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SHORT COMMUNICATION

Light-dependent microbial metabolisms drive carbon fluxes on glacier surfaces

Andrea Franzetti[1], Ilario Tagliaferri[1], Isabella Gandolfi[1], Giuseppina Bestetti[1], Umberto Minora[2,4], Christoph Mayer[3], Roberto S Azzoni[2], Guglielmina Diolaiuti[2], Claudio Smiraglia[2] and Roberto Ambrosini[1]

[1]*Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Milano, Italy;* [2]*"A. Desio" Department of Earth Sciences, Università degli Studi di Milano, Milano, Italy and* [3]*Bavarian Academy of Sciences and Humanities, Munich, Germany*

Biological processes on glacier surfaces affect glacier reflectance, influence surface energy budget and glacier response to climate warming, and determine glacier carbon exchange with the atmosphere. Currently, carbon balance of supraglacial environment is assessed as the balance between the activity of oxygenic phototrophs and the respiration rate of heterotrophic organisms. Here we present a metagenomic analysis of tiny wind-blown supraglacial sediment (cryoconite) from Baltoro (Pakistani Karakoram) and Forni (Italian Alps) glaciers, providing evidence for the occurrence in these environments of different and previously neglected metabolic pathways. Indeed, we observed high abundance of heterotrophic anoxygenic phototrophs, suggesting that light might directly supplement the energy demand of some bacterial strains allowing them to use as carbon source organic molecules, which otherwise would be respired. Furthermore, data suggest that CO_2 could be produced also by microbiologically mediated oxidation of CO, which may be produced by photodegradation of organic matter.
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Climate change is determining a global cryosphere shrinkage and mountain glacier environments are declining (IPPC, 2014). The consequent loss of biodiversity is yet to be fully assessed, particularly the loss of functional biodiversity in extreme environments (Stibal *et al.*, 2012; Boetius *et al.*, 2015). Cryoconite holes, that is, small depressions on glacier surfaces whose formation is because of wind-borne debris (cryoconite), are the most biologically active environments on glaciers (Boetius *et al.*, 2015).

We used whole-metagenomic sequencing to investigate the main functions of six cryoconite holes from Forni (Italian Alps) and six from Baltoro (Pakistani Karakoram) glaciers. We focused on carbon and energy metabolisms by comparing the total coverage of marker genes for photosynthesis, use of inorganic and organic compounds as energy source and autotrophy/heterotrophy. We also used metagenomic sequences for the taxonomic attribution of microorganisms carrying specific metabolic genes (Supplementary Figures S3 and Supplementary data set 1). Supplementary Table S4 reports the marker genes whose coverage (mean number per base of reads mapping the genes) was used to infer the abundance of each metabolism. Supplementary Table S5 and Figure 1 report their normalized coverages. Finally, we measured chemical/physical parameters of cryoconite holes and oxygen consumption rates on the days of sampling, both under light and dark conditions (Supplementary Table S3 and Supplementary Figure S2). The main hypothesis tested was whether oxygenic phototrophy and organotrophic respiration represent the only significant metabolisms affecting carbon balance on glacier surface, as currently conceived, or other microbial processes could contribute to it.

On the basis of 16S rRNA gene sequencing, Cyanobacteria represented 22 and 3% of the microbial community on Forni and Baltoro, respectively (Supplementary Figure S3). High abundance of cyanobacteria has been already observed in polar and alpine cryoconite (Segawa *et al.*, 2014; Stibal *et al.*, 2014), whereas low abundance was reported on Rotmoosferner Glacier (Tirol, Austria; Edwards *et al.*, 2013). Consistently, coverage of *psb*D (photosystem II P680 reaction center D2 protein gene) was (mean ± s.e.) 128.0 ± 9.5 on Forni and 74.5 ± 12.5 on Baltoro, similar to that reported for Arctic and Antarctic supraglacial microbial mats (Varin *et al.*, 2013).

