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Bacteria contribute to pesticide degradation in cryoconite holes in an Alpine glacier☆

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ABSTRACT

Organic contaminants deposited on glacier snow and ice are subject to partitioning and degradation processes that determine their environmental fate and, consequently, their accumulation in ice bodies. Among these processes, organic compound degradation by supraglacial bacteria has been investigated to a lesser extent than photo- and chemical degradation. We investigated biodegradation of the organophosphorus insecticide chlorpyrifos (CPF), a xenobiotic tracer that accumulates on glaciers after atmospheric medium- and long-range transport, by installing *in situ* microcosms on an Alpine glacier to simulate cryoconite hole systems. We found that biodegradation contributed to the removal of CPF from the glacier surface more than photo- and chemical degradation. The high concentration of CPF (2–3 μg g^{-1} w.w.) detected in cryoconite holes and the estimated half-life of this compound (35–69 days in glacier environment) indicated that biodegradation can significantly reduce CPF concentrations on glaciers and its runoff to downstream ecosystems. The metabolic versatility of cryoconite bacteria suggests that these habitats might contribute to the degradation of a wide class of pollutants. We therefore propose that cryoconite acts as a "biofilter" by accumulating both pollutants and biodegradative microbial communities. The contribution of cryoconite to the removal of organic pollutants should be included in models predicting the environmental fate of these compounds in cold areas.

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1. Introduction

It is widely documented that organic contaminants are present in polar and mountain regions far from their emission sources (Bidleman et al., 2010; Carrera et al., 2001). High mountains, acting as cold condensers (Calamari et al., 1991), interfere with the atmospheric transport and global cycling of semi volatile organic compounds (SVOCs) (Carrera et al., 2001; Villa et al., 2003). These organic pollutants can be efficiently scavenged from the atmosphere by snow (Grannas et al., 2013), along with aerosols, microorganisms and nutrients (Price et al., 2009). When deposited on glaciers, pollutants undergo partitioning among different environmental matrices (e.g. snow, ice, water, interstitial atmospheric gases and supraglacial sediments) and post-depositional alteration processes.

Among those environmental matrices, cryoconite, a wind-borne fine debris deposited on glacier surfaces, represents a potential sink for organic pollutants because of its high content of organic matter (OM). Indeed, recent studies showed that cryoconite can accumulate organic aromatic pollutants like polycyclic aromatic hydrocarbons, polychlorinated biphenyls (PCBs) and organochlorine pesticides (Li et al., 2017; Weiland-Bräuer et al., 2017). This occurs particularly in cryoconite holes, small depressions on glacier surfaces filled with water and added with a layer of cryoconite at the bottom, which are considered the most biologically active habitats on glaciers (Cook et al., 2015).

Currently, studies on the post-depositional alteration of pollutants in glacier environments have considered mostly physical-chemical processes, such as photodegradation, hydrolysis and revolatilization of contaminant burdens in snowpack and ice (Grannas et al., 2007; Herbert et al., 2006). Conversely, although the

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pollutant biodegradation by microbial communities has been proved in different cold environments (Margesin, 2007), their microbial degradation on glaciers has received less attention (Hodson, 2014). For instance, Cappa and colleagues investigated the effect of pollutants on microbial communities on glaciers (Cappa et al., 2014). Moreover, the microbial potential to degrade PCBs on a glacier has been assessed by a laboratory microcosm study (Weiland-Bräuer et al., 2017) and a metagenomics approach (Hauptmann et al., 2017) whereas pesticide biodegradation has been quantified in laboratory by microcosms simulating glacier surface (Stibal et al., 2012a), followed by a comparison with the results of *in situ* metabolomic analyses (Cook et al., 2016). However, to the best of our knowledge, no studies have been conducted to quantitatively evaluate pollutant biodegradation rate in cryoconite holes through *in situ* experiments.

In this work, we tested the hypothesis that cryoconite might act as a "biofilter" for organic pollutants on glaciers by both accumulating them and promoting their biodegradation, thus significantly contributing to their removal. To this end, we conducted *in situ* microcosm experiments on an Alpine glacier simulating a cryoconite hole system exposed to the organophosphorus insecticide chlorpyrifos (CPF). CPF is one of the most widely used pesticides (George et al., 2014) and represents an ideal model compound because it is frequently detected in matrices from both Arctic (air, water, sediment and biota; Hageman et al., 2010; Hoferkamp et al., 2010; Landers et al., 2008) and Alpine areas (glacial meltwater; Ferrario et al., 2017). Although not officially classified as a Persistent Organic Pollutant (POP) or Persistent, Bioaccumulative and Toxic Substance (PBT), CPF shows great potential to undergo long-range atmospheric transport and to reach cold areas (Mackay et al., 2014). The experiments were carried out on the Forni Glacier, one of the largest Italian valley glaciers, where CPF was found in meltwater in the order of ng L^{-1} (Ferrario et al., 2017).

