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Deliverable – WP1, D1.5, D9

An estimation of the microbial biomass and activity originating from the atmosphere and deposited on glacier surfaces

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Introduction

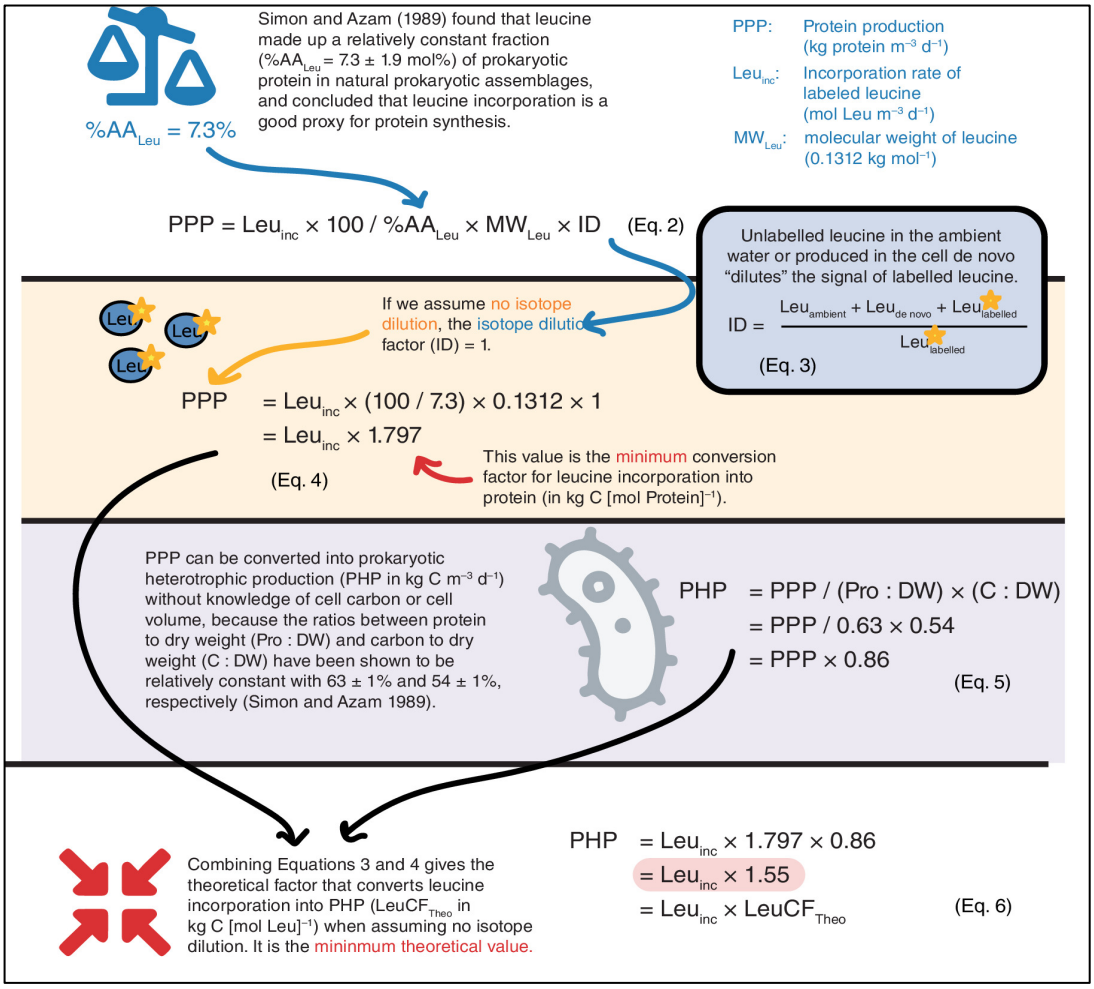
Despite commonly depicted as desolate and inhospitable, glacial environments are characterized by a plethora of living microorganisms associated to virtually every habitat and matrix identifiable (Boetius et al., 2015). In this context, microbes are mediators in nutrient cycling and release and can establish complex ecological communities based on mutual reliance (e.g. biofilms, Smith et al., 2016) or competition for resources, which can be scarce. Due to these complex dynamics, glacial habitats have been recently recognized as biomes (Anesio et al., 2012), and their biogeochemical profiling constitutes a key interest in modern environmental sciences. Prominent among these habitats are the so-called cryoconite holes, consisting of small (up to a meter) supraglacial depressions filled with water and eolian sediment at the bottom, which have been described as microbial biodiversity hotspots (Cook et al., 2016). Despite glaciers having a status as biomes, information about carbon fluxes within microbial communities is still scarce. Primary production typical of these habitats is estimated to be around 64 thousand tons of carbon on the global scale (excluding Antarctica's contribution) per year (Anesio et al., 2009), which represent a non-marginal influence on the entire carbon cycle.

The atmosphere is arguably even more inhospitable for lifeforms than glacial habitats: UV radiation, nutrient scarcity, desiccation and free radicals, among many others, threaten the survival of microorganisms that happen to be (re)suspended into it. Despite such harsh conditions, it is well known that the air matrix plays a key role in microbial dispersal, with the first published detailed observations dating back to the fifties (Gislen, (1948). Almost eighty years later, bioaerosols, like clouds, have been identified as a more favorable atmospheric habitat for the "microforms", to the point that we are aware of their phylogenies (Miaskiewicz-Peska et al., 2012), growth (Sattler et al., 2001), adaptations and seasonal trends (Zhong et al., 2016).

