Snow ecosystem, microbial community structure and function in artic snowpacks

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L'écosystème neige, structure et fonctionnement des communautés microbiennes du manteau neigeux en Arctique

soutenue le 18 Septembre 2015 devant le jury composé de:

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Résumé (Français)

La couverture neigeuse arctique peut atteindre jusqu'à un tiers de la surface terrestre. Cet environnement, chimiquement très dynamique, est en interaction avec tous les compartiments environnementaux : l'atmosphère, le sol, les aquifères, et ce influence la biosphère toute entière. Durant les dernières décennies, la neige a été reconnue comme étant un réservoir de microorganismes. Pourtant l'écologie des microbes du manteau neigeux reste mal comprise. L'objectif principal de cette thèse est donc de caractériser le manteau neigeux en tant qu'écosystème fonctionnel, par définition une communauté d'organismes vivants, en conjonction avec la composante non vivante de leur environnement et agissant comme un système. Pour cela, la composition taxonomique et fonctionnelle des communautés microbiennes a été analysée via la technologie de séquençage haut débit pour deux types de modèles de manteau neigeux : une neige saisonnière d'eau douce d'un manteau neigeux terrestre (Ny-Ålesund, Svalbard) et une couverture neigeuse saline sur la glace de mer (Nuuk, Greenland).

Le premier objectif est de caractériser l'hétérogénéité des communautés microbiennes en relation avec les fluctuations conditions environnementales. La composition des communautés microbiennes du manteau neigeux est très variable en fonction de l'avancement dans la saison du printemps vers l'été et en fonction de la profondeur. La corrélation entre les fonctions microbiennes et les conditions environnementales soutient l'hypothèse que les communautés microbiennes interagissent avec les fluctuations des conditions en abiotiques de leur habitat. Le second objectif concerne la spécificité des communautés microbiennes du manteau neigeux ; si le manteau neigeux est un écosystème fonctionnel alors les communautés microbiennes le composant devraient présenter des caractéristiques spécifiques liées à leur adaptation aux conditions de cet habitat, malgré la variabilité. La comparaison de la distribution fonctionnelle entre la neige et des environnements distants (polaires ou non) ainsi que des environnements en interaction proche permet de confirmer une spécificité des communautés microbiennes de la neige. Le troisième objectif se concentre sur la sélection environnementale ; étant donné que l'existence d'une communauté microbienne spécifique implique que des processus de sélection se réalisent au sein du manteau neigeux. La comparaison de la distribution de la structure (quels microorganismes sont présents) et la fonction (que sont-ils capables de faire ?) des communautés microbiennes en fonction de la sources des microorganismes au sein d'un manteau neigeux couvrant la glace de mer révèle que la communauté est largement influencée mais diffère de leur source en réponse aux conditions environnementales spécifiques. Les résultats préliminaires des analyses metagenomiques et metatranscriptomiques ont révélées qu'il existe une grande variabilité entre les communautés présentes et potentiellement actives au sein du manteau neigeux. Bien que des limitations conceptuelles et techniques persistent, les méthodes de séquençages haut-débit basées sur les molécules d'ARN sont des outils prometteurs pour décrire les réponses à court terme des communautés microbiennes du manteau neigeux aux variations des conditions environnementales. Finalement, une approche mécanistique préliminaire basée sur la mise en place de microcosmes de neige artificielle et des microorganismes modèles a été développée afin de déterminer les processus de colonisation au sein du manteau neigeux. Alors que de nombreuses questions demeurent concernant l'activité microbiennes et les interactions complexes de communautés, les études menées durant cette thèse ont permis de soutenir l'hypothèse que la neige est un écosystème fonctionnel.

Abstract

The Arctic seasonal snowpack can extend at times over a third of the Earth's land surface. This chemically dynamic environment interacts with different environmental compartments such as the atmosphere, soil and meltwater, and thus, strongly influences the entire biosphere. During the last decades, snow has been recognized as a microbial reservoir. The ecology of snow microorganisms however remains poorly understood. The main goal of this thesis was to investigate the snow as a functional ecosystem; *i.e.* a community of living organisms in conjunction with the non-living component of their environment and interacting as a system. In order to do so, microbial community taxonomic and functional composition of snow samples from two arctic snowpack models: seasonal snow from terrestrial fresh water snowpack (Ny-Ålesund, Svalbard) and sea ice snow cover (Nuuk, Greenland) was analyzed using high throughput sequencing technologies.

The first objective addressed microbial community heterogeneity in relation with fluctuating environmental conditions. Snow microbial community composition was highly variable during spring season and depth. The relationship between microbial functions and environmental conditions supports the hypothesis that the snow microbial community interacts with the abiotic variability characteristic of their habitat. The second objective addressed snow community specificity; if the snowpack is a functional ecosystem, then the microbial communities inhabiting it should have specific features related to their adaptation to the conditions of this environment, despite variability. The comparison of functional distribution between snow and both remote (polar and non polar) and closely interacting environments provided evidence of snowpack microbial community specificity. The third objective focused on environmental selection, given that the existence of a specific snow microbial community implies that one or more selective processes occur in the snowpack. Comparing the distribution of microbial community structure and function as related to the source of the microorganisms in a sea ice snow cover revealed that snow microbial communities were largely influenced by, yet differed from their seeding sources in response to specific environmental conditions. Mechanistic approaches with model microorganisms in snow microcosms were developed during this thesis and, based on preliminary results, will help to determine colonization processes within snowpack. Finally, preliminary results in the first section of Chapter 4 also showed that a high variability exists between the microorganisms present within the snowpack, and those that are active. Although technical and conceptual issues remain, RNA based high throughput sequencing was evaluated as an encouraging tool to evaluate short-term responses of microbial communities to environmental fluctuations. While numerous questions remain about microbial activity and complex community interactions, the results from this thesis support the hypothesis that snow is a functional ecosystem.

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List of abbreviations

BDL= below detection limit

BioHg= bioavailable mercury

MeHg= methylmercury

MSA= methyl sulfonic acid

DOC= dissolved organic carbon

Ppt= parts per thousand (salinity measure)

BLAST= basic local alignment search tool

MG-RAST= metagenomic rapid annotations using subsystems technology

nr= non redundant protein database

PCA= principal component analysis

STAMP= software package for analyzing taxonomic or metabolic profiles

IR= infrared red (light spectra)

PAR= photosynthetically active radiation (light spectra)

UV= ultra-violet (light spectra)

EPS= extracellular polymeric substances

HGT= horizontal gene transfer

MAA= mycosporine like amino acids

ROS= reactive oxygen species

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Part 2: Freshwater colonization model by Chlamydomonas nivalis

List of peer-reviewed publications

Maccario, L., Vogel, T. M., and Larose, C. (2014). Potential drivers of microbial community structure and function in Arctic spring snow. *Front. Microbiol.* 5, 413. doi:10.3389/fmicb.2014.00413.

Delmont, T. O., Eren, A. M., Maccario, L., Prestat, E., Esen, Ö. C., Pelletier, E., Le Paslier, D., Simonet, P., and Vogel, T. M. (2015). Reconstructing rare soil microbial genomes using in situ enrichments and metagenomics. *Front. Microbiol.* 6, 358. doi:10.3389/fmicb.2015.00358.

Maccario L, Sanguino L, Vogel TM, Larose C. Snow and ice ecosystems: not so extreme. Res Microbiol 2015. doi:10.1016/j.resmic.2015.09.002.

Synthèse (Français)

L'écosystème neige, structure et fonctionnement des communautés microbiennes du manteaux neigeux en Arctique.

La couverture neigeuse arctique peut atteindre jusqu'à un tiers de la surface terrestre. Cet environnement, chimiquement très dynamique, est en interaction avec tous les compartiments environnementaux : l'atmosphère, le sol, les aquifères, et ce influence la biosphère toute entière. Durant les dernières décennies, la neige a été reconnue comme étant un réservoir de microorganismes. Pourtant l'écologie des microbes du manteau neigeux reste mal comprise. L'objectif principal de cette thèse est donc de caractériser le manteau neigeux en tant qu'écosystème fonctionnel, par définition une communauté d'organismes vivants, en conjonction avec la composante non vivante de leur environnement et agissant comme un système. Pour cela, la composition taxonomique et fonctionnelle des communautés microbiennes a été analysée via la technologie de séquençage haut débit pour deux types de modèles de manteau neigeux : une neige saisonnière d'eau douce d'un manteau neigeux terrestre (Ny-Ålesund, Svalbard) et une couverture neigeuse saline sur la glace de mer (Nuuk, Greenland).

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conditions en abiotiques de leur habitat. Le second objectif concerne la spécificité des communautés microbiennes du manteau neigeux ; si le manteau neigeux est un écosystème fonctionnel alors les communautés microbiennes le composant devraient présenter des caractéristiques spécifiques liées à leur adaptation aux conditions de cet habitat, malgré la variabilité. La comparaison de la distribution fonctionnelle entre la neige et des environnements distants (polaires ou non) ainsi que des environnements en interaction proche permet de confirmer une spécificité des communautés microbiennes de la neige. Le troisième objectif se concentre sur la sélection environnementale ; étant donné que l'existence d'une communauté microbienne spécifique implique que des processus de sélection se réalisent au sein du manteau neigeux. La comparaison de la distribution de la structure (quels microorganismes sont présents) et la fonction (que sont-ils capables de faire ?) des communautés microbiennes en fonction de la sources des microorganismes au sein d'un manteau neigeux couvrant la glace de mer révèle que la communauté est largement influencée mais diffère de leur source en réponse aux conditions environnementales spécifiques. Les résultats préliminaires des analyses metagenomiques et metatranscriptomiques ont révélées qu'il existe une grande variabilité entre les communautés présentes et potentiellement actives au sein du manteau neigeux. Bien que des limitations conceptuelles et techniques persistent, les méthodes de séquençages haut-débit basées sur les molécules d'ARN sont des outils prometteurs pour décrire les réponses à court terme des communautés microbiennes du manteau neigeux aux variations des conditions environnementales. Finalement, une approche mécanistique préliminaire basée sur la mise en place de microcosmes de neige artificielle et des microorganismes modèles a été développée afin de déterminer les processus de colonisation au sein du manteau neigeux. Alors que de nombreuses questions demeurent concernant l'activité microbiennes

et les interactions complexes de communautés, les études menées durant cette thèse ont permis de soutenir l'hypothèse que la neige est un écosystème fonctionnel.

La cryosphère se définit comme la portion de la terre où l'eau est présente sous forme solide [1]. Elle inclut la glace de mer, les glaciers et calottes glacières, le pergélisol et la couverture neigeuse. La neige, dont la couverture peut atteindre jusqu'à un tiers de la surface terrestre, influence le bilan énergétique et d'humidité global et de ce fait le climat [2]. La neige est également une interface entre tous les compartiments de la biosphère de la Terre : les sols, les aquifères, la glace de mer et l'atmosphère [3–6]. Par exemple, le manteau neigeux agit comme un réacteur photochimique et est impliqué dans les échanges gazeux avec l'atmosphère, jouant un rôle clef dans le cycle des contaminants organiques volatiles dans les régions froides [7]. Bien que la couverture neigeuse apparaît comme étant un élément critique de la cryosphère, elle disparaît. En effet, la réduction de la couverture neigeuse a été estimé à 17,8% dans l'hémisphère Nord de 1979 à 2011 [8], avec un impact potentiel sur la régulation du climat à l'échelle mondiale, comme décrit pour le climat estivale subarctique eurasien [9]. La réduction de la couverture neigeuse pourrait également impacter différents écosystèmes tant micro- que macrobiotiques. Par exemple, au niveau des sols couverts de neige, l'augmentation de température liée à la fonte précoce du manteau neigeux durant le printemps affecte fortement les processus microbiens dans les sols [10]. Pourtant, la perturbation des processus microbiens au sein du manteau neigeux lui-même est rarement inclus dans les modèles de changement climatique du fait que la neige en tant qu'entité biologique est largement sous-estimée (figure 1).

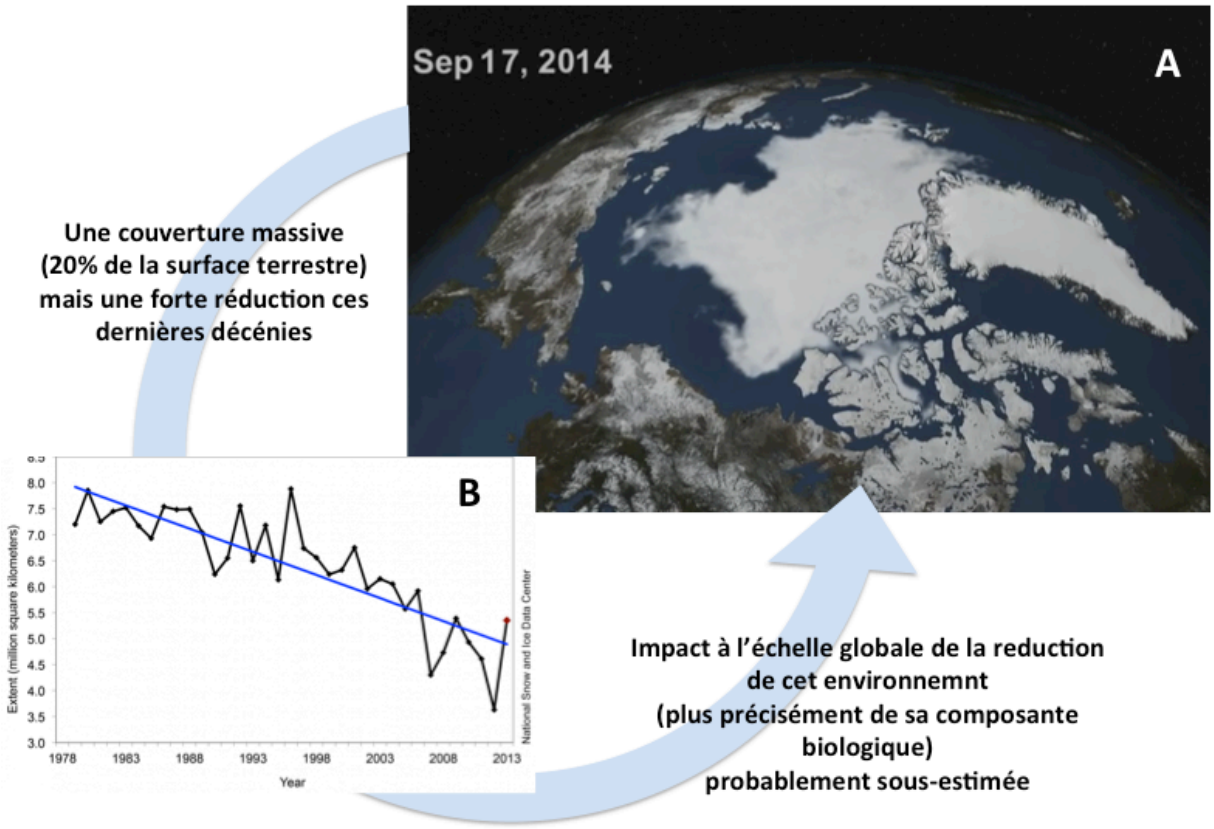

Figure 1 : Couverture des environnements d'eau gelée, massive mais menacée. A : Photo satellite de la couverture de la glace de mer sur l'hémisphère Nord (Ocean Arctique) lorsqu'elle est la plus faible (fin d'été - septembre) (NASA Science News, 2014). B : Entendue de la glace de mer (en millions de km^2) à cette même période comparée à ces 30 dernières années (Snow and Ice National Data Center, 2015)

La neige est formée par l'accumulation de cristaux de glace déposés sur une surface puis subit des modifications post-déposition [11]. Dans ce milieu poreux et froid, les microorganismes sont confrontés à des conditions environnementales spécifiques telles que des concentrations faibles en nutriments, dessiccation, cycles de gel-dégel, de fortes radiations solaires et de ce fait un photochimie hautement réactive, en plus d'une température basse [1,12–14]. Dans les régions polaires, le régime de lumière impacte également les communautés présentes avec des périodes de lumières de 24h durant l'été et une obscurité complète durant l'hiver. C'est pourquoi, la neige a été considérée comme peu propice au développement de la vie et seulement un piège de microbes à l'état végétatif avant leur redistribution vers d'autres environnements durant la fonte [15]. Cette thèse s'inscrit dans les recherches récentes contestant cette assertion. Les microorganismes contenus dans la neige arctique ont été partiellement caractérisés via différentes approches dépendant ou non de la culture de ces microbes, bien que leur activité et fonctions potentielles restent largement méconnues. Des études récentes, basées sur l'extraction et séquençage d'ADN d'échantillons de neige, ont conduit à l'identification de gènes fonctionnels spécifiques, tels que les gènes ribosomiques (codant l'ARN ribosomique 16s), des gènes de résistance au mercure ou des gènes impliqués dans le cycle de l'azote [16–18]. Ces études ont mis en lumière que divers microorganismes sont présents dans la neige avec des densités cellulaires variables et potentiellement impliqués dans différents processus biologiques. Pourtant, le concept de manteau neigeux en tant qu'écosystème reste largement inexploré et constitue l'objectif principal de ce travail de thèse. La notion d'écosytème a été définit pour la première fois en 1934 par A.G Tansley en tant qu'une communauté d'organismes vivants (plantes, animaux et microbes) en conjonction avec la composante non vivante de leur environnement et interagissant comme un système [19].

Basée sur cette définition, l'étude du manteau neigeux en tant qu'écosystème engendre trois grands objectifs avec des questions de recherche spécifiques au centre de travail de thèse.

Due à sa conformation chimique et physique, le manteau neigeux est décrit comme un environnement hautement réactif et hétérogène [20]. Le premier objectif concerne donc l'appréhension de l'hétérogénéité des communautés microbiennes en relation avec les conditions environnementales. Quelle est la variabilité des communautés microbiennes de la neige? Cette variabilité est-elle corrélée aux changements des conditions environnementales. Quelles sont les échelles spatiales et temporelles de cette variabilité. Dans le but de répondre à ces questions, des échantillons de neige ont été collectés et la composition taxanomique et fonctionnelle ainsi que les paramètres environnementaux ont été comparés. Si le manteau neigeux est une écosystème fonctionnel alors les communautés microbiennes le composant devraient présenter des caractéristiques spécifiques du fait de leur adaptation aux conditions spécifiques de cet habitat. Le second objectif est de caractériser la structure et le fonctionnement des communautés microbiennes : Si la communauté microbienne du manteau neigeux est spécifiques, quels son les facteurs physico-chimiques induisant la structure de la communauté ? Quelles sont les fonctions spécifiques sélectionnées ? La méthode employée consiste à comparer la composition des communautés microbiennes, tant en termes de taxonomie que de fonctions, avec des environnements variés. L'existence d'une communauté spécifique de la neige implique que des phénomènes de sélection se produisent au sein du manteau neigeux. Ainsi, appréhender comment les microorganismes sont sélectionnés au sein du manteau neigeux constitue le troisième objectif de cette thèse. Ici, deux approches ont été développées :i) comparer la distribution des communautés microbiennes en relation avec leur source d'origine ii) des

expériences en laboratoire permettant de retranscrire la colonisation de la neige via des organismes modèles en microcosmes de neige artificielle stérile. Finalement toutes ces questions impliquent que les microorganismes du manteau neigeux soient actifs. Le dernier objectif est donc d'examiner l'activité microbienne du manteau neigeux : Quelle est la fraction de microorganismes actifs et quelle est l'échelle de temps de leur réponse physiologique au sein du manteau neigeux ?

Ces questions, centrales à ce travail de thèse ont été abordées selon différents aspects : différentes échelles dans le temps et l'espace, techniques et aussi de modèles de manteaux neigeux (Figure 2). Deux types de modèles de manteaux neigeux saisonniers ont été décrits durant cette thèse : soit une couverture neigeuse au dessus de la glace de mer, soit manteau neigeux terrestre composé de neige d'eau douce. Le manteau neigeux couvrant la glace de mer est en interaction proche avec les environnements connectés, à savoir l'atmosphère et la glace de mer sous-jacente. Il représente donc un excellent modèle pour examiner la distribution spatiale des microorganismes de la neige en fonction de leur source d'origine et des processus de modifications post-déposition. En revanche, ce type de manteau neigeux est extrêmement fragile et est sujet à la dispersion via le vent, les craquements de la glace et une courte persistance durant la saison du printemps. Les manteaux neigeux terrestres sont relativement plus stables et sont donc plus adaptés pour l'étude des caractéristiques des communautés microbiennes et leur variabilité sur une saison entière et en fonction des modifications des conditions environnementales. La plupart des résultats présentés dans cette thèse correspondent à un assignement taxonomique (qui ?) et fonctionnel (quoi ?) de séquences d'ADN génomique total obtenues via les technologies de séquençage haut débit appliqués à des échantillons de neige arctique printanière issus de ces deux modèles de manteaux neigeux : un manteau neigeux terrestre à proximité de la base scientifique de Ny-

Alesund (Svalbard) et une couverture neigeuse surmontant la glace recouvrant un fjord à proximité de Nuuk (Groenland). Des données préliminaires basées sur l'analyse du séquençage de transcrits ARN est également discuté en tant que parallèle à l'activité microbienne. De plus une approche mécanistique a été conduite pour étudier les processus de colonisation microbienne via des organismes modèles en microcosmes de neige artificielle stérile. Ce manuscrit est donc organisé au travers des différentes expériences menées durant les trois années de thèse pour aborder les aspects précédemment énoncés.

Le premier chapitre est une étude bibliographique donc l'objectif est de décrire les caractéristiques abiotiques des manteaux neigeux ainsi que leurs habitants, et ce, comparées à tous les environnements constitués d'eau glacée, glace de mer, glaciers et calottes glacières. Le second chapitre explore la signature microbienne d'un manteau neigeux terrestre en région arctique, comparé à des environnements non glacés et dans le but d'étudier le lien entre structure et fonction de la communauté microbienne et la variabilité de l'environnement chimique au cours de la saison printanière. Le troisième chapitre s'intéresse lui à la distribution de la communauté microbienne dans différentes couches de neige d'une couverture neigeuse surplombant la glace de mer en comparaison avec leur différentes sources d'origine, c'est-à-dire l'atmosphère et la glace de mer sous-jacente. Le dernier chapitre correspond à une discussion sur l'utilisation des approches méta-omiques pour accéder aux communautés microbiennes actives et basée sur des résultats préliminaires sur la composition en microorganismes présents (analyses ADN) et actifs (analyses ARN) à l'échelle d'une journée et sur toute une saison printanière dans un manteau neigeux terrestre de la région arctique. Une deuxième section de ce chapitre décrit les expériences mécanistiques préliminaires menées en laboratoire afin d'appréhender les

processus de colonisation manteau neigeux vie des microorganismes modèles en microcosmes.

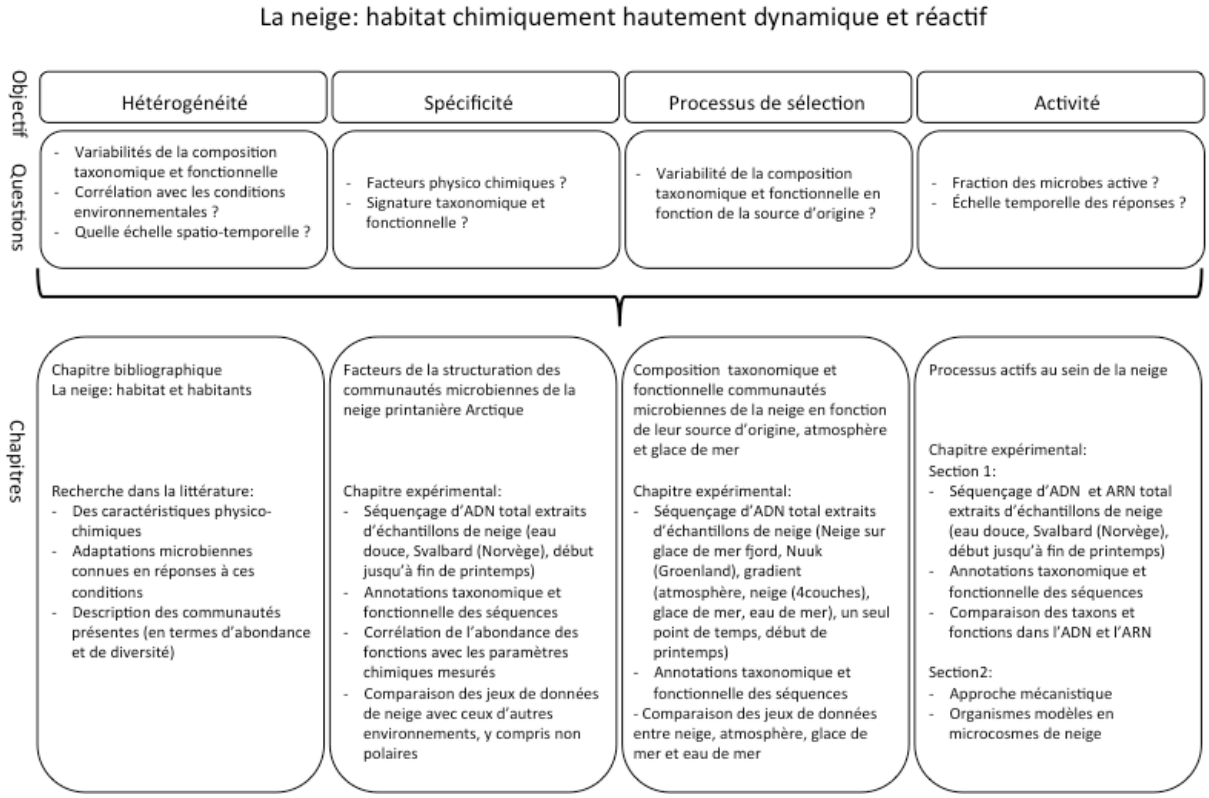

Figure 2 : Diagramme des objectifs et questions de recherches au centre de la thèse et la répartition des expériences et recherches bibliographiques menées pour aborder ces questions

Les environnements extrêmes sont définis comme des zones où un ou plusieurs des paramètres abiotiques, à savoir les concentrations en nutriments, le pH, la température, la teneur en eau, l'irradiation solaire, la pression, etc., atteignent des valeurs extrêmes [21]. Ces conditions difficiles sont considérées comme limitantes pour le développement de la vie. Néanmoins, la gamme des valeurs qui sont considérées comme extrêmes sont souvent considérées du point de vue des micro-organismes, alors que la limite pour le développement de la vie microbienne sur Terre n'a pas encore été totalement établie. Les paramètres cités ci-dessus influencent tous les organismes à l'échelle de l'individu et peuvent à différents degrés, être la cause un stress physiologique de la cellule. Cependant, les microorganismes ont une remarquable capacité d'adaptation à un large éventail d'habitats. À ce jour, aucun endroit sur Terre n'a été démontré comme étant stérile, même les environnements les plus "inhospitaliers", tels que les cheminées hydrothermales des profondeurs marines, les eaux de drainage des mines acides et les sources chaudes, qui contiennent de nombreux micro-organismes viables. Dans le cas des sources d'eau chaude, l'existence d'une communauté microbienne viable et active a été reconnue rapidement [22]. De nombreuses études ont porté sur l'écologie de ces environnements à la fois à des fins fondamentales et appliquées: comme un modèle pour le développement de la vie dans des conditions terrestres primitives et la recherche d'enzymes avec intérêt industriel potentiel en raison de leur activité à des températures élevées.

A l'extrémité opposée du spectre de la température, la neige et la glace ont reçu beaucoup moins d'attention (Figure 3). En dehors de basses températures, la neige et la glace ont une faible disponibilité de l'eau, la teneur en nutriments est faible et l'irradiation solaire élevée pendant l'été et l'obscurité totale pendant l'hiver, mais des microbes sont présents dans ces habitats à l'apparence vierge. La neige, les glaciers et la glace de mer, les principales

composantes de la cryosphère, couvrent jusqu'à 1,06 x 108 km2 de la surface de la Terre. La neige en hiver peut couvrir jusqu'à 12% de la surface de la Terre, soit environ 61 millions de km2 [23]. Environ 10% des terres, environ 15 millions de kilomètres carrés, sont recouvertes par les calottes glaciaires, qui avec les les glaciers stockent 75% de l'eau douce du monde [24]. Compte tenu de leur couverture massive à l'échelle mondiale, la neige et la glace pourraient avoir un rôle important et sous-estimé dans les cycles biogéochimiques global. Par conséquent, les lacunes dans les connaissances concernant l'écologie microbienne de la neige et de la glace écosystèmes doivent être évalués pour des recherches futures.

Le but du chapitre bibliographique est d'examiner les mécanismes connus pour permettre aux micro-organismes de résister aux conditions potentiellement délétères caractéristiques des environnements constitués de neige et de glace. L'abondance et la diversité des communautés microbiennes de la neige et de la glace ainsi que les interactions qui ont lieu au sein de la communauté sont également présentées.

Figure 3 : Diagramme des contraintes rencontrées lors de l'étude de l'environnement neige. La neige est considérée comme un environnement extrême du fait des conditions drastiques qui le caractérise en particulier. Ceci influence fortement la manière d'étudier l'écologie de cet environnement, avec de nombreuses contraintes techniques. Pourtant ces conditions pourraient ne pas être limitant à l'établissement de communautés microbiennes diverses et dynamiques.

La neige et la glace soutiennent une vie microbienne et pourraient ne pas être si extrême d'un point de vue microbien. Bien que la neige et la glace partagent de multiples caractéristiques abiotiques en raison de leur structure constituée de cristaux de glace et leur aspect homogène à un niveau macroscopique, ils sont composés d'une multitude de niches avec des conditions différentes. Au niveau microscopique pour chaque micro-habitat, les contraintes, telles que de faibles nutriments ou disponibilité de l'eau, pourraient ne pas être aussi extrêmes. De plus, de nombreuses stratégies à différents niveaux de la physiologie cellulaire pourrait être utilisées par les cellules individuelles pour résister au stress potentiel dans la neige et la glace. Par exemple les microbes peuvent contrer le froid via l'augmentation fluidité de la membrane (synthèse d'acide gras insaturé, désaturases), la protection contre le gel (synthèse de solutés compatibles, anti-gel et protéines de liaison à la glace), et le maintien de l'efficacité catalytique des protéines (réduction des interactions hydrophobes internes, contenu en proline et arginine, l'augmentation de longueur des boucles externes et de l'accessibilité au site actif) [25,26]. Les micro-organismes de la neige et la glace ne sont pas seulement en mesure de faire face aux conditions physico-chimiques de leur habitat, mais ils sont aussi capables de façonner leur environnement abiotique. La production de pigments, créant un importante coloration (rouge ou grise) au niveau des surfaces des glaciers et des manteaux neigeux), diminue considérablement l'albédo de surface et augmentent la température locale et la teneur en eau [27,28]. Dans la glace de glacier de glace de lac, la production de protéines de liaison de la glace par des bactéries peut modifier la structure de glace en inhibant la recristallisation, ce qui semble réduire la diffusion dans les veines [29,30]. De même, la production d'EPS par les algues et les bactéries de glace de mer modifient la structure de la glace en réduisant sa perméabilité et est considérée comme essentielle pour la création de canaux d'eau liquide salée connectés

et complexes au sein de la glace de mer [31,32]. Toutes ces modifications physiques et chimiques peuvent faciliter le développement de communautés microbiennes complexes.

Les bactéries semblent être ubiquistes dans neige et la glace, puisque elles sont observées systématiquement dans tous les environnements de neige et de glace étudiés, quelles que soient les approches méthodologiques utilisées (dépendant de la culture ou non). Les bactéries identifiées à partir de ces études appartiennent à de nombreux taxons, bien que la plupart appartiennent aux Proteobacteria (alpha, bêta et Gamma), au groupe des Cytophaga-Flexibacter-Bacteroides, des Actinobacteria, et des cyanobactéries. La diversité bactérienne estimée varie entre les différents écosystèmes étudiés. Par exemple, l'indice de diversité de Shannon est compris entre H '= 0,18 la neige de la calotte glaciaire de l'Arctique canadien et près de H' = 4.0 pour les neiges des plateaux tibétains [33] [34]. La diversité bactérienne moyenne mesurée dans la neige et la glace est similaire à celle des sols tempérés (2.4 <H '<3.6) [35]. Contrairement aux bactéries, les archées sont rarement observées. Des séquences associées au domaine des archées ont été détectées avec une abondance relative inférieure à 1% dans des échantillons de neige terrestre de l'Arctique, la glace de mer et de la banquise.

La neige et la glace fournissent également des habitats pour une large gamme de micro-organismes eucaryotes tels que les champignons, les microalgues ou les protistes hétérotrophes [36]. Des concentrations élevées d'algues de neige (*Chlamydomonas nivalis* et les espèces apparentées) sont trouvées dans les accumulations de neige de divers endroits (de la neige de la banquise en Arctique [37,38] et les systèmes alpins comme les montagnes Rocheuses [39] ou les Alpes autrichiennes [40]) produisant le phénomène de neige sanguinolente. Dans la neige, la communauté eucaryote est largement dominée par des

champignons (Ascomycota et Basidiomycota) suivie par Alveolata (dinoflagellés) [33,41,42]. Cependant, les champignons sont rarement détectés dans la glace de mer, qui est dominé par les micro-algues, les diatomées étant les plus abondants (Bacillariophyceae) à la fois dans les échantillons arctique et antarctique. Les protistes hétérotrophes y compris ciliés et flagellés sont également fréquents dans la glace de mer. Les communautés microbiennes de la neige et la glace sont largement variables en termes de taille et de composition. Ceci peut s'expliquer par l'existence d'une grande variété de niches avec des conditions abiotiques différentes. Les microbes de la neige et la glace sont plus nombreux et diversifiés que précédemment évalué et peuvent répondre de façon dynamique aux changements de leurs conditions environnementales. De plus, ils semblent être intégré dans un réseau très complexe d'interactions.

L'activité microbienne dans le manteau neigeux, telle que mesurée par l'incorporation de thymidine et de leucine marquées, peut être variable en fonction de la température, l'emplacement et la proximité des activités anthropiques [43], allant de 4,2 fmolL-1.h-1 dans la neige fraîchement tombée à plus de 160 pmol L-1.h-1 dans des sites antarctiques, avec un clair effet anthropique [44–46]. La surface de la glace des glaciers est un élément important des glaciers et présente une activité microbienne élevée [47–49]. Par exemple, l'activité microbienne mesurée à plusieurs µg de carbone par gramme d'agrégats cryconite par jour pour la photosynthèse et de la respiration pendant l'été est similaire à celle de sols et des sédiments dans les régions tempérées [47]. Dans la glace glaciaire profonde, l'activité microbienne est supposée très faible, mais suffisante pour maintenir les cellules et le métabolisme des organismes méthanogènes [47]. Bien que leur taux de production soit faible par rapport à d'autres grands compartiments de la biosphère, comme l'océan et le sol,

la neige et la glace pourraient soutenir une partie sous-estimée de l'activité biogéochimique globale.

Dans la partie précédente, nous avons vu que la neige supporte divers micro-organismes, dont certains présentent des caractères spécifiques aux conditions environnementales caractéristiques de cet habitat d'eau gelée. Si le manteau neigeux de l'Arctique est un écosystème fonctionnel, alors la communauté microbienne l'habitant devrait avoir une signature génomique fonctionnelle liée à leur adaptation aux conditions spécifiques de cet environnement. Plusieurs adaptations physiologiques ont été décrites pour les microorganismes survivants dans des conditions froides basées sur des isolats microbiens psychrophiliques [50–53]. Bien que l'augmentation de la fluidité membranaire et la synthèse d'enzymes adaptés au froid sont critiques, les autres paramètres physiques et chimiques pourraient être tout aussi critique dans le manteau neigeux arctique. Par exemple, les rayonnements de photons sont également une reconnue comme une condition extrême [54]. Ces réactions photochimiques et la capacité oxydative associée ont été décrites comme jouant un rôle majeur dans la chimie manteau neigeux [13], mais l'impact sur la communauté microbienne de la neige reste inconnu. L'objectif est d'étudier la capacité fonctionnelle, la diversité et la dynamique des micro-organismes dans la neige et de tester l'hypothèse que leur signature fonctionnelle reflète l'environnement de la neige. Notre approche a consisté à comparer les séquences d'ADN fonctionnelles annotées à d'autres communautés et de familles de gènes connus associés à différentes contraintes telles que le stress oxydatif lié à un rayonnement UV important. Cette étude a permis d'explorer les gènes fonctionnels des communautés microbiennes dans le manteau neigeux arctique. Cette communauté microbienne, y compris les membres représentatifs associés à des environnements froids, subit des changements majeurs au cours de la saison de printemps.

Les données fonctionnelles en corrélation avec les paramètres chimiques soutiennent l'hypothèse que cette variation dans la structure de la communauté microbienne et leur fonction pourraient être expliquée par des fluctuations dans les conditions environnementales. En outre, dans cette étude, nous avons testé l'existence d'une signature fonctionnelle spécifique de la communauté microbienne du manteau neigeux. L'irradiation de lumière UV pourrait être un facteur déterminant dans la définition de l'écologie microbienne de l'écosystème du manteau neigeux arctique. Un échantillonnage au cours de la période de pénombre ainsi que des études metatranscriptomiques et de comparaison de l'atmosphère permettrait d'établir comment les microorganismes sont sélectionnés dans le manteau neigeux et le rôle de la lumière comme un facteur majeur de structure et de la fonction de la communauté microbienne du manteau neigeux.

A une plus petite échelle spatiale et de temps, nous avons également observé la distribution de la communauté microbienne dans les différentes couches de la couverture de neige de glace de mer par rapport à leurs sources d'origine, c'est-à-dire l'atmosphère environnante et la glace de mer.

La neige interagit avec tous les compartiments de la biosphère: les océans, les sols, la glace et l'atmosphère, et influence fortement leurs processus abiotiques et biotiques [3][4]. Par exemple, le manteau neigeux en tant que bioréacteur photochimique est impliqué dans de nombreux échanges de gaz avec l'atmosphère et joue un rôle clé dans le devenir des contaminants organiques volatils dans les régions froides [7]. Les micro-organismes sont transportés par flux atmosphériques, parfois sur de longues distances et sont déposés durant les chutes de neige et des événements de dépôt sec. D'autre part, la neige sur la glace de mer peut également être mouillé par l'eau salée de la glace de mer qui est

fortement concentrée en microbes [55]. Les dépôts atmosphériques et flux capillaires de liquides de la glace de mer modifient aussi l'échelle microscopique, ce qui peut avoir un impact sur le fonctionnement microbien. Toutefois, les processus de sélection et la réponse des communautés microbiennes à des changements rapides des conditions environnementales induites par des événements d'ensemencement restent méconnues. Ici, en utilisant un manteau neigeux couvrant la glace de mer comme un système modèle, nous pouvons déterminer dans quelle mesure les communautés microbiennes de la neige sont influencées par leurs sources, l'atmosphère et la glace de mer, et identifier les fonctions spécifiques impliquées dans la réponse aux changements des conditions abiotiques du manteau neigeux. L'hypothèse est que la couche saline de la neige basale sera largement influencée par la teneur en saumure, avec la colonisation par des micro-organismes d'origine marine et une réponse fonctionnelle à une salinité élevée, alors que la neige de surface sera dominée par des fonctions liées au stress photochimiques, tels que les réponses au rayonnement UV et au stress oxydatif. La communauté microbienne de la neige couvrant la glace de mer d'un fjord au Groenland est largement influencée par ses sources d'origine i) l'atmosphère avec des particules et des organismes provenant de différentes sources terrestres ou marines d'origine locale ou distante ii) les injections d'eau saline de la glace de mer avec une apport microbien élevé dans la couche basale et saline du manteau neigeux. Des processus de sélection post-déposition pourraient alors se produire dans la neige pour former une communauté microbienne adaptée à la neige. Ces processus de sélection impliquent des caractéristiques importantes de la neige couvrant la glace de mer tels que la photochimie et les gradients chimiques et conduisent à des adaptations microbiennes spécifiques au stress photochimique dans les communautés microbiennes de la neige de surface et au stress osmotique dans les couches plus profondes du manteau neigeux.

Cette étude soutient l'hypothèse selon laquelle le manteau neigeux est un écosystème avec les communautés microbiennes qui présentent des capacités sous-estimées.

La variation dynamique de la structure des communautés microbiennes et de leurs fonctions en fonction de la saison, l'emplacement dans le manteau neigeux et l'emplacement géographique, déterminée via l'extraction d'ADN total, est un argument crucial en faveur de la nature active de ces communautés microbiennes dans le manteau neigeux, mais ne supprime pas tous les doutes que ces changements sont les résultats d'autres phénomènes. Afin de fournir de nouveaux arguments en faveur de l'activité de ces microorganismes du manteau neigeux dans la neige, d'autres expériences ont été mises en place durant cette thèse, c'est-à-dire de la variation du contenu en ARN messager dans le manteau neigeux et la mise en place de microcosmes de neige avec des organismes modèles dans des conditions de laboratoire.

La neige abrite des habitants microbiens avec une grande diversité et en abondance relativement élevée. En outre, cette communauté microbienne semble refléter une signature spécifique de la neige en termes de composition taxonomique et de capacités fonctionnelles par rapport à d'autres environnements. Malgré cette signature fonctionnelle et taxonomique, la neige a une forte variabilité de la structure et de la fonction communauté à toutes les échelles spatiales et temporelles. La couverture de neige mondial, avec une couverture importante de la surface de la Terre, est largement hétérogène car elle varie en fonction du type de conditions de l'habitat: l'irradiation solaire, la disponibilité et la concentration des éléments nutritifs, la température, la disponibilité de l'eau en fonction du lieu, de l'altitude, des environnements et même la profondeur. Cela conduit à une distribution hétérogène des microbes.

Ces conditions de l'habitat varient également avec le temps et avec le renforcement de l'effet de saisonnalité dans les régions polaires. Les événements de dépôts atmosphériques occasionnels peuvent conduire à un apport massif de produits chimiques, tels que les nutriments et de contaminants d'origine locale ou distante. Ces composés sont ensuite transformés et peuvent conduire à des changements dans les conditions physico-chimiques environnantes. La modification de la composition de la communauté microbienne et la corrélation de la fonction avec l'abondance intrants chimiques soutiennent l'hypothèse que la communauté microbienne est dynamique et répond efficacement aux changements des conditions environnementales. Cependant, les membres actifs de cette communauté et l'échelle de temps de la réponse microbienne restent inconnus. Dans cette section les avantages mais aussi les limites de séquençage à haut débit pour répondre à ces questions en utilisant les résultats préliminaires obtenus à partir de l'Arctique neige de printemps seront discutés conjointement avec les résultats décrits précédemment dans ce manuscrit.

Tout d'abord, d'importantes limitations techniques existent liées à l'utilisation des approches méta-omiques dans la détermination des communautés microbiennes actives, en particulier pour l'environnement neige. La faible abondance des organismes et la structure de glace de la neige sont certainement les plus évidentes. Comme examiné dans le chapitre 1, la neige est loin d'être stérile, mais l'abondance microbienne est généralement faible avec environ 10^2 à 10^4 cellules/ml et atteignant parfois 10^5 cellules/ml. En termes de logistique, il semble irréaliste d'augmenter de façon exponentielle la taille des échantillons. En outre, le processus de fonte peut influencer le taux récupération de l'ADN; par exemple, lors de la fonte la plasmolyse pourrait augmenter la perte de l'ADN extracellulaire lors de l'étape de filtration. L'ADN extracellulaire peut persister sur le long terme dans l'environnement, adsorbé à des particules d'argile dans les sols par exemple [56].

Dans la banquise Arctique canadien, les mesures de l'ADN extracellulaire indiquent des concentrations allant jusqu'à 135 mg/L [57]. La persistance de l'ADN et les facteurs influençant la répartition de l'ADN, telles que la température et les radiations UV, ont été examinés pour différents environnements, y compris le sol, l'eau douce et marine [58]. Dans les accumulations de neige, les températures basses pourraient conduire à la préservation de l'ADN, puisque l'activité des nucleases est fortement réduite à 4 ° C (comparée à celle à 37 ° C) dans les sédiments marins [59]. Cependant, la sensibilité de l'ADN au rayonnement UV [60] pourrait fortement limiter la persistance de l'ADN dans la neige. Bien que l'ADN extracellulaire persiste dans la neige et certaines molécules soient maintenu dans le film de particules sur le filtre, la majorité devrait être exclu lors de l'étape de filtration. Dans ces conditions, il peut être difficile d'atteindre le rendement de l'ADN nécessaire pour les technologies de séquençage (3 µg pour 454 technologies de pyroséquençage). Voilà pourquoi nous avons décidé d'utiliser l'amplification du génome entier pour l'exploration de la fonction de la communauté microbienne dans le chapitre 2, malgré les biais potentiels liés à cette technique. La récupération de l'ADN, la préservation pendant le transport et le stockage ainsi que l'efficacité de l'amplification du génome entier renforcent les difficultés dans la gestion des échantillons déjà rares. Avec les améliorations récentes dans les technologies de séquençage, il est possible d'obtenir des données de séquençage avec seulement 1 ng de matériel d'ADN de départ. Il a alors été possible de construire des métagénomes pour les échantillons à faible abondance comme la neige superficielle et l'atmosphère pour de la couverture de neige de la glace de mer au Groenland présentés dans le chapitre 3. Une autre limitation de la métagénomique est fonction de la qualité des bases de données, pour la plupart dominées par des environnements bien étudiés, ce qui influence l'efficacité de l'annotation. L'efficacité de l'annotation pour la neige est variable,

mais semblable à celle de la glace de mer et les habitats marins. Dans le chapitre 1, la notion d'environnement extrême et pourquoi la neige pourrait ne pas être si extrême ont été discutés. Bien que la majorité des séquences ne soit pas identifiés et que nous ne pouvons pas exclure la possibilité que des extrêmophiles hautement spécifiques de la neige dominent la neige, la métagénomique est un outil puissant pour explorer la communauté microbienne de la neige. L'utilisation des technologies de séquençage à haut débit nous a permis de poser des hypothèses sur les facteurs potentiels impliqués dans la structuration des processus communautaires et de la sélection se produisant dans le manteau neigeux. L'exploration de la neige communautés via une approche metatranscriptomique présente des problèmes communs et divergents avec la métagénomique, en termes de préparation des échantillons et des analyses de séquences. La teneur en ARNm d'une cellule bactérienne, en fonction de l'état de croissance, est difficile à estimer. Le nombre de molécules d'ARNm dans *Escherichia coli* lors de la croissance exponentielle en culture a été estimée à ≈1380, un petit nombre comparé à plus de 43 000 gènes et 42 000 000 protéines [61]. Via l'ajout de molécules d'ARNm artificiels à des échantillons environnementaux pour estimer le contenu cellulaire en ARNm, il a été estimé que chaque microbe marin dans les eaux côtières du sud-est des États-Unis et le panache du fleuve Amazone peut contenir seulement 200 molécules d'ARNm [62]. ARN rendement d'extraction à partir d'échantillons environnementaux est donc attendu o soit très faible. Les techniques de quantification disponibles ne sont pas adaptées à ces faibles concentrations. Les molécules d'ARNm sont aussi extrêmement labiles, ce qui implique que i) contrairement à l'ADN, la persistance de molécules extracellulaires est peu probable ii) la préparation des échantillons nécessite des procédures propres. La demi-vie intracellulaire de l'ARNm est faible et est de l'ordre de quelques minutes et indépendante du taux de croissance [62], ce qui implique que, même dans les écosystèmes où les organismes

ont un faible taux de croissance, ce qui est probablement le cas avec de la neige, la transcription de la réponse à des fluctuations rapides dans les conditions environnementales pourrait être un signal très court. Dans le cas du manteau neigeux, aussi bien pour les habitats de glace, la fonte peut influencer grandement le pool de transcrits dans les échantillons, du fait de la libération et la biodisponibilité des nutriments et le stress hypo-osmotique créé. La conservation des échantillons représentatifs de la communauté in situ dans les conditions spécifiques observées est donc une préoccupation majeure. Au cours de cette thèse, nous avons essayé de développer deux procédures alternatives pour éviter le biais lié au processus de fonte, à savoir une solution de stabilisation chimique (RNA later comme solution tampon) et la préservation physique avec la lyophilisation. L'influence de cette procédure alternative sur la composition taxonomique basée sur une analyse de la transcription de l'ARNr 16S sont en cours. Toutefois, la nécessité d'une étude approfondie pour évaluer l'efficacité de ces procédures et leur mise en œuvre avec une stratégie réaliste (nombre d'échantillons à traiter dans une temps raisonnable, le coût et les déchets, par exemple 700g de sulfate d'ammonium pour 1.5L de neige fondue n'est pas une option durable) ont limité leur utilisation dans les campagnes de terrain de 2014. En termes d'analyses bioinformatique, la metatranscriptomique présentent les mêmes questions que la métagénomique. En outre, la forte dominance de l'ARNr dans les jeux de données de séquençage réduit couverture de séquençage nécessaire pour l'exploration fonctionnelle des réponses en ARNm, en fonction des questions de recherche. Pourtant, la production ou la présence de l'ARN comme un proxy de l'activité microbienne pourraient confirmer que les microbes sont actifs dans le manteau neigeux et pas seulement stockés à l'état congelé ou dormant. L'analyse des gènes transcrits et notamment ARN ribosomique 16S ont été proposées pour identifier les bactéries qui sont les plus susceptibles d'être présents dans un

état métaboliquement actif dans la neige [46]. Dans cette étude, les auteurs ont identifié des taxons très abondant dans les transcrits d'ARNr 16S et considérés comme endogènes habitants de neige de l'Antarctique, comme Janthinobacterium, Pseudomonas, Sphingomonas et Variovorax. Le genre Variovorax, souvent décrit dans les environnements à basse température basse, y compris la neige, a également été détecté dans nos jeux de données à la fois l'ADN et l'ARN. Un nombre élevé de familles ont été détectées uniquement dans les jeux de données metatatranscriptomiques. Parmi ces taxons, nous pouvons citer la famille Halithiobacillaceae, une bactérie pourpre sulfureuse appartenant aux gamma-protéobactéries et déjà rapportée dans les lacs en Arctique et Antarctique [63,64] et dans les bulles de glace dans un lac de glace subarctique [65]. Cela suggère que divers taxons présent en faible abondance, mais sous la limite de détection dans le pool ADN, pourrait contribuer à la production molécule d'ARN et donc être actif dans les conditions d'observation. En revanche, certains taxons ont été détectés seulement dans le pool ADN, telles que des familles appartenant au Ascomyceta, et peuvent ne pas être des membres actifs de la communauté de la neige, au moins à cette époque de l'échantillonnage. Parmi les microbes apportés par le vent et déposés dans la neige avec de la poussière ou les flocons, certains pourraient ne pas être en mesure de croître dans la neige, dont les caractéristiques sont trop différentes par rapport aux environnements dont ils sont issus. Par exemple, les agents pathogènes de plantes tels que des organismes associés à Agrobacterium ont été détectés dans la neige fraîchement tombée, mais ne sont plus détectés après le dépôt [66]. Ces processus de sélection post-déposition ont également été étudiés dans le chapitre 3, indiquant qu'une proportion de Rhizobiaceae pourrait diminuer entre l'atmosphère et la neige. Toutefois, la proportion réelle de ces cellules dormantes et leur persistance dans les manteaux neigeux restent inconnues. Cette différence entre annotation métagénomique et

metatranscriptomic a également été observée dans nos données à un niveau fonctionnel, avec un écart plus élevé entre les fonctions présentes et potentiellement exprimées au début du printemps. Ces observations conduisent à se poser plusieurs questions. L'écart entre la communauté présente et active dans le manteau neigeux est-il plus important lorsque les conditions sont plus sévères avec potentiellement seulement quelques microorganismes actifs? La différence entre les microbes actifs et les microbes les plus abondants dans la neige et la glace est elle plus importante par rapport à des environnements plus classiques. En raison de l'apport régulier de nouveaux microorganismes, imposé par sa nature d'interface? En raison de ses conditions environnementales difficiles? Si oui, quelle est l'échelle de temps de la sélection microbienne qui conduit à une communauté adaptée par rapport à l'entrée de nouveaux microbes par dépôt? Et de manière plus générale, dans quelle mesure la production de l'ARNm est une indication de l'activité microbienne?