Correspondence: A Franzetti, Department of Earth and Environmental Sciences (DISAT), University of Milano-Bicocca, Piazza della Scienza 1, Milano 20126, Italy.
E-mail: andrea.franzetti@unimib.it
[4]Present address: Dipartimento di Ingegneria Civile e Ambientale, Politecnico di Milano, Milano, Italy.
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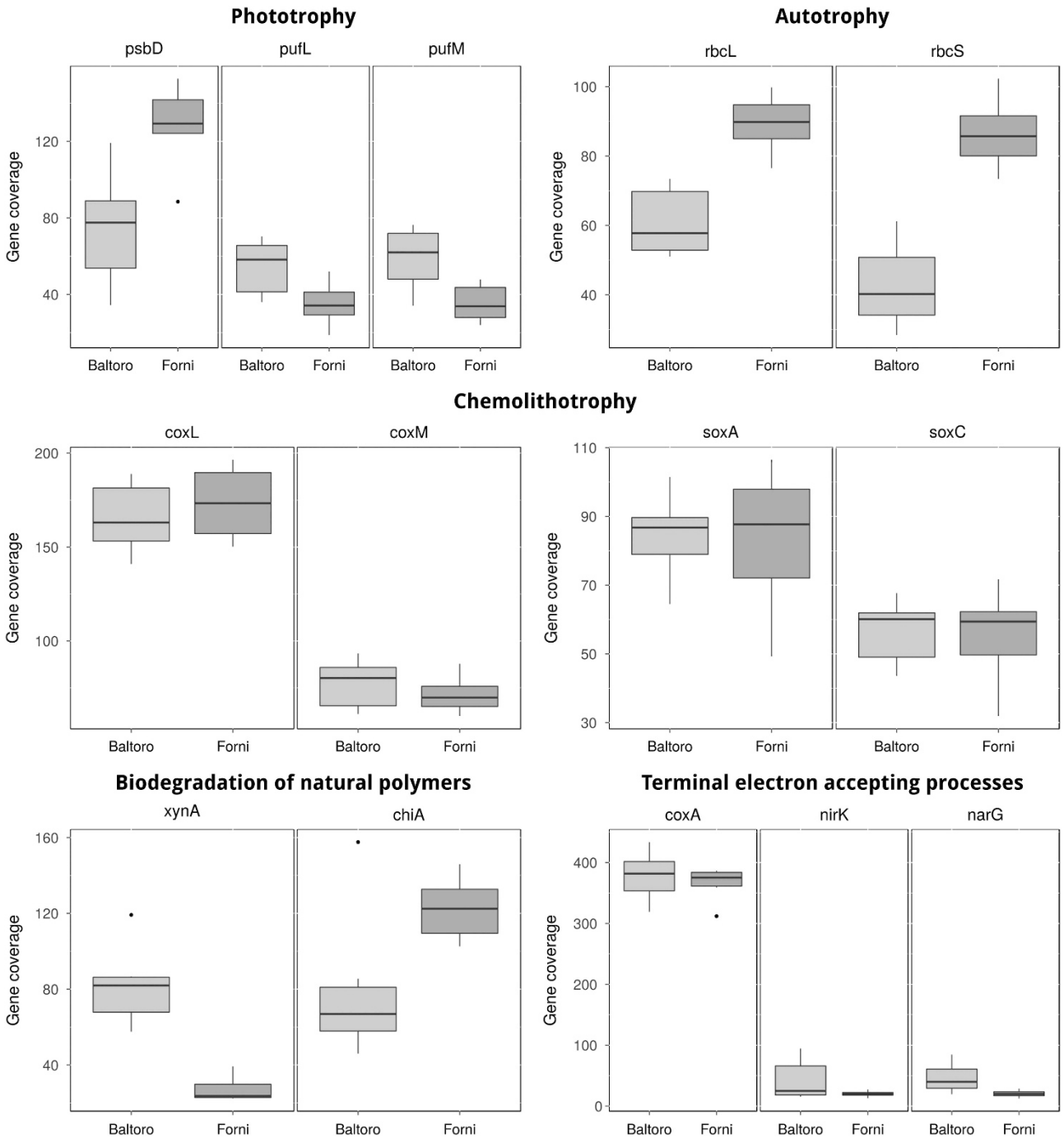

Figure 1 Box plots of marker gene coverages in Forni and Baltoro cryoconite metagenomes. *chi*A, chitinase; *cox*A, cytochrome *c* oxidase subunit I; *cox*ML, carbon-monoxide dehydrogenase medium and large subunits; *nar*G, nitrate reductase alpha subunit; *nir*K, nitrite reductase; *psb*D, photosystem II P680 reaction center D2 protein; *puf*LM, photosynthetic reaction center L and M subunits; *rbc*LS, ribulose bisphosphate carboxylase (RubisCO); *sox*AC, sulfur-oxidizing proteins SoxAC; *xyn*A, endo-1,4-beta-xylanase.

