber tops. The UV lamps (A) are oriented so that they face towards the irradiated chamber sub-surface snow while facing away from the non-irradiated control chamber.

Figure 3: Comparisons of trace gas fluxes from "non-irradiated controls" (stippled, light grey columns) and irradiated snowpack (dark grey columns) (in ng m^{-2} day^{-1}) and between Signy and Svalbard. Negative fluxes connote degradation or consumption within the snowpack while positive fluxes indicate production within the snowpack. Note change of scale between Signy and Svalbard fluxes. Error bars show ± 1 standard error.


Svalbard
Spitsbergen
Larsbreen
(Longyearbyen)
Coronation Island
Signy Island
South Orkney
Islands


A)
B)


Signy Island
Flux in ng m-2 day-1
500
0
-500
-1000
-1500
-2000
-2500
Methyl iodide
Methyl bromide
Methyl chloride
Dimethyl sulfide
Svalbard
Non-irradiated controls
UV irradiated snow
Flux in ng m-2 day-1
300
200
100
0
-100
-200
-300
-400
-500
Methyl iodide
Methyl bromide
Methyl chloride
Dimethyl sulfide