Comme mentionné précédemment dans la section sur les limitations techniques, la demi-vie intracellulaire de l'ARNm a été montré pour être aussi faible que quelques minutes et indépendante du taux de croissance [62], ce qui implique que, même dans les écosystèmes où les organismes ont une croissance lente, comme cela est probablement le cas avec de la neige, la réponse de la transcription pourrait être un signal très court. Le manque d'informations environnementales à propos de l'ARN et le renouvellement des protéines, l'expression constitutive ou induite et l'étendue des modifications post-transcriptionnelles [67] sont communs à tous les types d'environnements et constituent une préoccupation majeure rencontrée par les écologistes microbiens concernant le proxy de la présence ou la production de l'ARN pour l'activité microbienne avec des approches metatranscriptomiques. La metatranscriptomique est alors un outil intéressant pour évaluer les réponses des

microbes aux stimuli environnementaux spécifiques plutôt que l'activité en soi. Le manteau neigeux est très dynamique, avec un apport régulier en minéraux et en composés organiques, dont la distribution et les concentrations sont modifiées par des processus physiques dans le manteau neigeux (la photochimie, pompage éolien, cycles de gel-dégel, et la neige métamorphisme). Ces conditions chimiques sont très variables à l'échelle quotidienne, les concentrations d'ions par exemple peuvent augmenter d'un facteur 30 en une journée. La metatranscriptomique pourrait être un outil qui peut aider à évaluer les réponses à court terme des communautés microbiennes de la neige. Quel est le délai pour l'échantillonnage afin d'attraper une réponse spécifique? Encore une fois la notion d'échelle de temps semble critique.

Références

[1] Miteva V. Bacteria in Snow and Glacier Ice. In: Margesin, RosaSchinner F, Marx J-C, Gerday C, editors. Psychrophiles from Biodivers. to Biotechnol., 2008.

[2] Hinkler J, Hansen BU, Tamstorf MP, Sigsgaard C, Petersen D. Snow and Snow-Cover in Central Northeast Greenland. Adv Ecol Res 2008;40:175–95. doi:10.1016/S0065-2504(07)00008-6.

[3] Pomeroy JW, Brun E. Physical Properties of Snow. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW, editors. Snow Ecol. An Interdiscip. Exam. Snow-Covered Ecosyst., Cambridge University Press; 2001, p. 45–126.

[4] Schmidt SK, Lipson D a. Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil 2004;259:1–7. doi:10.1023/B:PLSO.0000020933.32473.7e.

[5] Schimel JP, Bilbrough C, Welker JM. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biol Biochem 2004;36:217–27.

[6] Dominé F, Shepson PB. Air-snow interactions and atmospheric chemistry. Science 2002;297:1506–10. doi:10.1126/science.1074610.

[7] Daly GL, Wania F. Simulating the influence of snow on the fate of organic compounds. Environ Sci Technol 2004;38:4176–86.

[8] Derksen C, Brown R. Arctic Report Card - Snow -. 2013.

[9] Matsumura S, Yamazaki K. Eurasian Subarctic Summer Climate in Response to Anomalous Snow Cover. J Clim 2012;25:1305–17. doi:10.1175/2011JCLI4116.1.

[10] Edwards AC, Scalenghe R, Freppaz M. Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. Quat Int 2007;162-163:172–81. doi:10.1016/j.quaint.2006.10.027.

[11] Dominé F, Lauzier T, Cabanes A, Legagneux L, Kuhs WF, Techmer K, et al. Snow metamorphism as revealed by scanning electron microscopy. Microsc Res Tech 2003;62:33–48. doi:10.1002/jemt.10384.

[12] Grannas AM, Shepson PB, Filley TR. Photochemistry and nature of organic matter in Arctic and Antarctic snow. Global Biogeochem Cycles 2004;18:n/a – n/a. doi:10.1029/2003GB002133.

[13] Grannas AM, Jones AE, Dibb J, Ammann M, Anastasio C, Beine HJ, et al. An overview of snow photochemistry : evidence , mechanisms and impacts 2007:4329–73.

[14] Techel F, Pielmeier C. Point observations of liquid water content in wet snow - Investigating methodical, spatial and temporal aspects. Cryosphere 2011;5:405–18. doi:10.5194/tc-5-405-2011.

[15] Cowan D a, Tow LA. Endangered antarctic environments. Annu Rev Microbiol 2004;58:649–90. doi:10.1146/annurev.micro.57.030502.090811.

[16] Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, et al. Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. Extremophiles 2010;14:205–12. doi:10.1007/s00792-009-0299-2.

[17] Møller AK, Barkay T, Abu Al-Soud W, Sørensen SJ, Skov H, Kroer N. Diversity and characterization of mercury-resistant bacteria in snow, freshwater and sea ice brine from the High Arctic. FEMS Microbiol Ecol 2011;75:390–401. doi:10.1111/j.1574-6941.2010.01016.x.

[18] Larose C, Dommergue A, Vogel TM. Microbial nitrogen cycling in Arctic snowpacks. Environ Res Lett 2013;8:035004. doi:10.1088/1748-9326/8/3/035004.

[19] Tansley AG. The Use and Abuse of Vegetational Concepts and Terms. Ecology 1935;16:284. doi:10.2307/1930070.

[20] Domine F, Albert M, Huthwelker T, Jacobi H-W, Kokhanovsky AA, Lehning M, et al. Snow physics as relevant to snow photochemistry. Atmos Chem Phys 2008;8:171–208. doi:10.5194/acp-8-171-2008.

[21] Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001;409:1092–101. doi:10.1038/35059215.

[22] Brock TD. Life at high temperatures. Science 1985;230:132–8. doi:10.1126/science.230.4722.132.

[23] Marshall SJ. The cryosphere. Princeton University Press; 2011.

[24] GLIMS, and National Snow and Ice Data Center. GLIMS Glacier Database 2012:Boulder, Colorado USA: National Snow and Ice Data . doi:http://dx.doi.org/10.7265/N5V98602.

[25] Feller G, Gerday C. Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 2003;1:200–8. doi:10.1038/nrmicro773.

[26] De Maayer P, Anderson D, Cary C, Cowan DA. Some like it cold: understanding the survival strategies of psychrophiles. EMBO Rep 2014;15:508–17. doi:10.1002/embr.201338170.

[27] Vincent WF, Mueller DR, Bonilla S. Ecosystems on ice: The microbial ecology of Markham Ice Shelf in the high Arctic. Cryobiology 2004;48:103–12. doi:10.1016/j.cryobiol.2004.01.006.

[28] Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu JC, et al. Glacial Ecosystems. Ecol Monogr 2008;78:41–67.

[29] Brown JR, Seymour JD, Brox TI, Skidmore ML, Wang C, Christner BC, et al. Recrystallization inhibition in ice due to ice binding protein activity detected by nuclear magnetic resonance. Biotechnol Reports 2014;3:60–4. doi:10.1016/j.btre.2014.06.005.

[30] Achberger AM, Brox TI, Skidmore ML, Christner BC. Expression and partial characterization of an ice-binding protein from a bacterium isolated at a depth of 3,519 m in the Vostok ice core, Antarctica. Front Microbiol 2011;2:1–8. doi:10.3389/fmicb.2011.00255.

[31] Krembs C, Eicken H, Deming JW. Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc Natl Acad Sci U S A 2011;108:3653–8. doi:10.1073/pnas.1100701108.

[32] Ewert M, Deming J. Sea Ice Microorganisms: Environmental Constraints and Extracellular Responses. Biology (Basel) 2013;2:603–28. doi:10.3390/biology2020603.

[33] Harding T, Jungblut AD, Lovejoy C, Vincent WF. Microbes in high arctic snow and implications for the cold biosphere. Appl Environ Microbiol 2011;77:3234–43. doi:10.1128/AEM.02611-10.

[34] Liu Y, Yao T, Jiao N, Kang S, Xu B, Zeng Y, et al. Bacterial diversity in the snow over Tibetan Plateau Glaciers. Extremophiles 2009;13:411–23. doi:10.1007/s00792-009-0227-5.

[35] Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 2006;103:626–31. doi:10.1073/pnas.0507535103.

[36] Arrigo KR. Sea ice ecosystems. Ann Rev Mar Sci 2014;6:439–67. doi:10.1146/annurev-marine-010213-135103.

[37] Gradinger R, Nurnberg D. Snow algal communities on Arctic pack ice floes dominated by Chlamydomonas nivalis (Bauer) Wille 1996:35–43.

[38] Stibal M, Elster J, Sabacká M, Kastovská K. Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. FEMS Microbiol Ecol 2007;59:265–73. doi:10.1111/j.1574-6941.2006.00264.x.

[39] Williams WE, Gorton HL, Vogelmann TC. Surface gas-exchange processes of snow algae. Proc Natl Acad Sci U S A 2003;100:562–6. doi:10.1073/pnas.0235560100.

[40] Remias D, Lütz-meindl U, Lütz C, Lu U. Photosynthesis, pigments and ultrastructure of the alpine snow alga Chlamydomonas nivalis. Eur J Phycol 2005;40:259–68. doi:10.1080/09670260500202148.

[41] Bachy C, López-García P, Vereshchaka A, Moreira D. Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the North Pole at the end of the polar night. Front Microbiol 2011;2:1–12. doi:10.3389/fmicb.2011.00106.

[42] Cameron K a, Hagedorn B, Dieser M, Christner BC, Choquette K, Sletten R, et al. Diversity and potential sources of microbiota associated with snow on western portions of the Greenland Ice Sheet. Environ Microbiol 2014. doi:10.1111/1462-2920.12446.

[43] Felip M, Sattler B, Psenner R, Catalan J. Highly active microbial communities in the ice and snow cover of high mountain lakes. Appl Environ Microbiol 1995;61:2394–401.

[44] Carpenter EJ, Lin S, Capone DG. Bacterial activity in South Pole snow. Appl Environ Microbiol 2000;66:4514–7.

[45] Sattler B, Puxbaum H, Psenner R. Bacterial growth in supercooled cloud droplets. Geophys Res Lett 2001;28:239–42. doi:10.1029/2000GL011684.

[46] Lopatina A, Krylenkov V, Severinov K. Activity and bacterial diversity of snow around Russian Antarctic stations. Res Microbiol 2013;164:949–58. doi:10.1016/j.resmic.2013.08.005.

[47] Anesio AM, Hodson AJ, Fritz A, Psenner R, Sattler B. High microbial activity on glaciers: importance to the global carbon cycle. Glob Chang Biol 2009;15:955–60. doi:10.1111/j.1365-2486.2008.01758.x.

[48] Anesio AM, Laybourn-Parry J. Glaciers and ice sheets as a biome. Trends Ecol Evol 2012;27:219–25. doi:10.1016/j.tree.2011.09.012.

[49] Stibal M, Telling J, Cook J, Mak KM, Hodson A, Anesio AM. Environmental controls on microbial abundance and activity on the greenland ice sheet: a multivariate analysis approach. Microb Ecol 2012;63:74–84. doi:10.1007/s00248-011-9935-3.

[50] Cloutier J, Prévost D, Nadeau P, Antoun H. Heat and cold shock protein synthesis in arctic and temperate strains of rhizobia. Appl Environ Microbiol 1992;58:2846–53.

[51] Kumar GS, Jagannadham M V, Ray MK. Low-temperature-induced changes in composition and fluidity of lipopolysaccharides in the antarctic psychrotrophic bacterium *Pseudomonas syringae.* J Bacteriol 2002;184:6746–9. doi:10.1128/JB.184.23.6746-6749.2002.

[52] Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, et al. Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 2004;8:475–88. doi:10.1007/s00792-004-0409-0.

[53] Gilbert JA, Davies PL, Laybourn-Parry J. A hyperactive, Ca2+-dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 2005;245:67–72. doi:10.1016/j.femsle.2005.02.022.

[54] Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001;409:1092–101. doi:10.1038/35059215.

[55] Ewert M, Carpenter SD, Colangelo-Lillis J, Deming JW. Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. J Geophys Res Ocean 2013;118:726–35. doi:10.1002/jgrc.20055.

[56] Paget E, Monrozier LJ, Simonet P. Adsorption of DNA on clay minerals: protection against DNaseI and influence on gene transfer. FEMS Microbiol Lett 1992;97:31–9. doi:10.1111/j.1574-6968.1992.tb05435.x.

[57] Collins RE, Deming JW. Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. Polar Biol 2011;34:1819–30. doi:10.1007/s00300-011-1041-y.

[58] Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D. Release and persistence of extracellular DNA in the environment. Environ Biosafety Res 6:37–53. doi:10.1051/ebr:2007031.

[59] Lorenz MG, Aardema BW, Krumbein WE. Interaction of marine sediment with DNA and DNA availability to nucleases. Mar Biol 1981;64:225–30. doi:10.1007/BF00397113.

[60] Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B 2001;63:88–102.

[61] Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE. *Escherichia coli* and *Salmonella typhimurium* - Cellular and molecular biology. American Society for Microbiology; 1987.

[62] Moran MA, Satinsky B, Gifford SM, Luo H, Rivers A, Chan L-K, et al. Sizing up metatranscriptomics. ISME J 2013;7:237–43. doi:10.1038/ismej.2012.94.

[63] Burke CM, Burton HR. The ecology of photosynthetic bacteria in Burton Lake, Vestfold Hills, Antarctica. Hydrobiologia 1988;165:1–11. doi:10.1007/BF00025569.

[64] Antoniades D, Veillette J, Martineau M-J, Belzile C, Tomkins J, Pienitz R, et al. Bacterial dominance of phototrophic communities in a High Arctic lake and its implications for paleoclimate analysis. Polar Sci 2009;3:147–61. doi:10.1016/j.polar.2009.05.002.

[65] Barsdate RJ, Alexander V. Photosynthetic Organisms in Subarctic Lake Ice. ARCTIC 1970;23:201. doi:10.14430/arctic3174.

[66] Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. PLoS One 2013;8:e79972. doi:10.1371/journal.pone.0079972.

[67] Prosser JI. Dispersing misconceptions and identifying opportunities for the use of “omics” in soil microbial ecology. Nat Rev Microbiol 2015;13:439–46. doi:10.1038/nrmicro3468.

Introduction

The cryosphere is defined as the portion of the Earth where the water is in solid form [1]. It includes sea ice, freshwater ice, glaciers, ice sheets, permafrost and snow cover. Snow, which can cover over 25% of the Earth's surface and up to one third of the terrestrial surface, influences the global energy and moisture budget and, thereby, climate [2]. Snow is also an interface between other biosphere compartments such as soil, aquifers, sea ice, and the atmosphere [3–6]. For example, snowpack acts as a photochemical bioreactor and is involved numerous gas exchanges with the atmosphere, playing a key role in the fate of volatile organic contaminants in cold regions [7]. While the snowpack appears to be a critical component of the cryosphere, it is disappearing. Snow cover was estimated to be reduced by 17.8 % in the Northern Hemisphere from 1979 to 2011 [8], with potential impacts on climate regulation [9]. The snow cover reduction may also influence macrobiotic and microbial ecosystems in snow-covered environments. For instance, increased temperature linked to early spring melt strongly affects ground temperature and soil processes [10]. The perturbation of biological processes in the snowpack however, is rarely included in cryospheric climate change models because snow as a biological entity is largely underestimated.

Snowpack is formed by the accumulation of deposited ice crystals that encountered post-depositional modifications [11]. In this cold porous medium, microorganisms are subjected to environment-specific physical and chemical conditions such as low nutrient concentrations, desiccation, freeze-thaw cycles, solar irradiation and therefore highly reactive photochemistry, in addition to low temperatures [1,12–14]. In the polar regions, light regime may also impact communities with periods of 24 h of sunlight during the

summer and darkness during the winter. Snow has not been considered suitable for supporting life, but rather as a trap for microorganisms in a vegetative state that releases them to other environments upon melting [15]. This thesis is part of the recent research challenging this view. Microorganisms in arctic snow have been partially characterized using various culture-dependent and -independent approaches. Recent molecular approaches, based on the extraction of DNA from snow samples, led to the identification of specific functional genes, such as ribosomal genes (coding for 16S rRNA), mercury resistance genes and nitrogen cycling genes [16–18]. These studies highlighted that diverse microorganisms are present in the snow at variable cell densities and might be involved in different biological processes. However, the snowpack as a functional ecosystem remains largely unexplored and this concept is central to this thesis. The notion of an ecosystem was first defined in 1934 by A. G. Tansley as a community of living organisms (plants, animals and microbes) in conjunction with the non-living component of their environment and interacting as a system [19]. Based on this definition, the investigation of snow as an ecosystem raised three major objectives with more specific research questions that were addressed in this thesis.

Due to its physical and chemical conformation, snowpack is described as a highly reactive and heterogeneous environment [20]. The first objective is thus related to understanding microbial community heterogeneity in relation to environmental conditions: how variable are snow communities? Is this variability related to changes in environmental conditions? What are the temporal and spatial scales of this microbial community variability in snowpacks? In order to address these questions, samples of snow microbial communities (present and potentially active) were collected over time and at different spatial scales and their taxonomical and functional composition as related to environmental parameters were compared. If the snowpack is a functional ecosystem, then the microbial communities

inhabiting it should have specific features related to their adaptation to the conditions of this environment, despite their variability. The second objective of this thesis relates to understanding microbial community structure and functioning: if the snow microbial community is specific, what are the physico-chemical drivers of snowpack microbial community structure? What are the specific functions that are selected in snowpack microbial communities? The method applied was to compare snow microbial community composition both in terms of taxa and function with other diverse environments, local and remote. The specificity of a microbial community implies that selection processes occur within its environment. How microbes are selected for in the snowpack constituted the third objective addressed in this thesis. Here two approaches were carried out: i) comparing the distribution of microbial community structure and function as related to the source of the microorganisms and ii) using laboratory experiments to examine the colonization of snow with model microorganisms. Finally, all these questions imply that snow microorganisms need to be active. The last objective was thus to investigate snow microbial activity: What is the active fraction of snow microbes and what is the time scale of microbial activity in snow?

These questions were addressed in various ways: at different time and spatial scales, using multiple techniques, and on different snowpack models. Two types of snowpack models were studied in this thesis: sea ice snow cover and seasonal snow from a terrestrial fresh water snowpack. Sea ice snow cover is a shallow snowpack that closely interacts with connected environments: *i.e*., sea ice and the atmosphere. It thus represents an excellent model for examining spatial distribution of snow microorganisms as related to seeding source effects and post-deposition selection processes. However, this type of snowpack is physically fragile and subjected to wind dispersion, ice cracks and low persistence during the spring season. Terrestrial freshwater snowpack are relatively more stable, and are more

suitable for investigating snow microbial community features and variability over an entire season as a function of environmental conditions. Most of the results presented in this manuscript are in the form ofmicrobial community taxonomic and functional assignments of genomic DNA sequences obtained by shotgun high-throughput sequencing technologies applied to samples from arctic spring snow from these two models: fjord sea ice snow cover in the vicinity of Nuuk, Greenland, and terrestrial snowpack in the vicinity of Ny-Ålesund in Svalbard. Preliminary data from RNA-based sequencing are also discussed as a proxy for microbial activity and a mechanistic approach to studying colonization processes, conducted using model microorganisms in laboratory experiment is presented. This manuscript is thus organized as the different experiments addressing these various aspects accomplished during these three years of thesis work. The first chapter of this thesis is a bibliography chapter, with the objective to highlight the abiotic characteristics and inhabitants of snow, as compared to other known frozen water habitats. The second chapter explores the microbial signature of arctic spring terrestrial snow in comparison to non-icy environments and addresses the link between variability in community structure and function and chemical fluctuations on a seasonal time scale. Chapter 3 focuses on the distribution of the microbial community in the different layers of sea ice snow cover as compared to the seeding sources of surrounding atmosphere and underlying sea ice. The last chapter addresses active processes in snowpacks in two distinct sections. The first one corresponds to a discussion of the use of meta-omic approaches to assess active microbial communities, based on preliminary results of present (DNA) and the variability ofactive (RNA) microbial community structure and function on a daily and spring season scale. The second section of Chapter 4 describes preliminary mechanistic laboratory experiments with model organisms that could help understand the mechanisms underlying the colonization processes of snow.

References

[1] Miteva V. Bacteria in Snow and Glacier Ice. In: Margesin, RosaSchinner F, Marx J-C, Gerday C, editors. Psychrophiles from Biodivers. to Biotechnol., 2008.

[2] Hinkler J, Hansen BU, Tamstorf MP, Sigsgaard C, Petersen D. Snow and snow-cover in central northeast Greenland. Adv Ecol Res 2008;40:175–95. doi:10.1016/S0065-2504(07)00008-6.

[3] Pomeroy JW, Brun E. Physical Properties of Snow. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW, editors. Snow Ecol. An Interdiscip. Exam. Snow-Covered Ecosyst., Cambridge University Press; 2001, p. 45–126.

[4] Schmidt SK, Lipson D a. Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil 2004;259:1–7. doi:10.1023/B:PLSO.0000020933.32473.7e.

[5] Schimel JP, Bilbrough C, Welker JM. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. Soil Biol Biochem 2004;36:217–27.

[6] Dominé F, Shepson PB. Air-snow interactions and atmospheric chemistry. Science 2002;297:1506–10. doi:10.1126/science.1074610.

[7] Daly GL, Wania F. Simulating the influence of snow on the fate of organic compounds. Environ Sci Technol 2004;38:4176–86.

[8] Derksen C, Brown R. Arctic Report Card - Snow -. 2013.

[9] Matsumura S, Yamazaki K. Eurasian subarctic summer climate in response to anomalous snow cover. J Clim 2012;25:1305–17. doi:10.1175/2011JCLI4116.1.

[10] Edwards AC, Scalenghe R, Freppaz M. Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. Quat Int 2007;162-163:172–81. doi:10.1016/j.quaint.2006.10.027.

[11] Dominé F, Lauzier T, Cabanes A, Legagneux L, Kuhs WF, Techmer K, et al. Snow metamorphism as revealed by scanning electron microscopy. Microsc Res Tech 2003;62:33–48. doi:10.1002/jemt.10384.

[12] Grannas AM, Shepson PB, Filley TR. Photochemistry and nature of organic matter in Arctic and Antarctic snow. Global Biogeochem Cycles 2004;18:n/a – n/a. doi:10.1029/2003GB002133.

[13] Grannas AM, Jones AE, Dibb J, Ammann M, Anastasio C, Beine HJ, et al. An overview of snow photochemistry: evidence, mechanisms and impacts 2007:4329–73.

[14] Techel F, Pielmeier C. Point observations of liquid water content in wet snow - Investigating methodical, spatial and temporal aspects. Cryosphere 2011;5:405–18. doi:10.5194/tc-5-405-2011.

[15] Cowan DA, Tow LA. Endangered antarctic environments. Annu Rev Microbiol 2004;58:649–90. doi:10.1146/annurev.micro.57.030502.090811.

[16] Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, et al. Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. Extremophiles 2010;14:205–12. doi:10.1007/s00792-009-0299-2.

[17] Møller AK, Barkay T, Abu Al-Soud W, Sørensen SJ, Skov H, Kroer N. Diversity and characterization of mercury-resistant bacteria in snow, freshwater and sea ice brine from the High Arctic. FEMS Microbiol Ecol 2011;75:390–401. doi:10.1111/j.1574-6941.2010.01016.x.

[18] Larose C, Dommergue A, Vogel TM. Microbial nitrogen cycling in Arctic snowpacks. Environ Res Lett 2013;8:035004. doi:10.1088/1748-9326/8/3/035004.

[19] Tansley AG. The use and abuse of vegetational concepts and terms. Ecology 1935;16:284. doi:10.2307/1930070.

[20] Domine F, Albert M, Huthwelker T, Jacobi H-W, Kokhanovsky AA, Lehning M, et al. Snow physics as relevant to snow photochemistry. Atmos Chem Phys 2008;8:171–208. doi:10.5194/acp-8-171-2008.

[21] Carpenter EJ, Lin S, Capone DG. Bacterial activity in South Pole snow. Appl Environ Microbiol 2000;66:4514–7.

Chapter 1: Bibliography - frozen water habitats and inhabitants

Abstract

Snow and ice environments cover up to 25% of the Earth's surface. They have been regarded as extreme environments because of their low temperatures, high UV irradiation, low nutrients and low water availability, and thus, their microbial activity has not been considered relevant from a global microbial ecology viewpoint. In this chapter, we focused on the characteristics of frozen water habitats and inhabitants and why snow and ice habitats might not be extreme from a microbiological perspective. Microorganisms interact closely with the abiotic conditions imposed by snow and ice habitats; their diverse adaptations include genetic resistance mechanisms to different types of stresses in addition to an ability to inhabit various niches where these potential stresses may be reduced. The microbial communities inhabiting snow and ice are not only abundant and taxonomically diverse, but complex in terms of their interactions. Altogether, snow and ice seem to be true ecosystems with a role in global biogeochemical cycles that has likely been underestimated. Future work should expand past resistance studies to understanding the functioning of these ecosystems.

Introduction

Extreme environments are defined as areas where one or more of the abiotic parameters of pH, temperature, water content, nutrient levels, solar irradiation, pressure, etc., reach extreme values [1]. These harsh conditions are considered to be limiting for the development of life. The range of values that are considered extreme, however are often regarded from a macro-organism point of view, whereas the limits for the development of microbial life on Earth have not yet been fully established. The parameters cited above

influence all organisms at the individual scale and could, to different extents, cause cellular physiological stress. However, microorganisms harbor a remarkable capacity to adapt to a wide range of habitats. To date, no place on Earth has been shown to be sterile, for even the most “inhospitable” environments, such as deep sea hydrothermal vent, acid mine drainage and hot springs, contain many viable microorganisms. In the case of hot springs, the occurrence of a viable and active microbial community has been recognized relatively early in the history of microbial work on extreme environnments [2].

At the opposite end of the temperature spectrum, snow and ice have received much less attention. Apart from low temperatures, seemingly pristine snow and ice (freshwater in particular) have low water availability, low nutrient contents and high solar irradiation during summer and darkness during winter, but they still support microbial inhabitants. Snow, glacial ice and sea ice, main components of the cryosphere, cover up to 1.06 x 10^8 km2 of the Earth’s surface. Snow in the winter can cover up to 12% of the Earth’s surface, which is about 61 million km^2 [3]. Approximately 10% of the planet’s land, about 15 million km^2, is covered by glacial ice in the form of ice caps, ice sheets or glaciers, storing 75% of the world’s fresh water [4]. Given their massive coverage at a global scale, snow and ice could have a major and underestimated role in global biogeochemical cycling (Figure 1). Therefore, the relevant knowledge and knowledge gaps concerning the microbial ecology of snow and ice ecosystems need to be evaluated for future research.

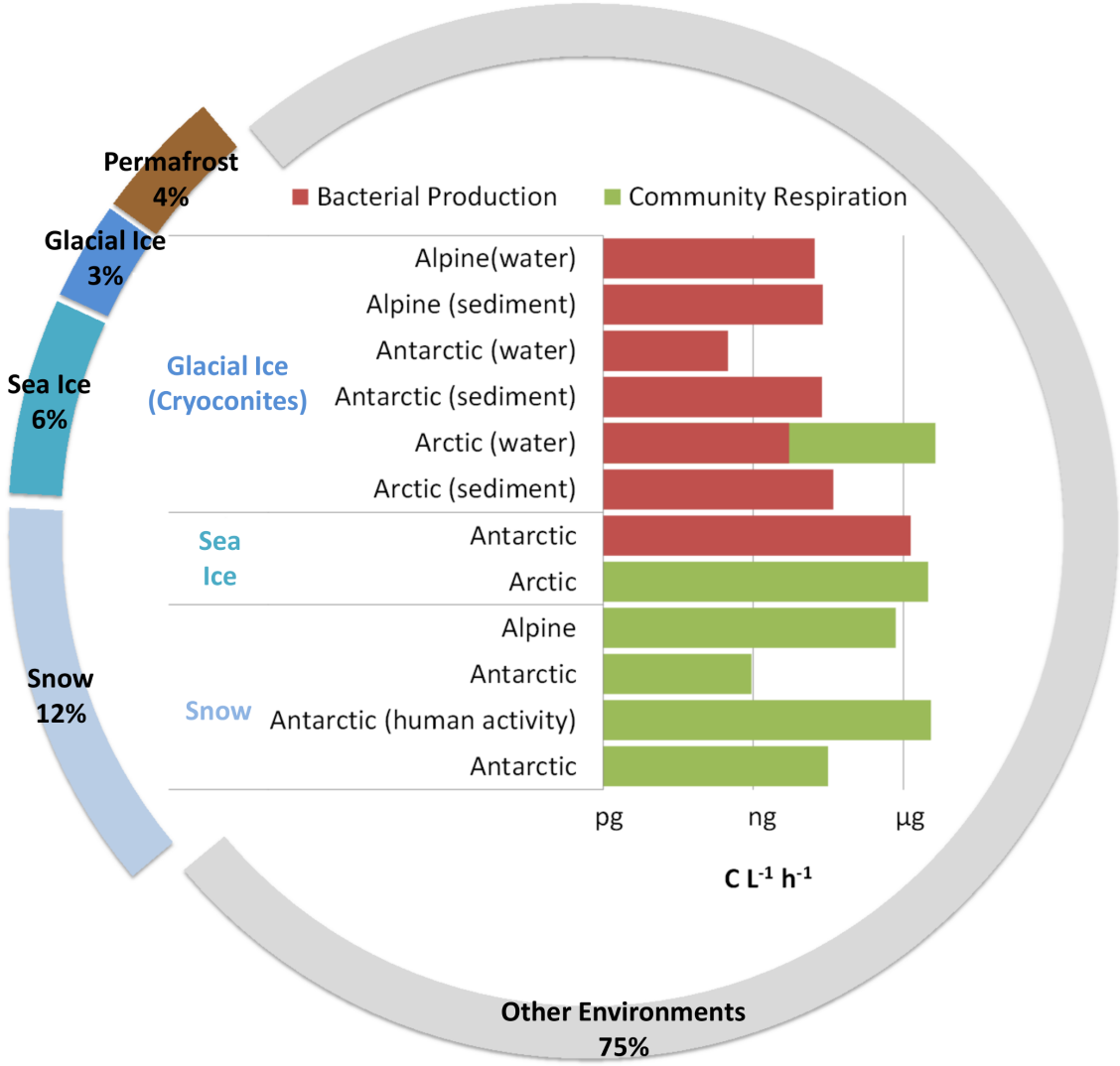

Figure 1. Coverage and microbial productivity of snow and ice. The graph on the outer ring depicts the percentage of the surface of the Earth covered by the different environments of the cryosphere. The bar chart shows bacterial production and community respiration rates (in Carbon per litre per hour) found in the literature for these environments. Sediment or water represent the different parts of cryoconite. Human activity refers to snow samples taken near a scientific station and potentially impacted by human activity.

In this chapter, we will review the mechanisms known to enable microorganisms to circumvent the potentially deleterious conditions found in snow and ice environments. We will also address the abundance and diversity of the snow and ice microbial communities as well as the interactions that take place within the community assisting them to inhabit snow and ice. Given that an ecosystem is defined as the compendium of the organisms present, the physical system that hosts them, and the interactions of the organisms with the abiotic factors as well as the interactions between organisms [5], the available data support the idea that snow and ice are true ecosystems and actually not so extreme from the microbial perspective.

Abiotic interactions

Snow and ice are habitats on Earth whose matrices are partly composed of frozen water, and thus, impose specific conditions on microbial life. The driving forces for microbial community structure and function might be the result of these physical-chemical conditions: low temperatures, scarcity of nutrients and high UV radiation. In this section, we compare the different abiotic attributes of snow and ice ecotypes and the related microbial adaptations, as illustrated in figure 2. The objective is to understand how the microbial community actively interacts with its physical-chemical environment.

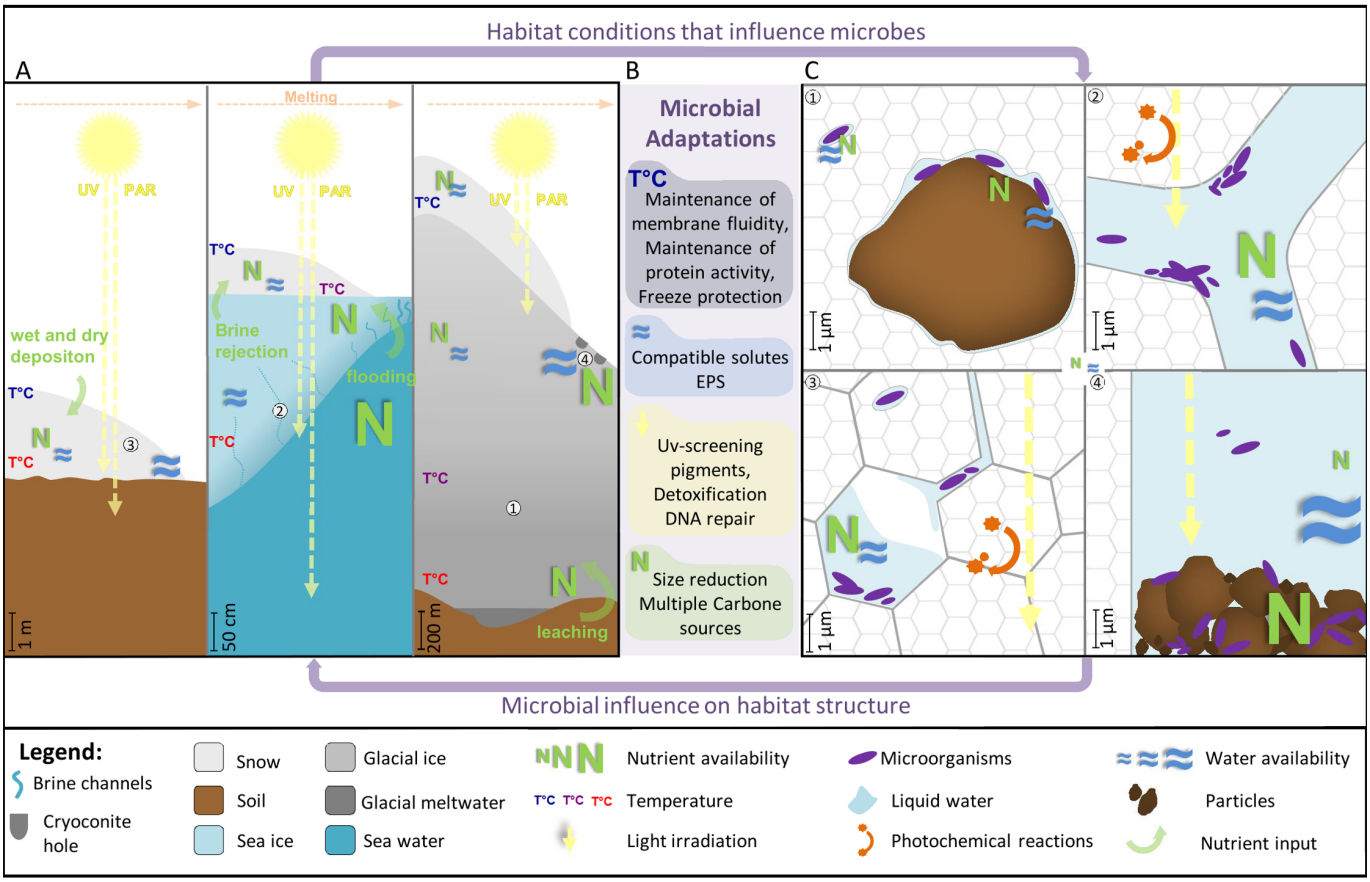

Figure 2. Microbial interactions with abiotic conditions in the different habitats within snow and ice environments. A: habitat structure and abiotic conditions at macroscopic level. B: Microbial adaption in response to potential stress caused by low temperature, water and nutrient availability and high UV-radiation. C: Examples of niches at micro-scale (1: particle or microbial cell trapped within glacial ice, 2: Sea ice brine channel, 3: Snow, 4: cryoconite hole)

Temperature

Snow and ice, by definition, are found in the coldest places on the planet and fall within group E of the Köppen climate classification or in polar and alpine climates, which are characterized by monthly mean temperatures below 10°C [6]. The term cryosphere, first introduced by Dobrowolski in 1923 [7], derives from the Greek *cryos,* which means "cold" or "ice" and refers to areas where water is in a frozen state [8]. Mean temperatures observed in snow and ice environments can be highly variable at different depths, sites or seasons. For example, surfaces directly exposed to wind are influenced by air temperature (as low as

–50°C and –70°C during the winter in the Arctic and Antarctic, respectively and can be as warm as 0°C during the melt period in summer) and therefore exhibit the coldest and most variable temperature. Top snow layers work as insulators leading to an increase in temperatures in the layers below. The low temperatures can be deleterious for cells due to increases in membrane rigidity that will limit substrate exchange, reduction in enzyme activity and damage to cells from cytoplasmic water crystal formation.

In laboratory experiments, the coldest temperature where bacterial growth has been observed is -15°C [9] while metabolic activity has been detected at temperatures as low as -40 °C [10]. Many studies on microbial life in the cryosphere have focused on understanding how microorganisms can tolerate the cold. Cold-adapted microorganisms are termed psychrophiles if their optimal growth temperatures fall at or below 15°C, and psychrotolerants areable to grow at or below 0°C but have optimal growth temperatures between 20 and 25°C [11]. They use strategies at different levels of cell physiology, including increased membrane fluidity facilitated by unsaturated fatty acid synthesis, desaturases, echanisms for freeze protection use of compatible solutes, synthesis of anti-freeze and ice-binding proteins, and maintenance of protein catalytic efficiency (reduction of internal hydrophobic interaction, proline and arginine content, increased length of external loops and accessibility to active site) (see detailed reviews on psychrophilic adaptations [12,13]) , also recently highlighted by sequencing technologies [14]. Many of the organisms with specific cold adaptations are found in snow and ice environments. Sequences associated with cold-adapted organisms have been systematically detected in snow and ice sequencing datasets [15–17]. The psychrophilic lifestyle of some snow and ice microorganisms has been confirmed via selective cultivation of isolates at low temperature [18,19].

While cold-adapted microorganisms or cold adaptations seem to be ubiquitous in snow and ice habitats, close relatives to psychrophilic or psychrotolerant microorganisms have also been detected in warmer environments. For example, strains isolated from a shallow estuary in Portugal with mean temperatures around 20°C formed an operational taxon unit (OTU), whose cluster consensus sequence showed 100% similarity with the partial 16S rRNA gene sequence of *Psychrobacter glacincola*, originally isolated from Antarctic sea ice and ice shelf [20]. Athough all strains of *Psychrobacter* were able to grow at 4 and 28°C, most of them also grew at 37°C, suggesting a higher optimal growth temperature than for the genera described in colder environments [21]. These findings suggest that cold adaptation or adapted microorganisms have not been restricted to cold environments. In addition, many of the microorganisms identified in snow and ice have not been described as psychrophiles. This might suggest that these microorganisms, including mesophiles, are trapped in ice and snow in a vegetative non-adapted state until release upon snow and ice melting [22], but could also explain this observation by a bias towards mesophilic representatives in genome databases. Indeed, at the time of writing, only 194 genomes, which is less than 2% of the Gold genome database (updated in real time), corresponded to cold-adapted microorganisms [23]. Therefore, sequences obtained from an unknown psychrophilic or psychrotolerant organisms could be matched against genomes of a closely related mesophile leading to an erroneous physiological classification. An alternative hypothesis is that microbial genome plasticity that enables temperature adaptation could be another hypothesis. Experiments have shown that after exposing mesophiles, including *E. coli*, to temperatures of -1.8°C for 48 days, their optimal growth temperature decreased and they even lost their ability to form colonies at 37°C [24].

On the other hand, the application of supra-optimal temperatures induced differentiation to heat tolerant phenotypes in psychrophilic prokaryotes and eukaryotes [25,26]. We can hypothesize that although specific mechanisms like psychrophilicity might occur in snow and ice they do not represent the entire community function. Therefore, psychrophilic life style might not be a unique criteria defining microbial ecology within snow and ice.

Low water activity / salinity

One physical consequence of freezing temperature is the low liquid water activity, as frozen water is not available for chemical reactions. Cooling of the sea surface due to cold atmospheric temperatures induces the creation of ice crystals, leading to sea ice formation. Formation and characteristics of sea ice depend on environmental conditions, such as temperature, sea turbulence and ice flooding, and brine volume will vary as a consequence of the formation conditions [27]. Channel sizes can vary from several to hundreds of micrometers in diameter [28]. As the water freezes, liquid water containing all elements excluded from ice crystals is concentrated in brine channels and pockets. Temperature determines solute concentrations and salinity varies from 30 ppt at freezing point temperature of -2°C and up to 230 ppt at -30°C [29]. Glacial ice is formed by layers of snow that accumulate over winter with each snowfall. As snow is transformed and frozen into ice crystals, impurities are excluded from the ice creating hyper-saline and hyper-acidic vein networks [30,31]. As the pressure exerted by the upper layers increases, ice crystals grow while air spaces and vein size decreases. Melting of snow and ice on the surface of glaciers can deliver water to englacial and maybe even basal areas of the glacier through crevasses, moulins and other channels [32–35]. Several detailed reviews of the different types of glacial

ice and their hydrology have been published recently [36,37]. The thermal structure of a glacier regulates the distribution of liquid water, and thus, might define the different microbial habitats that can be found in glaciers. Microorganisms within glacial and sea ice veins are confronted with desiccation and relatively high osmotic pressure, resulting in cytoplasmic water loss and intracellular ionic imbalance. Potential microbial adaptations to low water availability have been detected in sea ice and glacial ice. For example, synthesis and uptake of compatible solutes acting as osmolytes might help microorganisms to face the osmotic pressure. Genes encoding the synthesis of some osmoprotectants, such as glycine, betaine, choline, sarcosine, and glutamate, have been detected in alpine glacier ice core metagenomes [15]. Glycine-betaine (and proline), were shown to offer protection against fluctuating temperatures and salinity in two sea ice model organisms *Colwellia psychrerythraea strain 34H* and *Psychrobacter P7E* [38]. Moreover, proteomic comparisons from three sea ice halophilic *Gamma-Proteobacteria Alteromonadales* against non-halophilic and extremely halophilic organisms showed a higher ratio of acidic membrane proteins, which may prevent protein precipitation under high salt conditions [39]. Extracellular polymeric substances are implicated in various aspects of life in sea ice [29] and can also help with tolerating osmotic stress as is the case for the sea ice diatom *Fragilariopsis cylindrus* [40] and for *Alteromonadales,* a sea ice isolate from the *Pseudoalteromonas* genera [41]. Osmoadaptation has also been observed with eukaryotic ice inhabitants. *Chlamydomonas sp. ARC*, isolated from Arctic sea ice, tolerates a broad range of salinities from 2.5 to 100 ppt [42]. Transforming its vacuoles into tubular complexes called spongiomes, the algae expels excess water when exposed to decreasing salinity correlated with sea ice melt [42]. Using selective hyperosmotic (sugar or salt) media, up to 9.10^3 CFU of

xerophilic fungi identified as black yeast, yeast or a large variety of *Penicillium* were isolated from an Arctic glacial ice core [43].