This suggests that oxygenic photosynthesis is among the dominant metabolisms in cryoconite holes. The significantly higher coverage of the *psb*D gene on Forni than on Baltoro (Mann–Whitney *U*-test: $U = 34$, $P = 0.009$) is consistent with the higher oxygen concentrations in cryoconite hole water observed on this glacier ($U = 36$, $P = 0.005$). However, on the days of sampling, we observed strong oxygenic phototrophic activities in only two out of six holes on Forni (Supplementary Table S3). The high *puf*LM (photosynthetic reaction center L and M subunits) gene coverage, mostly affiliated to Proteobacteria (Supplementary Figures S3), suggests that aerobic anoxygenic phototrophs (AAPs) may contribute to energy input of the ecosystem (Yurkov and Hughes, 2013). AAPs are obligate heterotrophic phototrophs

whose presence has been recently documented in cold environments. AAPs use light to supplement their metabolic demands and organic molecules as carbon source: under light conditions, they replace oxidative respiration with photophosphorylation, thus saving carbon, which is used in anabolic reactions for building cell biomass (Čuperová *et al.*, 2013; Caliz and Casamayor, 2014). Interestingly, *puf* gene coverages were significantly higher on Baltoro than on Forni ($U \geqslant 31$, $P \leqslant 0.041$), where oxygen consumption rates were also higher in dark conditions ($U = 21$, $P = 0.041$), when AAPs switch to a more respiratory metabolism. In contrast, oxygen consumption rates in light conditions were similar on the two glaciers ($U = 23$, $P = 0.485$).

CO_2 fixation was also widespread and almost completely achieved through the Calvin–Benson cycle, as revealed by the high coverages of *rbc*LS genes (ribulose bisphosphate carboxylase (RubisCO)), mostly affiliated to Proteobacteria (Supplementary Figures S3), and by the negligible presence of reductive acetyl-CoA pathway and reductive citric acid cycle (details not shown). Although the taxonomic attribution of *rbc*LS genes showed that autotrophs mainly belonged to Cyanobacteria, it also revealed that Proteobacteria substantially contribute to CO_2 fixation. Indeed, Proteobacteria represented 16–22% of bacteria harboring *rbc*L genes, respectively, on Forni and Baltoro (Supplementary Figures S3). This suggests that autotrophic chemolithotrophs might also occur in cryoconite. Indeed, high coverages of sulfur oxidation genes (*sox*AC) and carbon-monoxide (CO) oxidation genes (*cox*LM) were detected on both glaciers. Conversely, no presence of *amo*AB was detected, suggesting the low relevance of ammonia oxidation and nitrification processes. *sox* gene clusters have been described in both anoxygenic sulfur-oxidizing phototrophs and chemolitotrophs (Friedrich *et al.*, 2005). CO oxidizers are a phylogenetically diverse group of bacteria inhabiting different environments (King and Weber, 2007). Interestingly, CO can form rapidly from organic carbon (OC) in melting snow exposed to light (Haan *et al.*, 2001; Xie and Zafiriou, 2009). We speculate that CO oxidizers occur in the melting snow cover of glaciers, and then persist on glacier surfaces, where photochemical CO production may occur, owing to high light intensity and abundant organic matter (OM) in cryoconite (Supplementary Table S3 and Supplementary Figure S1). Direct measurements of CO in the holes are not available; however, CO photochemical formation rates were found to be correlated with the concentrations of dissolved OC (DOC) in the snow (Haan *et al.*, 2001). DOC, in turn, seems more abundant in mountain glacier cryoconite (0.71 mg l^{-1}; Hood *et al.*, 2015) than in the snow (0.07–0.30 mg l^{-1}; Legrand *et al.*, 2013). Therefore, despite we have no direct evidence that CO actually forms in cryoconite holes and to which amount, it might represent a bioavailable substrate on glacier surface, which supplements the energy demand of microbial populations through its oxidation to CO_2 (King and Weber, 2007). Importantly, cyanobacteria produce extracellular polymeric substances (EPSs) that represent an important DOC component (Bhatia *et al.*, 2010). Hence, oxygenic phototrophs may contribute to the amount of DOC in the cryoconite, which in turn might be photodegraded to CO and sustain CO oxidizers. Surprisingly, CO oxidizers have not been reported in glacier environments previously, probably because of the lack of functional studies on these environments. However, phylogenetic assignment of the *cox*M gene revealed that CO oxidizers mostly belonged to Actinobacteria (up to 26%), α- (up to 25%) and β-Proteobacteria (up to 69%), particularly Comamonadaceae (34% on Baltoro; Supplementary Figures S3), which are known to occur on glacier surfaces (Edwards *et al.*, 2014; Boetius *et al.*, 2015). To gain more insight into the role of Comamonadaceae, we reconstructed from metagenomic data 10 partial genomes of *Polaromonas* (Supplementary Figure S4), a genus of Comamonadaceae, which is ubiquitous in glacial environments (Darcy *et al.*, 2011; Michaud *et al.*, 2012; Franzetti *et al.*, 2013). Genome annotation revealed widespread presence of CO dehydrogenase and absence of a complete CO_2 fixation pathway (Supplementary Table S6), consistently with the complete genome of *Polaromonas* JS666. This suggests that *Polaromonas* in cryoconite might use CO as energy source in presence of OC (mixotrophy) (Mattes *et al.*, 2008).