2. Methods

2.1. Study area and field methods

The experiments were carried out on the Forni Glacier (Italian Alps, coordinates of the approximate center of the Glacier: 46°12′30″ N, 10°13′50″ E), one of the largest Italian valley glaciers. It is about 3 km long, stretches over an elevation range of 2600 to 3670 m a.s.l. (Senese et al., 2012b) and its surface area was 11.34 km^2 in 2007 (Smiraglia et al., 2015). The microcosms were placed on the Eastern ablation tongue of the Forni Glacier at about 2700 m a.s.l. near an Automatic Weather Station (named AWS1 Forni; 46°23′51.96″ N, 10°35′29.16″ E) (Azzoni et al., 2016) in a flat area with no crevasses.

Microcosms were prepared using cryoconite and water collected on the Forni Glacier on 17 July 2015. Sediment was taken from cryoconite holes with a spoon sterilized with alcohol and flame and transferred into sterile Falcon tubes (50 mL). The meltwater was gathered from a supraglacial stream in a sterilized bottle. After collection, 42 transparent Pyrex bottles (50 mL) were filled with 2 g of sediment and 36 mL of meltwater.

Eighteen bottles were covered with tinfoil ("dark" condition, hereafter) while the other ones were kept transparent ("light" condition, hereafter). Nine of these dark and nine of these light bottles were also sterilized in a pressure-cooker. In each bottle, 8 μg of CPF, dissolved in DMSO, were spiked. We stress that CPF was added after bottle sterilization, while taking care to avoid sterilized microcosm contamination. Six light bottles were immediately brought to the lab for the analyses, which occurred within 4 h of CPF injection, and served as a control at time zero (t_0). The remaining 36 bottles were then placed in three Plexiglas racks, covered with a plastic net to avoid bottle overturning and dispersal, and placed on the glacier surface bound to a wooden stake drilled in the ice (Fig. S1). Thus we placed on the glacier microcosms belonging to four experimental groups ("light biotic", "dark biotic", "light sterile", "dark sterile") including nine microcosms each. Three bottles from each experimental group were then collected on July 27, August 10, August 26, and brought to the lab within 4 h for analyses. At each visit, all non-collected bottles were aerated by quickly opening the lid to assure aerobic conditions. CPF mass present in natural cryoconite was determined taking sediment from cryoconite holes on 2 July 2015 and on 5 July 2016. Samples were collected with a precleaned spoon, transferred into Falcon tubes (50 mL) and brought to the lab within 4 h for analyses.

2.2. Chemical analyses

Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) was purchased from Sigma-Aldrich (product number: 45395-100 MG, Saint Louis, MO, purity > 99.7%). All solvents were checked by gas chromatography (GC) before use.

Water samples were filtered on a glass fiber filter (0.45 μm; Whatman, Maidstone, UK). Prior to the extraction of suspended solid samples, glass fiber filters were cleaned with *n*-hexane (Carlo Erba, Milan, Italy; purity N99.8%). 10 mL aliquots of filtered water of each sample were separately extracted using OASIS HLB cartridges (Oasis HLB, 6 cc/500 mg, LP Extraction cartridge, 60 μm; Waters Corporation Milford, MA). Cartridges were conditioned with 5 mL of methanol (J.T. Baker, Center Valley, PA; purity N99.8%) and 5 mL of deionized water. Samples were drawn under vacuum through the cartridges at a regulated flow rate of 10 mL min^{-1}. After extraction, cartridges were dried using a vacuum pump and subsequently eluted (under gravity) with 3 + 3 mL of ethyl acetate (Carlo Erba, Milan, Italy; purity N99.8%) and 4 mL of acetone (Carlo Erba, Milan, Italy; purity N99.8%). The extracts were then concentrated to 0.5 mL under a gentle stream of nitrogen.

The extracted suspended solids were dried by adding anhydrous sodium sulfate (Granular 12–60 Mesh; J.T. Baker Center Valley, PA; purity Ultra Resi-Analyzed), transferred into cellulose extraction thimbles (19 × 90 mm; Albet Labscience, Seville, Spain) and extracted with *n*-hexane for 24 h. The extracts were then concentrated to 0.5 mL under a gentle stream of nitrogen.

Both water and suspended solid extracts were transferred into GC micro-vials. An internal standard (PCB 40, lot: 40714; Dr. Ehrenstorfer GmbH, Augsburg, Germany; purity 99.0%) was added for subsequent analysis by GC–MS (Agilent Technologies, Santa Clara, CA), in SIM (Single Ion Monitoring) mode. Identification and quantification ions were 314 and 316 for CPF, and 290 and 291 for PCB 40. The GC analysis was performed with an Agilent Technologies 6890N Series gas chromatograph equipped with a 30-m long, 0.25-mm internal diameter capillary column (Zebron™ Capillary GC Column, ZB-SemiVolatiles Guardian; Phenomenex, Torrance, CA). Samples were injected by an Agilent Technologies (Santa Clara, CA) 7683 Series autoinjector, with the injection port at 250 °C in splitless mode. Samples were run in splitless mode using helium as a carrier gas (flow = 1 mL min^{-1}). The oven program started with a temperature of 70 °C and increased of 15 °C per min to 280 °C, hold for 2 min.

Procedural blanks were included during analyses, generated and handled in a manner identical to that of water samples, with no CPF detected.

Two different fortification levels were used to validate the analytical procedure, and average recoveries were of 107% for 0.4 μg mL^{-1}, and 96% for 0.004 μg mL^{-1}. The method detection limit was determined as the instrument detection limit of the lowest

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