Within glacial ice, ice veins might not be the only habitat able to support microbial life. Microbes might be also present within ice cores, like on the surface of trapped minerals [44]. Laboratory ice formation simulation suggested that while larger particles (5µm and 10 µm) get trapped in the bulk ice, microbial cells and smaller particles are excluded from the ice and into the veins [45]. However, Rohde and Price proposed that microorganisms could also survive trapped directly in ice crystals by a coat (1 nm) of water that would be sufficient for nutrient exchange [46]. Osmotic stress in these habitats, not subjected to solute accumulation, might be lower than in ice veins. Morever, liquid water niches are created by the melting of snow and ice on the surface of glaciers, such as supra-glacial lakes, ponds and streams, and cryoconite holes. Cryoconites, from the greek *kruos* (ice) and *konis* (dust), are small aggregate granules of mineral particles, organic matter, and microorganisms [47]. These granules absorb light leading to the melting of snow and ice and the creation of cryoconite holes that are filled with liquid water and a bottom layer of sediment. In addition, the combination of friction created by the movement of the glaciers and the geothermal heat from the ground beneath them can melt the ice at the base of the glaciers creating a layer of liquid water between the ice and the bedrock [48–50]. In these habitats that originate from melting, water content and activity might be less limiting than in the vein systems. However, metagenome datasets of samples from microbial mats that formed on Arctic and Antarctic ice sheet ponds appear to have numerous genes involved in compatible solute synthesis and uptake. Antarctic mats metagenome datasets had a higher representation of genes encoding a general stressor regulon Sigma B, which has been

suggested to reflect a greater osmotic pressure at freeze-up in the more saline waters from Antarctic ponds compared to Arctic ponds [16].

Snowpack is a porous media formed by accumulation of deposited snowflakes. Its water content can vary from less than 0.5% in dry snow to above 15% in soaked snow [51] depending in part on temperature. This variation appears to affect water distribution at the microscale. At low water content, water forms isolated patches in concave areas such as at grain boundaries. When water content reaches above 10%, the water film is continuous with no interface between the ice crystal and interstitial air [52]. Snowpack physics influences chemical distribution and reactions [53]. However interaction of these physical parameters with microbial inhabitants within the snow matrix is not well understood. For example, the exact location of microorganisms within the snowpack, and the size and configuration of their microscale habitat within the water film is largely unknown. Some microorganisms might swim through the snowpack as a mechanism to escape from stress, such as high light [54], but the motility of microorganisms in the snow matrix has never been verified. Snow microbes contained in the thin water film might be exposed to high osmotic pressure. Yet, mechanisms of microbial response to low water activity in snow matrices have not been demonstrated.

Snow and ice water availability as well as the potential osmotic stress exerted on microorganisms might be variable within the different snow and ice ecotypes. Some niches, due to melting processes, can benefit of increased water availability, such as water ponds and streams and cryoconite holes. However these structures are mostly ephermal and freeze again during cold season [32,33]. Mechanisms from osmoadaptation that allow life at

low water activity have largely been described, mostly involving the production or accumulation of compatible solutes that are used by a majority of halophiles and all xerophiles [55]. As seen above, this mechanism has been reported in both sea ice and glacial ice, but not in snow. Given the large variety of compatible solutes (free amino acids, sugars and polyol) used by a wide range of microorganisms (Archaea, Bacteria and Eukaryotes) [56–58], further research could help determine how important osmoprotection is in snow and ice ecosystems.

Solar irradiation

Snow and ice located in the polar zones are subjected to continuous light during the summer, while alpine ecosystems are exposed to high light levels due to altitude. Solar radiation reaching the surface is composed of ultraviolet (UV-B and A), photosynthetically active (PAR) and infrared (IR) radiation. The optical properties of snow and ice are intimately related to their physical structure (air, water and dust content), but generally snow and ice are highly scattering media with a highly reflective surface (high albedo) [59]. Due to recent ozone depletion, polar regions have been subjected to increased UV exposure. Snowpacks are especially reactive to UV light and have been described as natural photochemical bioreactors due to a multitude of ice grains resulting in an ice-air interface increasing the scattering effect [60][53]. The sequestered chemical compounds are photolyzed and reactive trace gases are released in the snow boundary layer. The processes are increased by higher gas diffusion due to low temperatures. These photochemically-induced reactions may result in the accumulation of reactive species within the snowpack, and thus, a hyper oxidative stressed habitat. However, UV-B transmittance is efficiently attenuated by the snow [59] , protecting underlying communities [61]. For instance, levels have been measured one order

of magnitude lower at a depth of 8 cm and photochemical reactions were estimated to occur for 85% within the ten first centimeters in two Arctic snowpacks [62] [63]. Uncovered sea ice has been shown to be an efficient medium for photochemical reactions with 85% occurring in the first meter of the sea ice column [64]. Deep ice core in glaciers are thought to be completely shielded from direct exposure to sunlight, but they are exposed to cosmic radiation, which may initiate ionizing transformations, although this phenomenon is poorly understood [65].

UV radiation is known to be deleterious for cell physiology [66] and the subsequent damage and microbial resistance are wavelength dependent and variable at the species level [67]. Generally, UV-B is involved in direct damage to absorbing biomolecules, such as DNA, by forming pyrimidine dimers that impede replication and transcription, whereas UV-A has indirect effects by producing reactive oxidative species (ROS) that cause oxidative damage to lipids, DNA and proteins. The production of UV-screening compounds, mycosporine-like amino acids, was observed in several ice samples exposed to high levels of UV radiation in Arctic first year sea ice [68], the surface layers of Baltic sea ice [69] and in Antarctic sea ice [70]. Astaxanthin, a secondary carotenoid with multifunctional stress response characteristics, also acts as a detoxifier of ROS produced by photochemical reactions and UV-screen in various algae [71]. This pigment, cytoplasmic or in polymer structure in cell wall, has been identified in snow algae like *Chlamydomonas nivalis* [72] [73] and *Chloromonas polyptera* [74] and its production is increased after exposure to high light [75] [76]. Other algae, such as members of the Zygnematophyceae class inhabiting glacial surface bare ice do not harbor any mycosporine-like compounds or secondary carotenoids, but accumulates a brownish UV-absorbing pigment identified as purpurogallin-derived [77]. Laboratory studies showed that when exposed to lower irradiance levels, *Mesotaenium*

berggrenii reduced or even lost the pigment-containing vacuoles [78]. This polyphenol was only previously observed in higher plants. These examples illustrate that specific and unique strategies to face UV-radiation might exist in snow and ice communities, but the total community response to UV is probably not restricted to pigment in photosynthetic algae. Metabolic stress, with cell activity reduction has been observed in Antarctic sea ice bacteria after initial UV-B exposure, but the possible resistance mechanism was not elucidated [79]. Although, arctic glacier ice core [15] and microbial mats from arctic and antarctic ice sheets ponds [16], highlighted the occurrence of some genetic traits for response to photo-oxidative stress, UV-radiation and photo-oxidative stress response mechanisms specific to snow and ice communities as a whole remain largely undescribed. A variety of defense mechanisms against oxidative and photooxidative stress are known for microorganisms, including heterotrophic representatives, from various habitats. For instance, melanin and mannitol are involved in antioxidant and UV protection in fungi [80] [81] also from Antarctic endophytic [82] or rock [83] communities. Detoxification systems (OxyR and SoxRS regulons) involving enzymatic oxidant elimination and DNA repair were described in a wide range of bacteria [84]. DNA repair mechanisms are also widespread in all microbial kingdoms based on base or nucleotide excision [85]. Searching for these mechanisms in snow and ice should be addressed in the future as microbial communities could have developed a wide diversity of adaptations given the levels of UV-light in these environments.

PAR radiation, used by phototrophic organisms during the process of photosynthesis, could also have deleterious effects. Snow and ice microbial communities are able to optimize their metabolism to maximize light-harvesting and protect against damaging light levels. Adaptation and acclimation of photosynthetic microorganisms to permanently cold

environments including snow and ice has been reviewed in detail [86]. In sea ice, diatom algae adjust the pigmentation by increasing the ratio of xanthophyll diaxanthin with increasing levels of irradiance [87]. Antarctic sea ice microalgae dissipate extra light energy via non photochemical quenching to avoid short term photo-inhibition [88]. Light intensities could influence the vertical distribution of photosynthetic microorganisms in snow [54] or in the ice matrix [89]. Other mechanisms such as the Circadian clock, that permit Cyanobacteria to regulate metabolism depending on light, may also be crucial for light dependent metabolisms under constant light conditions [90]. Light is a major factor driving microorganism ecology in a wide range of processes and thus determining various life styles; photosynthesis, photoacclimatation, phototactism but also attachment and biofilm formation [91].

Nutrients

Snow and ice environments are generally oligotrophic, therefore, sources of nutrients could be very critical to their inhabiting communities. One carbon source might be inorganic carbon fixation and autotrophic energy production by primary producers [92] [93] [30]. Photosynthetic activity might be important in several cryosphere ecosystems such as colored snow colonized by snow algae [94] [74], ice sheet surfaces [95], ice lake covers [96], sea ice [97] and cryoconite holes [98]. Highly active cells of *Ancylonema nordenskiöldii* algae were isolated from arctic glacial ice [77], which is consistent with these photosynthetic microbes being relevant in carbon input in snow and ice habitats. However, algal blooms are periodic, probably restrained to specific conditions. Moreover, polar snow and ice microorganisms are subjected to total absence of light during winter. How the phototrophic members of the community survive through winter and are impacted by this low light regime is still largely

unknown. The occurrence of mixotrophic algae, able to sustain their metabolism through bacterivory, has been described in Arctic sea ice samples at the end of the polar night as an alternative pathway [99]. Photosynthetic organisms and activity in deeper layers of glacier ice cores are rarely detected, likely due to lower levels of irradiation [15]. Yet, non-photochemical autotrophic processes could also be involved in nutrient dynamics within these habitats. The importance of this lifestyle was supported by the presence in glacial ice metagenomes of a significant number of genes involved in carbon fixation [15]. In subglacial areas, water, organic carbon and minerals sustain the development of microbial communities where chemolithotrophic primary production could be an important process [100–102].

Another source of organic compounds for microorganisms in glaciers and ice sheets could be carbon released from dead cells and allochthonous carbon from local or remote origins [103]. Numerous genes detected in glacier ice core metagenomes related to xenobiotics, biopolymers and other carbon sources suggest that glacial ice microorganisms have the potential to degrade a wide range of substrates [15]. Microbial degradation of xenobiotic compounds from distant allochtonous sources has been observed on the Greenland Ice sheet [104]. Microbial preferences for different carbon classes were also studied in Antarctic snow and results showed a higher rate of carbon uptake when snow microcosms were amended with a combination of simple and complex carbon sources [105]. In the same study, snow isolates were capable of oxidizing a broad spectrum of low and high molecular weight carbon sources including amino acids, amines, amides, carboxylic acids, carbohydrates, and complex polymers. Altogether these results highlight the potential for high metabolic versatility of microorganisms in snow and ice habitats with low

concentrations of many different carbon sources. One strategy to cope with low nutrients is to reduce cell size, since small size increases the surface to volume ratio, thereby improving the ability to efficiently scavenging for nutrients [106]. Miteva and Brenchley in 2005 observed a dominance of cells with a size inferior to 0.1µm in addition to numerous isolates that were closely related to the oligotrophic ultramicrobacterium *Sphingopyxis alaskensis* in a Greenland glacial ice core [107].

However, certain niches in snow and ice ecosystems could actually be nutrient-rich environments. For example, given that nutrients and particles are excluded from the ice crystal structure and are concentrated in pore spaces, concentrations of nitrate and sulfate in ice veins from Greenland and Antarctica were 10^3 to 10^5 times more concentrated than in bulk ice [30]. These levels of nutrients are similar to what can be found in rich growth media [45] and are likely sufficient to support microbial activity in deep glacial ice [46,108]. Even in the bulk ice, microorganisms might be associated with mineral particles and may extract energy from redox reactions with putative methane production based on observations from ice cores [109]. Cryoconite holes might also be nutrient-rich areas, as these semi-stagnant aquatic habitats have been considered to be eutrophic [110,111]. In sea ice, DOC concentration is often correlated to algal biomass and can be higher than the underlying seawater [112,113]. The high culturability of sea ice microorganisms observed using both organic-rich and-poor media is consistent with the microorganisms coming from nutrient-rich conditions [18]. As for the snow, crystal formation can be induced by particles named ice nuclei, which can be suspended in the atmosphere. As snowflakes fall, they scavenge atmospheric particles including microorganisms and pollutants [114]. Thus, snow has been defined as a nutrient and microbial reservoir [115]. Due to dry and wet atmospheric

deposition events, snowpack inhabitants can be exposed to occasional inputs of particles and nutrients, such as nitrogen compounds, derived from natural or anthropogenic sources. Several reports suggested that snow microorganisms are able to metabolize these nitrogen compounds. For example, nitrogen cycling pathway genes were apparently increased in microbial communities during spring and were correlated to different nitrogen compounds [116]. Fluxes of nitrogen species in snow during polar night (*i.e.* in the absence of photochemical reactions) were observed and attributed to microbial activity [117]. Other sources of nutrients for snow over sea ice could be sea ice brines and seawater. When snow accumulates on the sea ice, the added weight can cause seawater to flood over the ice [118]. Also, during sea ice freeze-up, liquid brine is rejected to the surface, wetting the overlaying snow layer [119].

In this section we have seen that snow and ice could be suitable to sustain microbial life and might not be so extreme from a microbial point of view. Although snow and ice share multiple characteristics due to their ice crystal backbone structure and their homogeneous appearance at a macroscopic level, they are composed of a multitude of niches under different conditions. At a microscopic level for each micro-habitat, stresses, such as low nutrients or water availability, might not be as extreme as expected. Moreover, a wide range of strategies could be used by individual cells to resist the potential stress in snow and ice. Snow and ice microorganisms are not only able to face the physicochemical conditions of their habitats, but they are also shaping their biotic environment. Pigment producers developing at surfaces, and forming watermelon snow or brown, red and grey ice, drastically decrease surface albedo and increase local temperature and water content [37,120]. In glacier and lake ice, the production of ice-binding proteins by bacteria can modify ice vein structure by inhibiting recrystallization, which seems to reduce diffusion in veins [121,122].

Likewise, EPS production by sea ice algae and bacteria modify the structure of the ice by reducing permeability, considered essential for the creation of connected and complex brine channels within sea ice [29,123]. All of these physical and chemical modifications can facilitate the development of complex microbial communities.

Snow and ice inhabitants

Over the past twenty years, microorganisms inhabiting the cryosphere have been increasingly studied for the potential discovery of enzymes with biotechnological interest and for fundamental research on the ecology of "extreme" environments [8,27,124–126]. Among these cold environments, snow and ice have unexpectedly high microbial abundance and diversity. In general, microbial cell counts registered in the snowpack range from 10^3 to 10^4 cells per ml of melted snow collected over soil and ice in Arctic, Antarctic and Alpine environments. A few reports described counts up to 10^5 cells per ml in some Alpine sites [127,128]. In debris-rich sea and glacier ice, the microbial abundance varies between 10^4 to 10^7 cells per ml of melted bulk ice. Among the cryosphere ecosystems, the lowest microbial abundance was observed in Antarctic snowpack samples from Concordia, which had only hundreds of cells per ml of melted snow. At the bottom of the sea ice, the concentration of microorganisms, especially algae, appears to increase. Subglacial lakes, which are formed by accumulation of glacial melt water at topographic depressions, can have two types of ice; the top layer is glacial ice formed through the deposition of snow, while the lower layer is accretion ice (refrozen lake water) [129]. In Lake Vostok, observed microbial abundances in accretion ice were two to seven times higher than in overlying glacial ice [130]. Also, while microbial abundance is expressed by total volume of melted samples and therefore

averaged over the sample size, cells are probably concentrated into the liquid phase of snow and ice. Models of particle exclusion in glacial ice veins predict that the concentration of cells could be a thousand times higher in the veins than the bulk melt water [45]. Due to the potential small cell sizes of cryosphere microorganisms, recent studies have suggested that the microbial abundance of frozen water ecosystems like snow and ice might be underestimated, given that the methods used for cell count measurements generally involve filtration and the entire community may not be retained by the filters [107,131]. The greatest abundance of microbial cells registered in cryoconite hole sediments were up to 10^8 cells/g, which is equivalent to that of soil communities [132].

Bacteria seem to be ubiquitous in snow and ice, as they have been observed systematically in all snow and ice environments studied, regardless of the methodological approaches used (culture dependent or not) (figure 3). The bacteria identified from these studies belong to numerous taxa, although mostly from Proteobacteria (Alpha-, Beta- and Gamma-), the Cytophaga-Flexibacter-Bacteriodes group, Actinobacteria, and Cyanobacteria. The estimated bacterial diversity varied among the different ecosystems studied. For example, the Shannon diversity index ranges from H'=0.18 in Canadian high Arctic ice sheet snow to near H'=4.0 in Tibetan plateau snow [133] [128]. The average bacterial diversity measured in snow and ice is similar to that of temperate soils ($2.4<H'<3.6$) [134].

Unlike Bacteria, Archaea are only rarely observed. Sequences associated with the Archaeal domain were detected with a relative abundance below 1% in Arctic spring snow samples over soil, sea ice and ice sheet. These sequences were annotated to Thaumarchaeota and Euryarchaeota [135,136]. Thaumarchaeota were also detected at low levels in cryconite

holes from Arctic and Antarctic glaciers [135,136]. In contrast, Archaea comprise 3-7% of the microbial community in both arctic [137,138] and Antarctic sea ice [139], which is explained by their clear presence in the source seawater. Snow and ice also provide habitats for a wide range of eukaryotic microorganisms such as fungi, microalgae or heterotrophic protists (see review by Arrigo, 2014 [27]). High concentrations of snow algae (*Chlamydomonas nivalis* and related species) can be found in snowpacks from various locations (from Arctic pack ice snow [54,140] to alpine systems like the Rocky Mountains [141] or the Austrian Alps [94]) producing the red snow or watermelon snow phenomenon. In sea ice/ice sheet snow cover, the eukaryotic community is largely dominated by fungi (Ascomycota and Basidiomycota) followed by Alveolata (Dinoflagellata) [99,133,142]. However, fungi are rarely detected in sea ice, which is dominated by microalgae, the most abundant taxa being diatoms (Bacillariophyceae) in both in Arctic and Antarctic samples. Heterotrophic protists including ciliates and flagellates are also common in sea ice. Algae can be found within cryoconite holes or on the surface of glaciers, creating what has been named “grey ice” [143]. Little is known about the biodiversity in supraglacial lakes and streams and how their ephemeral nature might affect their habitability. Nevertheless, the presence of *Proteobacteria*, *Bacteroidetes* and algal phototrophs was described in a stream system of Antarctica with low dissolved organic matter content [144]. Another study reported the presence of green algae in two superglacial lakes of Antarctica, although biomass was considered to be low [145].

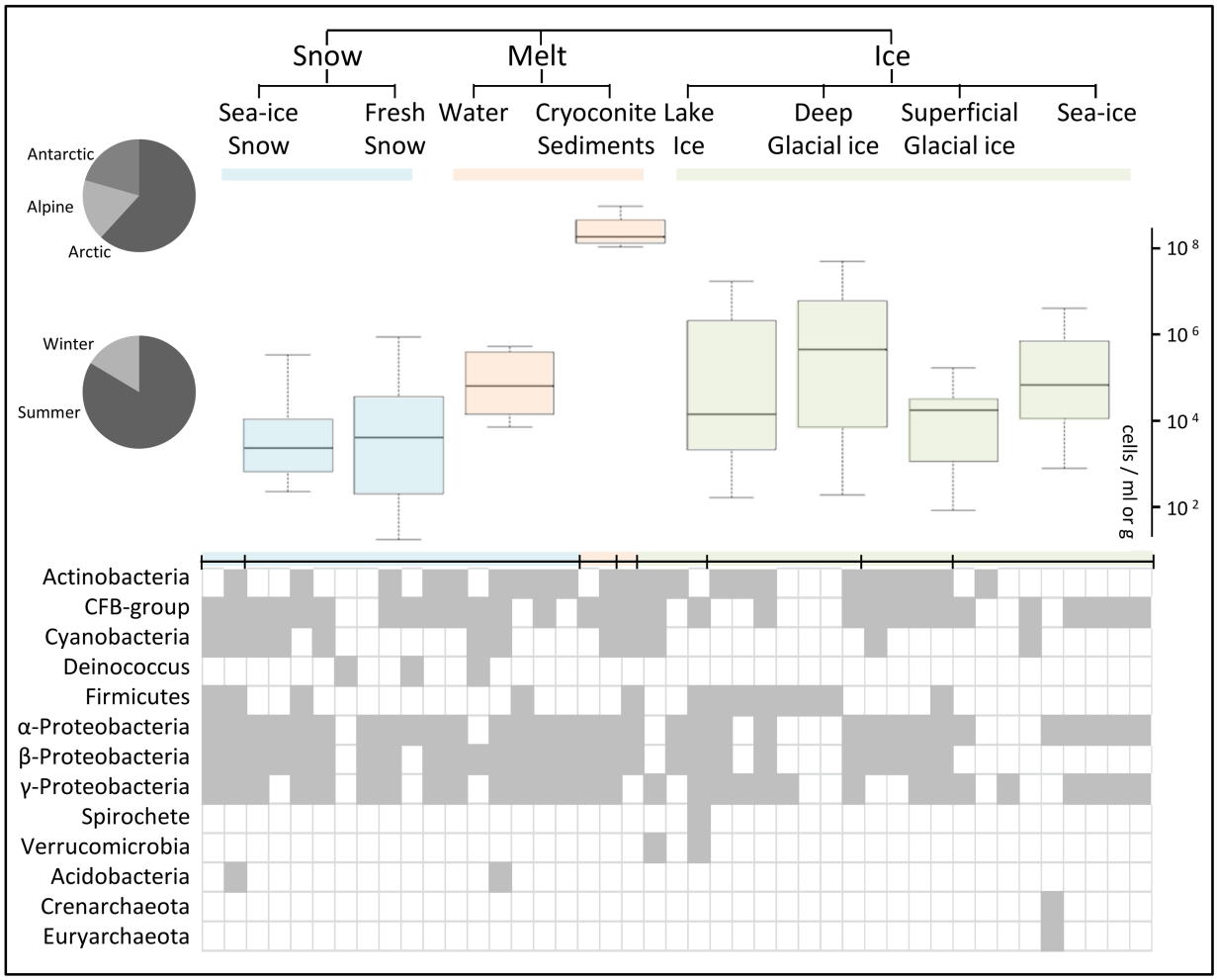

Figure 3. **Prokaryotic inhabitants of snow and ice.** Abundance and community composition data were extracted from available literature. Pie charts depict geographical distribution (Artic, Antarctic or Alpine) and sampling season (summer/spring or winter/fall) of the data used. Data were grouped into eight snow and ice ecotypes: snow over sea ice, fresh snow over soil or glacier, melt water (ponds, streams, cryoconite hole water), lake ice, deep glacial ice, superficial glacial ice and sea ice. Prokaryotic abundance is illustrated by boxplots of cell counts per milliliter of melted snow or ice (or gram for cryoconite sediments) for each ecotype. Community composition is assessed by a heatmap of presence (in grey) of most detected taxa at phylum level in the following studies. Data from [15–19,45,99,119,127,128,131,133,140,142,144,146–185,138]

Snow and ice microbial communities appear to vary in size and composition (Figure 3). This variability can be explained by the various niches with different abiotic conditions found in snow and ice (figure 2). Even in the same niche, conditions can fluctuate with time. This is reflected in the composition and size variation of microbial communities from the same sample type. This might be an indication of local changes in the microbial community in response to temporal environmental modifications. The light regime changes during season transitions might have a major impact on polar snow and ice microbial communities. Little is known about microbial communities in snow and ice during dark periods due in part to the difficulties in sampling. In one high Canadian Arctic winter study on sea ice, most of the 16S rRNA gene clone library sequences were identified as SAR11 clade *Alphaproteobacteria* and Marine Group I.1a *Crenarchaeota* [138]. These groups are rarely found during other seasons. *Alphaproteobateria* (*e.g.*, *Roseobacter*), *Gammaproteobacteria* and *Flavobacteria* predominate in spring communities, but were not detected in winter samples. No eukaryotic 18S rRNA gene sequences were detected whereas Eukaryotes and mostly photosynthetic Algae contributed to a major part of the microbial community in the spring-summer sea ice samples [27]. Snow community composition has been shown to vary with depth [163] and time with snow warming and melting during spring [176]. Melt water flow also strongly influence vertical distribution of soluble ions and microbial cells in snowpack [186]. Snow chemistry, such as pH, organic acids and mercury, has been correlated with bacterial community dynamics and higher relative abundance of associated genes [187]. These examples illustrate how snow and ice communities are not only able to resist harsh conditions but might also respond dynamically to modifications in their surrounding physical and chemical conditions.

The majority of microorganisms that inhabit any given ecosystem are rarely alone as they often live in communities and share their niche with other organisms. Biological interactions between the members of a community can shape and partly define an ecosystem [188]. In the cryosphere, these relationships could be an important asset in the colonization of the different habitats. For instance, production and excretion of certain proteins by bacteria can help in the development of microbial communities. An example of this phenomenon in snow and ice is the production of extracellular polymeric substances (EPS) or ice-binding proteins [189–191]. In glacier and lake ice, the production of ice-binding proteins by bacteria can modify ice vein structure by inhibiting recrystallization, which seems to reduce diffusion in veins [121,122]. Likewise, EPS production by algae and bacteria can alter the structure of sea ice, by reducing permeability and retaining salt [123]. In both cases, microbial activity leads to a retention of molecules and microorganisms, and thus actively promotes the formation of a stable microbial community.

Horizontal gene transfer (HGT) between the members of a community can also promote adaptation to environmental conditions [192]. Some studies suggest that conjugation events could be taking place in the cryosphere. One of these studies found plasmids encoding for a conjugal transfer system and plasmids with UV tolerance operons in a bacterial strain isolated from glacier ice [193]. Another study reported plasmids containing mercury resistance genes in snow and sea ice [194]. Scavenging of atmospheric mercury and its concentrations in the snowpack have been noted to be high [195], as are the UV radiation levels in the cryosphere (see previous section), thus HGT through conjugation could help bacteria adapt to environmental perturbations. In addition, ice-binding protein genes might be transferred from bacteria to algae through horizontal gene transfer in sea ice and snow

[196,197]. This putative HGT within the snow and ice microbial community still needs confirmatory experiments in order to firmly establish the causation.

Viral-host interactions and transduction events might also take place in snow and ice. In the last twenty years, a variety of reports have pointed to an unforeseen high abundance and high diversity of viruses in cold environments [198–203]. Abundance and diversity values seem to be highly variable with cryoconite hole sediments having the highest viral like particle (VPL) numbers (2.5×10^9 VLP g^{-1})[147]. The scarcity of viral sequences in databases negatively influences accurate viral taxonomy in environmental samples. Several studies have found newly undescribed viral groups for sea ice and supra-glacial systems [201,204]. In sea ice, siphovirus and myoviruses were identified as phages of *Flavobacterium sp*. and *Shewanella sp*., respectively [205]. In glacial and sea ice, with most bacteria and viruses in the liquid phase, contact rates between viruses and bacteria might be higher than estimated in bulk ice [206]. Indeed, a recent study suggested that viral interactions with their host in arctic glacial ice were more dynamic that in nearby soil [207], while high concentrations of extracellular DNA and viruses have been described in sea ice [208]. Thus, based on high viral abundance, diversity, infection rates [146], and broad host ranges [209], viruses have been promoted as drivers of evolution in glaciers and, in general, in cold environments [210]. The interactions leading to HGT appear to be present in the cryosphere and might affect its microbial communities. In addition, viral interactions with their hosts could still play another role in the shaping of ecosystems in the cryosphere. Viruses influence nutrient and organic carbon recycling through the lysis of microbial cells in what has been named the “viral shunt” [211]. This process might have an increased relevance in oligotrophic environments such as snow and ice. Although no study has attempted to determine the viral diversity in

snow, prophages were induced from bacteria (*Paenibacillus spp*.) recovered from the snow [212]. All bacteria tested had up to 3 morphologically different inducible phages.

Interactions with algae seem to be present in all snow and ice environments. Cryoconite aggregates on the surface of glaciers are an example of algal interactions with other organisms in the cryosphere. Filamentous cyanobacteria along with algal and bacterial EPS production determine the size and stability of these aggregates [213]. These aggregates trap mineral particles inside for potential use as nutrient sources and photosynthetic primary production sustain heterotrophic bacteria [110] along with fungi and archaea [17,136]. In snow, algal-bacterial interactions have been described where bacteria benefit from the organic carbon excreted by the algae [214]. This commensalistic relationship has also been reported in sea ice [215]. For example, bacteria were seen to consume the toxic hydrogen peroxide produced by diatoms during photosynthesis [216].

A large diversity of non-microbial organisms has also been observed in snow and ice. Meiofauna identified in glacial and lake ice as well as in sea ice includes nematodes, copepods, rotifers and tardigrades among others [217–219]. Even macrofauna, such as several species of the genus *Mesenchytraeus,* a genus of annelids (snow or ice worms) that has recurrently been found inhabiting glacial ice and snow, actually live their entire lifespans in these systems [220]. A recent study noted that ice worms from the species *Mesenchytraeus solifugus* have bacteria associated with their gut walls in what seems to be a symbiotic relationship [221]. Thus, the macrofauna also provide yet another niche for microorganisms in snow and ice. Additionally, annelids inhabiting snow and ice graze on microbial algae [222] and maybe also bacteria, and thus, control their populations. As depicted above, interactions between organisms can promote the settlement of

communities and can assist in the adaptation of its members and a hypothetic network of trophic interactions in snow and ice ecosystems is presented in figure 4. So in addition to the mechanisms reviewed in the previous section, community interactions can reinforce the capacity of microbial inhabitants to face the stresses of snow and ice ecosystems.

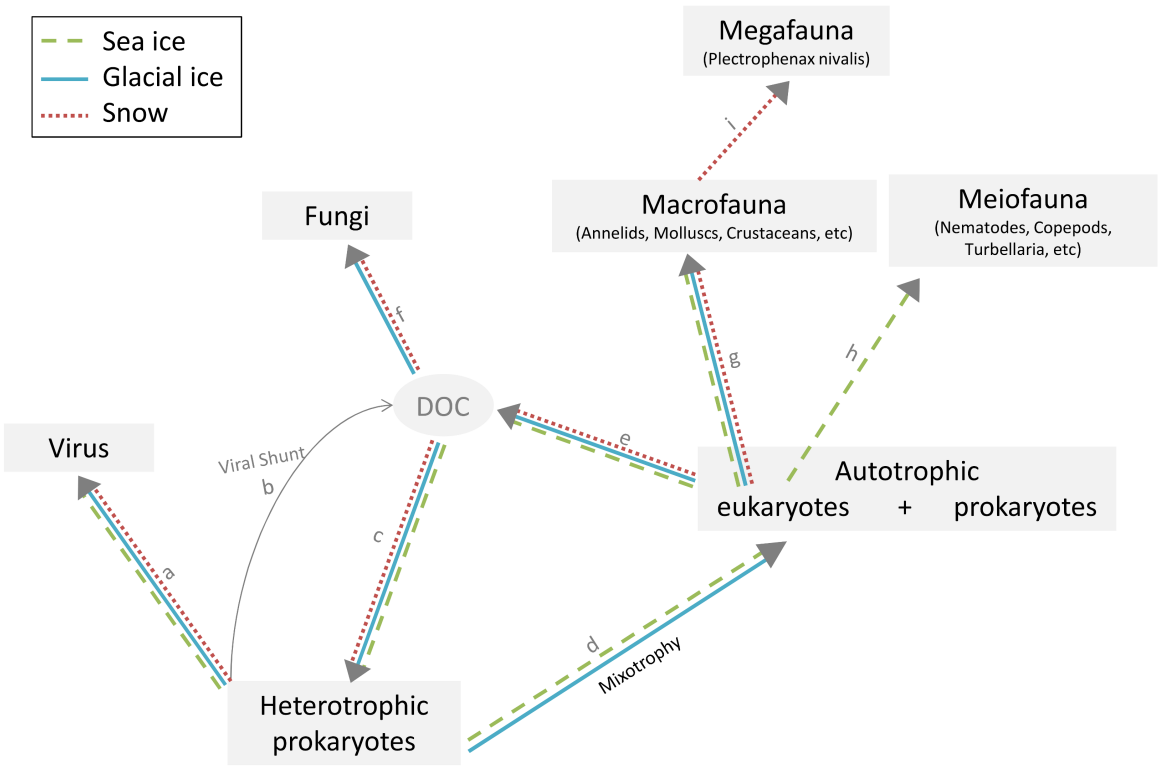

Figure 4. Food web in snow and ice environments.Dashed green arrows represent the interactions present in sea ice, while continuous blue arrows present the interactions in glacial ice and doted red arrows represent those in snow. The grey arrow represents theoretical assumptions not yet described in these environments. Data from a. [146,204,212] b. [211] c. [111][27,133] d. [99,223] e. [77][94] [97] f. [111][99] g. [220,224] h. [225] i. [226]

Snow and ice microbes are more numerous and diverse than expected and respond dynamically to the changes of their environmental conditions. Moreover, they seem to be integrated into a highly complex network of interactions. Thus, snow and ice appear to be functioning ecosystems untethered by limitations in temperature, UV radiation and nutrients.

Snow and ice habitats – not so extreme

Microorganisms employ a multitude of adaptive strategies endowing them with a potential for acclimation and adaptation. They efficiently colonize all habitats, even those characterized as extreme, such as the deep sea, hot springs, deep-sea vents, ultra-dry soil, and icy habitats. The field of research in cryospheric microbial ecology is directly influenced by extreme conditions on humans doing the sampling and analyses due to technical and logistical issues more than on the microorganisms themselves. Access to the sampling site and the sampling itself can be complicated and difficult. This is even more critical during the polar winter as illustrated by the strong bias towards spring and summer microbial characterization of snow and ice polar habitats (Figure 3). Sample management can also greatly interfere with natural community condition observations. i) Deep ice core studies can be limited by drilling difficulties such as contamination. ii) Melting procedures destroy 3D structure of samples, which might be a major factor in snow and ice microbial distribution, and induce cell losses due to osmotic shock. Methodologies such as genomic reference based comparisons are limited by database content with a lack of sufficient representatives from these environments. These potential biases are major concerns for cryosphere microbial ecologists. Non-destructive methods, such as intact ice section microscopy, have

shown that the majority of active cells are attached to surfaces within the brine channel network of winter arctic sea ice [169,227]. Recently, fluorescent microspheres were developed as tracers for assessing potential microbial contamination from indigenous microorganisms in glacial ice during drilling [228,229]. Technological and methodological improvements would increase the extent to which we can probe the snow and ice and their biologically diverse communities.

Here, we focused on the characterization of snow and ice ecosystems and especially how microorganisms interact with their complex abiotic habitat under specific conditions in terms of irradiation, water activity, salinity and nutrient content. Although many adaptive mechanisms might remain to be described, snow and ice inhabitants possess a large set of tools to deal with these variable and intense conditions, which might not be so extreme from an adapted microbial point of view. In addition, the possibility that this icy life style could have a remote or ancient origin cannot be discarded. Although the origin of life and the emergence of microorganisms on Earth remain unknown, some proposals for a cold origin of life have been made [44,230]. This hypothesis is supported by various studies including assays on prebiotics polymerisation in icy matrix [231–234] and bacterial phylogeny [235]. If this was to be the case then native conditions might not be considered as extreme. A truly extreme environment might sustain only highly specialized microorganisms, perfectly adapted to their specific environment and unable to exist elsewhere. This does not seem to be the case for all microorganisms in snow and ice ecosystems. Cold-adaptation, the most described adaptation in icy environments is a good example since mesophiles have been recovered from cold environments and cold adaptation is even detected in temperate environments. Although highly specialized microorganisms exist, their actual proportion,

what their role is and whether they are essential to the entire community function and activity in snow and ice habitats is unknown.

Microbial activity in snowpack, as measured by labelled thymidine and leucine incorporation, can be variable depending on temperature, location and the proximity of anthropogenic activities [236], ranging from 4.2 fmol$L^{-1}.h^{-1}$ in fresh fallen snow to more than 160 pmol $L^{-1}.h^{-1}$ in Antarctic sites with clear anthropogenic effect[166,237,238]. Due to variations in temperature and water content in laboratory measurements, these measurements might not provide evidence of in situ metabolism for extremely low temperature habitat such Antarctic plateau snow fields [237,239]. However, microbial activity in snow slush layer of alpine lake has been shown to be much higher than the underlying lake water [236]. Actively respiring cells, labelled using the fluorescent electron transport system-specific reagent CTC, were observed within brine in winter sea ice section at -20°C [169]. In the same study, proportion of active cells that were attached increased with decreasing temperature compared to free cells, suggesting an important role of attachment in survival at cold temperature in ice. Glacial ice surface is an important component of glacier and ice sheet with high microbial activity [98,240,241]. For instance, microbial activity measured as several microgram of carbon per gram of cryconite aggregates per day for photosynthesis and respiration during summer is similar to that in soils and sediments in temperate regions [98]. Within deep glacial ice, microbial activity is supposed to be very low but sufficient to maintain cells components and methanogens metabolism [98]. Comparison of temperature dependence of metabolic rates for microbial communities from diverse environments suggest far below the freezing point, liquid water inside ice is available for metabolism [242].

Conclusions

Snow and ice microbial communities do not seem limited by their specific conditions, at least in superficial environments, and have numerous and diverse members involved in a complex network of interactions. Although their global production rates might be low compared to other major biosphere compartments, such as ocean and soil, snow and ice ecosystems might sustain an underestimated part of global biogeochemical activity. Future research focusing on snow and ice ecology should expand past observational studies to function studies of these ecosystems.

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References

[1] Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001;409:1092–101. doi:10.1038/35059215.

[2] Brock TD. Life at high temperatures. Science 1985;230:132–8. doi:10.1126/science.230.4722.132.

[3] Marshall SJ. The Cryosphere. Princeton University Press; 2011.

[4] GLIMS, and National Snow and Ice Data Center. GLIMS Glacier Database 2012:Boulder, Colorado USA: National Snow and Ice Data . doi:http://dx.doi.org/10.7265/N5V98602.

[5] Tansley A. The use and abuse of vegetational concepts and terms. Ecology 1935;16:284–307.

[6] Peel MC, Finlayson BL, McMahon TA. Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci 2007;11:1633–44. doi:10.5194/hess-11-1633-2007.

[7] Barry RG, Jania J, Birkenmajer K. Review article “A. B. Dobrowolski – the first cryospheric scientist – and the subsequent development of cryospheric science.” Hist Geo Space Sci 2011;2:75–9. doi:10.5194/hgss-2-75-2011.

[8] Miteva V. Bacteria in Snow and Glacier Ice. In: Margesin, RosaSchinner F, Marx J-C, Gerday C, editors. Psychrophiles from Biodiversity to Biotechnology, 2008.

[9] Mykytczuk NCS, Foote SJ, Omelon CR, Southam G, Greer CW, Whyte LG. Bacterial growth at -15 °C; molecular insights from the permafrost bacterium Planococcus halocryophilus Or1. ISME J 2013;7:1211–26. doi:10.1038/ismej.2013.8.

[10] Panikov NS, Flanagan PW, Oechel WC, Mastepanov M a., Christensen TR. Microbial activity in soils frozen to below -39°C. Soil Biol Biochem 2006;38:785–94. doi:10.1016/j.soilbio.2005.07.004.

[11] Morita RY. Psychrophilic bacteria. Bacteriol Rev 1975;39:144–67.

[12] Feller G, Gerday C. Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 2003;1:200–8. doi:10.1038/nrmicro773.

[13] De Maayer P, Anderson D, Cary C, Cowan DA. Some like it cold: understanding the survival strategies of psychrophiles. EMBO Rep 2014;15:508–17. doi:10.1002/embr.201338170.

[14] Casanueva A, Tuffin M, Cary C, Cowan D a. Molecular adaptations to psychrophily: the impact of “omic” technologies. Trends Microbiol 2010;18:374–81. doi:10.1016/j.tim.2010.05.002.

[15] Simon C, Wiezer A, Strittmatter AW, Daniel R. Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. Appl Environ Microbiol 2009;75:7519–26. doi:10.1128/AEM.00946-09.

[16] Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J. Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the High Arctic. Appl Environ Microbiol 2012;78:549–59. doi:10.1128/AEM.06354-11.

[17] Edwards A, Pachebat J a, Swain M, Hegarty M, Hodson AJ, Irvine-Fynn TDL, et al. A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. Environ Res Lett 2013;8:035003. doi:10.1088/1748-9326/8/3/035003.

[18] Junge K, Imhoff F, Staley T, Deming JW. Phylogenetic diversity of numerically important Arctic sea ice bacteria cultured at subzero temperature. Microb Ecol 2002;43:315–28. doi:10.1007/s00248-001-1026-4.

[19] Amato P, Hennebelle R, Magand O, Sancelme M, Delort A-M, Barbante C, et al. Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol Ecol 2007;59:255–64. doi:10.1111/j.1574-6941.2006.00198.x.

[20] Bowman JP, Nichols DS, McKeekin TA. Psychrobacter glacincolia sp. nov., a halotolerant, psychrophilic bacterium isolated from antarctic sea ice. Syst Appl Microbiol 1997;20:209–15.

[21] Azevedo JSN, Correia A, Henriques S. Molecular analysis of the diversity of genus Psychrobacter present within a temperate estuary. FEMS Microbiol Ecol 2013;84:451–60.

[22] Cowan D a, Tow LA. Endangered antarctic environments. Annu Rev Microbiol 2004;58:649–90. doi:10.1146/annurev.micro.57.030502.090811.

[23] Reddy TBK, Thomas AD, Stamatis D, Bertsch J, Isbandi M, Jansson J, et al. The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. Nucleic Acids Res 2014;43:D1099–106. doi:10.1093/nar/gku950.

[24] Smith JJ, Howington JP, McFeters GA. Survival, physiological response and recovery of enteric bacteria exposed to a polar marine environment. Appl Environ Microbiol 1994;60:2977–84.

[25] Preyer JM, Oliver JD. Starvation-induced thermal tolerance as a survival mechanism in a psychrophilic marine bacterium. Appl Envir Microbiol 1993;59:2653–6.

[26] Possmayer M, Berardi G, Beall BFN, Trick CG, Hüner NP a, Maxwell DP. Plasticity of the psychrophilic green alga *Chlamydomonas raudensis* (UWO 241) (Chlorophyta) to supraoptimal temperature stress. J Phycol 2011;47:1098–109. doi:10.1111/j.1529-8817.2011.01047.x.

[27] Arrigo KR. Sea ice ecosystems. Ann Rev Mar Sci 2014;6:439–67. doi:10.1146/annurev-marine-010213-135103.

[28] Krembs C, Gradinger R, Spindler M. Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. J Exp Mar Bio Ecol 2000;243:55–80. doi:10.1016/S0022-0981(99)00111-2.

[29] Ewert M, Deming J. Sea Ice Microorganisms: Environmental constraints and extracellular responses. Biology (Basel) 2013;2:603–28. doi:10.3390/biology2020603.

[30] Barletta RE, Priscu JC, Mader HM, Jones WL, Roe CH. Chemical analysis of ice vein microenvironments: II. Analysis of glacial samples from Greenland and Antarctica. J Glaciol 2012;58:1109–18. doi:10.3189/2012JoG12J112.

[31] Dani KGS, Mader HM, Wolff EW, Wadham JL. Modelling the liquid-water vein system within polar ice sheets as a potential microbial habitat. Earth Planet Sci Lett 2012;333-334:238–49. doi:10.1016/j.epsl.2012.04.009.

[32] Leeson AA, Shepherd A, Briggs K, Howat I, Fettweis X, Morlighem M, et al. Supraglacial lakes on the Greenland ice sheet advance inland under warming climate. Nat Clim Chang 2015;5:51–5. doi:10.1038/NCLIMATE2463.

[33] McMillan M, Nienow P, Shepherd A, Benham T, Sole A. Seasonal evolution of supra-glacial lakes on the Greenland Ice Sheet. Earth Planet Sci Lett 2007;262:484–92. doi:10.1016/j.epsl.2007.08.002.

[34] Smith LC, Chu VW, Yang K, Gleason CJ, Pitcher LH, Rennermalm AK. Efficient meltwater drainage through supraglacial streams and rivers on the southwest Greenland ice sheet 2015:1–6. doi:10.1073/pnas.1413024112.

[35] Chu VW. Greenland ice sheet hydrology: A review. vol. 38. 2013. doi:10.1177/0309133313507075.

[36] Irvine-fynn TDL, Hodson AJ, Moorman BJ, Vatne G, Hubbard AL. Polythermal glacier : a review. Rev Geophys 2011;49:1–37. doi:10.1029/2010RG000350.1.INTRODUCTION.

[37] Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu JC, et al. Glacial Ecosystems. Ecol Monogr 2008;78:41–67.

[38] Ewert M, Deming JW. Bacterial responses to fluctuations and extremes in temperature and brine salinity at the surface of Arctic winter sea ice. FEMS Microbiol Ecol 2014;89:476–89. doi:10.1111/1574-6941.12363.

[39] Saum SH, Pfeiffer F, Palm P, Rampp M, Schuster SC, Müller V, et al. Chloride and organic osmolytes: a hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride-dependent bacterium *Halobacillus halophilus*. Environ Microbiol 2013;15:1619–33. doi:10.1111/j.1462-2920.2012.02770.x.

[40] Aslam SN, Cresswell-Maynard T, Thomas DN, Underwood GJC. Production and characterization of the intra- and extracellular carbohydrates and polymeric substances (EPS) of three sea ice diatom species, and evidence for a cryoprotective role for EPS. J Phycol 2012;48:1494–509. doi:10.1111/jpy.12004.

[41] Liu S-B, Chen X-L, He H-L, Zhang X-Y, Xie B-B, Yu Y, et al. Structure and ecological roles of a novel exopolysaccharide from the arctic sea ice Bacterium *Pseudoalteromonas* sp. Strain SM20310. Appl Environ Microbiol 2012;79:224–30. doi:10.1128/AEM.01801-12.

[42] Eddie B, Krembs C, Neuer S. Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. Mar Ecol Prog Ser 2008;354:107–17. doi:10.3354/meps07243.

[43] Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš a. Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Phys Chem Earth, Parts A/B/C 2003;28:1273–8. doi:10.1016/j.pce.2003.08.056.

[44] Price PB. Microbial life in glacial ice and implications for a cold origin of life. FEMS Microbiol Ecol 2007;59:217–31. doi:10.1111/j.1574-6941.2006.00234.x.

[45] Mader HM, Pettitt ME, Wadham JL, Wolff EW, Parkes RJ. Subsurface ice as a microbial habitat. Geology 2006;34:169. doi:10.1130/G22096.1.

[46] Rohde RA, Price PB. Diffusion-controlled metabolism for long-term survival of single isolated microorganisms trapped within ice crystals. Proc Natl Acad Sci U S A 2007;104:16592–7. doi:10.1073/pnas.0708183104.

[47] MacDonell S, Fitzsimons S. The formation and hydrological significance of cryoconite holes. Prog Phys Geogr 2008;32:595–610. doi:10.1177/0309133308101382.

[48] Llubes M, Lanseau C, Rémy F. Relations between basal condition, subglacial hydrological networks and geothermal flux in Antarctica. Earth Planet Sci Lett 2006;241:655–62. doi:10.1016/j.epsl.2005.10.040.

[49] Kyrke-Smith TM, Katz RF, Fowler AC. Subglacial hydrology and the formation of ice streams. Proc R Soc A 2014;470:20130494. doi:10.1098/rspa.2013.0494.

[50] Dowdeswell J a., Siegert MJ. The physiography of modern Antarctic subglacial lakes. Glob Planet Change 2003;35:221–36. doi:10.1016/S0921-8181(02)00128-5.

[51] Techel F, Pielmeier C. Point observations of liquid water content in wet snow - Investigating methodical, spatial and temporal aspects. Cryosphere 2011;5:405–18. doi:10.5194/tc-5-405-2011.

[52] Colbeck SC. An overview of seasonal snow metamorphism. Rev Geophys 1982;20:45. doi:10.1029/RG020i001p00045.

[53] Domine F, Albert M, Huthwelker T, Jacobi H, Kokhanovsky AA, Lehning M, et al. Snow physics as relevant to snow photochemistry 2008;10:171–208.

[54] Stibal M, Elster J, Sabacká M, Kastovská K. Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. FEMS Microbiol Ecol 2007;59:265–73. doi:10.1111/j.1574-6941.2006.00264.x.

[55] Grant WD. Life at low water activity. Philos Trans R Soc Lond B Biol Sci 2004;359:1249–66; discussion 1266–7. doi:10.1098/rstb.2004.1502.