We observed high coverages of genes coding for enzymes involved in the use of several OC sources,

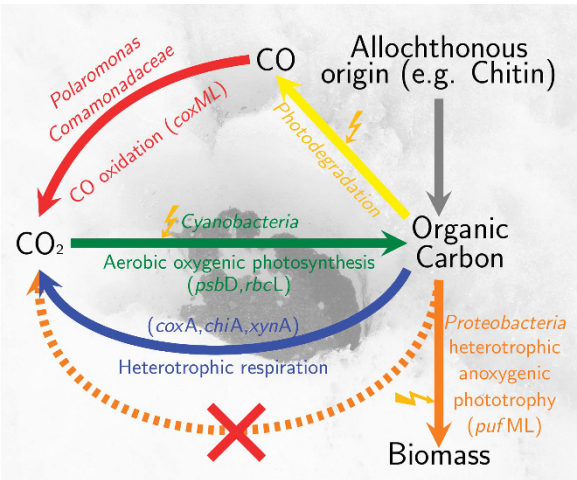

Figure 2 Proposed cryoconite carbon interactome. The main inferred carbon metabolisms and reactions are reported with the taxonomic affiliation of the microorganisms carrying out these metabolisms. Microorganisms involved in heterotrophic respiration are not reported because they belong to many different taxa. Lightning symbols represent light energy involvement into the reaction. Heterotrophic anoxygenic phototrophs use light as energy source in anabolic reactions (orange arrow), thus producing biomass from organic carbon, which otherwise would be respired to CO_2 (dashed orange arrow with a red cross).

such as cellulose, EPSs produced by cyanobacteria (*xyl*A) and chitin (*chi*A). Furthermore, measures revealed that cryoconite holes were fully oxygenated environments. This condition explains the very high coverages of cytochrome *c* oxidase subunit I gene (*cox*A). However, we observed also the presence of *nir*K/*nar*G genes, which suggest the occurrence of anaerobic conditions. Conversely, the abundance of marker genes for dissimilatory sulfate reduction (*dsr*AB) was negligible.

In summary, supraglacial bacterial communities exhibit a high functional biodiversity as they can exploit OC both as energy and carbon source. The high solar radiation at glacier surface may favor both oxygenic and anoxygenic photosynthesis, likely by organisms with both pure autotrophic and mixotrophic lifestyles. Light might also support photochemical CO production and its microbiologically mediated consumption (Figure 2). Despite the presence of the genetic potential for the above-described metabolisms does not necessarily imply that they are actually active, we propose that models of carbon fluxes on glacier surfaces should integrate also alternative metabolisms that has been overlooked so far, such as anoxygenic photosynthesis and CO oxidation.

Conflict of Interest

The authors declare no conflict of interest.

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Data Accessibility

Sequence data of metagenomes were submitted to European Nucleotide Archive (ENA), study accession number PRJEB12327 (http://www.ebi.ac.uk/ena/data/view/PRJEB12327).

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