This information is especially relevant when remote ecosystems, such as glaciers, are the on receiving end of such a "conveyor belt", as their functionality relies primarily on allochthonous inputs (Franzetti et al., 2017). These can in fact contribute to the microbial dynamics observed in the above-mentioned surface habitats. It was indeed found that near-glacial environments represent an inoculation source for cryoconite communities (Franzetti et al., 2017), although to our knowledge this never led to any further production-based comparison between ice dwelling and airborne bacterial communities.

Along with carbon fixation, heterotrophic biomass production is another key parameter to understand how these microbe-based systems operate. Heterotrophs often constitute the core of glacier-dwelling microbial communities (Sanyal et al., 2024). A particularly effective method to assess prokaryotic cell growth and metabolic function consists of using [^{3}H]-Leucine as a tracer. This amino acid is incorporated into proteins mostly by heterotrophic bacteria, which are outcompeting cyanobacteria and phytoplankton in the uptake, as observed in aquatic systems (Kirchman et al., 1985). Due to proteins being the "macromolecules of life"

constituting most of the cells' dry weight, they were chosen as the primary quantification target when conducting assays aimed at estimating bacterial growth. By applying empirically derived conversion factors (figure below) it is then possible to obtain the prokaryotic heterotrophic production of the investigated communities. These conversion factors are calculated under the assumptions of (i) negligible intra and extra-cellular leucine concentrations (no isotope dilution), (ii) equal leucine proportion between the target community and the factors and (iii) that the entirety of the incorporated leucine is conveyed into protein synthesis, as opposed to other metabolic pathways (Giering et al., 2022).



Example of conversion factors derivation (from Giering, S. L., & Evans, C., 2022).

Framework implementation

In the current research framework that aims at characterizing microbial communities transported through the atmosphere and deposited on glacier surfaces, the [^{3}H]-Leucine incorporation assay has been consistently part of the analyses. Here we present preliminary results from alpine cryoconites (Jamtalferner, Silvretta Alps, Austria; n = 60), representing surface glacial environments along the ablation season (June to September), as opposed to air samples collected at Lake Untersee (Queen Maud Land, East Antarctica; n = 16) near and far from scientific/logistics settlements, and compare them to the existing literature.

Methods

From each cryoconite sample, three sub-samples were prepared into 2 ml micro-centrifuge tubes, pipetting 1.7 ml of sediment, and adding the labelled leucine up to the concentration of 40nM, including two controls inactivated with 90 µl of 100% trichloroacetic acid (TCA) to test for abiotic uptake. Since the incubation was not conducted in the field due to logistical reasons, the samples have been incubated in a water bath (Haake) in the lab with gentle shaking, kept a 0,5-1°C for 24 hours, to simulate the natural conditions, and terminated adding 90 µl of TCA. Ideal incubation time has been tested in the course of a previous project by saturation curves. Samples have been centrifuged at 13.000 G for 10 min and the supernatant was discarded. For protein extraction, 90µl of ice cold 50% TCA has been added for 5 min, centrifuged as above and the supernatant discarded. To obtain the proteins, 90 µl of 96% ethanol was added for 5 min, centrifuged as indicated above and the supernatant was again discarded. For liquid scintillation counting, 1 ml of scintillation cocktail (Packard Gold Safe) was added, and the disintegrations per minute (dpm) were counted using a Packard Tricarb 4910TR Liquid Scintillation Counter. Due to high sample density and high quench, the measurement time was set to 50 minutes to correct for quenching and reach the 2% standard deviation threshold.
A similar protocol was applied to air samples, which were collected in triplicates in standard salt solution with a Coriolis µ liquid impinger (Bertin). After thawing at 4°C, each sample was divided into four 3 ml sub-samples (3 technical replicates and one TCA inactivated control) and treated as previously detailed.

Results highlights

While the measurements from different matrices are not directly comparable, preliminary results suggest discrete levels of bacterial productivity for both the air and the cryoconite environments. Specifically, air samples from Antarctica were, on average, in line with what is reported in a previous study on super-cooled cloud water droplets in high alpine environments by Sattler et al. (2001), exhibiting bacterial production of 0.7 $ngC \cdot L^{-1} \cdot h^{-1}$ and 0.5 $ngC \cdot L^{-1} \cdot h^{-1}$ respectively. Interestingly, when accounting for site remoteness (i.e. distance from (semi)permanent human settlements such as research stations), samples collected in their vicinity showed an intra-group mean of 1.4 $ngC \cdot L^{-1} \cdot h^{-1}$ which is nearly three times as much as the alpine counterpart, potentially indicating a substantial anthropogenic influence on bacterial growth in the air just above it. Conversely, air samples collected further than 150km from these settlements (Lake Untersee campsite) are characterized by levels of average productivity around 0.2 $ngC \cdot L^{-1} \cdot h^{-1}$, in this case well reflecting the extreme remoteness of the study area.

Cryoconite holes are the surface habitats of choice for this investigation. These are unique micro-environments characterized by levels of primary productivity comparable to temperate soils (Anesio et al., 2009). For this reason, we investigated whether it was possible to extend such comparison in terms of secondary productivity, using soil data from the existing literature. Notably, Bååth E. (1994) quantified the productivity of soil bacteria at $1.4x10^5$ $ngC \cdot h^{-1}$ per gram of soil, which is comparable to the average results of the current study ($0.3x10^5$ $ngC \cdot h^{-1}$ per gram of wet cryoconite sediment). In our time series we also observe a productivity peak in the latter half of the season, although the driver of such phenomenon remains elusive. For this reason, while secondary productivity data can provide crucial information on these habitats, we strongly recommend sequencing the extracts from the same samples. For instance, coupling

16S rRNA gene amplicon analysis as a routinary technique may allow to explain underlying dynamics that it wouldn't be possible to disentangle otherwise.

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