[56] Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems 2005;1:5. doi:10.1186/1746-1448-1-5.

[57] Brown AD. Compatible solutes and extreme water stress in eukaryotic micro-organisms. Adv Microb Physiol 1978;17:181–242.

[58] Kirst GO. Osmotic Adjustment in phytoplankton and macroAlgae. In: Keller, M.D., Kiene, R.P., Kirst, G.O., Visscher PT, editor. Biol. Environ. Chem. DMSP Relat. Sulfonium Compd. | Springer, Plenum Press; 1998, p. 121–9.

[59] Perovich D. Ultraviolet Radiation and the Optical Properties of Sea Ice and Snow. In: Hessen DO, editor. UV Radiat. Arct. Ecosyst., vol. 153, Berlin, Heidelberg: Springer Berlin Heidelberg; 2002, p. 73–89. doi:10.1007/978-3-642-56075-0.

[60] Grannas AM, Jones AE, Dibb J, Ammann M, Anastasio C, Beine HJ, et al. An overview of snow photochemistry : evidence , mechanisms and impacts 2007:4329–73.

[61] Cockell CS, Rettberg P, Horneck G, Wynn-Williams DD, Scherer K, Gugg-Helminger A. Influence of ice and snow covers on the UV exposure of terrestrial microbial communities: dosimetric studies. J Photochem Photobiol B Biol 2002;68:23–32. doi:10.1016/S1011-1344(02)00327-5.

~~[62] Perovich DK. UV and the optocal Properties of Sea Ice and Snow. In: Hessen D, editor. UV Radiat. Arct. Ecosyst. Ecological, Springer; 2002, p. 73–87.~~ See 59

[63] King MD, Simpson WR. Extinction of UV radiation in Arctic snow at Alert, Canada (82°N). J Geophys Res 2001;106:12499. doi:10.1029/2001JD900006.

[64] King MD, France JL, Fisher FN, Beine HJ. Measurement and modelling of UV radiation penetration and photolysis rates of nitrate and hydrogen peroxide in Antarctic sea ice: An estimate of the production rate of hydroxyl radicals in first-year sea ice. J Photochem Photobiol A Chem 2005;176:39–49. doi:10.1016/j.jphotochem.2005.08.032.

[65] Anastasio C, Hoffmann M, Klán P, Sodeau J. Photochemistry in Terrestrial Ices. In: Gudipati MS, Castillo-Rogez J, editors. Sci. Sol. Syst. Ices, vol. 356. Astrophysi, New York, NY: Springer New York; 2013. doi:10.1007/978-1-4614-3076-6.

[66] Moan J, Peak MJ. Effects of UV radiation on cells. J Photochem Photobiol B Biol 1989;4:21–34. doi:10.1016/1011-1344(89)80099-5.

[67] Santos AL, Oliveira V, Baptista I, Henriques I, Gomes NCM, Almeida A, et al. Wavelength dependence of biological damage induced by UV radiation on bacteria. Arch Microbiol 2013;195:63–74. doi:10.1007/s00203-012-0847-5.

[68] Mundy CJ, Gosselin M, Ehn JK, Belzile C, Poulin M, Alou E, et al. Characteristics of two distinct high-light acclimated algal communities during advanced stages of sea ice melt. Polar Biol 2011;34:1869–86. doi:10.1007/s00300-011-0998-x.

[69] Uusikivi J, Vähätalo A V, Granskog MA, Sommaruga R. Contribution of mycosporine-like amino acids and colored dissolved and particulate matter to sea ice optical properties and ultraviolet attenuation. Limnol Oceanogr 2010;55:703–13.

[70] Ryan KG, McMinn A, Mitchell KA, Trenerry L. Mycosporine-like amino acids in Antarctic sea ice algae, and their response to UVB radiation. Z Naturforsch C 2002;57:471–7.

[71] Lemoine Y, Schoefs B. Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. Photosynth Res 2010;106:155–77. doi:10.1007/s11120-010-9583-3.

[72] Duval B, Shetty K, Thomas WH. Phenolic compounds and antioxidant properties in the snow alga Chlamydomonas nivalis after exposure to UV light 2000:559–66.

[73] Gorton HL, Vogelmann TC. Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. Photochem Photobiol 2003;77:608–15.

[74] Remias D, Wastian H, Lütz C, Leya T. Insights into the biology and phylogeny of *Chloromonas polyptera* (Chlorophyta), an alga causing orange snow in Maritime Antarctica. Antarct Sci 2013;25:648–56. doi:10.1017/S0954102013000060.

[75] Leya T, Rahn A, Lütz C, Remias D. Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. FEMS Microbiol Ecol 2009;67:432–43. doi:10.1111/j.1574-6941.2008.00641.x.

[76] Remias D, Albert a., Lütz C. Effects of realistically simulated, elevated UV irradiation on photosynthesis and pigment composition of the alpine snow alga *Chlamydomonas nivalis* and the arctic soil alga *Tetracystis sp*. (Chlorophyceae). Photosynthetica 2010;48:269–77. doi:10.1007/s11099-010-0033-4.

[77] Remias D, Holzinger A, Aigner S, Lütz C. Ecophysiology and ultrastructure of *Ancylonema nordenskiöldii* (Zygnematales, Streptophyta), causing brown ice on glaciers in Svalbard (high arctic). Polar Biol 2012;35:899–908. doi:10.1007/s00300-011-1135-6.

[78] Remias D, Schwaiger S, Aigner S, Leya T, Stuppner H, Lütz C. Characterization of an UV- and VIS-absorbing, purpurogallin-derived secondary pigment new to algae and highly abundant in *Mesotaenium berggrenii* (Zygnematophyceae, Chlorophyta), an extremophyte living on glaciers. FEMS Microbiol Ecol 2011;79:638–48. doi:10.1111/j.1574-6941.2011.01245.x.

[79] Martin A, Hall J, Ryan K. Low salinity and high-level UV-B radiation reduce single-cell activity in antarctic sea ice bacteria. Appl Environ Microbiol 2009;75:7570–3. doi:10.1128/AEM.00829-09.

[80] Gessler NN, Egorova AS, Belozerskaya TA. Melanin pigments of fungi under extreme environmental conditions (Review). Appl Biochem Microbiol 2014;50:105–13. doi:10.1134/S0003683814020094.

[81] Solomon PS, Waters ODC, Oliver RP. Decoding the mannitol enigma in filamentous fungi. Trends Microbiol 2007;15:257–62. doi:10.1016/j.tim.2007.04.002.

[82] Rosa LH, Vaz ABM, Caligiorne RB, Campolina S, Rosa CA. Endophytic fungi associated with the Antarctic grass *Deschampsia antarctica Desv.* (Poaceae). Polar Biol 2008;32:161–7. doi:10.1007/s00300-008-0515-z.

[83] Selbmann L, Isola D, Zucconi L, Onofri S. Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. Fungal Biol 2011;115:937–44. doi:10.1016/j.funbio.2011.02.016.

[84] Ziegelhoffer EC, Donohue TJ. Bacterial responses to photo-oxidative stress 2009;7:856–63. doi:10.1038/nrmicro2237.Bacterial.

[85] Sinha RP, Häder DP. UV-induced DNA damage and repair: a review. Photochem Photobiol Sci 2002;1:225–36.

[86] Morgan-kiss RM, Priscu JC, Pocock T, Gudynaite-savitch L, Huner NPA. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiolohy Mol Biol Rev 2006;70:222–52. doi:10.1128/MMBR.70.1.222.

[87] Falkowski PG, LaRoche J. Acclimation to spectral irradiance in algae. J Phycol 1991;27:8–14. doi:10.1111/j.0022-3646.1991.00008.x.

[88] Ralph PJ, McMinn A, Ryan KG, Ashworth C. Short-term effect of temperature on the photokinetics of microalgae from the surface layers of antarctic pack ice.. J Phycol 2005;41:763–9. doi:10.1111/j.1529-8817.2005.00106.x.

[89] Kashino Y, Fujimoto K, Akamatsu A, Koike H, Satoh K, Kudoh S. photosynthetic pigment composition of ice algal and phytoplankton assemblages in early spring in Saroma Ko Lagoon, Hokkaido, Japan (19th Symposium on Polar Biology). Proc NIPR Symp Polar Biol 1998;11:22–32.

[90] Ng KW, Pointing SB, Dvornyk V. Patterns of nucleotide diversity of the ldpA circadian gene in closely related species of cyanobacteria from extreme cold deserts. Appl Environ Microbiol 2013;79:1516–22. doi:10.1128/AEM.03439-12.

[91] Gomelsky M, Hoff WD. Light helps bacteria make important lifestyle decisions. Trends Microbiol 2011;19:441–8. doi:10.1016/j.tim.2011.05.002.

[92] Thomas DN, Papadimitriou S. Biogeochemistry of Sea Ice. In: Thomas DN, Dieckmann GS, editors. Sea Ice An Introd. to its Physics, Chem. Biol. Geol., Blackwell publishing; 2008, p. 267–302.

[93] Larose C, Dommergue A, De Angelis M, Cossa D, Averty B, Marusczak N, et al. Springtime changes in snow chemistry lead to new insights into mercury methylation in the Arctic. Geochim Cosmochim Acta 2010;74:6263–75. doi:10.1016/j.gca.2010.08.043.

[94] Remias D, Lütz-meindl U, Lütz C, Lu U. Photosynthesis, pigments and ultrastructure of the alpine snow alga Chlamydomonas nivalis. Eur J Phycol 2005;40:259–68. doi:10.1080/09670260500202148.

[95] Yallop ML, Anesio AM, Perkins RG, Cook J, Telling J, Fagan D, et al. Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. ISME J 2012;6:2302–13. doi:10.1038/ismej.2012.107.

[96] Fritsen CH, Priscu JC. CYANOBACTERIAL ASSEMBLAGES IN PERMANENT ICE COVERS ON ANTARCTIC LAKES: DISTRIBUTION, GROWTH RATE, AND TEMPERATURE RESPONSE OF PHOTOSYNTHESIS. J Phycol 1998;34:587–97. doi:10.1046/j.1529-8817.1998.340587.x.

[97] Koh EY, Martin AR, McMinn A, Ryan KG. Recent Advances and Future Perspectives in Microbial Phototrophy in Antarctic Sea Ice. Biology (Basel) 2012;1:542–56. doi:10.3390/biology1030542.

[98] Anesio AM, Hodson AJ, Fritz A, Psenner R, Sattler B. High microbial activity on glaciers: importance to the global carbon cycle. Glob Chang Biol 2009;15:955–60. doi:10.1111/j.1365-2486.2008.01758.x.

[99] Bachy C, López-García P, Vereshchaka A, Moreira D. Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the North Pole at the end of the polar night. Front Microbiol 2011;2:1–12. doi:10.3389/fmicb.2011.00106.

[100] Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD. Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. Appl Environ Microbiol 2005;71:6986–97. doi:10.1128/AEM.71.11.6986-6997.2005.

[101] Boyd ES, Hamilton TL, Havig JR, Skidmore ML, Shock EL. Chemolithotrophic primary production in a subglacial ecosystem. Appl Environ Microbiol 2014;80:6146–53. doi:10.1128/AEM.01956-14.

[102] Stibal M, Hasan F, Wadham JL, Sharp MJ, Anesio AM. Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates. Extremophiles 2012;16:255–65. doi:10.1007/s00792-011-0426-8.

[103] Stibal M, Šabacká M, Žárský J. Biological processes on glacier and ice sheet surfaces. Nat Geosci 2012;5:771–4. doi:10.1038/NGEO1611.

[104] Stibal M, Bælum J, Holben WE, Sørensen SR, Jensen A, Jacobsen CS. Microbial degradation of 2,4-dichlorophenoxyacetic acid on the Greenland ice sheet. Appl Environ Microbiol 2012;78:5070–6. doi:10.1128/AEM.00400-12.

[105] Antony R, Mahalinganathan K, Krishnan KP, Thamban M. Microbial preference for different size classes of organic carbon: a study from Antarctic snow. Environ Monit Assess 2012;184:5929–43. doi:10.1007/s10661-011-2391-1.

[106] Christner BC, Skidmore ML, Priscu JC, Tranter M, Foreman CM. Bacteria in Subglacial Environments 2008:51–71.

[107] Miteva VI, Brenchley JE. Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. Appl Environ Microbiol 2005;71:7806–18. doi:10.1128/AEM.71.12.7806-7818.2005.

[108] Price PB. A habitat for psychrophiles in deep Antarctic ice. Proc Natl Acad Sci U S A 2000;97:1247–51.

[109] Tung HC, Bramall NE, Price PB. Microbial origin of excess methane in glacial ice and implications for life on Mars. Proc Natl Acad Sci U S A 2005;102:18292–6. doi:10.1073/pnas.0507601102.

[110] Takeuchi N. Structure, formation, and darkening process of albedo-reducing material cryoconite on a Himalayan glacier: a granular algal mat growing on the glacier. Arctic, Antarct Alp Res 2001;33:115–22.

[111] Margesin R, Zacke G, Schinner F. Characterization of heterotrophic microorganisms in alpine glacier cryoconite. Arctic, Antarct Alp Res 2002;34:88–93.

[112] Grossmann S, Dieckmann GS. Bacterial standing stock, activity, and carbon production during formation and growth of sea ice in the Weddell Sea, Antarctica. Appl Environ Microbiol 1994;60:2746–53.

[113] Vancoppenolle M, Meiners KM, Michel C, Bopp L, Brabant F, Carnat G, et al. Role of sea ice in global biogeochemical cycles: emerging views and challenges. Quat Sci Rev 2013;79:207–30. doi:10.1016/j.quascirev.2013.04.011.

[114] Barrie LA. Snow Formation and Processes in the Atmosphere that influence its chemical composition. In: Davies TD, Tranter M, Jones HG, editors. Seas. Snowpacks SE - 1, vol. 28, Springer Berlin Heidelberg; 1991, p. 1–20. doi:10.1007/978-3-642-75112-7_1.

[115] Jones HG. The ecology of snow-covered systems: a brief overview of nutrient cycling and life in the cold. Hydrol Process 1999;13:2135–47. doi:10.1002/(sici)1099-1085(199910)13:14/15<2135::aid-hyp862>3.3.co;2-p.

[116] Larose C, Dommergue A, Vogel TM. Microbial nitrogen cycling in Arctic snowpacks. Environ Res Lett 2013;8:035004. doi:10.1088/1748-9326/8/3/035004.

[117] Amoroso a, Domine F, Esposito G, Morin S, Savarino J, Nardino M, et al. Microorganisms in dry polar snow are involved in the exchanges of reactive nitrogen species with the atmosphere. Environ Sci Technol 2010;44:714–9. doi:10.1021/es9027309.

[118] Saenz BT, Arrigo KR. Simulation of a sea ice ecosystem using a hybrid model for slush layer desalination. J Geophys Res 2012;117:C05007. doi:10.1029/2011JC007544.

[119] Ewert M, Carpenter SD, Colangelo-Lillis J, Deming JW. Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. J Geophys Res Ocean 2013;118:726–35. doi:10.1002/jgrc.20055.

[120] Vincent WF, Mueller DR, Bonilla S. Ecosystems on ice: The microbial ecology of Markham Ice Shelf in the high Arctic. Cryobiology 2004;48:103–12. doi:10.1016/j.cryobiol.2004.01.006.

[121] Brown JR, Seymour JD, Brox TI, Skidmore ML, Wang C, Christner BC, et al. Recrystallization inhibition in ice due to ice binding protein activity detected by nuclear magnetic resonance. Biotechnol Reports 2014;3:60–4. doi:10.1016/j.btre.2014.06.005.

[122] Achberger AM, Brox TI, Skidmore ML, Christner BC. Expression and partial characterization of an ice-binding protein from a bacterium isolated at a depth of 3,519 m in the Vostok ice core, Antarctica. Front Microbiol 2011;2:1–8. doi:10.3389/fmicb.2011.00255.

[123] Krembs C, Eicken H, Deming JW. Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc Natl Acad Sci U S A 2011;108:3653–8. doi:10.1073/pnas.1100701108.

[124] Margesin R, Miteva V. Diversity and ecology of psychrophilic microorganisms. Res Microbiol 2011;162:346–61. doi:10.1016/j.resmic.2010.12.004.

[125] Deming JW. Sea ice bacteria and viruses. In: Thomas DN, Dieckmann GS, editors. Sea Ice An Introd. to Its Physics, Chem- istry, Biol. Geol., Oxford, UK: Wiley-Blackwell; 2009, p. 247–82. doi:10.1002/9781444317145.

[126] Larose C, Dommergue A, Vogel T. The dynamic arctic snowpack: An unexplored environment for microbial diversity and activity. Biology (Basel) 2013;2:317–30. doi:10.3390/biology2010317.

[127] Segawa T, Miyamoto K, Ushida K, Agata K, Okada N, Kohshima S. Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains , Japan , analyzed by 16S rRNA gene sequencing and real-Time PCR 2005;71:123–30. doi:10.1128/AEM.71.1.123.

[128] Liu Y, Yao T, Jiao N, Kang S, Xu B, Zeng Y, et al. Bacterial diversity in the snow over Tibetan Plateau Glaciers. Extremophiles 2009;13:411–23. doi:10.1007/s00792-009-0227-5.

[129] Souchez R, Petit JR, Tison JL, Jouzel J, Verbeke V. Ice formation in subglacial Lake Vostok, Central Antarctica. Earth Planet Sci Lett 2000;181:529–38. doi:10.1016/S0012-821X(00)00228-4.

[130] Christner BC, Royston-Bishop G, Foreman CM, Arnold BR, Tranter M, Welch K a., et al. Limnological conditions in Subglacial Lake Vostok, Antarctica. Limnol Oceanogr 2006;51:2485–501. doi:10.4319/lo.2006.51.6.2485.

[131] Karl D., Bird DF, Björkman K, Houlihan T, Shackelford R, Tupas L. Microorganisms in the accreted Ice of lake Vostok , Antarctica. Science (80-) 1999;286:2144–7.

[132] Whitman WB, Coleman DC, Wiebe WJ. Perspective Prokaryotes : The unseen majority 1998;95:6578–83.

[133] Harding T, Jungblut AD, Lovejoy C, Vincent WF. Microbes in high arctic snow and implications for the cold biosphere. Appl Environ Microbiol 2011;77:3234–43. doi:10.1128/AEM.02611-10.

[134] Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 2006;103:626–31. doi:10.1073/pnas.0507535103.

[135] Cameron K a, Hodson AJ, Osborn a M. Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic. FEMS Microbiol Ecol 2012;82:254–67. doi:10.1111/j.1574-6941.2011.01277.x.

[136] Zarsky JD, Stibal M, Hodson A, Sattler B, Schostag M, Hansen LH, et al. Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea. Environ Res Lett 2013;8:035044. doi:10.1088/1748-9326/8/3/035044.

[137] Junge K, Eicken H, Deming JW. Wintertime Sea Ice Bacterial Activity at ⁒ 2 to ⁒ 20 ° C in Arctic Wintertime Sea Ice 2004;70. doi:10.1128/AEM.70.1.550.

[138] Collins RE, Rocap G, Deming JW. Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 2010;12:1828–41. doi:10.1111/j.1462-2920.2010.02179.x.

[139] Cowie ROM, Maas EW, Ryan KG. Archaeal diversity revealed in Antarctic sea ice. Antarct Sci 2011;23:531–6. doi:10.1017/S0954102011000368.

[140] Gradinger R, Nurnberg D. Snow algal communities on Arctic pack ice floes dominated by *Chlamydomonas nivalis* (Bauer) Wille 1996:35–43.

[141] Williams WE, Gorton HL, Vogelmann TC. Surface gas-exchange processes of snow algae. Proc Natl Acad Sci U S A 2003;100:562–6. doi:10.1073/pnas.0235560100.

[142] Cameron K a, Hagedorn B, Dieser M, Christner BC, Choquette K, Sletten R, et al. Diversity and potential sources of microbiota associated with snow on western portions of the Greenland Ice Sheet. Environ Microbiol 2014. doi:10.1111/1462-2920.12446.

[143] Lutz S, Anesio AM, Jorge Villar SE, Benning LG. Variations of algal communities cause darkening of a Greenland glacier. FEMS Microbiol Ecol 2014;89:402–14. doi:10.1111/1574-6941.12351.

[144] Foreman CM, Cory RM, Morris CE, SanClements MD, Smith HJ, Lisle JT, et al. Microbial growth under humic-free conditions in a supraglacial stream system on the Cotton Glacier , Antarctica. Environ Res Lett 2013;8:1–11. doi:10.1088/1748-9326/8/3/035022.

[145] Keskitalo J, Leppäranta M, Arvola L. First records of primary producers of epiglacial and supraglacial lakes in western Dronning Maud Land, Antarctica. Polar Biol 2013;36:1441–50. doi:10.1007/s00300-013-1362-0.

[146] Säwström C, Granéli W, Laybourn-Parry J, Anesio AM. High viral infection rates in Antarctic and Arctic bacterioplankton. Environ Microbiol 2007;9:250–5. doi:10.1111/j.1462-2920.2006.01135.x.

[147] Bellas CM, Anesio AM, Telling J, Stibal M, Tranter M, Davis S. Viral impacts on bacterial communities in Arctic cryoconite. Environ Res Lett 2013;8:045021. doi:10.1088/1748-9326/8/4/045021.

[148] Doyle S, Montross S, Skidmore M, Christner B. Characterizing microbial diversity and the potential for metabolic function at −15 °C in the Basal Ice of Taylor glacier, Antarctica. Biology (Basel) 2013;2:1034–53. doi:10.3390/biology2031034.

[149] Miteva VI, Sheridan PP, Brenchley JE. Phylogenetic and physiological diversity of microorganisms isolated from a deep greenland glacier ice core. Appl Environ Microbiol 2004;70:202–13. doi:10.1128/AEM.70.1.202.

[150] Miteva V, Teacher C, Sowers T, Brenchley J. Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates. Environ Microbiol 2009;11:640–56. doi:10.1111/j.1462-2920.2008.01835.x.

[151] Miteva V, Burlingame C, Sowers T, Brenchley J. Comparative evaluation of the indigenous microbial diversity vs. drilling fluid contaminants in the NEEM Greenland ice core. FEMS Microbiol Ecol 2014:1–19. doi:10.1111/1574-6941.12286.

[152] Irvine-Fynn TDL, Edwards a, Newton S, Langford H, Rassner SM, Telling J, et al. Microbial cell budgets of an Arctic glacier surface quantified using flow cytometry. Environ Microbiol 2012;14:2998–3012. doi:10.1111/j.1462-2920.2012.02876.x.

[153] Zeng YX, Yan M, Yu Y, Li HR, He JF, Sun K, et al. Diversity of bacteria in surface ice of Austre Lovénbreen glacier, Svalbard. Arch Microbiol 2013;195:313–22. doi:10.1007/s00203-013-0880-z.

[154] Cappa F, Suciu N, Trevisan M, Ferrari S, Puglisi E, Sandro P. Bacterial diversity in a contaminated Alpine glacier as determined by culture-based and molecular

approaches. Sci Total Environ 2014;497-498:50–9. doi:10.1016/j.scitotenv.2014.07.094.

[155] Ball MM, Gómez W, Magallanes X, Rosales R, Melfo A, Yarzábal LA. Bacteria recovered from a high-altitude, tropical glacier in Venezuelan Andes. World J Microbiol Biotechnol 2014;30:931–41. doi:10.1007/s11274-013-1511-1.

[156] Zhang XF, Yao TD, Tian LD, Xu SJ, An LZ. Phylogenetic and physiological diversity of bacteria isolated from Puruogangri ice core. Microb Ecol 2008;55:476–88. doi:10.1007/s00248-007-9293-3.

[157] Priscu JC, Adams EE, Lyons WB, Voytek MA, Mogk DW, Brown RL, et al. Geomicrobiology of subglacial ice above Lake Vostok , Antarctica. Science (80-) 1999;286:2141–4.

[158] Foreman CM, Dieser M, Greenwood M, Cory RM, Laybourn-Parry J, Lisle JT, et al. When a habitat freezes solid: microorganisms over-winter within the ice column of a coastal Antarctic lake. FEMS Microbiol Ecol 2011;76:401–12. doi:10.1111/j.1574-6941.2011.01061.x.

[159] Jungblut AD, Vincent WF, Lovejoy C. Eukaryotes in Arctic and Antarctic cyanobacterial mats. FEMS Microbiol Ecol 2012;82:416–28. doi:10.1111/j.1574-6941.2012.01418.x.

[160] Werner I, Ikävalko J, Schünemann H. Sea ice algae in Arctic pack ice during late winter. Polar Biol 2007;30:1493–504. doi:10.1007/s00300-007-0310-2.

[161] Fiala M, Kuosa H, Kopczyńska EE, Oriol L, Delille D. Spatial and seasonal heterogeneity of sea ice microbial communities in the first-year ice of Terre Adélie area (Antarctica). Aquat Microb Ecol 2006;43:95–106. doi:10.3354/ame043095.

[162] Booth BC, Horner RA. Microalgae on the arctic ocean section, 1994: species abundance and biomass. Deep Sea Res Part II Top Stud Oceanogr 1997;44:1607–22. doi:10.1016/S0967-0645(97)00057-X.

[163] Møller AK, Søborg DA, Al-soud WA, Sørensen SJ, Kroer N. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. Polar Res 2013;1:1–11.

[164] Møller AK, Barkay T, Abu Al-Soud W, Sørensen SJ, Skov H, Kroer N. Diversity and characterization of mercury-resistant bacteria in snow, freshwater and sea ice brine from the High Arctic. FEMS Microbiol Ecol 2011;75:390–401. doi:10.1111/j.1574-6941.2010.01016.x.

[165] Carpenter EJ, Lin S, Capone DG. Bacterial activity in South Pole snow 2000;66. doi:10.1128/AEM.66.10.4514-4517.2000.Updated.

[166] Lopatina A, Krylenkov V, Severinov K. Activity and bacterial diversity of snow around Russian Antarctic stations. Res Microbiol 2013;164:949–58. doi:10.1016/j.resmic.2013.08.005.

[167] Hauptmann AL, Stibal M, Bælum J, Sicheritz-Pontén T, Brunak S, Bowman JS, et al. Bacterial diversity in snow on North Pole ice floes. Extremophiles 2014. doi:10.1007/s00792-014-0660-y.

[168] Bowman JS, Rasmussen S, Blom N, Deming JW, Rysgaard S, Sicheritz-Ponten T. Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. ISME J 2012;6:11–20. doi:10.1038/ismej.2011.76.

[169] Junge K, Eicken H, Deming JW. Wintertime sea ice bacterial activity at -2 to -20 ° C in arctic wintertime sea ice 2004;70. doi:10.1128/AEM.70.1.550.

[170] Bowman JP, McCammon S a, Brown M V, Nichols DS, McMeekin T a. Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 1997;63:3068–78.

[171] Petri R, Imhoff JF. Genetic analysis of sea ice bacterial communities of the Western Baltic Sea using an improved double gradient method. Polar Biol 2014;24:252–7. doi:10.1007/s003000000205.

[172] Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol 2003;69:6610–9.

[173] Brown M V., Bowman JP. A molecular phylogenetic survey of sea ice microbial communities (SIMCO). FEMS Microbiol Ecol 2001;35:267–75.

[174] Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, et al. Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. Extremophiles 2010;14:205–12. doi:10.1007/s00792-009-0299-2.

[175] Zhang S, Yang G, Wang Y, Hou S. Abundance and community of snow bacteria from three glaciers in the Tibetan Plateau. J Environ Sci 2010;22:1418–24. doi:10.1016/S1001-0742(09)60269-2.

[176] Hell K, Edwards A, Zarsky J, Podmirseg SM, Girdwood S, Pachebat J a, et al. The dynamic bacterial communities of a melting High Arctic glacier snowpack. ISME J 2013;7:1814–26. doi:10.1038/ismej.2013.51.

[177] Michaud L, Lo Giudice A, Mysara M, Monsieurs P, Raffa C, Leys N, et al. Snow Surface Microbiome on the High Antarctic Plateau (DOME C). PLoS One 2014;9:e104505. doi:10.1371/journal.pone.0104505.

[178] González-Toril E, Amils R, Delmas RJ, Petit J-R, Komárek J, Elster J. Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glacial areas (Antarctica, Alps and Andes). Biogeosciences Discuss 2008;5:1607–30. doi:10.5194/bgd-5-1607-2008.

[179] Chuvochina MS, Marie D, Chevaillier S, Petit J-R, Normand P, Alekhina I a., et al. Community variability of bacteria in alpine snow (Mont Blanc) containing saharan dust deposition and their snow colonisation potential. Microbes Environ 2011;26:237–47. doi:10.1264/jsme2.ME11116.

[180] Maccario L, Vogel TM, Larose C. Potential drivers of microbial community structure and function in Arctic spring snow. Front Microbiol 2014;5:413. doi:10.3389/fmicb.2014.00413.

[181] Naff CS, Darcy JL, Schmidt SK. Phylogeny and biogeography of an uncultured clade of snow chytrids. Environ Microbiol 2013. doi:10.1111/1462-2920.12116.

[182] Eric Collins R, Rocap G, Deming JW. Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 2010;12:1828–41. doi:10.1111/j.1462-2920.2010.02179.x.

[183] Collins RE, Carpenter SD, Deming JW. Spatial heterogeneity and temporal dynamics of particles, bacteria, and pEPS in Arctic winter sea ice. J Mar Syst 2008;74:902–17. doi:10.1016/j.jmarsys.2007.09.005.

[184] Bramucci A, Han S, Beckers J, Haas C, Lanoil B. Composition, Diversity, and Stability of Microbial Assemblages in Seasonal Lake Ice, Miquelon Lake, Central Alberta. Biology (Basel) 2013;2:514–32. doi:10.3390/biology2020514.

[185] Ma L-J, Rogers SO, Catranis CM, Starmer WT. Detection and characterization of ancient fungi entrapped in glacial ice. Mycologia 2000;92:286–95. doi:10.2307/3761562.

[186] Lazzaro A, Wismer A, Schneebeli M, Erny I, Zeyer J. Microbial abundance and community structure in a melting alpine snowpack. Extremophiles 2015. doi:10.1007/s00792-015-0744-3.

[187] Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. PLoS One 2013;8:e79972. doi:10.1371/journal.pone.0079972.

[188] Konopka A. What is microbial community ecology? ISME J 2009;3:1223–30. doi:10.1038/ismej.2009.88.

[189] Raymond J a., Christner BC, Schuster SC. A bacterial ice-binding protein from the Vostok ice core. Extremophiles 2008;12:713–7. doi:10.1007/s00792-008-0178-2.

[190] Singh P, Hanada Y, Singh SM, Tsuda S. Antifreeze protein activity in Arctic cryoconite bacteria. FEMS Microbiol Lett 2013:1–9. doi:10.1111/1574-6968.12345.

[191] Mancuso Nichols C a., Garon S, Bowman JP, Raguénès G, Guézennec J. Production of exopolysaccharides by Antarctic marine bacterial isolates. J Appl Microbiol 2004;96:1057–66. doi:10.1111/j.1365-2672.2004.02216.x.

[192] Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol 2005;3:722–32. doi:10.1038/nrmicro1235.

[193] Dziewit L, Grzesiak J, Ciok A, Nieckarz M, Zdanowski MK, Bartosik D. Sequence determination and analysis of three plasmids of Pseudomonas sp. GLE121, a psychrophile isolated from surface ice of Ecology Glacier (Antarctica). Plasmid 2013;70:254–62. doi:10.1016/j.plasmid.2013.05.007.

[194] Moller AK, Barkay T, Hansen M a., Norman A, Hansen LH, Sorensen SJ, et al. Mercuric reductase genes (merA) and mercury resistance plasmids in High Arctic snow, freshwater and sea ice brine. FEMS Microbiol Ecol 2014;87:52–63. doi:10.1111/1574-6941.12189.

[195] Dommergue A, Larose C, Faïn X, Clarisse O, Hintelmann H, Schneider D, et al. Deposition of mercury species in the Ny-Alesund area (79 degrees N) and their transfer during snowmelt. Environ Sci Technol 2010;44:901–7. doi:10.1021/es902579m.

[196] Raymond J a., Kim HJ. Possible role of horizontal gene transfer in the colonization of sea ice by Algae. PLoS One 2012;7. doi:10.1371/journal.pone.0035968.

[197] Raymond J a. The ice-binding proteins of a snow alga, Chloromonas brevispina: probable acquisition by horizontal gene transfer. Extremophiles 2014:987–94. doi:10.1007/s00792-014-0668-3.

[198] Kepner RL, Wharton R a, Suttle C a. Viruses in Antarctic lakes. Limnol Oceanogr 1998;43:1754–61. doi:10.4319/lo.1998.43.7.1754.

[199] López-Bueno A, Tamames J, Velázquez D, Moya A, Quesada A, Alcamí A. High diversity of the viral community from an Antarctic lake. Science 2009;326:858–61. doi:10.1126/science.1179287.

[200] Laybourn-Parry J, Marshall W a., Madan NJ. Viral dynamics and patterns of lysogeny in saline Antarctic lakes. Polar Biol 2007;30:351–8. doi:10.1007/s00300-006-0191-9.

[201] Bellas CM, Anesio AM. High diversity and potential origins of T4-type bacteriophages on the surface of Arctic glaciers. Extremophiles 2013;17:861–70. doi:10.1007/s00792-013-0569-x.

[202] Maranger R, Bird DF, Juniper SK. Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar Ecol Prog Ser 1994;111:121–8. doi:10.3354/meps111121.

[203] Zawar-Reza P, Argüello-Astorga GR, Kraberger S, Julian L, Stainton D, Broady P a, et al. Diverse small circular single-stranded DNA viruses identified in a freshwater pond on the McMurdo Ice Shelf (Antarctica). Infect Genet Evol 2014;26:132–8. doi:10.1016/j.meegid.2014.05.018.

[204] Senčilo A, Luhtanen A-M, Saarijärvi M, Bamford DH, Roine E. Cold-active bacteriophages from the Baltic Sea ice have diverse genomes and virus-host interactions. Environ Microbiol 2014. doi:10.1111/1462-2920.12611.

[205] Luhtanen A-M, Eronen-Rasimus E, Kaartokallio H, Rintala J-M, Autio R, Roine E. Isolation and characterization of phage-host systems from the Baltic Sea ice. Extremophiles 2014;18:121–30. doi:10.1007/s00792-013-0604-y.

[206] Wells LE, Deming JW. Brief report Modelled and measured dynamics of viruses in Arctic winter sea ice brines. Environ Microbiol 2006;8:1115–21. doi:10.1111/j.1462-2920.2005.00984.x.

[207] Sanguino L, Franqueville L, Vogel TM, Larose C. Linking environmental prokaryotic viruses and their host through CRISPRs. FEMS Microbiol Ecol 2015. doi:10.1093/femsec/fiv046.

[208] Collins RE, Deming JW. Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. Polar Biol 2011;34:1819–30. doi:10.1007/s00300-011-1041-y.

[209] Anesio AM, Mindl B, Laybourn-Parry J, Hodson AJ, Sattler B. Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). J Geophys Res 2007;112:G04S31. doi:10.1029/2006JG000350.

[210] Anesio AM, Bellas CM. Are low temperature habitats hot spots of microbial evolution driven by viruses? Trends Microbiol 2011;19:52–7. doi:10.1016/j.tim.2010.11.002.

[211] Wilhelm SW, Suttle CA. Viruses and Nutrient Cycles in the Sea aquatic food webs. Bioscience 1999;49:781–8.

[212] Van Houdt R, Deghorain M, Vermeersch M, Provoost A, Lo Giudice A, Leys N, et al. Characterization of culturable Paenibacillus spp. from the snow surface on the high Antarctic Plateau (DOME C) and their dissemination in the Concordia research station. Extremophiles 2013;17:565–73. doi:10.1007/s00792-013-0539-3.

[213] Langford HJ, Irvine-Fynn TDL, Edwards a., Banwart S a., Hodson a. J. A spatial investigation of the environmental controls over cryoconite aggregation on Longyearbreen glacier, Svalbard. Biogeosciences Discuss 2014;11:3423–63. doi:10.5194/bgd-11-3423-2014.

[214] Thomas WH, Duval B. Sierra Nevada , California , USA , Snow Algae : Snow Albedo changes , algal-bacterial Interrelationships , and ultraviolet radiation effects. Arct Alp Res 1995;27:389–99.

[215] Stewart FJ, Fritsen CH. Bacteria-algae relationships in Antarctic sea ice. Antarct Sci 2004;16:143–56. doi:10.1017/S0954102004001889.

[216] Hünken M, Harder J, Kirst GO. Epiphytic bacteria on the Antarctic ice diatom *Amphiprora kufferathii* Manguin cleave hydrogen peroxide produced during algal photosynthesis. Plant Biol 2008;10:519–26. doi:10.1111/j.1438-8677.2008.00040.x.

[217] Marquardt M, Kramer M, Carnat G, Werner I. Vertical distribution of sympagic meiofauna in sea ice in the Canadian Beaufort Sea. Polar Biol 2011;34:1887–900. doi:10.1007/s00300-011-1078-y.

[218] Shtarkman YM, Koçer Z a, Edgar R, Veerapaneni RS, D'Elia T, Morris PF, et al. Subglacial Lake Vostok (Antarctica) accretion ice contains a diverse set of sequences from aquatic, marine and sediment-inhabiting bacteria and eukarya. PLoS One 2013;8:e67221. doi:10.1371/journal.pone.0067221.

[219] Zawierucha K, Kolicka M, Takeuchi N, Kaczmarek Ł. What animals can live in cryoconite holes ? A faunal review. J Zool 2014:1–11. doi:10.1111/jzo.12195.

[220] Hartzell PL, Nghiem J V, Richio KJ, Shain DH. Distribution and phylogeny of glacier ice worms (Mesenchytraeus solifugus and Mesenchytraeus solifugus rainierensis). Can J Zool 2005;83:1206–13. doi:10.1139/z05-116.

[221] Murakami T, Segawa T, Bodington D, Dial R. Census of bacterial microbiota associated with the glacier ice worm Mesenchytraeus solifugus. FEMS Microbiol Ecol 2015:1–28.

[222] Goodman D. Ecological Investigations of Ice Worms on Casement Glacier, Southeastern Alaska. vol. Report No. 1971.

[223] Miezcan T, Górniak D, Swiatecki A, Zdanowski M, Tarkowska-Kukuryk M, Adamczuk M. Vertical microzonation of ciliates in cryoconite holes in Ecology Glacier, King George Island. Polish Polar Res 2013;34:201–12. doi:10.2478/popore.

[224] Brown T a., Belt ST. Identification of the sea ice diatom biomarker IP25 in Arctic benthic macrofauna: Direct evidence for a sea ice diatom diet in Arctic heterotrophs. Polar Biol 2012;35:131–7. doi:10.1007/s00300-011-1045-7.

[225] Gradinger R, Friedrich C, Spindler M. Abundance, biomass and composition of the sea ice biota of the Greenland Sea pack ice. Deep Res Part II Top Stud Oceanogr 1999;46:1457–72. doi:10.1016/S0967-0645(99)00030-2.

[226] Shain DH, Mason T a., Farrell AH, Michalewicz L a. Distribution and behavior of ice worms (*Mesenchytraeus solifugus*) in south-central Alaska. Can J Zool 2001;79:1813–21. doi:10.1139/cjz-79-10-1813.

[227] Junge K, Krembs C, Deming J, Stierle A, Eicken H. A microscopic approach to investigate bacteria under in situ conditions in sea ice samples. Ann Glaciol 2001;33:304–10. doi:10.3189/172756401781818275.

[228] Miteva V, Sowers T, Brenchley J. Penetration of fluorescent microspheres into the NEEM (North Eemian) Greenland ice core to assess the probability of microbial contamination. Polar Biol 2013;37:47–59. doi:10.1007/s00300-013-1409-2.

[229] Miteva V, Burlingame C, Sowers T, Brenchley J. Comparative evaluation of the indigenous microbial diversity vs. drilling fluid contaminants in the NEEM Greenland ice core. FEMS Microbiol Ecol 2014:1–19. doi:10.1111/1574-6941.12286.

[230] Price PB. Microbial genesis, life and death in glacial ice. Can J Microbiol 2009;55:1–11. doi:10.1139/W08-117.

[231] Miyakawa S, Cleaves HJ, Miller SL. The cold origin of life: Hydrogen cyanide and formamide. Orig Life Evol Biosph 2002;32:195–208.

[232] Trinks H, Schröder W, Biebricher CK. Ice and the origin of life. Orig Life Evol Biosph 2005;35:429–45. doi:10.1007/s11084-005-5009-1.

[233] Menor-Salván C, Marín-Yaseli MR. Prebiotic chemistry in eutectic solutions at the water-ice matrix. Chem Soc Rev 2012;41:5404–15. doi:10.1039/c2cs35060b.

[234] Lehman N. Origin of life: Cold-hearted RNA heats up life. Nat Chem 2013;5:987–9. doi:10.1038/nchem.1811.

[235] Galtier N, Tourasse N, Gouy M. A non hyperthermophilic common ancestor to extant life forms. Science 1999;283:220–1.

[236] Felip M, Sattler B, Psenner R, Catalan J. Highly active microbial communities in the ice and snow cover of high mountain lakes. Appl Environ Microbiol 1995;61:2394–401.

[237] Carpenter EJ, Lin S, Capone DG. Bacterial activity in South Pole snow. Appl Environ Microbiol 2000;66:4514–7.

[238] Sattler B, Puxbaum H, Psenner R. Bacterial growth in supercooled cloud droplets. Geophys Res Lett 2001;28:239–42. doi:10.1029/2000GL011684.

[239] Warren SG, Hudson SR. Bacterial Activity in South Pole Snow Is Questionable. 2003;69:6340–2. doi:10.1128/AEM.69.10.6340.

[240] Anesio AM, Laybourn-Parry J. Glaciers and ice sheets as a biome. Trends Ecol Evol 2012;27:219–25. doi:10.1016/j.tree.2011.09.012.

[241] Stibal M, Telling J, Cook J, Mak KM, Hodson A, Anesio AM. Environmental controls on microbial abundance and activity on the greenland ice sheet: a multivariate analysis approach. Microb Ecol 2012;63:74–84. doi:10.1007/s00248-011-9935-3.

[242] Price PB, Sowers T. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. Proc Natl Acad Sci U S A 2004;101:4631–6. doi:10.1073/pnas.0400522101.

Chapter 2: Potential drivers of microbial community structure and function in arctic spring snow

Abstract

The arctic seasonal snowpack can extend at times over a third of the Earth's land surface. This chemically dynamic environment interacts constantly with different environmental compartments such as atmosphere, soil and meltwater, and thus, influences the entire biosphere. However, the microbial community associated with this habitat remains poorly understood. Our objective was to investigate the functional capacities, diversity and dynamics of the microorganisms in snow and to test the hypothesis that their functional signature reflects the snow environment. We applied a metagenomic approach to nine snow samples taken over two months during the spring season. Fungi, *Bacteroidetes* and *Proteobacteria* were predominant in metagenomic datasets and changes in community structure were apparent throughout the field season. Functional data that strongly correlated with chemical parameters like mercury or nitrogen species supported that this variation could be explained by fluctuations in environmental conditions. Through inter-environmental comparisons we examined potential drivers of snowpack microbial community functioning. Photochemical reactions and oxidative stress seem to be decisive parameters in structuring microbial communities inside arctic snowpacks.

Introduction

In Chapter 1, we have seen that snow harbors diverse microorganisms that have characteristics specific to the environmental conditions of this frozen water habitat. If the arctic snowpack is a functional ecosystem, then the microbial community inhabiting it should have functional genomic signatures related to their adaptation to the specific conditions of this environment. Several physiological adaptations have been described for microorganisms surviving under cold conditions based on psychrophilic microbial isolates [1–4]. Although the described increased membrane fluidity and synthesis of cold-adapted enzymes are critical to life in the cold, other physical and chemical parameters might be equally critical in the arctic snowpack. For example, photon-induced radiation is also a recognized cause of extreme conditions [5]. These photochemical reactions and the associated oxidative capacity have been described as playing a major role in snowpack chemistry [6], but their impact on snow microbial community remains unknown. Our objective was to investigate the functional capacities, diversity and dynamics of the microorganisms in snow and to test the hypothesis that their functional signature reflects the snow environment. Our approach was to compare the annotated functional DNA sequences in the microbial community to other communities and to known gene families associated with different stresses such as oxidative stress in relation to high UV irradiance

Material and Methods

Sampling procedure

Sampling site and procedure is illustrated in figure 1. Samples were taken during a 2008 springtime field campaign in Ny-Ålesund (Svalbard, Norway, 78°56'N, 11°52'E). Shallow pits (total snow pack depth of 45 cm at the beginning of the field season, snow melt from mid-May) were dug between April and June at the same sampling site with a 50 m^2 perimeter with restricted access located along the south coast of the Kongsfjorden (please consult Larose et al., 2013 for a complete description of the samples [7]). Surface (3 first cm) and basal snow samples (10 cm above the ground) were collected in three 3 L sterile sampling bags using a sterilized Teflon shovel. To avoid contaminating the snow, Tyvex® body suits and latex gloves were worn during sampling and gloves were worn during all subsequent sample handling. The nine samples chosen in this study for metagenomic analyses were representative of 8 distinct groups defined by chemical and taxonomical analysis [8]. Snow chemistry was analysed as described previously [9]. Briefly, total mercury was measured with a Tekran Model 2600 using USEPA method 1631 revision E and bioavailable mercury was determined using a *mer-lux* biosensor at the field laboratory. . Samples for methylmercury and chemical analysis were shipped frozen to the laboratory in France where methylmercury was analysed by purge and cryotrapping gas chromatography and inorganic ions and organic acids were measured by suppressed ion chromatography using a Dionex ICS 300. Chemical data are provided in Table 1.

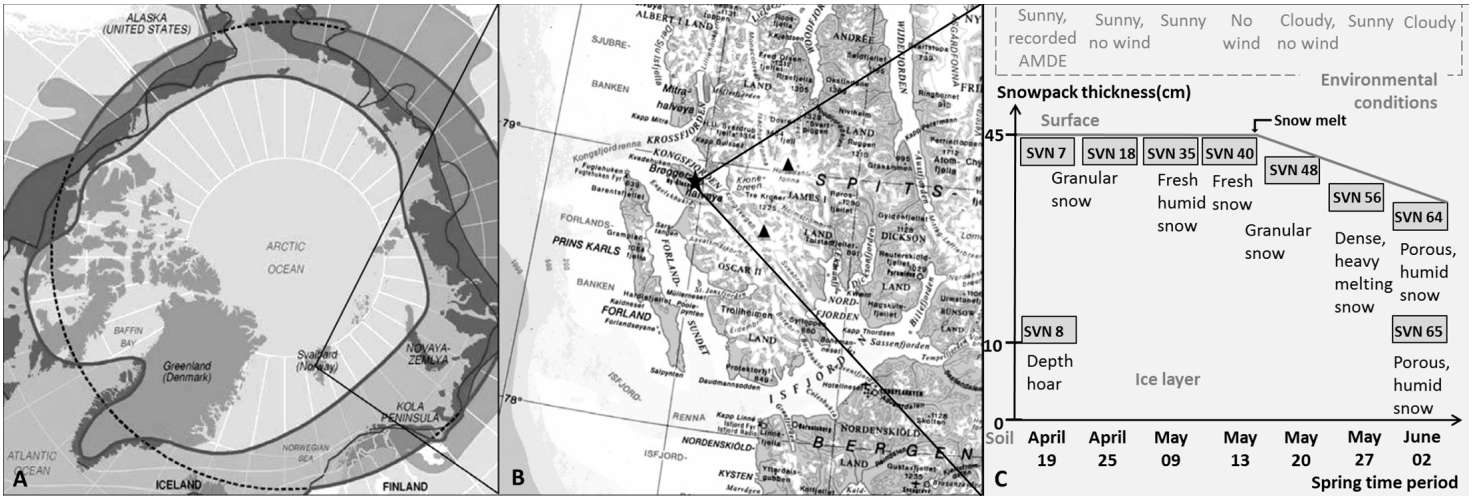

Figure 1: Description of sampling site and procedure– A: Svalbard Archipelago (Norway) – B: Sampling site along the south coast of the Kongsfjorden in Ny-Ålesund – C: Samples description (AMDE=atmospheric mercury deposition event) (scheme modified from Larose et al 2010 b)

Microbial sample processing

Samples were processed immediately after collection in the field laboratory. Samples were left to melt at room temperature prior to being filtered onto sterile 0.22 µM 47 mm filters (Millipore) using a sterile filtration unit (Nalge Nunc International Corporation) and filters were stored in sterile bead-beating tubes at -20°C until further analysis. Procedural blanks were carried out by filtering Nanopure water (Siemens) using the same procedure.

DNA extraction

DNA was extracted using the protocol outlined in David *et al*. (2009). Briefly, filters were chopped and placed in a Fastprep® bead-beating tube (Lysing matrix E, MP Biomedicals) to which 1 mL of DNA extraction buffer and 20 mg ml^{-1} lysing enzyme (*Trichoderma harzianum*, Sigma L1412) were added. Tubes were left at room temperature for 1 hour and then frozen at -20°C overnight. The frozen tubes were incubated at 65°C for 30 minutes and placed in a Fastprep® bead-beater (MP Biomedicals) set at speed 5.5 for 30 seconds. DNA was extracted from the water phase with an equal volume of chloroform:isoamyl alcohol (24:1) and precipitated with isopropanol.

Pyrosequencing

DNA extracted from environmental samples were sequenced by GATC (Constanz, Germany) using a Roche 454 Titanium pyrosequencer. Since the required DNA yield for pyrosequencing was 2µg/50µL, which could represent up to 1200 L of snow (DNA yield between 1.6 and 16 ng per L of snow), the DNA extracted from each sample was amplified using multiple displacement amplification with the illustra™ GenomiPhi™ HS DNA Amplification Kit (GE Healthcare).

Table 2: Snow Samples chemistry

Sample Number	SVN7	SVN8	SVN18	SVN35	SVN40	SVN48	SVN56	SVN64	SVN65
pH	6.4	4.9	5.5	4.7	5.1	4.0	4.2	5.1	6.4
Mercury (ngL^{-1})	40.7	1.9	58.5	1.2	3.7	7.7	0.8	1.1	3.3
MeHg (pgL^{-1})	BDL	BDL	BDL	BDL	BDL	BDL	0.5	BDL	0.2
BioHg (ngL^{-1})	6.7	1.6	7.3	2.1	1.5	1.2	1.1	NA	NA
MSA ($\mu molL^{-1}$)	BDL	BDL	BDL	1.0	1.4	1.1	1.6	BDL	0.6
Chloride ($\mu molL^{-1}$)	21456.5	156.1	623.3	107.8	30.7	37.4	51.4	54.9	1456.7
Natrium ($\mu molL^{-1}$)	19410.9	141.8	545.1	92.5	30.4	28.6	42	50.2	1258.2
Bromide ($\mu molL^{-1}$)	47.0	0.3	0.7	0.2	0.1	0.1	0.1	0.1	4.0
Sulfate ($\mu molL^{-1}$)	2105.6	11.6	64.2	9.6	38.8	13.7	11.4	0.5	76.4
Ammonium ($\mu molL^{-1}$)	50.7	1.3	4.4	3.6	6.9	4.3	7.2	1.4	14.2
Potassium ($\mu molL^{-1}$)	394.5	2.5	11.7	1.8	1.0	0.6	1.0	0.4	24.0
Magnesium ($\mu molL^{-1}$)	4341.5	32.0	129.5	20.5	6.8	5.5	11.2	3.5	276.1
Calcium ($\mu molL^{-1}$)	828.5	7.1	45.9	13.4	9.8	10.4	7.7	6.5	88.6
Nitrate ($\mu molL^{-1}$)	13.3	2.2	4	4.1	5.3	6.2	8.4	BDL	1.9
Nitrite ($\mu molL^{-1}$)	BDL	0.2	0.3	BDL	BDL	BDL	0.2	BDL	BDL
AGly ($\mu molL^{-1}$)	0.2	0.4	0.5	0.5	0.7	0.4	0.5	0.3	2.6

MeHG= methyl mercury; BioHG=bioavailable mercury [10]; MSA= methyl sulfonic acid; Agly= acetate-glycolate; NA= not available; BDL=bBelow detection limit)

Amplification was carried out according to the manufacturer's instructions and purified by addition of 3.5 volumes of both RA1 and 70% ethanol followed by centrifugation on Nucleospin Tissue XS columns. Further washing was carried out according to the manufacturer's instructions (Nucleospin). No amplification was obtained using extractions carried out on field blanks.

Sequence analysis

The fasta sequences obtained were filtered for errors using cd-hit, blasted against the NCBI-NR database using the BLASTX default settings [11,12] and analyzed using MEGAN4 [13]. In parallel, metagenomic datasets were analyzed using the Metagenome Rapid Annotation with Subsystem Technology (MG-RAST) [14]. Reads were taxonomically and functionally annotated by similarity searching against SEED database [15] with a maximum e-value of 10^{-5}. Annotated data were analyzed at a broad taxonomical level (Phylum/classes) and at the second level of hierarchical functional subsystems classification from SEED database including 477 subsystems representing the collection of functional roles that make up a metabolic pathway, complex, or a class of proteins. We compared the different snow samples taken during springtime for the global community composition. The relative abundance of reads in the fifty most represented functional subsystems in the snow datasets were tested for their correlation with chemical parameters (pH, mercury, methylmercury, etc, see Table 1). The resulting Pearson correlation matrix was then visualized in a heatmap from the R-Package "gplot" [16]. We compared functional subsystem distributions from snow metagenomes with other metagenomes from different ecosystems publically available on the MG-RAST platform (listed in table S1) [14,17–19].

Read distributions among the different functional subsystems for each ecosystem were then analyzed with the statistical software STAMP [20] using analysis of variance as the statistical test parameter. The relative distributions of annotated reads in functional subsystems between ecosystems were also analyzed by principal component analysis (PCA).

Results

Snow metagenomes characteristics:

Snow metagenomic datasets harbored on average 27 thousand sequences with an average length of 330 nucleotides. The smallest and the largest datasets were obtained from the samples SVN65 (12181 sequences) and SVN7 (42989 sequences) respectively. Taxonomic annotation efficiency at a broad taxonomic level (phylum/classes) was high; with a proportion of unannotated reads varying between 5 and 12 % of the total sequences. However, functional annotation efficiency was low; the percentage of reads with no occurrence with genes with known functions in the database varied between 60 and 88% (for SVN8 and SVN40, respectively). Detailed relative abundances of each functional or taxonomical group are directly available on MG-RAST software under the accession number indicated in table S2. The raw metagenomic dataset can be also downloaded from the MG-RAST website.

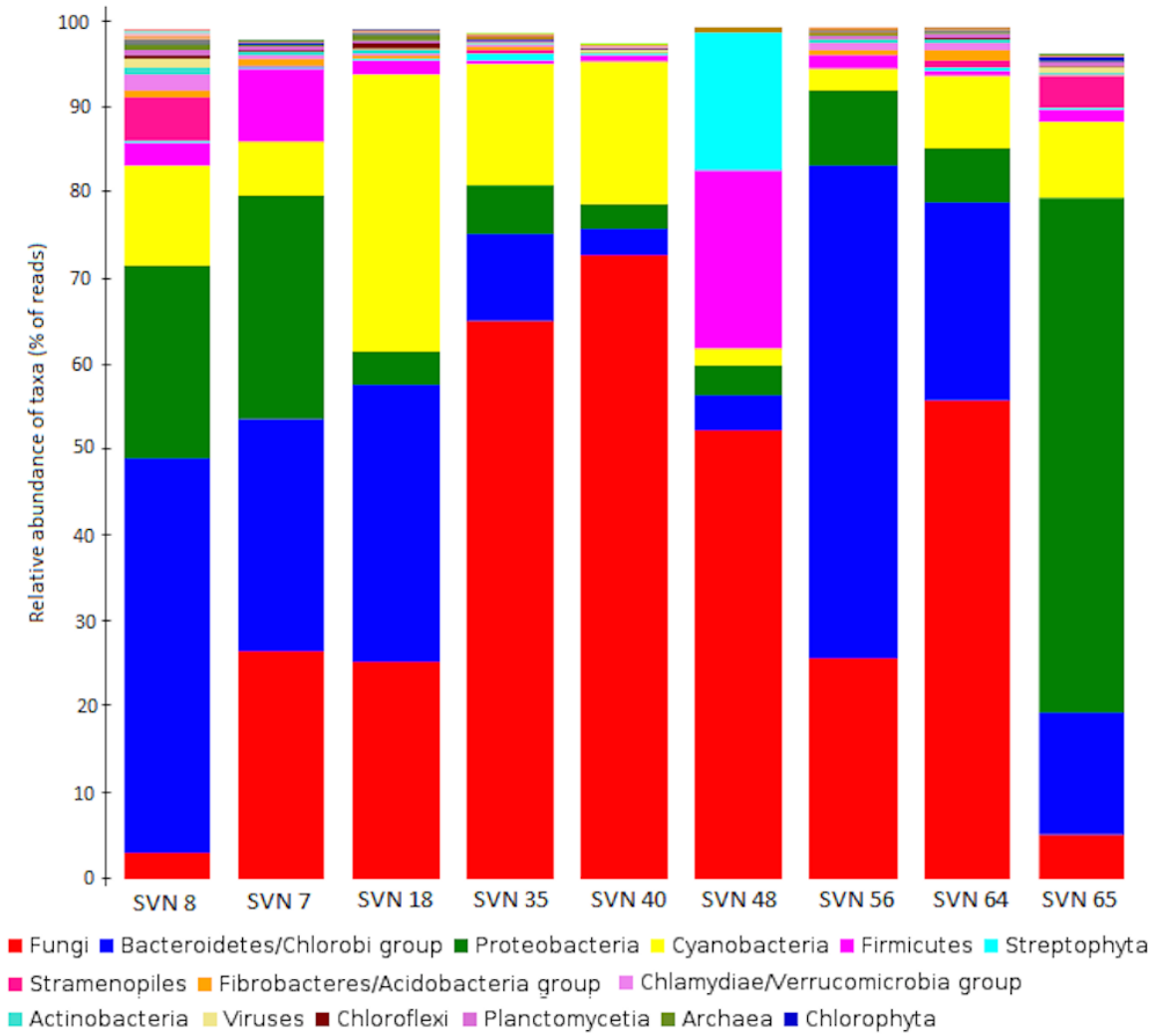

Figure 2: Comparison of the major phyla/classes (NCBI-Taxonomy in MEGAN) in all snow samples. Data are plotted as the percentage of sequence reads annotated to genomes within each phyla/class. The legend is classified in decreasing order of read numbers.

Functional and taxonomical dynamic of snowpack microbial community

Fungi represented the taxon with highest amount of annotated sequence reads, followed by *Bacteria* with major phyla *Bacteriodetes/Chlorobi (37%) and Proteobacteria (34%)* (Figure 2). *Cyanobacteria* (*Nostocales*, *Chroococcales*) represented approximately 10% of the classes, except for in one surface snow sample from May 20th (SVN48). *Archaea* domain had the least amount of annotated reads and was only detected in the early season snow samples (SVN7, SVN8, SVN18).

Some reads were similar to genomic sequences of species characterized as psychrophile or psycrotolerant with their highest relative abundance in basal samples and during late spring (sample SVN 65). We observed variability in community structure from samples throughout the field season. Reads related to *Fungi* were dominant in surface snow metagenomes sampled between the 25th of April (SVN18) and the 27th of May (SVN56) and reached up to 70 percent of annotated reads in the sequenced sample from the 13th of May (SVN40). We also observed differences in community composition between surface and basal snow (SVN8 and SVN65), where reads annotated to bacteria from *Proteobacteria*, *Bacteroidetes*/*Chlorobi* and *Cyanobacteria* were dominant relative to *Fungi* that were in relatively low annotated read abundance.

Sequence reads from the snow metagenomes were classified into metabolic functions using the SEED database of the annotated reads, most were classified as carbohydrate metabolism genes (10-19%), followed by virulence, amino acid, protein, DNA, cell wall, cofactors and respiration. The functional profile varied among snow samples. For example, the proportions of reads associated with virulence varied between 8.72% for the surface snow sample svn35 to up to 18.10 % for the surface snow sample svn56 sampled three weeks later. Among virulence associated reads, the majority corresponded to antibiotic and toxic compound resistance, and pollutant biodegradation and reach up to 91 % of the annotated reads in sequences from sample svn56. The chemical parameters measured in snow samples also varied between samples; surface versus basal samples and during the spring season (Table 1). Detailed analyses of these abiotic data have been published in a previous article (Larose et al. 2010). Based on the heatmap from the Pearson correlation matrix (Figure 3), many of these physico-chemical factors correlated with the functional annotation from the high throughput sequencing.

For example, total mercury (Hg), bio-available mercury (bio-Hg) concentrations were correlated to oxidative stress, tetrapyrroles (Cobalamin and coenzyme B12 biosynthesis and Heme/siroheme biosynthesis) and NAD/NADP metabolism. Methyl sulfonic acid (MSA) was also positively correlated to iron acquisition and quinine cofactors.

Functional signature of snowpack microbial community

The relative abundance of annotated reads of functional subsystems was compared among different ecosystem metagenomic datasets referenced in Table S2: snowpack, polar microbial mats for samples corresponding to the cryosphere, and soil (forest and grassland) together with oceans (coastal and open oceans) for mesophilic environments. All of the snow samples grouped together in the principal component analysis (PCA) of the functional read distributions and were separated from the other ecosystems (Figure 4). However, different snow samples from the same sampling site in Svalbard and from the same sampling season were more dispersed than samples collected for other environmental groups even those that included sequences from different sampling sites, time periods, extraction protocols, and sequencing technologies. Several functional subsystems were more represented in terms of normalized read numbers in the snowpack than in sequences from other environments. All subsystems at the second level of seed classification that were more abundant in snow samples are listed in table S2. As an example, we focused on four different subsystems, illustrated in Figure 5. For NAD/NADP metabolism, the proportion of reads represents on average 0.8 % of annotated sequences and was significantly higher in snow samples (p-value 1.72×10^{-3}).

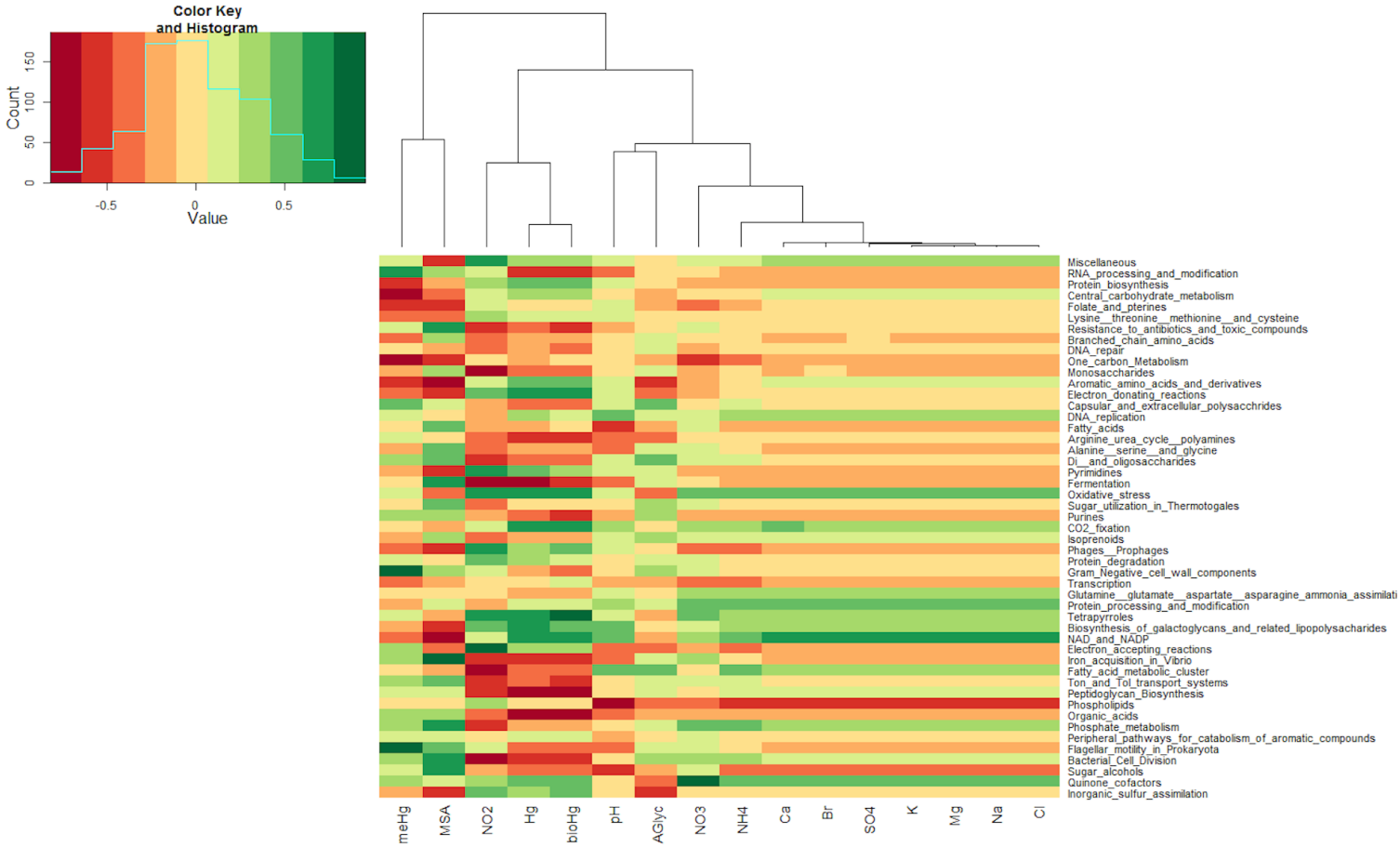

Figure 3: Heatmap from Pearson correlations between chemical data and the first 50 subsystems (in abundance) at the second level of seed classification. Functional subsystems are ordered from the most to the least abundant

Proportions of reads associated with biosynthesis of galactoglycans and associated polysaccharides were also globally more elevated in early spring snow samples (up to 1.2 %). In addition, the percentage of reads related to cyanobacterial circadian clock was significantly different among environments (p-value 1.05×10^{-3}) with a greater representation in polar microbial mat and snow samples (0.13 % of sequences on average for both). Bacterial hemoglobin associated reads also seem to be more represented in most snow samples despite the non-significant p-value 0.056 which is likely explained by snow sample heterogeneity. Among all functional subsystems, we also focused on those associated to cold-resistance mechanisms, whose distributions in the different environments are provided in Table S3. Although the associated genes were found in our snow samples, most of them were not statistically more represented in cryospheric environments (both snow and polar microbial mat) than in other environments. However, genes related to fatty acid desaturases and biosynthesis of galactoglycans that are involved in cold resistance were relatively more dominant in our snow samples and polar mats than in other ecosystems.

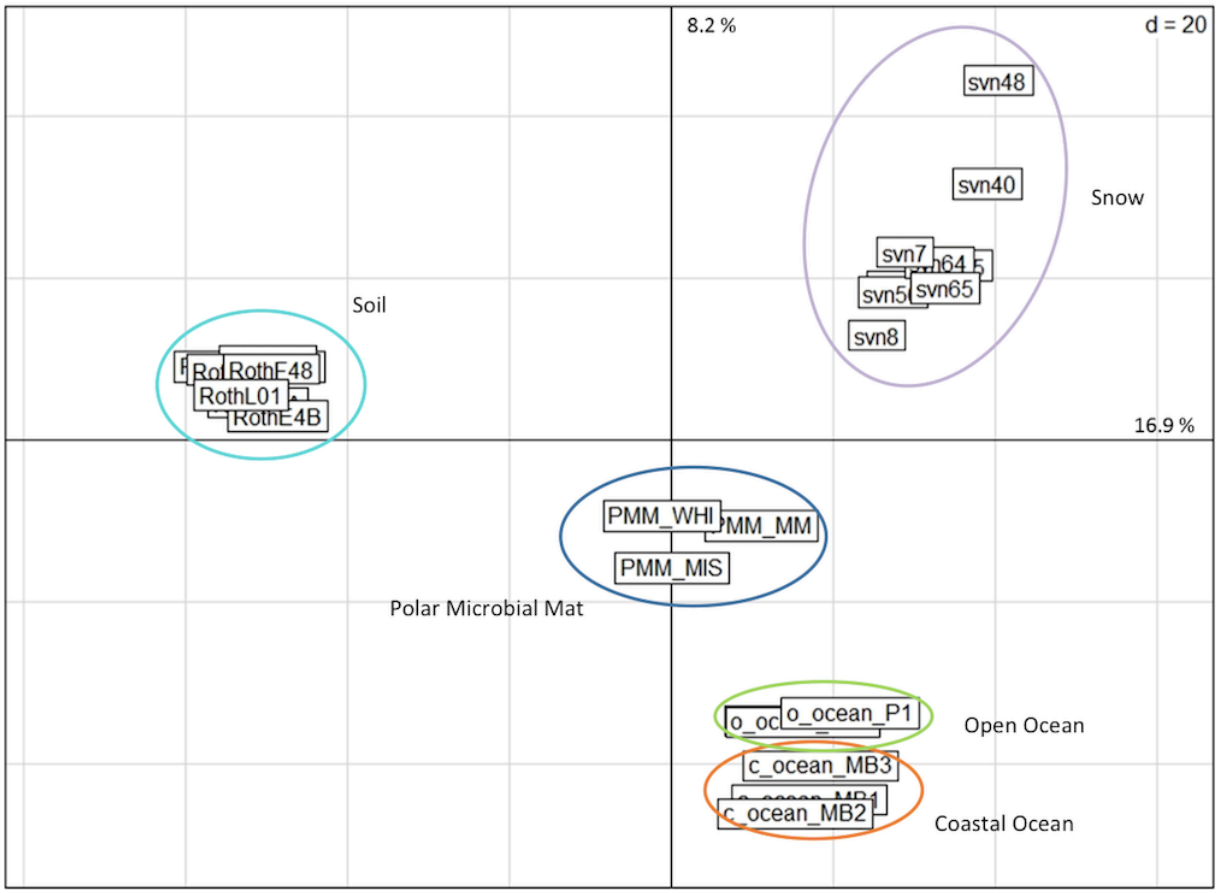

Figure 4: PCA based on the relative distribution of annotated sequences (E-value < 10^{-5}) classified in different functional subsystems by MG-RAST software. Distributions were normalized as a function of the number of annotated sequences for each metagenome. The snow samples were compared to metagenomes from different ecosystems. PMM: Polar Microbial Mats Arctic and Antarctic (Varin *et al*) [19], Roth:Rothamsted soil (Delmont *et al*.) [17], PR: Puerto Rico soil (DeAngelis *et al*.) [18], OOcean: Open Ocean (Delong *et al.*, Giovannoni et al.), C-ocean: Coastal Ocean (Delong *et al*.) [21]

Discussion

Snow is increasingly considered to be a diverse and active ecosystem, but the microbial community inhabiting this environment remains poorly understood [22]. High throughput sequencing was used to determine the composition and potential functional capability of the microbial community in the snow. We sequenced nine snow samples taken over a Spring sampling season. The resulting metagenomic datasets were analyzed for both their taxonomic and their functional profiles based on comparing the sequences to known microorganisms and proteins.

Bacterial community composition of our snow samples had been analyzed in detail previously using a 16S rRNA gene phylogenetic microarray [8]. Despite differences inherent to the methodologies used, the bacterial taxa detected were similar with both techniques (microarray and high throughput sequencing) and consistent with previous studies on snow in the Arctic and Antarctic [23–27]. Bacterial sequences retrieved from our snow metagenomes were mostly annotated to *Proteobacteria* and *Bacteroidetes* and include reads affiliated to known psychrophiles or psychrotolerant bacteria. Total microbial community sequencing increased our taxonomic description of snow communities and highlighted the abundance of eukaryotic representatives that constituted, in some of the snow metagenomes, a majority of the total reads (up to 70% in SVN40). Most of these sequences were annotated to *Fungi*, mainly *Ascomyceta*, which has representatives described in other cold environments like Canadian High Arctic snowpack and arctic Ice [28,29]. As short and highly conserved domains can lead to unspecific taxonomic assignment of metagenomics reads [30], we analyzed these data at broader taxonomical levels (phylum/classes).

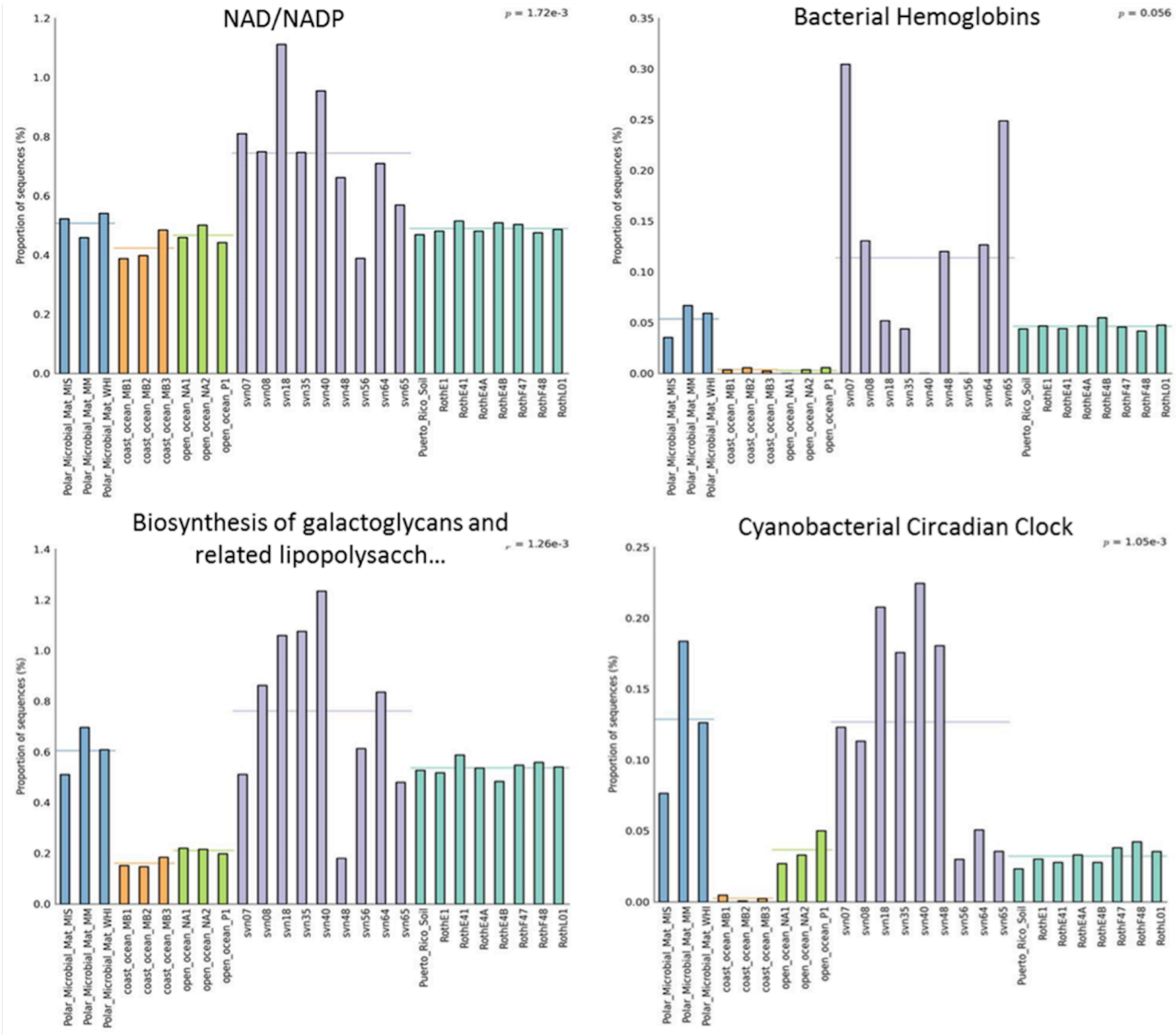

Figure 5: Relative distribution of different functional subsystems (in percentage of annotated sequences) (Annotation with MG-RAST Software). Distributions between different ecosystems were compared using multiple group analyses (ANOVA, Tukey-Kramer) in STAMP. PMM: Polar Microbial Mats Arctic and Antarctic (Varin *et al*) [19], Roth:Rothamsted soil (Delmont *et al*.) [17], PR: Puerto Rico soil (DeAngelis *et al*.) [18], Open Ocean (Delong *et al.*, Giovannoni et al.), Coastal Ocean (Delong *et al*.) [21]. Horizontal lines are mean value within the different ecosystems and purple coloration indicate snow samples.

To our knowledge, this is the first study to include prokaryotic and eukaryotic representatives together to produce a complete picture of snow microbial community structure and dynamics. We observed fluctuations in community structure between samples, with depth (surface versus basal snow) and at temporal scales (taken over a two-month period during the spring season). Since we did not replicate our metagenomic analysis within a same snow layer at the same time point, we cannot evaluate beta diversity and while we cannot exclude that these fluctuations reflect snowpack heterogeneity, microarray analysis presented in Larose et al. (2013) suggested that modifications in environmental conditions may also be involved [8]. For example, more constant conditions at the base of the snowpack might create more stable communities, including psychrotolerant/psychrophile organisms, to establish there. The high relative abundance of reads associated with *Fungi* in mid-May could be linked to spore deposition during snowfall and a fungal bloom due to snowpack wetting and warming. This heterogeneity in community structure between surface and basal snow due to variable conditions has been previously observed in Greenland snowpacks [26]. Environmental chemistry, such as pH, organic acids, nitrogen, sulfur cycling, and mercury, has been correlated with bacterial community dynamics measured by 16S rRNA gene phylogenetic microarray in the Svalbard snowpack [8]. Unfortunately, taxonomical data associated with ribosomal gene analyses cannot provide the details of the ecological role of these organisms, since functional potential can differ between species and within the same taxonomic species [31]. Functional metagenomic analysis has been proposed as a technique for assessing ecosystem ecology (*e.g* how microorganisms are adapted to a given environment and what role they play in this environment [32–34].

The relative abundance of functionally unannotated reads was very high in our snow metagenomes, illustrating the lack of environmental representatives in databases, especially from largely unexplored environments such as snowpacks. However our preliminary data indicate that the arctic snowpack harbors a specific functional signature based on gene annotation of sequenced DNA and that this functional potential is correlated to the varying environmental conditions during the spring season and with depth in the snowpack. We observed a correlation between specific functional gene abundance and chemical parameters. For example, mercury and bio-available mercury were correlated to the subsystem tetrapyrroles in which most of the reads are associated with heme and siroheme biosynthesis and cobalamin and coenzyme B12 biosynthesis. Cobalamin has been shown to be involved in mercury methylation in sulfate-reducing bacteria [35,36]. This supports the hypothesis that snowpack is a dynamic ecosystem that responds to changes in environmental conditions [8], but this functional exploration also uncovered some of the factors that might drive microbial community structure and function in the snowpack ecosystem. The arctic snowpack is an important component of the cryosphere [37] and shares many extreme environment characteristics with other frozen habitats, like sea ice and polar mats. These characteristics include low nutrient concentrations, desiccation due to low water activity, a freeze-thaw cycle, and intense UV irradiation during summer and darkness during winter as well as low temperatures [37]. Fungi using effective adaptation mechanisms might be able to grow and develop in such habitats and are not just windblown contaminant spores [29]. If this is the case, then *Fungi* might be carrying out several different metabolic activities in the snow. Unfortunately, the large majority of *Fungi* affiliated reads in our metagenomic datasets (up to 86%) were not functionally annotated due to a lack of fungal genomic and protein data, and therefore, we were unable to clearly characterize the

functional potential of the fungal microbial community. Some bacterial adaptation mechanisms have been described for how microorganisms in culture deal with these cold habitats [38,39]. These consist of polyunsaturated fatty acid biosynthesis involved in maintenance of membrane fluidity, production of exopolysaccharides, cold shock protein, DNA gyrase maintaining DNA topology or choline betain uptake. The vast amount of metagenomic sequence data could provide a rich source for the mining of psychrophilic adaptations from uncultured organisms [40]. We observed sequence reads associated with the genes considered to be related to these adaptation mechanisms in our snow metagenomes. Similar observations were carried out in glacier ice and polar mat microbial community metagenomes [19,41], which emphasized the role of these mechanisms for adaption in cold environments. But cold adaptation does not only occur in extreme environments and we detected these functions in all other ecosystem metagenomes. This implies that other adaptive mechanisms related to environmental factors may play a role in defining the snow microbial community structure and function relative to other ecosystems. One such environmental factor might include constant light irradiation during the summer months in the Arctic. This intense irradiation has been described as playing a major role in snowpack chemistry due to photochemical reactions [6]. Constant light irradiation could also affect regulation of light-dependent metabolisms, especially in photosynthetic microorganisms. Cyanobacterial circadian clock functions appeared more dominant in our snow metagenomes (and in polar microbial mat) than in other ecosystems. *Cyanobacteria* are the simplest organisms known to have endogenous circadian clock mechanisms [42]. The circadian input kinase (cikA) gene encodes a bacterio-phytochrome-like histidine kinase involved in the input signaling of the clock [43]. These mechanisms might be crucial for light-dependent metabolisms under constant light conditions.

In addition, UV-light irradiation might be an especially important factor in microbial snow community ecology in arctic snowpacks. Described as a natural photochemical bioreactor [6,44], snowpacks are highly reactive to UV light. Chemical compounds sequestered in snowpacks are photolyzed upon irradiation and reactive trace gases are released in the snow boundary layer. These photochemical-induced reactions may result in the accumulation of reactive species within the snowpack, and thus, in the emergence of a hyper oxidative stress habitat. Oxidative stress is defined by a physiological state produced when the concentration of reactive oxygen species exceeds cell defense capacity as expressed during aerobic metabolism. Free radicals are highly reactive chemical species that damage DNA, proteins or lipids via oxidation; lipid peroxidation, for example, causes cell membrane degradation [45]. This stress can be caused by the diffusion of radicals into the cell or by environmental agents, such as ions, near-UV radiation and other compounds that generate intracellular radicals [45]. Some functional subsystems associated with potential oxidative stress response, such as oxidative stress and NAD/NADP metabolism, were among the fifty most abundant subsystems in relative abundance of annotated reads. Moreover, the abundance of reads annotated within these oxidative stress subsystems showed a strong correlation with mercury, bio-available mercury, and nitrite concentrations in our samples. During mercury exposure, oxygen reactive species like hydrogen peroxide might increase due to the direct effect of Hg^{2+} on electron transport pathways [46] and to the Hg^{2+}-mediated activation of super oxide stress responses [47]. Reactive nitrogen species (RNS), like nitric oxide, are formed as metabolic side products when nitrate or nitrite is used as a terminal electron acceptor in denitrifying bacteria [48] or by the reduction of nitrite to $NO^{\cdot}$ by an oxidase [49] and also generated exogenously by nitrate or nitrite photolysis [6]. These RNS might be involved in the induction of oxidative stress response mechanisms like hemoglobins [50].

The NAD/NADP and quinine cofactors subsystem might be involved in oxidative stress response in the snowpack microbial community. NADH/NADPH pools represent essential non enzymatic antioxidants within bacterial cells [45]. In *Escherichia coli*, a fine regulation of NADP(H) homeostasis is necessary for proper deployment of the oxidative stress defensive response [51]. The widely distributed NAPH quinone reductase is implicated in the oxidative stress resistance during host colonization by *Helicobacter pylori* [52]. Reads associated with Sigma B factor, required for the induction of approximately 100 genes responding to a whole range of stresses [53], were more abundant in our snow metagenomes than in other ecosystems. In particular, oxidative stress response is mediated by sigma factor in a wide range of microorganisms such as *Bacillus subtilis* [53], *Streptomyces coelicolor* [54] and *Listeria monocytogenes* [55]. This function has also been identified in genera from nutritionally poor aquatic environments, such as *Caulobacter* [56], which were detected in our snow metagenomes. Moreover, part of these oxidative stress response associated functions (i.e. bacterial hemoglobins, NAD/NADP or Sigma B stress response regulation) were more highly represented in snow metagenomes than in other ecosystems. Although polar microbial mats in ice shelves are also exposed to high UV irradiation, high photosynthetically active radiation and photodamage [57,58], the highly structured multilayer microbial community contains a high amount of pigments such as Scytonemin and its reduced derivatives. These pigments could act as an effective sunscreen protecting the entire community against UV exposure in ice shelf polar mats [59]. Given that the microorganisms in snowpacks are not structured with comparable complexity and thickness, they likely do not benefit from similar protective effects. The results presented here support the possibility that photochemistry, given the high light exposure in snow that may result in oxidative stress conditions, might be an important factor for defining microbial community

structure in the arctic spring snowpack. However, one of the microbial sources for snowpacks is the atmosphere and microorganisms in clouds in the atmosphere are also exposed to high UV irradiance and some present corresponding adaptations such as pigments [60]. Cloud microflora exposed to UV light in microcosms also remained metabolically active in the presence of ·OH radicals [61]. While our data does not allow us to clearly discriminate between adaptation to stresses encountered during atmospheric transport and those selected for after deposition in snowpack, snow and atmosphere were shown to present distinct microbial assemblages in other reports [28,62]. In addition, the rapid selection of microorganisms after deposition to constitute a snow specific microbial community has been suggested [63] with, for example, some plant pathogens belonging to *Agrobacterium* that are likely wind transported and which are detected in fresh snow and not after the snowfall event [8]. Metagenomic comparison of different snow layers and the corresponding atmosphere samples will help to address how microorganisms are selected after deposition. In the same vein, metatranscriptomic analyses will also be useful to determine which part of this community is active at a given period and what their roles in the snow ecosystem are.

Conclusions

This study explored the microbial community functional genes in the arctic snowpack. This microbial community, including representative members associated with cold environments, underwent major changes during the spring season. Functional data that correlated with chemical parameters supported the hypothesis that this variation in microbial community structure and function could be explained by fluctuations in environmental conditions.

Moreover, in this study, we tested the occurrence of a specific functional signature from the snowpack microbial community. Intense UV-light irradiation might be a critical factor in defining the microbial ecology of the arctic snowpack ecosystem. Further sampling during the dark period as well as metatranscriptomic and atmosphere comparison studies year round would help establish how microorganisms are selected in snowpack and the role of light as a major driver of snowpack microbial community structure and function.

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References

[1] Cloutier J, Prévost D, Nadeau P, Antoun H. Heat and cold shock protein synthesis in arctic and temperate strains of rhizobia. Appl Environ Microbiol 1992;58:2846–53.

[2] Kumar GS, Jagannadham M V, Ray MK. Low-temperature-induced changes in composition and fluidity of lipopolysaccharides in the antarctic psychrotrophic bacterium *Pseudomonas syringae*. J Bacteriol 2002;184:6746–9. doi:10.1128/JB.184.23.6746-6749.2002.

[3] Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, et al. Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 2004;8:475–88. doi:10.1007/s00792-004-0409-0.

[4] Gilbert JA, Davies PL, Laybourn-Parry J. A hyperactive, Ca2+-dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 2005;245:67–72. doi:10.1016/j.femsle.2005.02.022.

[5] Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature 2001;409:1092–101. doi:10.1038/35059215.

[6] Grannas AM, Jones AE, Dibb J, Ammann M, Anastasio C, Beine HJ, et al. An overview of snow photochemistry : evidence , mechanisms and impacts 2007:4329–73.

[7] Larose C, Dommergue A, Vogel TM. Microbial nitrogen cycling in Arctic snowpacks. Environ Res Lett 2013;8:035004. doi:10.1088/1748-9326/8/3/035004.

[8] Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. PLoS One 2013;8:e79972. doi:10.1371/journal.pone.0079972.

[9] Larose C, Dommergue A, De Angelis M, Cossa D, Averty B, Marusczak N, et al. Springtime changes in snow chemistry lead to new insights into mercury methylation in the Arctic. Geochim Cosmochim Acta 2010;74:6263–75. doi:10.1016/j.gca.2010.08.043.

[10] Schroeder WH, Anlauf KG, Barrie LA, Lu JY, Steffen A, Schneeberger DR, et al. Arctic springtime depletion of mercury 1998;394:331–2. doi:10.1038/28530.

[11] Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. Nucleic Acids Res 2005;33:D34–8. doi:10.1093/nar/gki063.

[12] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403–10.

[13] Huson DH, Mitra S, Ruscheweyh H-J, Weber N, Schuster SC. Integrative analysis of environmental sequences using MEGAN4. Genome Res 2011;21:1552–60. doi:10.1101/gr.120618.111.

[14] Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, et al. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 2008;9:386. doi:10.1186/1471-2105-9-386.

[15] Overbeek R, Begley T, Butler RM, Choudhuri J V, Chuang H-Y, Cohoon M, et al. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 2005;33:5691–702. doi:10.1093/nar/gki866.

[16] Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. J Comput Graph Stat 1996;5:299–314. doi:10.1080/10618600.1996.10474713.

[17] Delmont TO, Robe P, Clark I, Simonet P, Vogel TM. Metagenomic comparison of direct and indirect soil DNA extraction approaches. J Microbiol Methods 2011;86:397–400. doi:10.1016/j.mimet.2011.06.013.

[18] DeAngelis KM, Allgaier M, Chavarria Y, Fortney JL, Hugenholtz P, Simmons B, et al. Characterization of trapped lignin-degrading microbes in tropical forest soil. PLoS One 2011;6:e19306. doi:10.1371/journal.pone.0019306.

[19] Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J. Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the High Arctic. Appl Environ Microbiol 2012;78:549–59. doi:10.1128/AEM.06354-11.

[20] Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic communities. Bioinformatics 2010;26:715–21. doi:10.1093/bioinformatics/btq041.

[21] DeLong EF. Microbial community genomics in the ocean. Nat Rev Microbiol 2005;3:459–69. doi:10.1038/nrmicro1158.

[22] Larose C, Dommergue A, Vogel T. The dynamic arctic snow Pack: an unexplored environment for microbial diversity and activity. Biology (Basel) 2013;2:317–30. doi:10.3390/biology2010317.

[23] Carpenter EJ, Lin S, Capone DG. Bacterial Activity in South Pole Snow 2000;66. doi:10.1128/AEM.66.10.4514-4517.2000.Updated.

[24] Amato P, Hennebelle R, Magand O, Sancelme M, Delort A-M, Barbante C, et al. Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol Ecol 2007;59:255–64. doi:10.1111/j.1574-6941.2006.00198.x.

[25] Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, et al. Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. Extremophiles 2010;14:205–12. doi:10.1007/s00792-009-0299-2.

[26] Møller AK, Søborg DA, Al-soud WA, Sørensen SJ, Kroer N. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. Polar Res 2013;1:1–11.

[27] Lopatina A, Krylenkov V, Severinov K. Activity and bacterial diversity of snow around Russian Antarctic stations. Res Microbiol 2013;164:949–58. doi:10.1016/j.resmic.2013.08.005.

[28] Harding T, Jungblut AD, Lovejoy C, Vincent WF. Microbes in high arctic snow and implications for the cold biosphere. Appl Environ Microbiol 2011;77:3234–43. doi:10.1128/AEM.02611-10.

[29] Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš a. Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Phys Chem Earth, Parts A/B/C 2003;28:1273–8. doi:10.1016/j.pce.2003.08.056.

[30] Huson DH, Auch AF, Qi J, Schuster SC. MEGAN analysis of metagenomic data. Genome Res 2007;17:377–86. doi:10.1101/gr.5969107.

[31] Polz MF, Alm EJ, Hanage WP. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. Trends Genet 2013;29:170–5.

[32] Dinsdale E a, Edwards R a, Hall D, Angly F, Breitbart M, Brulc JM, et al. Functional metagenomic profiling of nine biomes. Nature 2008;452:629–32. doi:10.1038/nature06810.

[33] Delmont TO, Malandain C, Prestat E, Larose C, Monier J-M, Simonet P, et al. Metagenomic mining for microbiologists. ISME J 2011;5:1837–43. doi:10.1038/ismej.2011.61.

[34] Simon C, Daniel R. Metagenomic analyses: past and future trends. Appl Environ Microbiol 2011;77:1153–61. doi:10.1128/AEM.02345-10.

[35] Choi SC, Chase T, Bartha R. Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. Appl Environ Microbiol 1994;60:1342–6.

[36] Parks JM, Johs A, Podar M, Bridou R, Hurt RA, Smith SD, et al. The genetic basis for bacterial mercury methylation. Science 2013;339:1332–5. doi:10.1126/science.1230667.

[37] Priscu JC, Christner BC. earth's icy biosphere. In: Bull AT, editor. Microbial Diversity. Bioprospecting, American Society for Microbiology, Washington, D.C; 2004, p. 130–45.

[38] Deming JW. Psychrophiles and polar regions. Curr Opin Microbiol 2002;5:301–9.

[39] Rodrigues DF, Tiedje JM. Coping with our cold planet. Appl Environ Microbiol 2008;74:1677–86. doi:10.1128/AEM.02000-07.

[40] Casanueva A, Tuffin M, Cary C, Cowan D. Molecular adaptations to psychrophily: the impact of "omic" technologies. Trends Microbiol 2010;18:374–81. doi:10.1016/j.tim.2010.05.002.

[41] Simon C, Wiezer A, Strittmatter AW, Daniel R. Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. Appl Environ Microbiol 2009;75:7519–26. doi:10.1128/AEM.00946-09.

[42] Kondo T, Strayer CA, Kulkarni RD, Taylor W, Ishiura M, Golden SS, et al. Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. Proc Natl Acad Sci U S A 1993;90:5672–6.

[43] Schmitz O. CikA, a Bacteriophytochrome That Resets the Cyanobacterial Circadian Clock. Science (80-) 2000;289:765–8. doi:10.1126/science.289.5480.765.

[44] Domine F, Albert M, Huthwelker T, Jacobi H-W, Kokhanovsky AA, Lehning M, et al. Snow physics as relevant to snow photochemistry. Atmos Chem Phys 2008;8:171–208. doi:10.5194/acp-8-171-2008.

[45] Cabiscol E, Tamarit J, Ros J. Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 2000;3:3–8.

[46] Lund BO, Miller DM, Woods JS. Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 1993;45:2017–24.

[47] Smith T, Pitts K, McGarvey JA, Summers AO. Bacterial oxidation of mercury metal vapor, Hg(0). Appl Environ Microbiol 1998;64:1328–32.

[48] Goretski J, Zafiriou OC, Hollocher TC. Steady-state nitric oxide concentrations during denitrification. J Biol Chem 1990;265:11535–8.

[49] Bonner FT, Hughes MN, Poole RK, Scott RI. Kinetics of the reactions of trioxodinitrate and nitrite ions with cytochrome d in *Escherichia coli*. Biochim Biophys Acta - Bioenerg 1991;1056:133–8. doi:10.1016/S0005-2728(05)80279-8.

[50] Frey AD, Farre J, Bollinger CJT, Kallio PT. Bacterial Hemoglobins and Flavohemoglobins for Alleviation of Nitrosative Stress in *Escherichia coli* 2002;68:4835–40. doi:10.1128/AEM.68.10.4835.

[51] Krapp AR, Humbert MV, Carrillo N. The soxRS response of *Escherichia coli* can be induced in the absence of oxidative stress and oxygen by modulation of NADPH content. Microbiology 2011;157:957–65. doi:10.1099/mic.0.039461-0.

[52] Wang G, Maier RJ. An NADPH quinone reductase of *Helicobacter pylori* plays an important role in oxidative stress resistance and host colonization. Infect Immun 2004;72:1391–6. doi:10.1128/IAI.72.3.1391-1396.2004.

[53] Völker U, Maul B, Hecker M. Expression of the sigmaB-dependent general stress regulon confers multiple stress resistance in *Bacillus subtilis*. J Bacteriol 1999;181:3942–8.

[54] Paget MS, Kang JG, Roe JH, Buttner MJ. sigmaR, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). EMBO J 1998;17:5776–82. doi:10.1093/emboj/17.19.5776.

[55] Ferreira A, O'Byrne CP, Boor KJ. Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. Appl Environ Microbiol 2001;67:4454–7.

[56] Alvarez-Martinez CE, Lourenço RF, Baldini RL, Laub MT, Gomes SL. The ECF sigma factor sigma(T) is involved in osmotic and oxidative stress responses in *Caulobacter crescentus.* Mol Microbiol 2007;66:1240–55. doi:10.1111/j.1365-2958.2007.06005.x.

[57] Roos JC, Vincent WF. temperature dependence of uv radiation effects on antarctic cyanobacteria. J Phycol 1998;34:118–25. doi:10.1046/j.1529-8817.1998.340118.x.

[58] Mueller DR, Vincent WF, Bonilla S, Laurion I. Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microbiol Ecol 2005;53:73–87. doi:10.1016/j.femsec.2004.11.001.

[59] Lionard M, Péquin B, Lovejoy C, Vincent WF. Benthic cyanobacterial mats in the high arctic: multi-layer structure and fluorescence responses to osmotic stress. Front Microbiol 2012;3:140. doi:10.3389/fmicb.2012.00140.

[60] Delort A-M, Vaïtilingom M, Amato P, Sancelme M, Parazols M, Mailhot G, et al. A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. Atmos Res 2010;98:249–60. doi:10.1016/j.atmosres.2010.07.004.

[61] Vaïtilingom M, Deguillaume L, Vinatier V, Sancelme M, Amato P, Chaumerliac N, et al. Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. Proc Natl Acad Sci U S A 2013;110:559–64. doi:10.1073/pnas.1205743110.

[62] Bowers RM, Lauber CL, Wiedinmyer C, Hamady M, Hallar AG, Fall R, et al. Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl Environ Microbiol 2009;75:5121–30. doi:10.1128/AEM.00447-09.

[63] Segawa T, Miyamoto K, Ushida K, Agata K, Okada N, Kohshima S. Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains , Japan , analyzed by 16S rRNA gene sequencing and real-time PCR 2005;71:123–30. doi:10.1128/AEM.71.1.123.

Chapter 3: Seeding and post deposition process leading to specific snow-adapted microbial communities in a Greenlandic sea ice snow cover model

Abstract

Snow interacts with all compartments of the biosphere: oceans, soils, ice and atmosphere, and strongly influences their abiotic and biotic processes. However, the selection processes and the response of microbial communities to rapid changes in environmental conditions induced by seeding events remain unclear. Here, using a sea ice snowpack as a model system, we address how the snow microbial communities are influenced by their different seeding sources, atmosphere and sea ice, and identify the specific functions involved in response to the changes in abiotic characteristics of the snowpack. Microbial communities in sea ice snow cover are influenced by their seeding as they include members from potential local and remote from terrestrial and marine origins and harbour adaptations related to specific conditions; i.e., photochemical stress in surface snow microbial communities and osmotic stress in deeper snow layers. However, the specific functional and taxonomical composition of all snow samples compared to their seeding sources implies that post-depositional selection processes might occur in the snow to form a snow-adapted microbial community.

Introduction

In Chapter 2, we have seen that snow microbial communities are highly variable during spring in correlation with changes of environmental conditions but are specific to the snow habitat compared to various other environments. This chapter, at a smaller time and spatial scale, focuses on microbial community distribution in the different layers of sea ice snow cover, as compared to seeding sources of surrounding atmosphere and underlying sea ice.

Snow interacts with all compartments of the biosphere: oceans, soils, ice and atmosphere, and strongly influences their abiotic and biotic processes [1,2]. For example, snowpack as a photochemical bioreactor is involved numerous gas exchanges with the atmosphere and plays a key role in the fate of volatile organic contaminants within cold regions [3]. Microorganisms are transported via atmospheric fluxes, sometimes over long ranges and are deposited during snowfall and dry deposition events. On the other hand, snow over sea ice can also be wetted by sea ice brine that is more highly concentrated in microbes [4]. Wet and dry atmospheric deposition and capillary fluxes of sea ice brine also modify on the microscale, which may in turn impact microbial functioning. However, the selection processes and the responses of microbial communities to rapid changes in environmental conditions induced by seeding events remain unclear. Here, using a sea ice snowpack as a model system, we address how the snow microbial communities are influenced by their different seeding sources of atmosphere and sea ice, and identify the specific functions involved in response to the changes in abiotic characteristics of the snowpack. We hypothesized that the surface snow will be dominated by photochemical stress-related functions such as UV radiation and oxidative stress responses, whereas basal saline snow layer will be largely influenced by brine content, with colonization by microorganisms of marine origin with a functional response to high salinity.

Methods

Site description and sampling procedure

Samples (ATM,SL0, SL1, SL2, SL3, BR, SW in triplicates) were collected in March 2014 from a sea ice covered fjord, Kobbefjord in the vicinity of Nuuk, Greenland (64°07'N / 51°21'W) with no close source of human activity. The climatic conditions were similar during the four hour sampling: no wind, clear sky and air temperature of - 15°C. Due to logistical issues, air sampling was performed on the adjacent coast using a 0.1 µm filter mounted on a swinnex connected to a remote downstream vacuum pump for the duration of sampling on the fjord. A 10 m^2 pristine snowfield with a homogeneous snow cover of 30 cm was selected for triplicate snow pit sampling. A vertical gradient of seawater, sea ice and snow was sampled under the conditions illustrated in Figure 1. Four layers of the overlying snowpack were identified by visual structure and sampled in 3L sterile bags: a thin hard top layer in direct contact with the atmosphere (SL0), a basal saline snow layer wetted by an ascending brine flow (SL3), and two intermediate layers (SL1, SL2). The snow-cleared sea ice was then drilled with a corer to form a 25 cm deep sack hole and left to fill with brine for one hour. 1 L of brine was recovered using a rinsed 1 L syringe and stored in a rinsed Nalgene pot. An adjacent hole was cored through the entire 60 cm ice cover to insert tubing, and the underlying seawater was pumped manually and stored in a rinsed 20 L carboy. Temperature and salinity were measured for each horizon using an electronic thermometer and a portable refractometer, respectively. Samples were then transported directly to the laboratory and processed at the Greenlang Center for Climate Research in Nuuk. Snow samples were melted at room temperature under constant agitation, to avoid warming as much as possible, and filtered immediately after melting. Melted snow from the four layers, seawater and brine, all in triplicate, were then filtered using 0.1 µm filters and a filtration unit connected to a vacuum pump. Filters were then stored at -20°C in an RNA-stable storage reagent and shipped to the laboratory in Lyon for further processing. Small fractions of liquid

samples were fixed with formaldehyde and shipped to University of Washington for cell microscopic enumeration using DAPI staining.

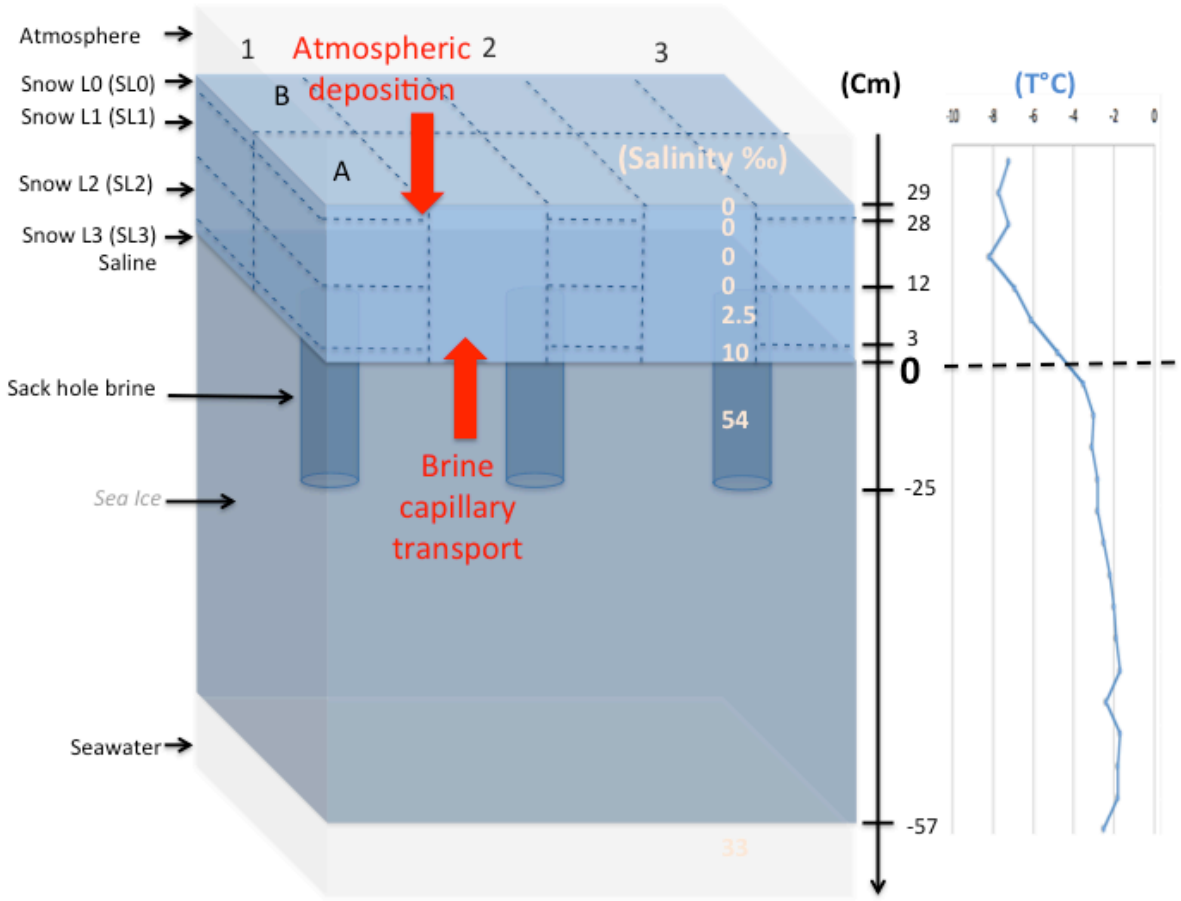

Figure 1: Sampling procedure: Samples were taken within a vertical gradient from the atmosphere to seawater (Atmopshere, four layers of snow, sea ice brine taken from a sack hole and seawater). Salinity (bulk for snow) and temperature were measured**.**

Molecular biology procedure

Filters and cells possibly detached from the filters were cleaned of RNA later reagent by three successive rinsing/centrifugation steps using a phosphate buffer solution (PBS). Total nucleic acids were then extracted using the Power Water RNA isolation kit (Machery-Nagel), following manufacturer instructions, except that the DNAse treatment step was omitted. Libraries of total genomic DNA were prepared using Nextera XT sampling preparation kit (Illumina) following the manufacturer's instructions, except that we increased the number of PCR amplifications to 13 cycles. Libraries were sequenced on a Miseq sequencer with 2x250 bp chemistry.

Bioinformatic analyses

Raw sequencing data were filtered using the base quality upon read length using the fastX toolkit. Due to high variability in sequenced fragment size leading to low quality pairing, forward and reverse reads were not pared and used as technical duplicates. Quality checked reads were then aligned against NR (Non-Redondant) protein reference database using Diamond BlastX algorithm. BlastX outputs were filtered to an e-value threshold of 10e-5. Taxonomic composition was analyzed with MEGAN 5, as well as functional annotations using SEED hierarchical subsystems. For specific functions, not indexed in the SEED database, such as melanin, scytonemin and mycosporine-like amino acids (MAAs) biosynthesis, metagenomes were aligned against specific databases created from the NCBI protein Database and retrieved using GERMLAB scripts. Best alignments with e-value < 10e -15 were selected. Abundance of reads matching against query databases, taxonomy, SEED or specific databases, were normalized by total abundance of annotated reads and statistical analysis was carried out using STAMP and R-packages as described for multivariate metagenomic analyses [5].

Results

Chemical and physical characterization

Temperature and salinity measurements are shown in Figure 1. Atmospheric temperatures, recorded at about -8°C, were similar to the temperature of the first 15 cm of the surface snow and then progressively increased to reach - 4°C at the snow-ice interface. Interior ice temperatures slowly increased from - 4°C at snow ice interface to - 2°C at bottom ice. Seawater salinity was 33 ppt whereas that for the sea ice brine was 54 ppt. Snow bulk salinity was 10 ppt in the basal saline snow layer, 2.5 ppt in the lower middle layer and below detection limit in the two uppers layers.

Microbial abundance by microscopic count

Microbial abundance varied among the horizons of the vertical gradient studied (Figure S1). The three most superficial snow layers (SL0, SL1 and SL2) had a microbial abundance of about 3.10^3 cells ml^{-1} of melted snow. Microbial abundance in the basal saline snow layer covering sea ice was a hundred times higher than the freshwater snow and thus more similar, though lower than those in sea ice brine and seawater, which were on the order of 10^5 cells ml^{-1}. Due to logistical issues, no bacterial counts were available for atmospheric samples.

Microbial community composition

Clustering of samples based on relative abundance of taxa retrieved (Figure 2A) shows three major groups of samples: seawater and brine, snow samples and atmosphere. Snow samples exhibited a more heterogeneous distribution of taxa within replicates as compared to brine and seawater. The twenty most detected taxa (Figure 2B) represented more than 70% of sequences taxonomically assigned for all datasets. Atmospheric samples were dominated by *Rhizobiales* (26 to 54% of sequences), mostly associated to *Bradirhizobium*. The relative abundance of *Rhyzobiales* was lower in snow and decreased with snow depth, with~17% in SL0 and SL1,~8% in SL2 and less than 2% in SL3. Moreover, taxonomic annotation in the *Rhizobiales* group showed a higher diversity with snow

depth, with increased relative abundance of *Phylabcatreiaceae* and *Methylobacteriaceae*. Superficial snow layers SL0, SL1 and SL2 were dominated by *Saccharomyceta*, *Actinomycetales, Alteromonadales, Acidobacteriales* and *Nostocales* including taxa statistically more abundant, compared to atmosphere, saline snow, brine and seawater, such as *Cytophagales, Ktedonobacterales, Leotiomyceta, Bacillales* and *Acidobacteriales*. The approximation of cell numbers for each taxon (based on proportion of reads and total prokaryotic cells) suggests that the number of cells belonging to these taxa migt be increased in the snow (Table S1). Although grouping together with the other snow samples, SL3 exhibited a unique taxonomic composition, highly dominated by *Alteromonadales* (71 to 78% of sequences), mostly associated with the *Glaciecola* genera. Taxa dominating in brine and seawater, such as *Rhodobacter* (about 26%), unclassified *Beta-Proteobacteria* (about 18%) and unclassified *Gamma/Alpha-Proteobacteria* (12 - 16%), *Thaumarchaeota* (8%), *Euryarcheota* (7%), had a lower relative abundance in the saline snow layer and upper snow layers. At a deeper taxonomic classification level, taxa detected in the different seeding sources of the atmosphere and brine/seawater were not detected in snow samples (such as *Rickettsiales, Longamoeba and Dermacoccaceae* for atmosphere and *Nitrosomonades, Nitrospinales* and unclassified *Flavobacteria* and *Planctomyctes* for brine/seawater and *Prochlorococcus* for atmosphere and seawater).

A high proportion of sequences were associated with viruses in seawater (about 2.5%) and in sea ice brine (about 5%) but were scarcely detected in snow samples. In both seawater and sea ice brine, viral sequences corresponded to Caudovirales, mostly unclassified Myoviridae, Podoviridae and Siphoviridae, but a high proportion of N4likevirus was observed only in sea ice brine.

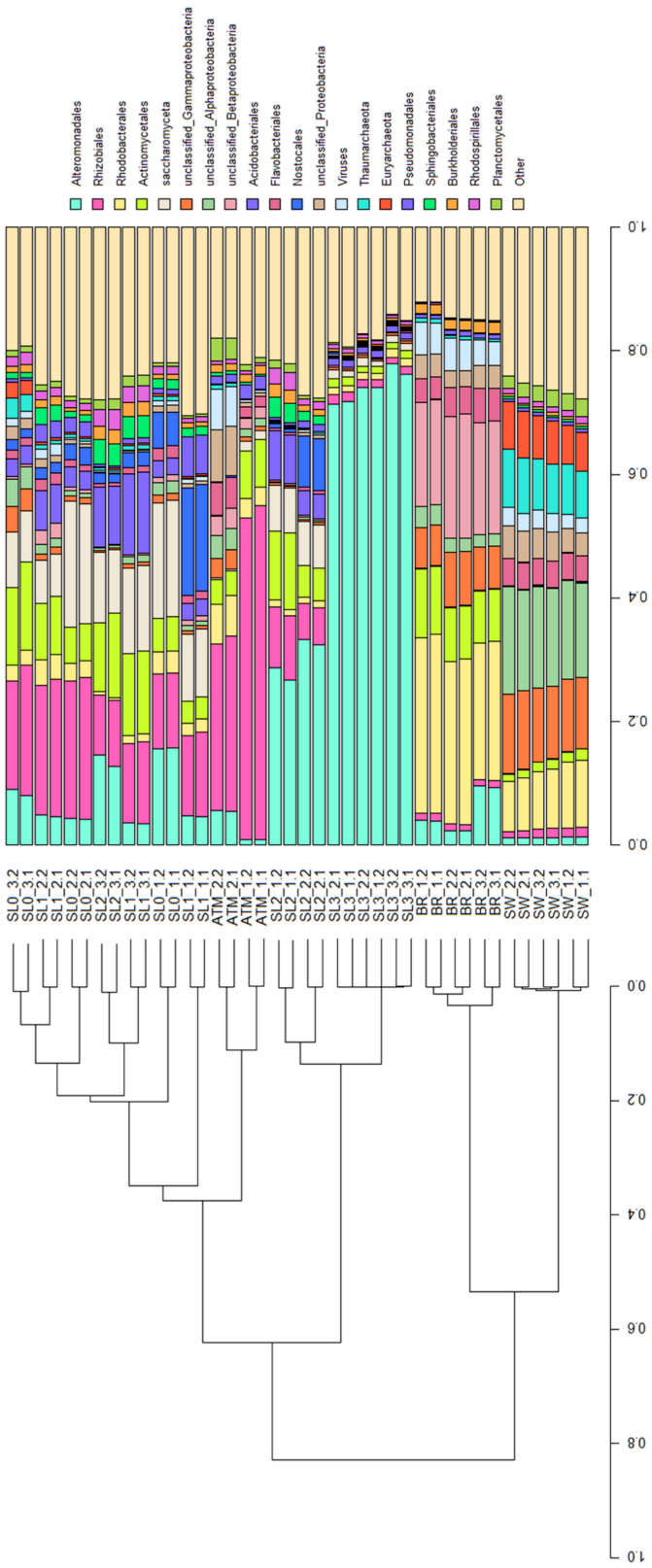

Figure 2: Taxonomical composition of Nuuk sea ice vertical profile. A: Hierarchical cluster using average clustering method and correlation as distance measure and 1000 bootstrap resampling based on relative abundance of all taxa detected. Distance between clusters are indicated by height bar. **B:** Barplot of relative abundance of 20 most abundant taxa (class or order) classed in decreasing order of abundance.

Functional assignments

Based on the principal component analysis (PCA) of the relative abundance (Level 3 of 4 of SEED classification) of the ten most variable functional groups (Figure 3), replicate samples grouped together for the different sample horizons. Similarly to taxonomical composition, functional distributions were more heterogeneous in superficial snow layers SL1, SL0 and the atmosphere. Brine and seawater were discriminated by functions associated with tricarboxylic acid cycle (TCA cycle) and the anabolic pathway for the serine-glyoxylate cycle, as well as Universal GTPases and Glutamine/ate and Asparagine/ate biosynthesis. Functions associated with flagellum biosynthesis and TonB dependent receptors were discriminant in the saline snow layer (SL3) clustering, with significantly higher proportion of reads in this layer. Superficial snow samples were grouped based on the relative abundance of Respiratory complex I, mycolic acid synthesis, metals resistance and Type4 secretion and conjugative transfer genes. Functions with higher relative abundance in superficial snow (Table S2) also included alternative metabolic pathways, such as maltose/maltodextrin and xylose utilization, glycogen metabolism, autotrophic lifestyle (CO_2 upatke, carboxysome, chlorophyll biosynthesis) and oxidative stress response. Genes encoding response to photochemical and osmotic stresses were also investigated (Table S3). The relative abundance of genes related to photochemical stress and other oxidative stress responses are presented in Figure 4. Enzymatic antioxidative mechanisms, including various enzymes such as catalase, superoxide dismutase/reductase, rubrerythrin, catalase, and glutaredoxin were significanty more abundant in superficial snow layers (SL0, SL1 and SL2) as compared to others horizons, especially in SL0 and SL1 where they represented up to 2% of total annotated reads. Same trend is observed for non-enzymatic response 1 and 2, including carotenoids, glutathione and tocopherol biosynthesis, scytonemin, melanin, mycosporine like amino acid (MAA) biosynthesis respectively. No genes coding for polyol mannitol (mdpa) and mycothiol niosynthesis were detected in any dataset. Alternative pathways, referring to the use of oxidant resistant isoenzymes or electron transfer pathways that help to remove excess electrons, were detected in similar relative abundances in all samples,

although with a higher variability in top surface snow (SL0). However, the ratio of the oxidant resistant isoenzymes fumarase CII and Aconitase A compared to sensitive forms was higher in superficial snow as compared to other horizons (Figure S2). DNA repair mechanisms are also well represented in all datasets (between 2 and 3%), with the highest relative abundance generally found in SL2, but otherwise relative abundance is higher in seawater and brine than in snow. However, at a deeper level of functional classification, DNA repair based on base excision mechanisms is statistically more abundant in superficial snow.

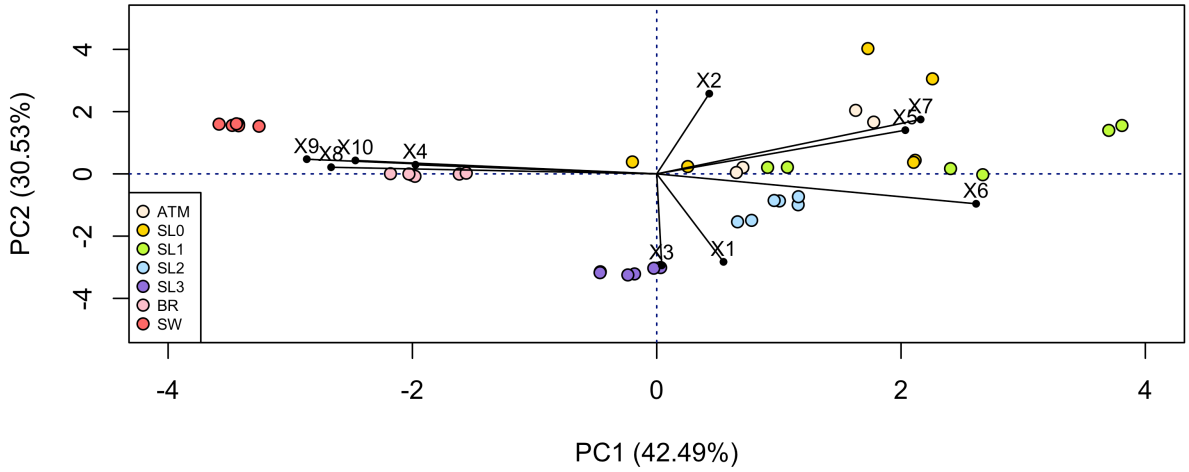

Figure 3: Functional variability within gradient. Principal component analysis (PCA) based on functional abundance of the ten most variable functions. Data were normalized by total abundance of annotated reads or by rRNA copies genes detected. Functions, in decreasing order of variability: X1 = TonB dependent receptor, X2 = Respiratory Complex I, X3 = flagellum, X4 = serine glyoxylate cycle, X5 = mycolic acid synthesis, X6 = cobalt zinc cadmium resistance, X7 = type4 secretion and conjugative transfer, X8 = universal GTPases, X9 = TCA Cycle and X10 = glutamine glutamate aspartate and asparagine biosynthesis.

On the other hand, osmotic stress response mechanisms were also detected with variable relative abundances in the different horizons (Figure 5). The general functional group for osmotic stress response mechanisms represents a larger number of sequences in saline horizons SL3, BR and SW as compared to freshwater horizons SL2, 1 and 0 (Figure 5A). However, the types of mechanisms involved in osmotic stress response within saline horizons differ (Figure 5B). Indeed, sarcosine metabolism was the predominant osmotic stress response in brine and seawater and relatively low in snow, which was mostly dominated by choline transport and catabolism. Less abundant ectoine biosynthesis genes were better represented in brine and saline snow compared to seawater.

Discussion

While the snowpack is increasingly recognized as a suitable habitat sustaining a taxonomically and functionally diverse microbial community [6], little is known about community function within the snow matrix, especially how microorganisms are selected after deposition. Here we addressed how the snow microbial communities are influenced by their different seeding sources and identified specific functions involved in response to snowpack habitat characteristics. For this purpose, we focused on a sea ice snow cover model to examine the influence from atmospheric deposition and from sea ice brine capillarity input.

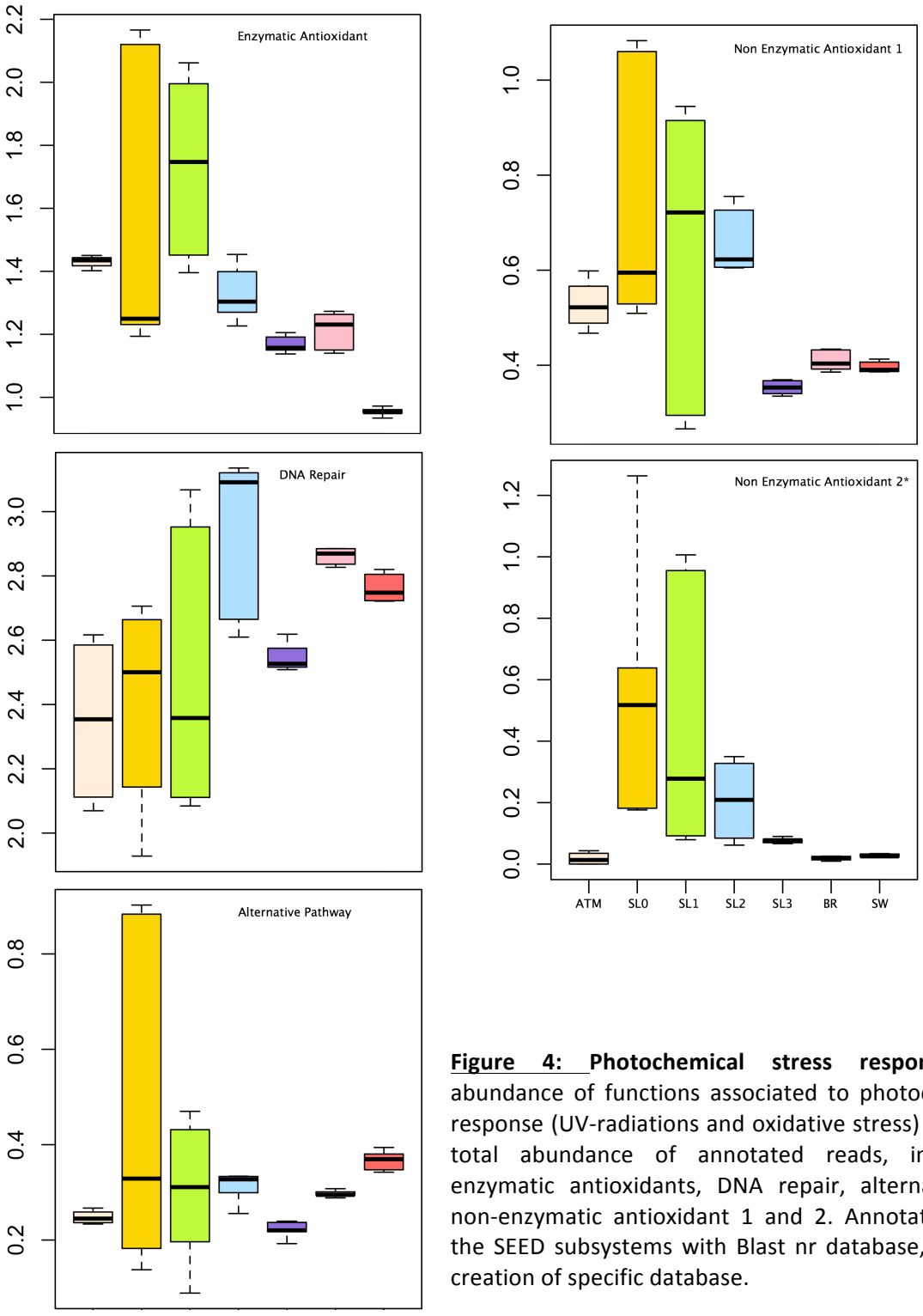

Figure 4: Photochemical stress responses: Relative abundance of functions associated to photochemical stress response (UV-radiations and oxidative stress) (normalized by total abundance of annotated reads, in percentage): enzymatic antioxidants, DNA repair, alternative pathway, non-enzymatic antioxidant 1 and 2. Annotations according the SEED subsystems with Blast nr database, * indicate the creation of specific database.

Seeding and post-depositional selective loss and gain of taxa in the snowpack

Microbial attributes, which correspond to database-associated metadata of isolated and sequenced strains (physiological characteristics such as shape, temperature growth range, arrangements and characteristics of isolation habitat) were analysed along with taxonomical annotations to determine potential sources of microorganisms in the vertical gradient sampled. Whereas seawater and sea ice datasets were clearly dominated by sequences from microorganisms originally isolated in marine environments, snow and atmosphere showed a similar proportion of sequences of potential terrestrial and marine origin. As recently reviewed, the atmosphere contains microorganisms in variable abundance, from 10^3 to 10^7 cells m^{-3}, with high taxonomic variability depending on season, elevation, temperature and meteorological events, among other factors [7]. In the present study, atmospheric samples were dominated by *Rhizobiales* (mostly the genud *Bradyrhizobium*). While atmospheric *Bradyrhizobium* is generally considered as plant associated of terrestrial origin [7], this taxon is still well represented in deeper snow layers, sea ice brine and seawater. Some members of this taxa, such as *Afipia,* which can grow with dimethyl sulphide as its sole carbon source of carbon, are commonly found in aquatic environments and have also been shown to represent up to 50% of the marine atmosphere microbial community [8,9]. *Rhizobiales* were also detected with high predominance in young arctic sea ice frost flowers [10]. Further functional characterization revealed that these Rhizobiales representatives were distinct from root-nodulating soil bacteria, given their lack of symbiosis plasmid but occurrence of genes coding metabolism of algal exudates, suggesting close association with aquatic phyotoplankton [11].

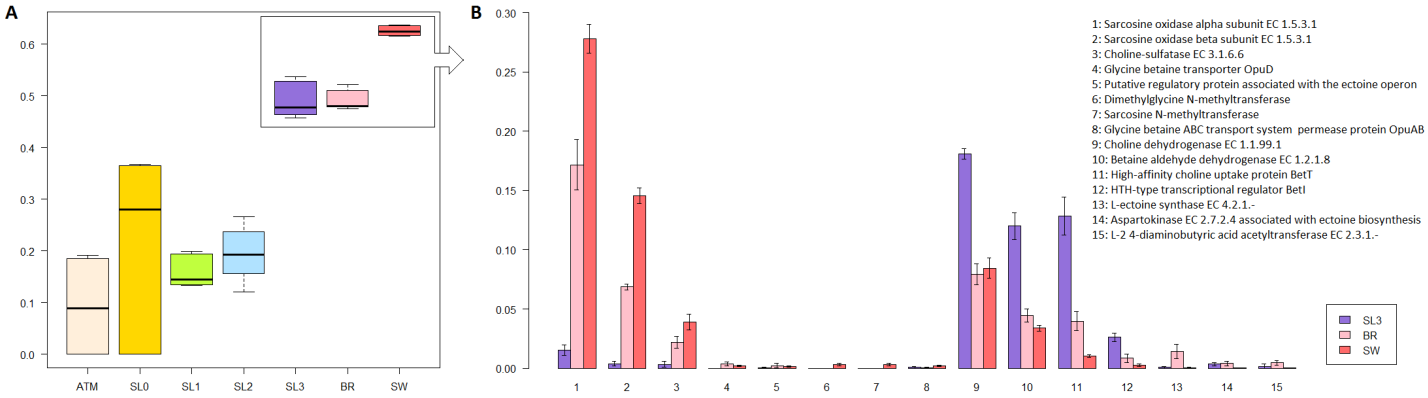

Figure 5: Osmotic stress responses. A: Relative abundance of functions associated with osmotic stress response (normalized to total abundance of annotated reads, as percentage). **B:** Relative abundance of specific osmotic stress responses within saline samples (SL3,BR and SW) (normalized to total abundance of annotated reads, in percentage).

In our datasets the community composition of *Rhizobiales* varied between the atmosphere and snow layers: In sow layers its diversity increased, as well as proportion of free-living soil and water organisms belonging to *Phyllobacteriaceaea, Methylobacteriaceae* and *Hyphomicrobiaceae.* Moreover, despite a significant drop compared to atmosphere, *Rhizobiales* associated sequences were still high in superficial snow layer and decreased with snow depth. The origin and fate of *Rhizobiales* within snowpack (as well for frost flowers) remain unclear but altogether these findings suggests that these bacteria may have occasionally a role in the ecology of sea ice cover. However, if aerially transported microorganisms are able to adapt to the snow environment, their abundance would increase relative to the atmosphere and contribute to establishing a snow microbial community within the snow habitat. A high number of sequences related to the *Alternomondales, Actinomycetales, Saccharomyceta, Acidobacteriales, Nostocales* and *Sphingobacteriales* groups was also observed in snow layers SL0, SL1 and SL2. They are statistically more abundant in superficial snow as compared to all other horizons, along with less abundant groups such as *Cyotphagales, Bacillales, Ktedonobacter*. The *Sphingobacteriales* were mostly dominated by *Chitinophagaceae* and *Segetibacter*, the most represented genus in our snow samples, has previously been isolated from

both the soil and atmosphere [12]. *Hymenobacter* and *Spirosoma,* previously isolated from permafrost and glacier habitats [12], were the major representatives in the *Cytophagales* fraction of our snow metagenomes and increased in snow compared to atmosphere. All of these organisms that likely originated from terrestrial sources and were transported by atmosphere fluxes could then potentially colonize snow. Cyanobacteria, presumably originating from sea spray, are often retrieved in snow samples from snowpacks over soil or sea ice [13]. The high abundance and increase proportions of cyanobacteria from the *Nostocales* group in our snow datasets support the hypothesis of the possible establishment of a prokaryotic photoautotrophic community within the snowpack. Based on our data and that of another study on microbial communities in late spring Greenland sea ice snow [14], cyanobacteria were most abundant in the middle layer, which suggests that they could develop at a depth corresponding to optimum light level. Distribution of cyanobacteria with snow depth has also been correlated to nitrogen availability in Arctic terrestrial spring snow [15].

Fungal spores can contribute significantly to the biomass and total carbon content of the atmosphere, cloud rain and snow precipitation [16]. Even less is known about fungal snow communities than bacterial communities [17]. However sequences annotated to fungal organisms belonging to *Ascomycota* or *Basidomycota* have been detected in datasets from terrestrial and sea ice snow, where they can, at times, represent over 70 % of the sequences (ribosomal gene marker or total shotgun sequences) [18,19]. Fungi are often retrieved from cold polar environments [20], including ice where they have been show to harbor numerous adaptations allowing them to develop in icy environments [21]. So the increase in our datasets of *Saccharomyceta* as compared to its atmospheric source supports the hypothesis that fungi could also be active members in the snowpack microbial community.

Once deposited on the snow through either wet or dry deposition, some passively transported microorganisms might not be able to adapt to the snow habitat and would not be maintained. This may be the case for members of numerous taxa such *Longameoba, Dermacoccaceae, Rickettsiales*

and *Prochlorococcus* whose relative abundance decreased between the atmosphere and the snow. For instance, *Prochlorococcus*, ultra-small cyanobacteria that are major contributors to biomass in the euphotic zone of temperate oceans, are rarely detected in polar zones [22]. In our datasets, this taxon was detected in low abundance but similar in atmosphere, seawater and sea ice brine, suggesting that members of this taxon could have been aerosolized in fjord air, but might not be able to develop in snow.

Air microbiota seeding snow via atmospheric deposition could then originate from mixed local and remote, terrestrial and marine sources, as it was already suggested for the High Canadian Arctic [17]. However, freshly fallen snow (wet deposition) is not truly representative of microorganisms residing in the atmosphere, as community composition can differ due to variable ice nucleation abilities in air microbiota leading to preferential precipitation [23]. Dry deposition could also lead to microbial input into snow and potential sources of snow colonizers may include long-range transported atmospheric dusts as has been demonstrated for non polar glaciers and remote pristine Antarctica stations [24,25] .

A high number of sequences related to the *Altermonodales* family were also observed in all samples, with an especially high proportion in the basal saline snow layer (SL3). The majority of sequences were associated to the genera *Glaciecola*, whose members have been isolated from

marine environments including both temperate and polar oceans, sea ice and sediments [12]. Isolated *Glaciecola* include numerous psychrophilic and halophilic representatives [26]. Sequences affiliated to the *Glaciecola* genus were also detected in high proportion in a Canadian high Arctic snowpack; the authors suggested regional marine aerosols as a source [17], which could also be the case for the superficial snow layers in our study. Sea ice brine injection through the basal saline layer constitutes another major input of microbes to the snow [4,13], as illustrated by the increase in microbial abundance in the basal snow layer as compared to more superficial ones (Figure S1). Organisms affiliated to *Glaciecola* in the basal saline snow layer may have also increased due to

injection through brine. As true extremophiles, they would present a selective advantage to resist to the harsh conditions imposed by a shift in habitat and would dominate the saline snow community.

Other than this predominant group, microorganisms belonging to common marine taxa such as *Oceanospirillales* were also detected with an increased abundance in the saline snow layer compared to brine and seawater, and at a bigger extent to the other snow samples. The Oceanospirillaceae family contains mostly halophilic genera retrieved from a wide of marine habitat [27], including arctic sea ice [28]. To our knowledge, our results provide the first evidence that taxa belonging to *Oceanospirillales* (mostly *Marinomonas* and *Neptunomonas*) are present in snow and suggest that they might be able to sustain within snow. In contrast, a drop in relative abundance of reads for unclassified marine *Beta-* and *Gammaproteabcteria* and *Rhodobacterales* groups was observed in snow compared to brine and seawater. Sequences associated with *Rhodobacterales* were dominated by *Planktomarina* in seawater samples whereas those in sea ice brine as well as in all snow samples were dominated by *Octadecabacter*, but the relative abundance of *Rhodobacterales* decreased dramatically between sea ice brine and snow (25% and lower than 5% of all annotated sequences respectively). The *Octadecabacter* genus, with known psychrophilic representatives, has been described in arctic and antarctic sea ice [29,30] and can be some times among the most dominant taxa in arctic sea ice [31]. Although many of the characteristic features found in the *Octadecabacter* genomes may represent adaptations to polar habitats [32], they may present more selective advantage in sea ice environments than in the snow.

SAR11 *Alpha-protebacteria* and *Archaea* belonging to *Thaumarcheaota* and *Euryarchaeaota* retrieved from polar oceans and sea ice display a dynamic distribution through depth in the water column and a strong seasonality [33,34]. In our dataset the relative abundance of reads associated with these taxa, low in sea ice brine and high in seawater, is consistent with assemblages described for early spring sea ice covered seawater. The proportions observed in snow are as low as those in sea ice, suggesting that they are not suitable snow or sea ice colonizers. These results are consistent

with a selection of snow-adapted microorganisms after seeding by brine wetting, although selective retention of microorganisms in sea ice might also occur [4] due to surface adhesion by bacterial coating [35,36].

Viral sequences are detected in higher relative abundance in sea ice brine compared to seawater, supporting the hypothesis that viral-host interactions are a major feature of sea ice microbial ecology [37,38]. However, very few sequences associated with virus are detected in snow, even in saline snow layer, so these types of interactions might be limited in snow.

The snow microbial community we examined appeared to be largely influenced by its seeding sources, the atmosphere above (with local and remote sources from marine and terrestrial origins) and to a lesser extent the sea ice brine from bottom up for saline snow. Selection processes may then occur in snow and lead to the formation of a snow specific community: microorganisms with increased abundance in our snow samples are similar to those identified as active in an antarctic snowpack (using 16s rRNA transcript analysis) and observed in other icy environments. This similarity further supports the establishment of active residents in the snowpack. To determine what drives this selection, we focused on microbial community functional responses to different conditions that are characteristic of the snow over sea ice model.

Factors driving microbial adaptation in snowpacks over sea ice

Salinity and osmotic stress response

Although the microbial community composition we detected reflects a snow-specific assemblage that differed from marine horizons, seawater and sea ice brine, snow microorganisms may also have been impacted by salinity due to the high salt concentrations delivered by the brine. The basal snow layer (SL3), in particular, exhibited higher bulk salinity than the overlying snow layers, which may have induced salinity stress in its microbial inhabitants. We observed a significant rise in the proportion of reads associated with

osmotic stress response in the saline snow layer as well as in seawater and sea ice brine as compared to superficial snow layers and atmosphere. The types of mechanisms involved in osmotic stress response associated with compatible solute metabolism varied among the saline snow layer, brine and seawater. Compatible solutes are small molecules that accumulate in the cytoplasm and act as osmolytes to help the cells cope with osmotic pressure, such as ectoine and glycine betaine [39]. Glycine betaine can accumulate via external uptake in a wide range of halotolerant and halophilic prokaryotes (both *Bacteria* and *Archaea*) using two types of transporters [40]. Genes encoding these transporters were detected in low abundance in seawater and sea ice brine but not in saline snow. Conversely, the proportion of reads annotated as genes encoding glycine betaine biosynthesis via choline uptake and oxidation (choline uptake protein BetT, transcriptional regulator BetI, choline dehydrogenase and betaine aldehyde dehydogrenase [41]) was significantly higher in saline snow. Successive methylation of glycine leading to the de novo production of glycine betaine involving sarcosine and dimethylglycine N-methyltransferase are described in only a few organisms [42] and were only detected in seawater samples in our datasets. Sea ice brine and seawater also showed a high abundance of reads affiliated to genes involves in catabolism of glycine betaine to glycine via sarcosine oxidase (alpha and beta subunits). Sarcosine oxidase was identified as originating from a horizontal gene transfer event in *Colwellia psychrerythraea* 34H in which the recycling of the osmoprotectant glycine betaine as a nutrient source could serve as a selective advantage in the sea ice environment [43]. Ectoine, considered as the major osmolyte in aerobic chemoheterotrophic bacteria, is produced by a wide range of bacteria, but not by eukaryotes [39] and was only detected in our saline snow and brine datasets.

Other than compatible solute accumulation, we also detected reads affiliated to Na+/H+ antiporters, with a higher proportion in saline snow as compared to sea ice brine and seawater, but also (although with more variability) in the upper snow layer and atmosphere. Antiporters catalyze the active efflux of Na+ and/or K+ in exchange for H+ protons from outside the cells and thus support key physiological functions, such as cytoplasmic pH homoeostasis, tolerance to alkalinity and fluctuations in osmolarity and resistance to toxic levels of Na+ in a wide range of organisms [44]. Antiporters might also be involved in tolerance of osmotic pressure imposed on microorganisms in the saline snow layer. The occurrence of reads associated wih antiporters in the upper snow might also be related to variations in pH that have been shown to be a driver of microbial community structure in snow [15].

Osmotic stress linked to salinity exposure or low water availability imposed to microorganisms in the snowpack has not yet been evaluated clearly. The exact location of microbes and the size and configuration of their microscale habitat in the thin water film is largely unknown, but they are likely exposed to high osmotic pressure. Moreover, previous studies focusing on physical characteristics of snow over land-fast first year sea ice indicated that whereas bulk salinity is low at the surface snow, brine salinity could decrease with snow depth and with the increase of the brine volume fraction [4,45].

Overall, microbial responses to osmotic stress involving compatible solute accumulation were predominant in the saline snow layer (SL3), mostly involving glyce betaine biosynthesis, but also ectoine biosynthesis and Na+/H+ antiporters, which differs from the strategies observed in seawater and sea ice brine microbial communities.

Photochemical stress response:

Snow, highly exposed and reactive to UV light, is defined as a photochemical bioreactor [46] [47], as described in Chapter 1. Inter-environmental comparisons have led to the hypothesis that photochemical stress could be a major driver of microbial community structure in snowpacks [19]. Here we investigated further adaptations that could help microbes cope with photochemical stresses associated with snowpack characteristics (direct UV radiation and resulting oxidative stress conditions), leading to the selection of an adapted microbial community.

Penetration of UV-light through snowpack has been shown to be highly variable between locations, snow grain size and dust concentration [48]. In our study UV extinction in relation with snow and sea ice depth was not evaluated, but genetic adaptations involved in response to photochemical stresses were detected in metagenomes from each of the horizons of the gradient, though in variable relative abundance depending both on depth and the mechanism observed. UV radiation is deleterious for cell physiology. UV-B is generally involved in direct damage to absorbing biomolecules (such as DNA) by forming pyrimidine dimers, whereas UV-A has indirect effects by producing intracellular reactive oxidative species that cause oxidative damage to lipids, DNA and proteins [49].

Non-enzymatic antioxidant and sunscreen biosynthesis, including carotenoids, tocopherol, glutathione, melanin and mycosporine like amino acids (MAA), were detected in higher abundance in the upper snow layers (SL0, SL1, SL2). MAA are small UV-absorbing molecules and were observed in several sea ice samples exposed to UV radiation [50,51], but to our knowledge they have not been isolated from snow. Accumulation of MAA might be involved in many biological processes, including omostic regulation and defense against oxidative stress [52]. Similarly tocopherol, produced by a wide range of oxygenic phototrophs and

providing (among others) protection against lipid peroxidation [53], has been identified in snow algae [54]. While MAA are widespread among prokaryotic and eukaryotic organisms, scytonemin is a UV-absorbing compound limited to *Cyanobacteria* [52], and its detection is linked to the high proportion of *Cyanobacteria* in superficial snow layers. Carotenoids such as astaxanthin act as detoxifiers of ROS and as UV-screens in various algae [55], including the snow algae *Chlamydomonas nivalis* and *Chloromonas polyptera* [54,56–59].

Other compounds to protect the cell against deleterious effects of photochemical stresses have also been identified in non-photosynthetic organisms. For example, the polyol mannitol has been shown to scavenge ROS in vitro and protect fungal cells from oxidative stress in vivo [60]. Although this compound has been described mostly in pathogenic fungi during host interactions, similar mechanisms could also exist in free-living organisms. However, we detected no reads with similarity to mdpa genes involved in mannitol biosynthesis in any of our datasets. Conversely, genes involved in melanin fungal biosynthesis [61], UV-screening pigments described from fungi under extreme environmental conditions [62], were detected in higher proportion in superficial snow, which might be linked to the increase of representatives of the *Ascomyceta* taxon.

DNA repair mechanisms regroup various genes that encode different sets of enzymes that are widespread among all kingdoms [63]. Reads associated with this function are retrieved in similar abundance from all horizons of the sampling gradient. At a finer scale, base excision repair (involved in indirect ROS mediated damage to DNA [64]), endonuclease V and Exonuclease SbcB seemed to be more predominant in superficial snow, reflecting the variation in community composition in between snow and sea ice/seawater and implying the use of different enzymes for DNA repair.

Moreover, we investigated the distribution of genes coding for metabolic enzymes with known oxidative resistant form, such as fumarase and aconitase, both involved in the citrate cycle. fumarase C and aconitase A isoenzymes, more resistant are used as a backup during oxidative stress conditions [65,66]. Although the relative abundance of reads associated with these isoenzymes was similar in all samples, the ratio of these forms compared to their sensitive isoform counterparts is higher in superficial snow. This implies that oxidative resistant isoforms might be more widespread in snow in response to selection in favour of photochemical stress-adapted microbes.

Mechanisms involved in response to photochemical reactions were detected in low proportion in atmosphere compared to snow datasets. Microorganisms must be subjected to high light radiation in atmosphere and some microorganisms isolated from atmosphere present associated adaptations such as pigments. Cloud microflora exposed to UV light in microcosms also remained metabolically active in the presence of $^{\cdot}OH$ radicals [67]. However, the mean global atmospheric residence time of particles including microorganisms (fluctuating with particle size, origin and season) has been evaluated to 3.4 to 7.5 days [68], which might not compatible with selection processes time scale. Our data thus suggest that selection towards photochemical adapted microorganisms would happen in snow after deposition.

To summarize, microbial communities in the snowpack apeared to harbour various strategies to cope with photochemical stress, a major factor in surface snow as compared to underlying sea ice and seawater communities that benefit from snow of UV-radiation attenuation [69]. All together our results support the hypothesis that organisms adapt to the specific conditions of the sea ice snowpack after deposition, such as photochemical stress in surface snow and osmotic stress in the bottom layer.

Other features of microbial snow functional signature

Apart from these gradient effects from surface to deep saline snow, microbial communities also harbour other features that allow them to thrive in the snow. For example, superficial snow samples harbour a higher proportion of reads associated with metal (cobalt, zinc, cadmium) resistance. Snowpack can accumulate considerable amount of metals from natural and anthropogenic sources [70,71]. Mercury contamination has been shown to impact snow microbial communities and a potential assimilation pathway has been proposed for Arctic snowpacks [15,72]. Unlike mercury, cobalt, zinc and cadmium cannot be reduced, therefore membrane proteins that act as efflux pumps help cells cope with metal toxicity [73].

As illustrated by figures depicting microbial functions distribution in this study, variability within replicates is much higher in snow compared to other samples, which may reflect the high variability of snow microbial communities even at local scale. However high variaility could be also due to technical issue of annotation linked to lower abundance of sequences of superficial snow samples.

Snowpacks are considered to be low nutrient environments [17]. For instance total phosphorus concentration Baltic sea ice snow cover varied between 1.55 and 9.92 μgL^{-1} [74], which corresponds to oligotrophic state (based on Phosphure concentration as a proxy of trophic state) [75]. One carbon source might be inorganic carbon fixation and autotrophic energy production by primary producers, consistent with the increased abundance of carboxysome, CO_2 and chlorophyll biosynthesis in our datasets in early spring snow samples compared to seawater and sea ice brine. Reads related to maltose/maltodextrine and xylose utilization as well as glycogen metabolism were detected in higher abundance in snow. Microbial preferences for different carbon classes were studied in antarctic snow and results

showed a higher rate of carbon uptake when microcosms were amended with a combination of simple and complex carbon sources [76]. In the same study, snow isolates were capable of oxidizing a broad spectrum of low and high molecular weight carbon sources including amino acids, amines, amides, carboxylic acids, carbohydrates, and complex polymers. Altogether these results highlight the potential for high metabolic versatility of microorganisms in snow with low concentrations of many different carbon sources. Interestingly, genes associated to flagellum synthesis are detected in higher abundance in saline snow layer compared to all other samples. The importance of motility processes in snow towards nutrients or salt concentrations remains unclear and will be discuss in the following chapter.

Conclusion

The Greenland sea ice snow microbial community we studied was strongly influence by its seeding sources i) the atmosphere, with particles and organisms originating from different terrestrial or marine sources of local or remote origin ii) sea ice brine with a high microbial input to the bottom saline snow layer. From the distribution of certain organisms and genes we infer that post-depositional selection processes occur in the snow to form a snow-adapted microbial community. These selection processes involve important characteristics of snow over sea ice particularly photochemistry and chemical gradients leading to specific microbial adaptations to photochemical stress in surface snow microbial communities and osmotic stress in deeper snow layers. This study further supports the hypothesis that the snowpack is an ecosystem with microbial communities that harbour underestimated abilities.

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References

[1] Pomeroy JW, Brun E. Physical Properties of Snow. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW, editors. Snow Ecology: An interdisciplinary examination of snow-covered ecosyst., Cambridge University Press; 2001, p. 45–126.

[2] Schmidt SK, Lipson D. Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil 2004;259:1–7. doi:10.1023/B:PLSO.0000020933.32473.7e.

[3] Daly GL, Wania F. Simulating the influence of snow on the fate of organic compounds. Environ Sci Technol 2004;38:4176–86.

[4] Ewert M, Carpenter SD, Colangelo-Lillis J, Deming JW. Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. J Geophys Res Ocean 2013;118:726–35. doi:10.1002/jgrc.20055.

[5] Dinsdale E, Edwards R, Bailey B, Tuba I, Akhter S, McNair K, et al. Multivariate analysis of functional metagenomes. Front Genet 2013;4:41. doi:10.3389/fgene.2013.00041.

[6] Larose C, Dommergue A, Vogel T. The dynamic arctic snow pack: An unexplored environment for microbial diversity and activity. Biology (Basel) 2013;2:317–30. doi:10.3390/biology2010317.

[7] Gandolfi I, Bertolini V, Ambrosini R, Bestetti G, Franzetti A. Unravelling the bacterial diversity in the atmosphere. Appl Microbiol Biotechnol 2013;97:4727–36. doi:10.1007/s00253-013-4901-2.

[8] Moosvi SA, Pacheco CC, McDonald IR, De Marco P, Pearce DA, Kelly DP, et al. Isolation and properties of methanesulfonate-degrading *Afipia felis* from Antarctica and comparison with other strains of *A. felis*. Environ Microbiol 2005;7:22–33. doi:10.1111/j.1462-2920.2004.00661.x.

[9] DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, Beyersdorf AJ, et al. Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc Natl Acad Sci U S A 2013;110:2575–80. doi:10.1073/pnas.1212089110.

[10] Bowman JS, Larose C, Vogel TM, Deming JW. Selective occurrence of Rhizobiales in frost flowers on the surface of young sea ice near Barrow, Alaska and distribution in the polar marine rare biosphere. Environ Microbiol Rep 2013;5:575–82. doi:10.1111/1758-2229.12047.

[11] Bowman JS, Berthiaume CT, Armbrust EV, Deming JW. The genetic potential for key biogeochemical processes in Arctic frost flowers and young sea ice revealed by metagenomic analysis. FEMS Microbiol Ecol 2014;89:376–87. doi:10.1111/1574-6941.12331.

[12] Euzebi JP. List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 1997;47:590–2. doi:10.1099/00207713-47-2-590.

[13] Hauptmann AL, Stibal M, Bælum J, Sicheritz-Pontén T, Brunak S, Bowman JS, et al. Bacterial diversity in snow on North Pole ice floes. Extremophiles 2014. doi:10.1007/s00792-014-0660-y.

[14] Møller AK, Søborg DA, Al-soud WA, Sørensen SJ, Kroer N. Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. Polar Res 2013;1:1–11.

[15] Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. PLoS One 2013;8:e79972. doi:10.1371/journal.pone.0079972.

[16] Bauer H, Kasper-Giebl A, Lo M. The contribution of bacteria and fungal spores to the organic carbon content of cloud water , precipitation and aerosols. 2nd International Conference on Fog and Fog Collection. 2002;64:109–19.

[17] Harding T, Jungblut AD, Lovejoy C, Vincent WF. Microbes in high arctic snow and implications for the cold biosphere. Appl Environ Microbiol 2011;77:3234–43. doi:10.1128/AEM.02611-10.

[18] Bachy C, López-García P, Vereshchaka A, Moreira D. Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the north pole at the end of the polar night. Front Microbiol 2011;2:106. doi:10.3389/fmicb.2011.00106.

[19] Maccario L, Vogel TM, Larose C. Potential drivers of microbial community structure and function in Arctic spring snow. Front Microbiol 2014;5:413. doi:10.3389/fmicb.2014.00413.

[20] Leonardo MR. Review of polar microbiology: Life in a deep freeze. J Microbiol Biol Educ 2013;14:283–4. doi:10.1128/jmbe.v14i2.666.

[21] Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B, Plemenitaš a. Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Phys Chem Earth, Parts A/B/C 2003;28:1273–8. doi:10.1016/j.pce.2003.08.056.

[22] Bano N, Hollibaugh JT. Phylogenetic composition of bacterioplankton Assemblages from the Arctic Ocean. Appl Environ Microbiol 2002;68:505–18. doi:10.1128/AEM.68.2.505-518.2002.

[23] Bowers RM, Lauber CL, Wiedinmyer C, Hamady M, Hallar AG, Fall R, et al. Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl Environ Microbiol 2009;75:5121–30. doi:10.1128/AEM.00447-09.

[24] Chuvochina MS, Alekhina I, Normand P, Petit J-R, Bulat S. Three events of Saharan dust deposition on the Mont Blanc glacier associated with different snow-colonizing bacterial phylotypes. Microbiology 2011;80:125–31. doi:10.1134/S0026261711010061.

[25] Cowan DA, Chown SL, Convey P, Tuffin M, Hughes K, Pointing S, et al. Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends Microbiol 2011;19:540–8. doi:10.1016/j.tim.2011.07.008.

[26] Bowman JP, McCammon SA, Brown JL, McMeekin TA. *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea ice habitats. Int J Syst Bacteriol 1998;48:1213–22. doi:10.1099/00207713-48-4-1213.

[27] Satomi M, Fujii T. The Family Oceanospirillaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. Prokaryotes - Gammaproteobacteria, Berlin, Heidelberg: Springer Berlin Heidelberg; 2014, p. 491–527. doi:10.1007/978-3-642-38922-1.

[28] Brinkmeyer R, Knittel K, Jürgens J, Amann R, Helmke E, Ju J, et al. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice diversity. 2003. doi:10.1128/AEM.69.11.6610.

[29] Gosink J, Staley J. Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. Appl Envir Microbiol 1995;61:3486–9.

[30] Gosink JJ, Herwig RP, Staley JT. *Octadecabacter arcticus* gen. nov., sp. nov., and *O. antarcticus*, sp. nov., nonpigmented, psychrophilic gas vacuolate bacteria from polar sea ice and water. Syst Appl Microbiol 1997;20:356–65. doi:10.1016/S0723-2020(97)80003-3.

[31] Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol 2003;69:6610–9.

[32] Vollmers J, Voget S, Dietrich S, Gollnow K, Smits M, Meyer K, et al. Poles apart: Arctic and Antarctic *Octadecabacter* strains share high genome plasticity and a new type of xanthorhodopsin. PLoS One 2013;8:e63422. doi:10.1371/journal.pone.0063422.

[33] Alonso-Sáez L, Sánchez O, Gasol JM, Balagué V, Pedrós-Alio C. Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. Environ Microbiol 2008;10:2444–54. doi:10.1111/j.1462-2920.2008.01674.x.

[34] Collins RE, Rocap G, Deming JW. Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ Microbiol 2010;12:1828–41. doi:10.1111/j.1462-2920.2010.02179.x.

[35] Junge K, Eicken H, Deming JW. Wintertime sea ice bacterial activity at -2 to -20 ° C in Arctic Wintertime Sea Ice 2004;70. 2004 Appl Environ Microbiol. ; 70(1): 550–557. doi:10.1128/AEM.70.1.550.

[36] Ewert M, Deming JW. Selective retention in saline ice of extracellular polysaccharides produced by the cold-adapted marine bacterium *Colwellia psychrerythraea* strain 34H 2011;52:111–7.

[37] Wells LE, Deming JW. Modelled and measured dynamics of viruses in Arctic winter sea ice brines. Environ Microbiol 2006;8:1115–21. doi:10.1111/j.1462-2920.2005.00984.x.

[38] Deming JW. Sea Ice Bacteria and Viruses. In: Thomas DN, Dieckmann GS, editors. sea ice an introduction to its Physics, Chemistry, Biology, Oxford, UK: Wiley-Blackwell; 2009, p. 247–82. doi:10.1002/9781444317145.

[39] Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems 2005;1:5. doi:10.1186/1746-1448-1-5.

[40] Ziegler C, Bremer E, Krämer R. The BCCT family of carriers: from physiology to crystal structure. Mol Microbiol 2010;78:13–34. doi:10.1111/j.1365-2958.2010.07332.x.

[41] Andresen PA, Kaasen I, Styrvold OB, Boulnois G, Strøm AR. Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. J Gen Microbiol 1988;134:1737–46.

[42] Nyyssola A, Kerovuo J, Kaukinen P, von Weymarn N, Reinikainen T. Extreme halophiles synthesize betaine from glycine by methylation. J Biol Chem 2000;275:22196–201. doi:10.1074/jbc.M910111199.

[43] Collins RE, Deming JW. An inter-order horizontal gene transfer event enables the catabolism of compatible solutes by *Colwellia psychrerythraea* 34H. Extremophiles 2013;17:601–10. doi:10.1007/s00792-013-0543-7.

[44] Krulwich TA, Hicks DB, Ito M. Cation/proton antiporter complements of bacteria: why so large and diverse? Mol Microbiol 2009;74:257–60. doi:10.1111/j.1365-2958.2009.06842.x.

[45] Langlois A, Mundy CJ, Barber DG. On the winter evolution of snow thermophysical properties over land-fast first-year sea ice 2007;716:705–16. doi:10.1002/hyp.

[46] Grannas AM, Jones AE, Dibb J, Ammann M, Anastasio C, Beine HJ, et al. An overview of snow photochemistry: evidence, mechanisms and impacts. 2007.

Atmos. Chem. Phys., 7, 4329-4373, 2007

[47] Domine F, Albert M, Huthwelker T, Jacobi H-W, Kokhanovsky AA, Lehning M, et al. Snow physics as relevant to snow photochemistry. Atmos Chem Phys 2008;8:171–208. doi:10.5194/acp-8-171-2008.

[48] King MD, Simpson WR. Extinction of UV radiation in Arctic snow at Alert, Canada (82°N). J Geophys Res 2001;106:12499. doi:10.1029/2001JD900006.

[49] Moan J, Peak MJ. Effects of UV radiation on cells. J Photochem Photobiol B Biol 1989;4:21–34. doi:10.1016/1011-1344(89)80099-5.

[50] Uusikivi J, Vähätalo A V, Granskog MA, Sommaruga R. Contribution of mycosporine-like amino acids and colored dissolved and particulate matter to sea ice optical properties and ultraviolet attenuation. Limnol Oceanogr 2010;55:703–13.

[51] Ryan KG, McMinn A, Mitchell KA, Trenerry L. Mycosporine-like amino acids in Antarctic sea ice algae, and their response to UVB radiation. Z Naturforsch C 2002;57:471–7.

[52] Balskus EP, Walsh CT. The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. Science 2010;329:1653–6. doi:10.1126/science.1193637.

[53] Maeda H, Sakuragi Y, Bryant DA, Dellapenna D. Tocopherols protect *Synechocystis* sp. strain PCC 6803 from lipid peroxidation. Plant Physiol 2005;138:1422–35. doi:10.1104/pp.105.061135.

[54] Leya T, Rahn A, Lütz C, Remias D. Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. FEMS Microbiol Ecol 2009;67:432–43. doi:10.1111/j.1574-6941.2008.00641.x.

[55] Lemoine Y, Schoefs B. Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. Photosynth Res 2010;106:155–77. doi:10.1007/s11120-010-9583-3.

[56] Duval B, Shetty K, Thomas WH. Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light 2000:559–66.

[57] Gorton HL, Vogelmann TC. Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. Photochem Photobiol 2003;77:608–15.

[58] Remias D, Wastian H, Lütz C, Leya T. Insights into the biology and phylogeny of Chloromonas polyptera (Chlorophyta), an alga causing orange snow in Maritime Antarctica. Antarct Sci 2013;25:648–56. doi:10.1017/S0954102013000060.

[59] Remias D, Schwaiger S, Aigner S, Leya T, Stuppner H, Lütz C. Characterization of an UV- and VIS-absorbing, purpurogallin-derived secondary pigment new to algae and highly abundant in *Mesotaenium berggrenii* (Zygnematophyceae, Chlorophyta), an extremophyte living on glaciers. FEMS Microbiol Ecol 2011;79:638–48. doi:10.1111/j.1574-6941.2011.01245.x.

[60] Missall TA, Lodge JK, Mcewen JE. Mechanisms of resistance to oxidative and nitrosative stress : implications for fungal survival in mammalian hosts 2004;3. doi:10.1128/EC.3.4.835.

[61] Eisenman HC, Casadevall A. Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 2012;93:931–40. doi:10.1007/s00253-011-3777-2.

[62] Gessler NN, Egorova AS, Belozerskaya TA. Melanin pigments of fungi under extreme environmental conditions (Review). Appl Biochem Microbiol 2014;50:105–13. doi:10.1134/S0003683814020094.

[63] Sinha RP, Häder DP. UV-induced DNA damage and repair: a review. Photochem Photobiol Sci 2002;1:225–36.

[64] Seeberg E, Eide L, Bjørås M. The base excision repair pathway. Trends Biochem Sci 1995;20:391–7. doi:10.1016/S0968-0004(00)89086-6.

[65] Varghese S, Tang Y, Imlay JA. Contrasting sensitivities of *Escherichia coli* aconitases A and B to oxidation and iron depletion. J Bacteriol 2003;185:221–30.

[66] Flint DH. Initial kinetic and mechanistic characterization of *Escherichia coli* fumarase A. Arch Biochem Biophys 1994;311:509–16. doi:10.1006/abbi.1994.1269.

[67] Vaïtilingom M, Deguillaume L, Vinatier V, Sancelme M, Amato P, Chaumerliac N, et al. Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. Proc Natl Acad Sci U S A 2013;110:559–64. doi:10.1073/pnas.1205743110.

[68] Burrows SM, Butler T, Jöckel P, Tost H, Kerkweg A, Pöschl U, et al. Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems. Atmos Chem Phys 2009;9:9281–97. doi:10.5194/acp-9-9281-2009.

[69] Cockell CS, Rettberg P, Horneck G, Wynn-Williams DD, Scherer K, Gugg-Helminger A. Influence of ice and snow covers on the UV exposure of terrestrial microbial communities: dosimetric studies. J Photochem Photobiol B Biol 2002;68:23–32. doi:10.1016/S1011-1344(02)00327-5.

[70] Boutron CF, Candelone J-P, Hong S. Past and recent changes in the large-scale tropospheric cycles of lead and other heavy metals as documented in Antarctic and Greenland snow and ice: A review. Geochim Cosmochim Acta 1994;58:3217–25. doi:10.1016/0016-7037(94)90049-3.

[71] Dommergue A, Larose C, Faïn X, Clarisse O, Hintelmann H, Schneider D, et al. Deposition of mercury species in the Ny-Alesund area (79 degrees N) and their transfer during snowmelt. Environ Sci Technol 2010;44:901–7. doi:10.1021/es902579m.

[72] Larose C, Dommergue A, De Angelis M, Cossa D, Averty B, Marusczak N, et al. Springtime changes in snow chemistry lead to new insights into mercury methylation in the Arctic. Geochim Cosmochim Acta 2010;74:6263–75. doi:10.1016/j.gca.2010.08.043.

[73] Nies DH. Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid 1992;27:17–28.

[74] Granskog MA, Kaartokallio H. An estimation of the potential fluxes of nitrogen, phosphorus, cadmium and lead from sea ice and snow in the Northern Baltic Sea. Water, Air, Soil Pollut 2004;154:331–47. doi:10.1023/B:WATE.0000022975.74321.27.

[75] Carlson R. A trophic state index for lakes. Limnol Oceanogr 1977;22:361–9.

[76] Antony R, Mahalinganathan K, Krishnan KP, Thamban M. Microbial preference for different size classes of organic carbon: a study from Antarctic snow. Environ Monit Assess 2012;184:5929–43. doi:10.1007/s10661-011-2391-1.

Chapter 4: Assessing the activity of snowpack microbial communities

The dynamic variation in microbial community structure and function as determined by DNA analyses, with regard to season, location in the snowpack and geographical location supports the active nature of these microbial communities in the snowpack, but does not remove all doubt that these changes could be attributed to other phenomena, such as passive redistribution of microorganisms along snow metamorphism and cumulative deposition. In order to provide further demonstration of the activity of these snowpack microorganisms in the snow, other experiments were initiated including the examination of messenger RNA variations in the snowpack and the development of snow microcosms in the laboratory conditions. These two sets of initial experiments are described below.

Section 1: The use of meta-omics to explore activities of snow microbial community

Introduction

Snow has been shown to harbour microbial inhabitants in significant abundance with a wide diversity and in as described in the previous chapters of this thesis. In addition, this microbial community seems to reflect a snow-specific pattern in terms of taxonomical composition and functional abilities when compared to neighboring environments. Despite detection of this functional and taxonomical signature, snow has a high variability of community structure and function at all spatial and temporal scales. Global snow cover, with significant coverage of the Earth's surface, is not homogeneous as it varies as a function of type of habitat conditions: solar irradiation, nutrient input, temperature, water availability depending on location, elevation, underlying environments and even the depth of the snowpack. This variability leads to heterogeneous spatial distribution of microbes. These habitat conditions also vary with time and with increased seasonal effects in polar regions. Occasional

atmospheric deposition events can lead to an input of chemicals, such as nutrients and contaminants from local or remote origin. These compounds could be further transformed and could lead to changes in physico-chemical surrounding conditions. Modification of microbial community composition and correlation of functional genes abundance with chemical input support the hypothesis that the microbial community is dynamic and responds effectively to changes in environmental conditions. However, the active members of this community and the time scale of microbial response remain unknown. In this short section, I discuss the advantages but also limitations of high throughput sequencing to address these questions using preliminary results obtained from Arctic spring snow in conjunction with results previously described in this thesis.

Methods

Samples were taken during a 2011 springtime field campaign in Ny-Ålesund (Svalbard, Norway, 78°56'N, 11°52'E). Surface snow layers were collected on a daily basis from mid-April to beginning of June 2011. Snow chemistry was analysed as described previously [1]. Samples were processed immediately after collection in the field laboratory. Samples were left to melt at room temperature prior to being filtered onto sterile 0.22 µM 47 mm filters (Millipore) using a sterile filtration unit (Nalge Nunc International Corporation) and filters were stored in Eppendorf tubes at -20°C for further analysis. Two sets of samples where chosen for microbial communities analyses based on chemical results: organic acids and particle concentration (Figure 1). Late spring samples (CH3N-40 to CH3N-66) had higher concentrations of organic and particles compared to early spring (CH3N-1 to CH3N-10), and we can hypothesize that microbial activity would be greater. Total nucleic acids were then extracted using Power Water RNA isolation kit following manufacturer instructions, except that the DNAse treatment step was omitted. The RNA fraction of nucleic acids was then further purified using RNeasy kit from Qiagen following the manufacturer's instructions. cDNA libraries were prepared from RNA using Tetro cDNA synthesis kit from Bioline. DNA and cDNA samples were then amplified using multiple displacement amplification with the illustra™ GenomiPhi™ HS DNA Amplification Kit (GE Healthcare) and sequenced using a Roche 454 Titanium pyrosequencer.

Metagenomic and metatranscriptomic datasets were analyzed for taxonomy and functional attributes using the Metagenome Rapid Annotation with Subsystem Technology (MG-RAST) [2]. The occurrence of taxa was compared in metagenomic and metatranscriptomic datasets in early spring samples. The relative abundance of functional attributes in metagenomic datasets versus the metatranscriptomic datasets was also compared for early and late spring. In parallel, general snowpack functional annotation efficiency was compared to a non-exhaustive set of metagenomes originating from various environments using annotation metadata available in MG-RAST metaganome analysis software.

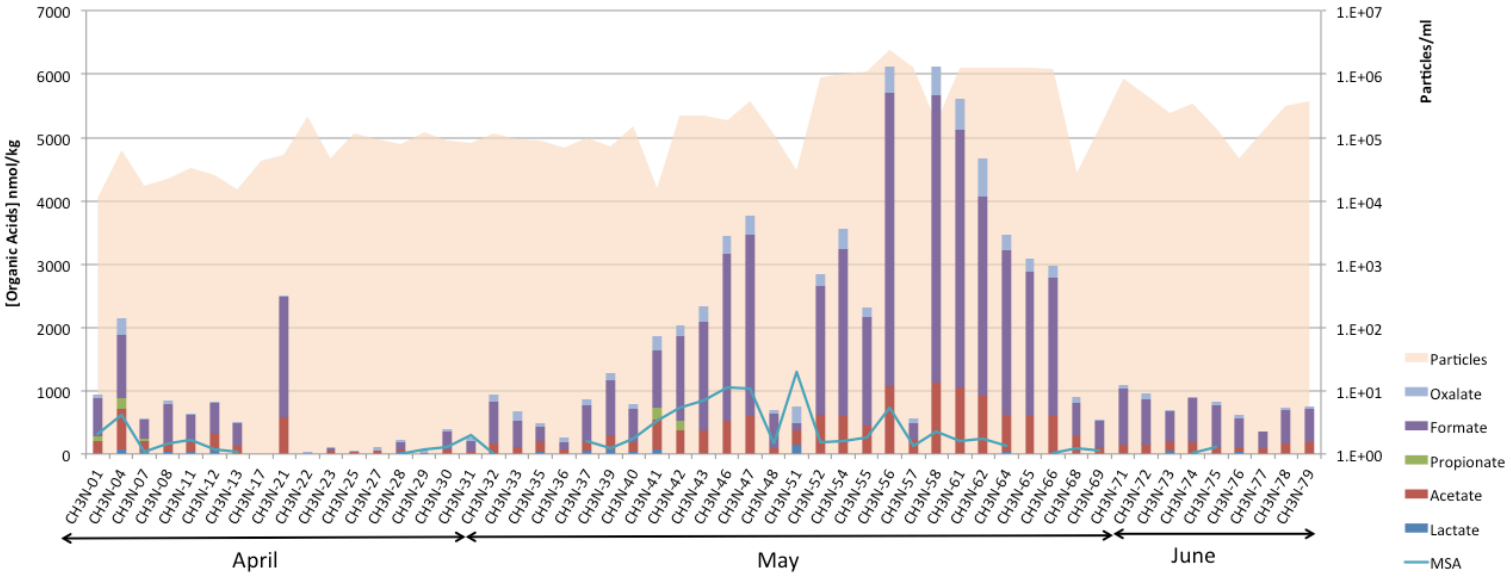


Figure 1: Samples chemistry. Snowpack daily concentrations of organic acids (in nmolkg^{-1}) and particles (in particles ml^{-1}) from mid-April to beginning of June during the 2011 Svalbard fiel trip campaign.

Preliminary Results

Differences between RNA and DNA datasets

The occurrence of taxa (presence/absence of a taxon at family level) did not completely overlap between metagenomic and metatranscriptomic datasets (Figure 2A). Some taxa were observed in both DNA and RNA, as the case for the family of *beta-Protebacteria*, *Comamonodaceae* including *Variovorax*. High numbers of families were only detected in metatatranscriptomic datasets, such as *Halithiobacillaceae* family. In contrast, some taxa were detected only in the DNA pool, such as families belonging to *Asocmyceta.* Functional distribution in metatransriptomic versus metagenomic datasets was also compared for early and late spring (Figure 2B). The pattern of functional distribution in terms of relative abundance is different between early and late spring. In late spring, a majority of functions is detected in the RNA pool as well in the DNA pool, with only a few functions highly abundant in RNA and not in DNA and vice versa. Conversely, in early spring samples, most of the functions do not overlap in the RNA and DNA pools; i.e., functions are detected only in metatranscriptomic or metagenomic datasets. The discrepancy between RNA and DNA annotation in our snow metagenome was compared with a set of marine mesocosms metagenomes to examine the proportion of taxa detected only in DNA datasets, only in RNA datasets or in both (Figure 2c). Similar as in Gigure 2b, the proportion of genera detected only in RNA was higher in early spring compared to late spring, but also much higher than in the marine mesocoms experiments. For marine mesocosms the proportion of genera detected only in RNA datasets was higher in mid-bloom samples than in post-bloom samples. The proportion of genera only detected in DNA in our snow metagenome was similar to that in mid-bloom marine mesocosms.

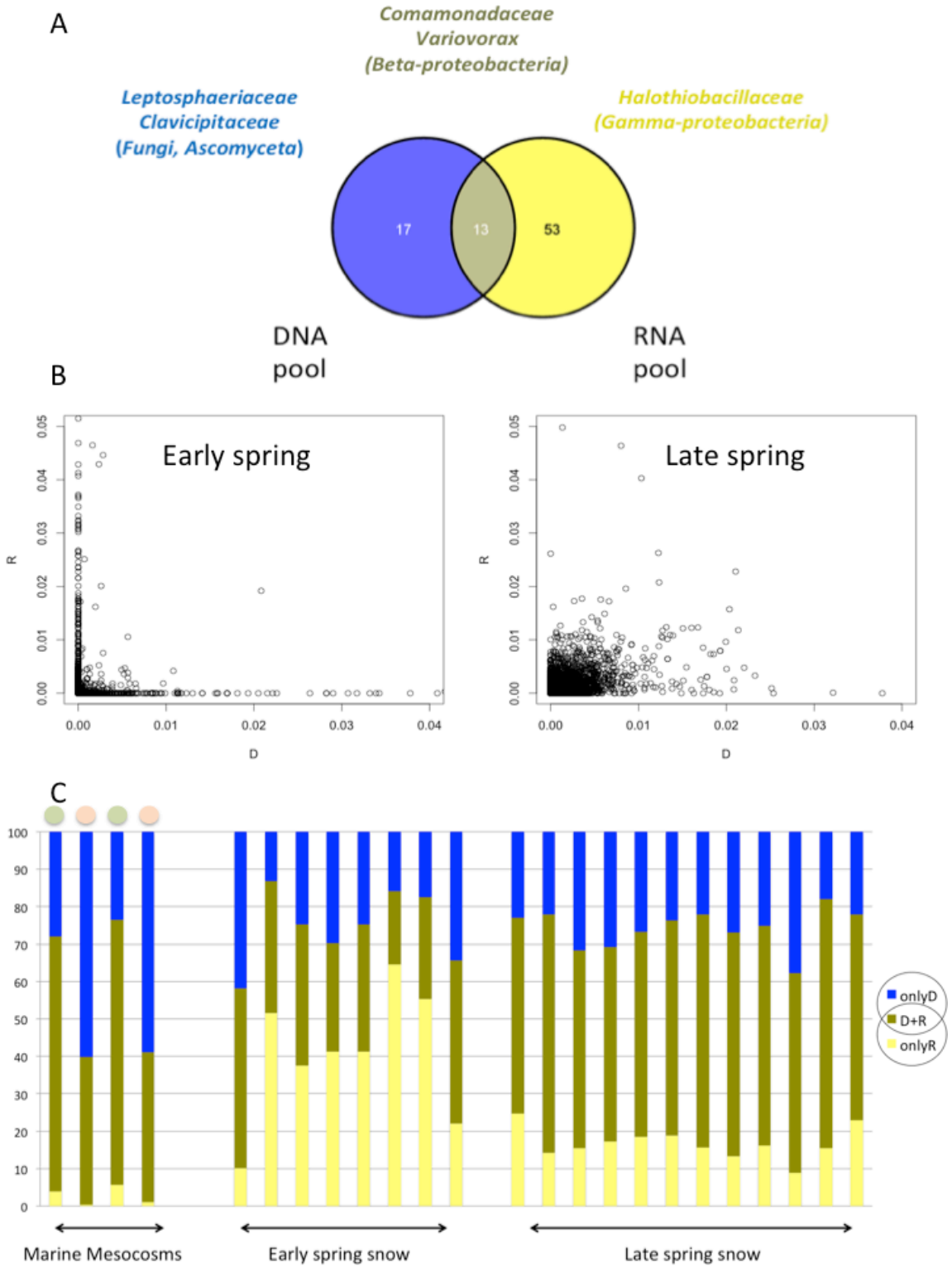


Figure 2: Discrepancy between metagenomes and metatranscriptomes. Taxonomical and functional annotations are based on assignment of shotgun sequences to M5nr database in Mgrast wit an evalue cut-off of 10^{-5}. **A:** Venn diagram of number of taxa detected in both types datasets, only in metagenome (blue) and only in metatranscriptomes (yellow). **B:** Relative abundance in mategenomic (D = X axis) and metatranscriptomic (R = Y axis) datasets of each function at deepest level of hierarchical classification of SEED database in early spring (left) compared to late spring (right). **C:** Percentage of genera detected only in metagenome (blue), only in metatranscriptome (yellow) or in both (brown) compared to total of genera detected per sample in our meta-omes compared to marine mesocosms (green dots indicate mid-bloom samples whereas orange dots indicate post-bloom samples)[3].

Database driven annotations

In the JGI Gold database (Genome online database) [4], most of the genomes sequenced originated from human-associated and aquatic environments, whereas only 15 and even 0 are originating from ice and snow, respectively (Figure 3A). Regarding microbial attributes, cold-adapted microorganism sequences represent only 2% of all sequences (Figure 3B). Metagenome annotation efficiency can be highly variable between and among environments (Figure 3C). Here we present a non-exhaustive description of annotation efficiency, using the percentage of sequences annotated (i.e. that have been assigned an annotation using at least one of M5NR protein databases) compared to total sequences with identified ORFs in the MgRast metagenomic database and analysis software [2]. These biases in annotation efficiency towards well-described environments are illustrated with soil (around 50%) and feces (up to 80%) metagenomes. Annotation efficiency for snow is variable but similar for sea ice and marine habitats.

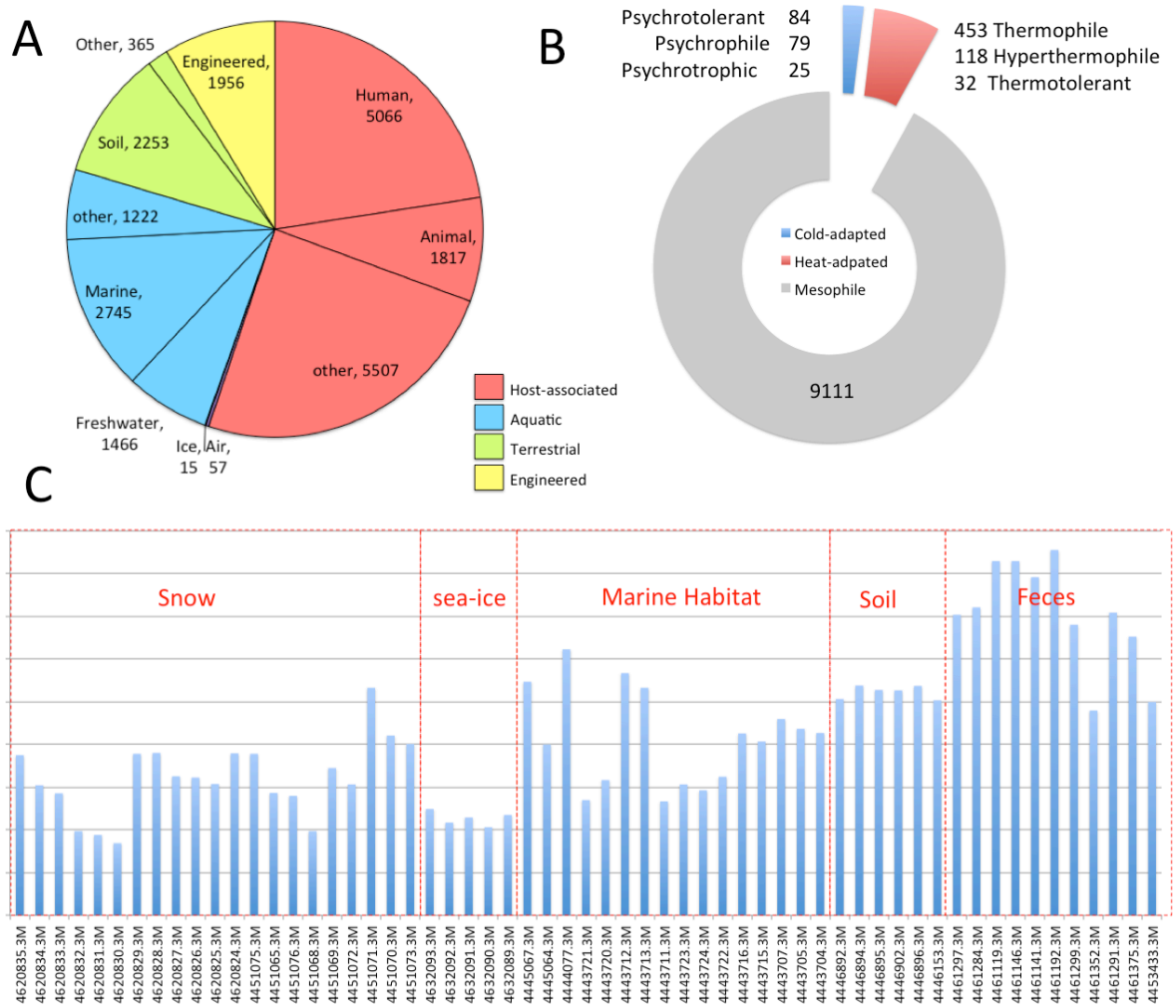
Discussion

Datasets accuracy

A complete separation between present and mRNA-synthesizing microorganisms seems unlikely. Conversely with to metatranscriptomic analyses in literature where rRNA can constitute up to 90% of sequences assignments, rRNA was surprisingly detected in low abundance in our snow RNA datastes [5–7]. Further characterization of the presented metagenomes and metatranscriptomes indicated the possibility of low quality dataset analyses, such as low assignment, high relative abundance of plasmid related functions and low abundance of reads associated with rRNA. This pattern can potentially be due to biased amplification towards small amplicons and creation of chimeric sequences, know for low material whole genome amplification via the multi-displacement technique used. Further bioinformatic analyses including additional quality filters are needed to evaluate dataset accuracy and thus no other analyses of these data will be described in this chapter. However,

it helps to illustrate issues, both technical and conceptual, related to the characterization of snow microbial communities, which have been underlying this thesis work.

Figure 3: Representation of snow and ice in metagenomic and genomic databases. A: Repartition of genomes entirely sequenced by environment of isolation. Genomes and metadata were assessed from JGI GOLD database (Genomes Online Database, is a World Wide Web resource for comprehensive access to information regarding genome and metagenome sequencing projects, and their associated metadata, around the world, Reddy et al., 2014) [4] **B :** From the same database, repartirtion of genomes sequenced by toptimal temperature growth described. **C :** Annotation



efficiency of non-exhaustive list of metagenomes publically avalable in Mgrast software compared to snow metagenomes, using the percentage of sequences annotated (i.e., that have been assigned an annotation using at least one of M5NR protein databases) compared to total sequences with identified ORF.

Technical issues linked with the use of meta-omics in snow environments

Among the technical limitations, low abundance (regarding detection limit of microbiology tool) of organisms and the icy structure of snow are certainly the most obvious. As reviewed in Chapter 1, snow is far from sterile, but microbial abundance is generally low with around 10^2 to 10^4 cells ml^{-1} and occasionally reaching 10^5 cells ml^{-1}. In terms of logistics, it seems unrealistic to exponentially increase samples size. In addition, the melting process might influence DNA recovery; for example, plasmolysis during melt could increase extracellular DNA loss during filtration step. Another issue might be extracellular DNA, which can persist in the environment, as adsorbed to clay particles in soils, for example [8]. In the Canadian Arctic shelf ice, extracellular DNA measurements indicated concentrations up to 135 μgL^{-1} [9]. The persistence of DNA and factors influencing DNA breakdown, such as temperature and radiation, were reviewed for different environments including soil, fresh and marine water [10]. In snowpacks, low temperature could lead to DNA preservation, as nuclease activity has been shown to be much lower at 4°C than at 37°C in marine sediments [11]. However the sensitivity of DNA to UV radiation [12] could greatly limit DNA persistence in snow. Although extracellular DNA might persist in snow and some molecules might be maintained in the particle film on the filter, the majority should be excluded during the filtration step.

under these conditions, it can be difficult to reach the DNA yield needed for sequencing technologies (3 µg for 454 pyrosequencing technologies). We therefore used whole genome amplification to explore microbial community function in Chapter 2, despite the potential biases related to this technique. DNA recovery, preservation during shipping and storage and efficiency of whole genome amplification are reinforcing the difficulties in management of already rare samples. With the recent improvements in sequencing technologies, it is possible to obtain sequencing datasets with as low as 1 ng of DNA starting material. We have thus able to construct metagenomes for samples with low abundance such as superficial snow and atmosphere from the Greenlandic fjord sea ice snow cover gradient presented in Chapter 3.

Another limitation of metagenomics is the dependence on the quality of databases, mostly dominated by well-studied environments, which influences annotation efficiency as described in the results section. This analysis of annotation efficiency depending on environment presented here is only given as an indication, as it includes a low number of metagenomes, since no other snow metagenomes are available for comparison and this percentage greatly relies on the e-value cut-off individually chosen on dataset submission. Although annotation efficiency for snow is variable, it is similar for sea ice and marine habitats. In Chapter 1, the notion of extreme environment and why snow might not be so extreme were discussed. Although a majority of sequences are not identified and we cannot exclude the possibility that highly snow-specific extremophiles dominate the snow, metagenomics is a powerful tool to explore the snow microbial community.

The use of high throughput sequencing technologies allowed us to develop hypotheses on potential drivers involved in structuring the community and selection processes occurring within the snowpack. Its results also support the hypothesis that snowpack is a real ecosystem, with complex microbial assemblages interacting with abiotic conditions of the snow habitat.

Snow community exploration via metatranscriptomic approach presented common and diverging issues with metagenomics, in terms of sampling preparation and sequences analyses. The mRNA content of a bacterial cell, depending on growth state, is difficult to estimate. The number of mRNA molecules in *Escherichia coli* during exponential growth in culture has been estimated to ≈1380, a small number compared to more than 43000 genes and 42 000 000 proteins [13]. Using the addition of artificial mRNA standards to environmental samples form mRNA content estimation, cells in marine microbial communities from southeastern US coastal waters and the Amazon River plume were estimated to contain as low as 200 mRNA molecules [14]. RNA extraction yield from environmental samples is thus expected to be very low. The quantification technics available are not suitable with these low concentrations. For instance, the detection limit using qBit assay based on fluophore quantification (life technologies) is $1ng\mu l^{-1}$, which was high for our samples. mRNA

molecules are also extremely labile, which implies that i) in contrast to DNA, the persistence of extracellular molecules is unlikely and ii) samples preparation requires clean procedures. The intracellular half-life of mRNA has been shown to be as low as a few minutes and independent of growth rate [14], implying that even in ecosystems where organisms have a slow growth rate, as is likely the case with snow, the transcriptional response could be a very short signal and rapidly fluctuating in changes in environmental conditions. In the case of snowpack, as well for icy habitats, melting might greatly influence transcript pools in samples, with release and bioavailability of nutrients and hypo-osmotic stress. The preservation of samples representative to *in situ* community in the specific conditions observed is thus a major concern. During this thesis, we tried to develop two alternative procedures to avoid the bias linked to melting process: *i.e,* chemical stabilization solution (RNA later-like buffer) and physical preservation with lyophilisation. Ammonium sulfate can precipitate ribonuclease (along with other solubilized proteins), thus preventing the degradation of labile RNA molecules [15]. This property has been used in the development of a tissue and sample storage buffer [16], that we also used to store filters from the sampling site to the laboratory. In order to limit RNA degradation and synthesis in response to melt, we added an RNA later like buffer, whose recipe is publically available in literature and joined in supplemental data of this manuscript [17], with a concentration for 0.5 L of equivalent melted snow prior to melting. On the other hand, freeze drying was also shown to be suitable for tissue storage without altering RNA [18] and has been used to lyophilized a liter of snow. Determining the influence of this alternative procedure on the taxonomical composition based on 16S rRNA transcript analysis are on going. However, the need for an in-depth study to evaluate the efficiency of these procedures and their implementation with a realistic strategy (numbers of samples to process in reasonable time, cost and waste; *e.g.,* 700 g of ammonium sulfate for 1.5 L of melted snow is not a sustainable option) limited their use in the field campaigns from 2014.

In terms of bioinformatics analyses, metatranscriptomics present the same issues as metagenomics. In addition the high dominance of rRNA in sequencing datasets can be challenging to obtain

sufficient coverage to explore mRNA functional response, depending on the research questions. Marine metagenome and metatranscrptome sequences similarity clusters revealed that RNA datasets present between 1 and 2 orders of magnitude more novel cluster than DNA datatsets [3], which suggests that unknown fraction of genes expressed might be high.

Metatranscriptomics as a proxy for microbial activity in snow

The production or presence of RNA as a proxy of microbial activity might be a step further in providing data that microbes are active in the snowpack and not just stored in a frozen or dormant state. The analysis of genes transcripts and in particular 16S rRNA have been proposed to identify the bacteria that are most likely to be present in a metabolically active state in the snow [19]. In this study, they identified highly abundant taxa in 16S rRNA transcript that might be considered as endogenous antarctic snow inhabitants, such as *Janthinobacterium*, *Pseudomonas*, *Sphingomonas* and *Variovorax*. *Variovorax*, genera often described in low temperature environments, including snow, has also been detected in our datasets both DNA and RNA. A high number of families were only detected in metatatranscriptomic datasets. Among these taxa we can cite the *Halithiobacillaceae* family, a purple sulfur bacteria belonging to *gamma-Proteobacteria* and already reported in arctic and antarctic lakes [20,21] and within ice bubbles in a subarctic lake ice [22]. These findings suggest that low abundant and diverse taxa, present but under the detection limit in the DNA pool, might contribute to RNA molecule production and thus be active in the conditions at the time of sampling. In contrast, some taxa were detected only in the DNA pool, such as families belonging to *Asocmyceta*, and might not be active members of the snow community, at least at this time of sampling. The occurrence of reads annotated as fungi and especially *Ascomyceta* in snow was observed in early spring snow with an increase at the end of the spring (mid-May). However the same trend was not observed in these late spring samples largely dominated by *Pseudomonodaceae*. Among microbes brought by wind and deposited in snow with dust or snowfall, some might not be able to grow in the snow, because the characteristics are too different as compared to the environments from which they originated. For instance, plant pathogens such as *Agrobacterium*

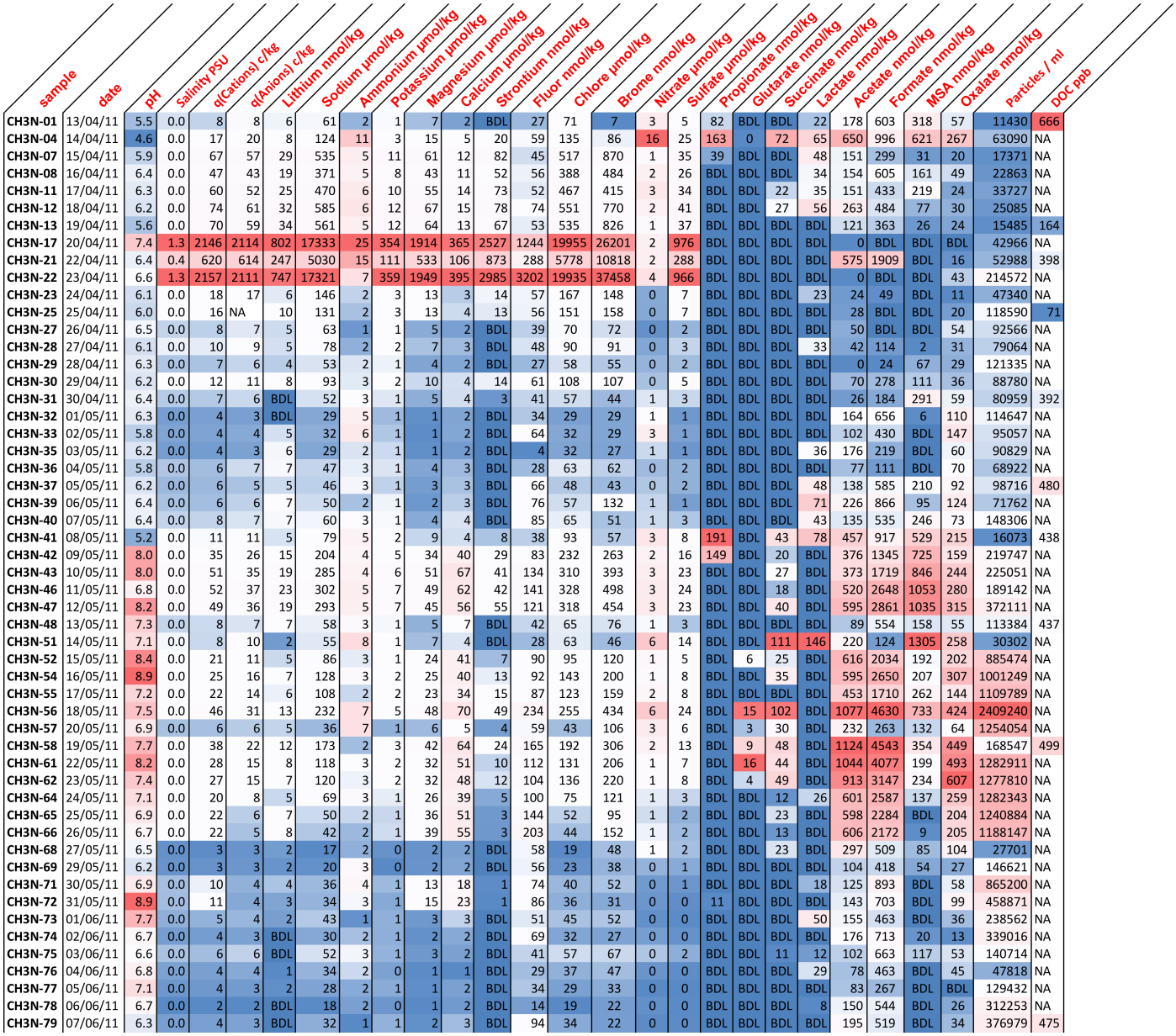
were detected in freshly fallen snow, but were no longer detected after deposition [23]. These post deposition selection processes have also been investigated in Chapter 3, indicating that a proportion of *Rhizobiaceae* could decrease between atmosphere and snow. However, the real proportion of these dormant cells and their persistence within snowpack remain unknown. This difference between metagenomic and metatranscriptomic annotation was also observed in our data at a functional level, with higher discrepancy between present and potentially expressed functions during early spring.

These observations lead to arise several questions. Is the discrepancy between the present and active community in snowpack more important when the conditions are harsher with potentially only few active microorganisms? Is the discrepancy between present and active microbes more important in snow and ice compared to more conventional environments, due to regular input and release imposed by its interface nature? Is it due to its harsh environmental conditions? If yes, then what is the time scale of microbial selection within them that leads to a snow-adapted community as compared to the input of new microbes via deposition? And more generally, in which measure is mRNA production an indication of microbial activity?

Here we proposed to compare the discrepancy between RNA and DNA from snow sequencing datasets with publically available sequencing datasets from eutrophic marine marine mesocosms at two different stages during algal bloom. The phase during mid-bloom might support high modifications of communities activities, with algal growth and concomitant influence on heterotrophic microbes, compared to more stable phase from post-bloom. Indeed, the proportion of genera potentially expressing genes (only in RNA, in DNA and RNA) was higher in the mid-bloom than in post-bloom phase. The fraction of present but potentially not transcribing genes (only DNA) in our snow metagenomes was comparable to mid-bloom phase marine mesocoms, whereas the fraction of genera detected ony in RNA was much more important. These results suggest that snow microbial community could be highly dynamic in terms of transcriptional response and that that low abundant

and diverse taxa might contribute to RNA molecule production and thus be active under the conditions at the time of sampling. However, this pattern of response might be also largely biased by microbial response triggered by melting procedure as described previously. As for annotation efficiency, this meta-ome comparison is not exhaustive but further analyses with more meta-omes from various conventional and non-conventional environments annotated in similar way would help to investigate the divergence between present and active members of snow microbial communities.

However, as previously mentioned, the intracellular half-life of mRNA has been shown to be as low as a few minutes and independent of growth rate [14], implying that even in ecosystems where organisms have a slow growth rate, as is likely the case with snow, the transcriptional response could be a very short signal. The lack of environmental information about RNA and protein turnover, the occurrence of constitutive or induced transcription and the extent of post-transcriptional modifications [14,24] and are common for all types of environments and constitute major issues for microbial ecologists who use RNA presence or production as a proxy for microbial activity. Metatranscriptomics is then an interesting tool to examine the changes responses of microbes to specific environmental stimuli rather than activity *per se*. Snowpack is highly dynamic, with regular input of minerals and organic compounds; their distributions and concentrations are modified by physical processes in the snowpack (photochemistry, wind pumping, freeze thaw cycles, snow metamorphism, etc). This chemical conditions are highly variable on a daily basis; *e.g,* ion concentrations can by increased by a factor 30 in one day (Figure 4). Metatranscriptomics might be a tool that can help to evaluate short-term responses of snow microbial communities. What is the time frame for sampling in order to catch a specific response? Again the notion of time scale appears critical.



sample	date	pH	Salinity PSU	q(Cations) c/kg	q(Anions) c/kg	Lithium nmol/kg	Sodium µmol/kg	Ammonium µmol/kg	Potassium µmol/kg	Magnesium µmol/kg	Calcium µmol/kg	Strontium nmol/kg	Fluor nmol/kg	Chlore µmol/kg	Brome nmol/kg	Nitrate µmol/kg	Sulfate µmol/kg	Propionate nmol/kg	Glutarate nmol/kg	Succinate nmol/kg	Lactate nmol/kg	Acetate nmol/kg	Formate nmol/kg	MSA nmol/kg	Oxalate nmol/kg	Particles / ml	DOC ppb
CH3N-01	13/04/11	5.5	0.0	8	8	6	61	2	1	7	2	BDL	27	71	7	3	5	82	BDL	BDL	22	178	603	318	57	11430	666
CH3N-04	14/04/11	4.6	0.0	17	20	8	124	11	3	15	5	20	59	135	86	16	25	163	0	72	65	650	996	621	267	63090	NA
CH3N-07	15/04/11	5.9	0.0	67	57	29	535	5	11	61	12	82	45	517	870	1	35	39	BDL	BDL	48	151	299	31	20	17371	NA
CH3N-08	16/04/11	6.4	0.0	47	43	19	371	5	8	43	11	52	56	388	484	2	26	BDL	BDL	BDL	34	154	605	161	49	22863	NA
CH3N-11	17/04/11	6.3	0.0	60	52	25	470	6	10	55	14	73	52	467	415	3	34	BDL	BDL	22	35	151	433	219	24	33727	NA
CH3N-12	18/04/11	6.2	0.0	74	61	32	585	6	12	67	15	78	74	551	770	2	41	BDL	BDL	27	56	263	484	77	30	25085	NA
CH3N-13	19/04/11	5.6	0.0	70	59	34	561	5	12	64	13	67	53	535	826	1	37	BDL	BDL	BDL	BDL	121	363	26	24	15485	164
CH3N-17	20/04/11	7.4	1.3	2146	2114	802	17333	25	354	1914	365	2527	1244	19955	26201	2	976	BDL	BDL	BDL	BDL	0	BDL	BDL	BDL	42966	NA
CH3N-21	22/04/11	6.4	0.4	620	614	247	5030	15	111	533	106	873	288	5778	10818	2	288	BDL	BDL	BDL	BDL	575	1909	BDL	16	52988	398
CH3N-22	23/04/11	6.6	1.3	2157	2111	747	17321	7	359	1949	395	2985	3202	19935	37458	4	966	BDL	BDL	BDL	BDL	0	BDL	BDL	43	214572	NA
CH3N-23	24/04/11	6.1	0.0	18	17	6	146	2	3	13	3	14	57	167	148	0	7	BDL	BDL	BDL	23	24	49	BDL	11	47340	NA
CH3N-25	25/04/11	6.0	0.0	16	NA	10	131	2	3	13	4	13	56	151	158	0	7	BDL	BDL	BDL	BDL	28	BDL	BDL	20	118590	71
CH3N-27	26/04/11	6.5	0.0	8	7	5	63	1	1	5	2	BDL	39	70	72	0	2	BDL	BDL	BDL	BDL	50	BDL	BDL	54	92566	NA
CH3N-28	27/04/11	6.1	0.0	10	9	5	78	2	2	7	3	BDL	48	90	91	0	3	BDL	BDL	BDL	33	42	114	2	31	79064	NA
CH3N-29	28/04/11	6.3	0.0	7	6	4	53	2	1	4	2	BDL	27	58	55	0	2	BDL	BDL	BDL	BDL	0	24	67	29	121335	NA
CH3N-30	29/04/11	6.2	0.0	12	11	8	93	3	2	10	4	14	61	108	107	0	5	BDL	BDL	BDL	BDL	70	278	111	36	88780	NA
CH3N-31	30/04/11	6.4	0.0	7	6	BDL	52	3	1	5	4	3	41	57	44	1	3	BDL	BDL	BDL	BDL	26	184	291	59	80959	392
CH3N-32	01/05/11	6.3	0.0	4	3	BDL	29	5	1	1	2	BDL	34	29	29	1	1	BDL	BDL	BDL	BDL	164	656	6	110	114647	NA
CH3N-33	02/05/11	5.8	0.0	4	4	5	32	6	1	1	2	BDL	64	32	29	3	1	BDL	BDL	BDL	BDL	102	430	BDL	147	95057	NA
CH3N-35	03/05/11	6.2	0.0	4	3	6	29	2	1	1	2	BDL	4	32	27	1	1	BDL	BDL	BDL	36	176	219	BDL	60	90829	NA
CH3N-36	04/05/11	5.8	0.0	6	7	7	47	3	1	4	3	BDL	28	63	62	0	2	BDL	BDL	BDL	BDL	77	111	BDL	70	68922	NA
CH3N-37	05/05/11	6.2	0.0	6	5	5	46	3	1	3	3	BDL	66	48	43	0	2	BDL	BDL	BDL	48	138	585	210	92	98716	480
CH3N-39	06/05/11	6.4	0.0	6	6	7	50	2	1	2	3	BDL	76	57	132	1	1	BDL	BDL	BDL	71	226	866	95	124	71762	NA
CH3N-40	07/05/11	6.4	0.0	8	7	7	60	3	1	4	4	BDL	85	65	51	1	3	BDL	BDL	BDL	43	135	535	246	73	148306	NA
CH3N-41	08/05/11	5.2	0.0	11	11	5	79	5	2	9	4	8	38	93	57	3	8	191	BDL	43	78	457	917	529	215	16073	438
CH3N-42	09/05/11	8.0	0.0	35	26	15	204	4	5	34	40	29	83	232	263	2	16	149	BDL	20	BDL	376	1345	725	159	219747	NA
CH3N-43	10/05/11	8.0	0.0	51	35	19	285	4	6	51	67	41	134	310	393	3	23	BDL	BDL	27	BDL	373	1719	846	244	225051	NA
CH3N-46	11/05/11	6.8	0.0	52	37	23	302	5	7	49	62	42	141	328	498	3	24	BDL	BDL	18	BDL	520	2648	1053	280	189142	NA
CH3N-47	12/05/11	8.2	0.0	49	36	19	293	5	7	45	56	55	121	318	454	3	23	BDL	BDL	40	BDL	595	2861	1035	315	372111	NA
CH3N-48	13/05/11	7.3	0.0	8	7	7	58	3	1	5	7	BDL	42	65	76	1	3	BDL	BDL	BDL	BDL	89	554	158	55	113384	437
CH3N-51	14/05/11	7.1	0.0	8	10	2	55	8	1	7	4	BDL	28	63	46	6	14	BDL	BDL	111	146	220	124	1305	258	30302	NA
CH3N-52	15/05/11	8.4	0.0	21	11	5	86	3	1	24	41	7	90	95	120	1	5	BDL	6	25	BDL	616	2034	192	202	885474	NA
CH3N-54	16/05/11	8.9	0.0	25	16	7	128	3	2	25	40	13	92	143	200	1	8	BDL	BDL	35	BDL	595	2650	207	307	1001249	NA
CH3N-55	17/05/11	7.2	0.0	22	14	6	108	2	2	23	34	15	87	123	159	2	8	BDL	BDL	BDL	BDL	453	1710	262	144	1109789	NA
CH3N-56	18/05/11	7.5	0.0	46	31	13	232	7	5	48	70	49	234	255	434	6	24	BDL	15	102	BDL	1077	4630	733	424	2409240	NA
CH3N-57	20/05/11	6.9	0.0	6	6	5	36	7	1	6	5	4	59	43	106	3	6	BDL	3	30	BDL	232	263	132	64	1254054	NA
CH3N-58	19/05/11	7.7	0.0	38	22	12	173	2	3	42	64	24	165	192	306	2	13	BDL	9	48	BDL	1124	4543	354	449	168547	499
CH3N-61	22/05/11	8.2	0.0	28	15	8	118	3	2	32	51	10	112	131	206	1	7	BDL	16	44	BDL	1044	4077	199	493	1282911	NA
CH3N-62	23/05/11	7.4	0.0	27	15	7	120	3	2	32	48	12	104	136	220	1	8	BDL	4	49	BDL	913	3147	234	607	1277810	NA
CH3N-64	24/05/11	7.1	0.0	20	8	5	69	3	1	26	39	5	100	75	121	1	3	BDL	BDL	12	26	601	2587	137	259	1282343	NA
CH3N-65	25/05/11	6.9	0.0	22	6	7	50	2	1	36	51	3	144	52	95	1	2	BDL	BDL	23	BDL	598	2284	BDL	204	1240884	NA
CH3N-66	26/05/11	6.7	0.0	22	5	8	42	2	1	39	55	3	203	44	152	1	2	BDL	BDL	13	BDL	606	2172	9	205	1188147	NA
CH3N-68	27/05/11	6.5	0.0	3	3	2	17	2	0	2	2	BDL	58	19	48	1	2	BDL	BDL	23	BDL	297	509	85	104	27701	NA
CH3N-69	29/05/11	6.2	0.0	3	3	2	20	3	0	2	2	BDL	56	23	38	0	1	BDL	BDL	BDL	BDL	104	418	54	27	146621	NA
CH3N-71	30/05/11	6.9	0.0	10	4	4	36	4	1	13	18	1	74	40	52	0	1	BDL	BDL	BDL	18	125	893	BDL	58	865200	NA
CH3N-72	31/05/11	8.9	0.0	11	4	3	34	3	1	15	23	1	86	36	31	0	0	11	BDL	BDL	BDL	143	703	BDL	99	458871	NA
CH3N-73	01/06/11	7.7	0.0	5	4	2	43	1	1	3	3	BDL	51	45	52	0	0	BDL	BDL	BDL	50	155	463	BDL	36	238562	NA
CH3N-74	02/06/11	6.7	0.0	4	3	BDL	30	2	1	2	2	BDL	69	32	27	0	0	BDL	BDL	BDL	BDL	176	713	20	13	339016	NA
CH3N-75	03/06/11	6.6	0.0	6	6	BDL	52	3	1	3	2	BDL	41	57	67	0	2	BDL	BDL	11	12	102	663	117	53	140714	NA
CH3N-76	04/06/11	6.8	0.0	4	4	1	34	2	0	1	1	BDL	29	37	47	0	0	BDL	BDL	BDL	29	78	463	BDL	45	47818	NA
CH3N-77	05/06/11	7.1	0.0	4	3	2	28	2	1	1	2	BDL	34	29	33	0	0	BDL	BDL	BDL	BDL	83	267	BDL	BDL	129432	NA
CH3N-78	06/06/11	6.7	0.0	2	2	BDL	18	2	0	1	2	BDL	14	19	22	0	0	BDL	BDL	BDL	8	150	544	BDL	26	312253	NA
CH3N-79	07/06/11	6.3	0.0	4	3	BDL	32	1	1	2	3	BDL	94	34	22	0	0	BDL	BDL	BDL	BDL	195	519	BDL	34	376979	475

Figure 4: Chemical variability of snowpack: Heatmap of chemical data (pH, salinity, ions, organic acids, DOC, particles) registered for the 2011 Svalbard field trip campaign. Highest values for each chemical concentration are in red, medium in white and lowest in blue (BDL = below detection limit).

Conclusion

Metatranscriptomics could definitely help investigate the microbial response to highly fluctuating conditions characteristic of their habitat, despite conceptual and technical issues, and should be further integrated in new experimental designs. The use of metagenomic tools allowed us to draw hypotheses on potential drivers involved in structuring the community and selection processes occurring within snowpack. However, in order to further investigate these hypotheses, these holistic and observation analyses should be complemented by mechanistic and controlled experiments.

Section 2: Snowpack colonization after seeding – a mechanistic approach

Introduction

Often described as an important and widespread interface, snow interacts with all compartments of the biosphere; oceans, soils, ice and atmosphere, and strongly influences their abiotic and biotic processes [25,26]. Snowpack ecosystems are likely influenced by the various seeding sources connected to the snow interface. This influence was investigated in Chapter 3, where we explored the influence of atmospheric deposition to the surface, and sea ice brine injection from the base on microbial communities in sea ice snow cover and with the hypothesized selection of a snow-specific microbial community after seeding. However, how microbes are colonizing the snow habitat remains largely unknown. In this short section, methodology proposed to investigate this question and preliminary results obtained will be presented. This work was carried out as collaborative project with the "Deming Ecosystem" group at the School of Oceanography at the University of Washington (Seattle, US) during summer 2014. Two models were explored: ii) a sea ice model with snow colonization and osmotic response after injection through brine of *Colwellia psychrerythraea* and *Psychrobacter sp. P7E* and ii) a freshwater model with snow colonization by the snow algae *Chlamydomonas nivalis* according to light.

1. Brine-wetted snow colonization model with *C. psychrerythraea* and *Psychrobacter sp. P7E*

The incorporation of sea ice microbes (as well as exopolysaccarides) in overlying snow cover due to sea ice brine injection can result in a high abundance of microorganisms, estimated at 1.5×10^4 cells ml^{-1}, of which 85% are alive based on live/dead staining [27]. As explored in Chapter 3, microbial community structure and function differ along a vertical profile in a Greenlandic spring sea ice model (atmosphere, snow, saline snow, sea ice brine, and seawater), with representative taxa and functions that varied as a function of environmental conditions (salinity in basal layer versus photochemistry in surface layer). However, how microorganisms are colonizing snow and how changes in

environmental conditions imposed by the shift from sea-brine to snow is not understood. Here, we proposed to monitor this shift under controlled conditions using two model organisms in microcosms and culture media: a) the migration of microbes as well their response to a shift of conditions from ice to snow b) the possibility of active movement within snow and between snow and ice (detailed protocol in the appendix).

The two model organisms used were *Colwellia psychrerythraea 34H* and *Psychrobacter sp. P7E*; abbreviations Cp34H and P7E are used in the following text. Cp34H is a psychrophilic and halotolerant gamma-Proteobacteria of the *Altereomonodales* order originally isolated from Arctic sediments [28], but also often detected in cold seawater and sea ice [29]. Representatives of the *Colwellia* genus have also been detected in high relative abundance in polynya sea ice and fjord saline snow layer in NE coast of Greenland [30]. In the taxonomical analyses of sea ice snow cover from the Greenlandic fjord presented in Chapter 3, datasets for the saline snow bottom layer were dominated by sequences affiliated to *Altermonodales*, mostly associated with the genus *Glaciecola* but the reads associated with *C. psychrerythraea* were also significantly higher in saline snow (up to 0.8 % in SL3, 0.3% in SL2 and at least ten times less in all other samples; data not shown). P7E is also a gamma-Proteobacteria from the order of *Pseudomonadales* and was isolated from upper sea ice brine with a high salinity (128 ppt) from first-year ice floe during the winter in the Beaufort Sea [31]. Representatives of the *Psychrobacter* genus have been isolated form various cold environments, such as Siberian permafrost [32] and antarctic sea ice [33]. The biogeography of *Psychrobacter* based on 16S rRNA detection, has suggested that cold-adapted microorganisms might not be restricted to cold environments, although *Psychrobacter* is only marginally successful in non polar-habitats [34]. *Psychrobacter* was also detected in our datasets with low relative abundance, but unlike *Colwellia*, the relative abundance of *Psychrobacter* sequences was higher in the surface layer (up to 0.12 and 0.14 % in SL0 and SL1, respectively, and less than 0.01 % in all other samples). P7E is able to grow over a wide range of salinity and temperatures (-7 to 25°C, 35 to 125 ppt) compared to Cp34H (-12 to

18°C, 35 to 65 ppt). This difference in resistance to salt and temperature stress of these two strains was used in the two experiments proposed above.

The migration of microbes and their response to shift of conditions from ice to snow.

Snow microcosms with artificial sterile snow were prepared in order to simulate snow wetting by sea ice brine. Artificial sterile snow was prepared with frozen sterilized tap water and reduced to flakes using an ice-shaving machine. The snow obtained was fluffier compared to natural snow and the snowflakes were most similar to big needle (around 1 mm) type snow grains from precipitation and thus different from round polycrystalline particles characteristic of snow machine made particles [35]. Due to the high air content, the density of snow columns prepared was not controlled. The snow column physics as well as mineral content needs to be optimized in order to obtain more realistic and replicable snow microcosms. In future experiments, migration of P7E and Cp34H within the snow will be monitored using the following parameters: i) migration front of cells: cell abundance estimated by microscopic count from a small section within the snow column from the bottom to the surface and ii) estimation of live and dead cells using live/dead staining in microscopy with depth and time.

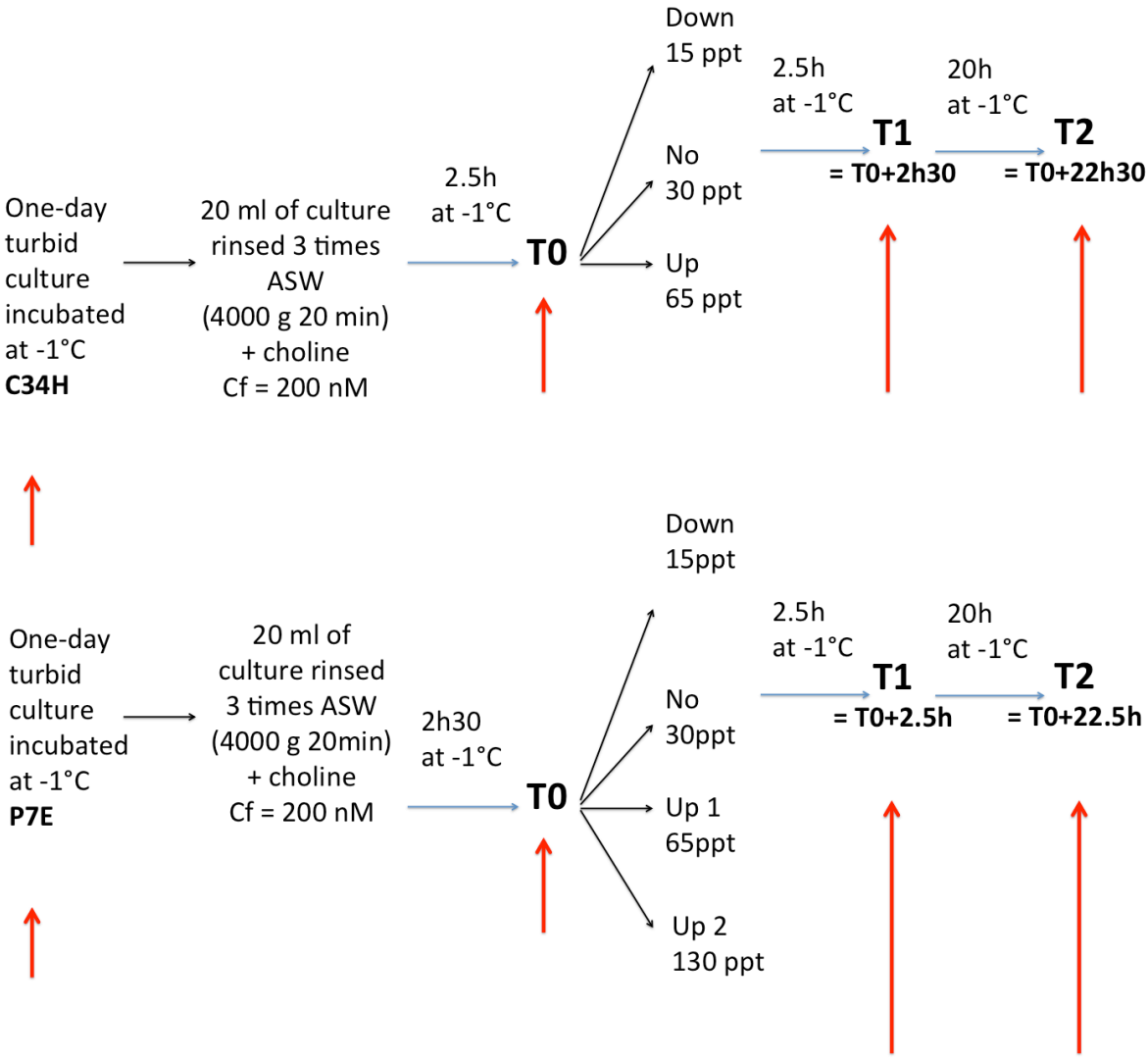


Figure 1: Transcriptional response of *Colwellia psychrerythraea* 34H and *Psychrobacter sp.* P7E to salinity shift: Turbid culture of Cp34H and P7E were rinsed with artificial seawater (ASW), incubated for 2.5 h with compatible solute solution (choline 200 nM) and subject to salinity upshift or downshift, or no shift as control . Red arrows indicate subsamples taken for microscopic observation and RNA extraction.

Microbes might be subjected to important changes in environmental conditions when moving up from sea ice brine to snow, especially in terms of salinity. Capillary movement from sea ice brine to snow over sea ice can result in a substantial input of microorganisms and chemicals, including salt from the brine liquid [27] (Chapter 3). Although the bulk salinity is quite low in saline snow, the small brine volume fraction suggests that salinity stress would be greater in saline snow compared to brine [27,36]. Compatible solutes are small molecules that accumulate in cytoplasm and act as osmolytes to help the cells cope with osmotic pressure. The availability of a compatible solute precursor, choline, was shown to reduce the mortality of Cp34H in fluctuating conditions [31]. The availability of compatible solutes and precursors in snowpack is unclear but sea ice might bring diverse sources, such as compounds released by diatom or virus-lysed bacterial cells [29,37] as well by bacterial cell lysis during the shift from ice to snow.

To address this, the transcriptional response and cell abundance of both strains to upshift and downshift in presence of choline were monitored from initial cultures at each time point (2.5 h and 22.5 h of incubation) (Figure 1). We can then hypothesize that the more halophilic P7E cells would better tolerate the salinity shifts than the narrower growth range Cp34H after injection into the snow microcosms. Expression patterns concerning general stress response (SOS system), compatible solute uptake and synthesis as well as energy requirement might be strongly influenced for the two strains after either downshift or upshift of salinity [31]. Transcriptional response in culture analyses are ongoing and would allow for a comparison of transcriptional response in snow microcosms.

This work was realized in collaboration with Evan Firth, a graduate student at the School of Oceanography of Washington University involved in a project on seasonal synergy between bacterial osmoprotection and algal production in sea ice.

The possibility of active movement within snow and between snow and ice

Snowpack, formed by the accumulation of ice crystals, is a porous medium with elevated air content [25]. Its water content can vary from less than 0.5% in dry snow to above 15% in soaked snow depending in part on temperature [38]. This variation appears to affect water distribution at the microscale. Microorganisms have been suggested to swim through the snowpack as a mechanism to escape from stress, such as from intense light [39], but the exact location in the microscale habitat as well the motility of microorganisms in the snow matrix has never been verified. However, since the snow is highly dynamic chemically, motility might be a useful feature to escape stresses and benefit from occasional input of nutrients. One of the most discriminant functional subsystem the for samples clustering in the sea ice snow vertical gradient (Chapter 3) was related to flagellum biosynthesis, with significant higher proportion of reads in basal saline snow layer compared to superficial snow and even seawater and sea ice. The group of genes associated with chemotactic responses, including proteins involved in signal transduction from the transmembrane receptor to the flagellar motor complex [40], was also detected in higher abundance in this saline layer. Are bacteria motile in saline snow layer and is this motility driven by nutrients distribution or salinity gradient between snow and ice? The objective of this study was to evaluate the potential of microbial movement between snow and ice, and within the snowpack.

Motility and directed movement (taxis) towards nutrient, oxygen or a light gradient is a major trait in microbial ecology and has been recognized in a wide variety of microorganisms from a range of environments, including animal pathogens *Esherichia coli* and *Salmonella thypimurium*, ubiquitous soil and freshwater *Rhodobacter sphaeroides* and the marine magnetotactic alpha-protebacterium *Magnetospirillum magnetotacticum* [41,42]. In most

cases, taxis involves a signal recognition by methyl-accepting chemotaxis proteins (MCPs), which have protein sequences (in particular ligand binding site) that can greatly vary between microorganisms in correlation to their habitat type [43]. Changes in salinity have been shown to trigger cyanobacterial migration causing color versatility in hypersaline microbial mats [44]. However, taxis has been poorly explored in cold environments [45]. Cp34H motility has been observed at -10°C using transmitted light microscopy [46], which suggests that microorganisms, at least cold-adapted ones, could maintain the energetic cost of motility at sub-zero temperatures. Here, a preliminary experiment was conducted to evaluate chemotaxis abilities of Cp34H towards both nutrients and salt (Figure 2). Turbid cultures (grown at 8°C in Difco Marine Broth Media 2216 ½) were washed and concentrated ten times (gentle centrifugation at 4000g for 2 min) and resuspended in 1 ml motility medium (EDTA 80 µM, HPMC 0,2%) at 50 ppt salinity. The cells preparations were then recovered with 1ml of motility medium at 35ppt salinity with or without nutrients (Marine Broth 2216 with adjusted salinity and the addition of HPMC solution). As previously described, Cp34H is a moderate halophile and has a narrow range of salinity tolerance for growth with an optimum around 35 ppt. If Cp34H were osmotactic, we would expect cells movement towards the 35 ppt salinity phase. However, no osmotactic response was observed under the conditions tested. In contrast, a chemotactic response towards nutrients was observed with the first visible migration observed after one hour with a complete change of phase still observable after two days. Osmotaxis assays with *E. coli* demonstrated that osmotatic response might not be influenced by type of chemorepellant but rather by concentration and might involve a mechanoreceptor [47]. In addition, osmotactic response depended on the experimental setup: positive with a spatial setup allowing swimming towards a gentle gradient; negative with a temporal setup where cells were suddenly

subjected to high concentrations, which is closer to the setup we used. A new experimental design would be needed to explore Cp34H osmotactic response in order to further evaluate up or down movement towards salt gradients between the saline layer and ice. Although osmoattractants remain to be determined, Cp34H is a good candidate for chemotaxis motility within the saline snow layer in snow microcosms.

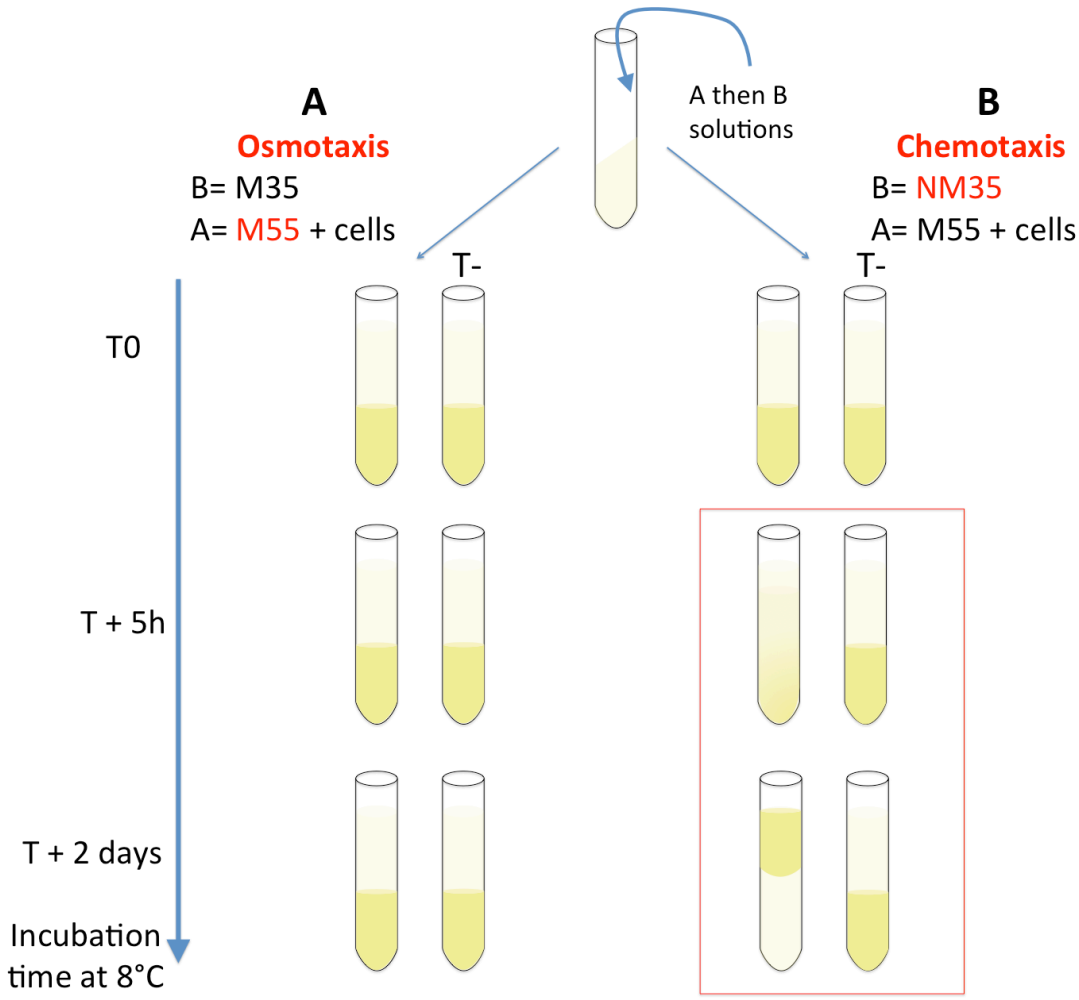


Figure 2: ***Colwellia psychrerythraea*** **34H chemotaxis**: Cp34H cells in motility medium at salinity of 55 ppt (M55) were deposited in small culture tube and gently recovered by motility medium at 35 ppt with (B – NM35) or without (A – M35) nutrients. In negative control (T-) cells were previously fixed with formaldehyde. Migration of cells in chemotaxis experiment were observed after 1 h of incubation and a complete migration from no nutrient to nutrient medium was still visible after 2 days.

This work was realized in collaboration with Gordon Max Showalter, a first-year graduate student at the School of Oceanography of Washington University involved in a project on bacterial swimming behavior (motility) in cold and subzero temperatures to help understand microbial colonization of sea ice and the use of motility as a biosignature in extreme and extraterrestrial environments.

2. Freshwater colonization model by *Chlamydomonas nivalis*

Watermelon or red snow is a phenomenon described in a variety of alpine and polar snowpacks caused by algal blooms [48–50] (Figure 1). Snow algae mostly belong to the genera *Chloromonas* and *Chlamydomonas*. *Chlamydomas nivalis*, a green flagellate alga that forms red aplanospore (Figure 2), is the most studied organism for physiology and snow adaptation ecology, such as the regulation of lipid metabolism in response to salt stress and especially pigment composition [51,52] and ultrastructure in response to UV radiation [53–56]. The life cycle has been hypothesized as following [57]: spores are present in soil after snowmelt and are covered by new snow or wind blown from soil to new snow; then during late spring/summer, when the snow is warmer and wetter and nutrients are more abundant, the spores germinate and flagellates move up to the upper layers of snow. While the flagellated stage might enable these organisms to change their position to reach optimal depth for their light and temperature requirements, in the upper layer, high radiation might cause them to stop and form the red aplanospores resulting in watermelon snow [54]. This particular behaviour of *C. nivalis* in snowpack was supported by field observations that show a seasonal and depth variability for algal distribution in snowpack from a valley glacier in Svalbard [39]. However, the actual motility of these flagellates within the snowpack and the factors triggering it (phototaxis, gravitaxis) are largely unknown. Phototaxis, a positive or negative displacement along a light gradient, involves different cell adaptations among all life kingdoms [58]. *Chlamydomonas reinhardtii* is an important model for understanding phototaxis mechanisms in green algae, with the isolation of a mutant with

altered phototactic responses [59]. Phototactic (as well as photophobic) response has been shown to involve a rhodopsin photoreceptor in *C. reinhardtii* [60]. Channelrhodopsin from different *Chlamydomonas* species were sequenced and phylogenetic analyses revealed that a remarkable diversity of channelrhodopsin exists even within the same genus *Chlamydomonas* [61].

Figure 1: Watermelon snow streak caused by accumulation of snow algae

(source = Wikipedia)

The objective of this work was to evaluate the phototactic response in *C. nivalis* in culture and within the snow matrix. *C. nivalis* strains were ordered from the UTEX culture collection of Algae (Texas University at Austin). The cells were cultured at 8°C with an alternate light cycle for three weeks using prepared Bold 1NV Medium (composition in Annex). Phototactic response was tested in three experimental set-ups illuminated with white fluorescent lamp at 8°C (Figure 3A): 1) culture in Petri dish with fluorescent light coming from one side, 2) culture in a vertical tube with only a small window (1 cm) of naked glass and 3) a smaller volume of culture in a pipette placed in a chamber with monitored PAR radiation. In none of these set-ups was the expected phototactic response observed (Figure 3B, from positive phototactic assays from literature [59,62]). During microscopic observation of cultures, cells were not motile despite the presence of flagella. Although the intense light from the microscope stopped cell movement during the time of observation, it is likely that the cells did not harbor a normal motile behaviour in the culture, maybe due to small variations in the medium. A number of factors can influence cell motility in response to light, such as light intensity, ion concentration and ion balance, pH and gas pressure [63,64]. For example, light motility response has been shown to be calcium-dependent. In addition, only five sequences are available for *C. nivalis*: chromosomal and chloroplastic rRNA genes small and large subunit, as well as photosystem II core protein D1 [65]. Thus, no genomic comparison regarding phototaxis response is possible at this time. Phototactic responses of *C. nivalis* might be parallel to highly specific physico-chemical parameters within the snowpack and remain to be elucidated. Although watermelon snow is a spectacular event, even if restricted to short-time scales, the response of an entire microbial community in the snow photochemical bioreactor might be crucial to the snow ecosystem.

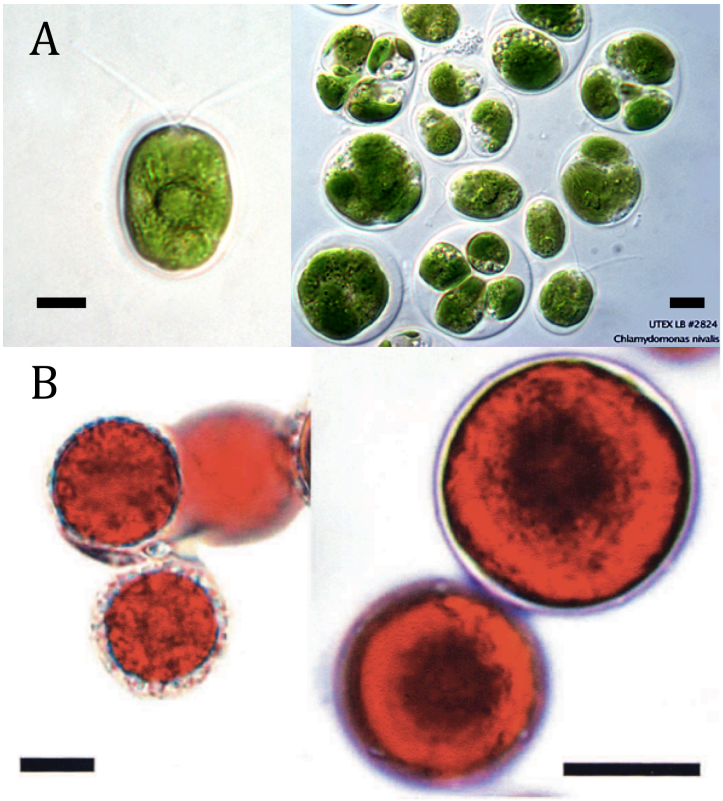


Figure 2: Microscopic observation of the green alga *Chlamydomonas nivalis*. A: On the left side, flagellated stage; on the right side cells at early stage of aplanospores formation. Pictures modified from UTEX collection centre, scale bar = 5 µm. **B:** Aplanospores inclusions accumulated secondary carotenoids with mucilage sheet (left) or smooth cell wall. Pictures modified from Remias et al. 2005, scale bar = 10µm) [54].

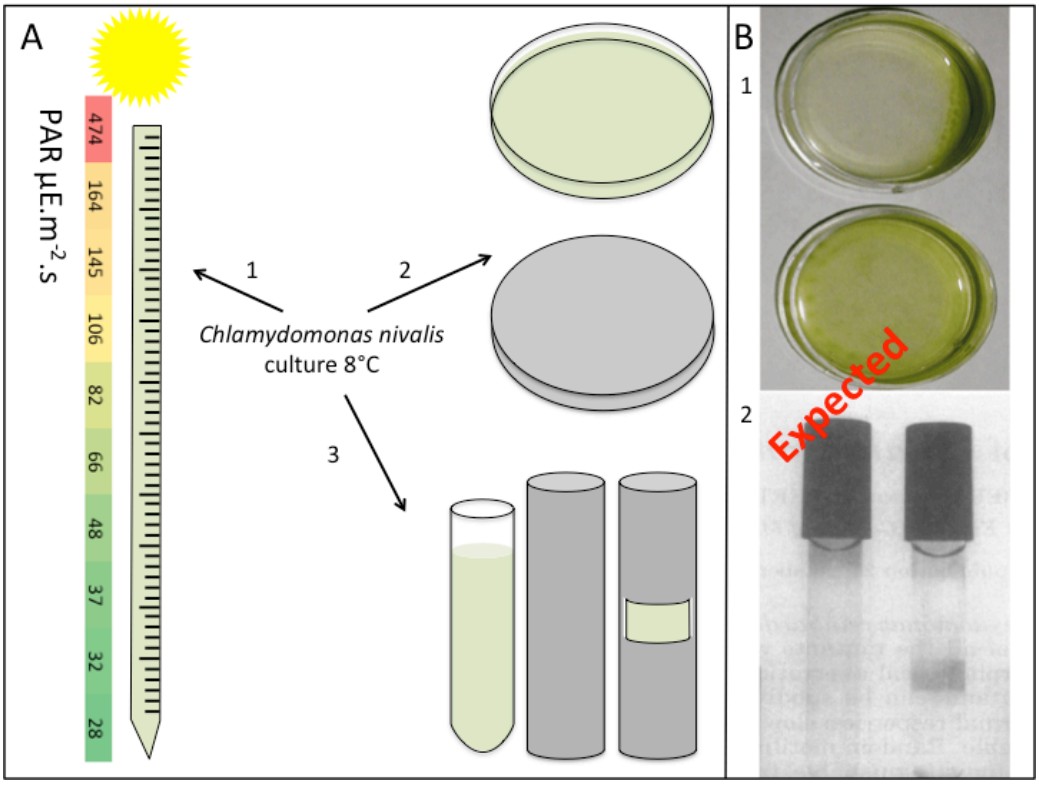


Figure 3: *Chlamydomonas nivalis* phototaxis experiments. A: Experimental setup used for qualitative assays in Petri dishes (1) and in culture tubes (2), and quantitative assay (taxis toward light optimum) (3). **B:** Expected results from the literature. (1) Phototactic *Chlamydomonas reinhardtii* in upper part and its non-phototactic mutant lacking nitrate reductase in bottom part. (modified from Okita et al. 2005) [66] (2) Phototactic *Chlamydomonas reinhardtii* in right part and its non phototactic in left part. (modified from Hirshberg and Stavis 1977) [59]

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References

[1] Larose C, Dommergue A, De Angelis M, Cossa D, Averty B, Marusczak N, et al. Springtime changes in snow chemistry lead to new insights into mercury methylation in the Arctic. Geochim Cosmochim Acta 2010;74:6263–75. doi:10.1016/j.gca.2010.08.043.

[2] Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, et al. The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 2008;9:386. doi:10.1186/1471-2105-9-386.

[3] Gilbert J a, Field D, Huang Y, Edwards R, Li W, Gilna P, et al. Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. PLoS One 2008;3:e3042. doi:10.1371/journal.pone.0003042.

[4] Reddy TBK, Thomas AD, Stamatis D, Bertsch J, Isbandi M, Jansson J, et al. The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. Nucleic Acids Res 2014;43:D1099–106. doi:10.1093/nar/gku950.

[5] Urich T, Lanzén A, Qi J, Huson DH, Schleper C, Schuster SC. Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One 2008;3:e2527. doi:10.1371/journal.pone.0002527.

[6] Poretsky RS, Hewson I, Sun S, Allen AE, Zehr JP, Moran MA. Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. Environ Microbiol 2009;11:1358–75. doi:10.1111/j.1462-2920.2008.01863.x.

[7] Stewart FJ, Ottesen E, DeLong EF. Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. ISME J 2010;4:896–907. doi:10.1038/ismej.2010.18.

[8] Paget E, Monrozier LJ, Simonet P. Adsorption of DNA on clay minerals: protection against DNaseI and influence on gene transfer. FEMS Microbiol Lett 1992;97:31–9. doi:10.1111/j.1574-6968.1992.tb05435.x.

[9] Collins RE, Deming JW. Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. Polar Biol 2011;34:1819–30. doi:10.1007/s00300-011-1041-y.

[10] Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D. Release and persistence of extracellular DNA in the environment. Environ Biosafety Res 6:37–53. doi:10.1051/ebr:2007031.

[11] Lorenz MG, Aardema BW, Krumbein WE. Interaction of marine sediment with DNA and DNA availability to nucleases. Mar Biol 1981;64:225–30. doi:10.1007/BF00397113.

[12] Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B 2001;63:88–102.

[13] Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umbarger HE. *Escherichia coli* and *Salmonella typhimurium* - Cellular and molecular biology. American Society for Microbiology; 1987.

[14] Moran MA, Satinsky B, Gifford SM, Luo H, Rivers A, Chan L-K, et al. Sizing up metatranscriptomics. ISME J 2013;7:237–43. doi:10.1038/ismej.2012.94.

[15] Allewell NM, Sama A. The effect of ammonium sulfate on the activity of ribonuclease A. Biochim Biophys Acta - Enzymol 1974;341:484–8. doi:10.1016/0005-2744(74)90240-X.

[16] Lader ES. Methods and reagents for preserving RNA in cell and tissue samples, 2003.

[17] De Wit P, Pespeni MH, Ladner JT, Barshis DJ, Seneca F, Jaris H, et al. The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. Mol Ecol Resour 2012;12:1058–67. doi:10.1111/1755-0998.12003.

[18] Wu Y, Wu M, Zhang Y, Li W, Gao Y, Li Z, et al. Lyophilization is suitable for storage and shipment of fresh tissue samples without altering RNA and protein levels stored at room temperature. Amino Acids 2012;43:1383–8. doi:10.1007/s00726-011-1212-8.

[19] Lopatina A, Krylenkov V, Severinov K. Activity and bacterial diversity of snow around Russian Antarctic stations. Res Microbiol 2013;164:949–58. doi:10.1016/j.resmic.2013.08.005.

[20] Burke CM, Burton HR. The ecology of photosynthetic bacteria in Burton Lake, Vestfold Hills, Antarctica. Hydrobiologia 1988;165:1–11. doi:10.1007/BF00025569.

[21] Antoniades D, Veillette J, Martineau M-J, Belzile C, Tomkins J, Pienitz R, et al. Bacterial dominance of phototrophic communities in a High Arctic lake and its implications for paleoclimate analysis. Polar Sci 2009;3:147–61. doi:10.1016/j.polar.2009.05.002.

[22] Barsdate RJ, Alexander V. Photosynthetic organisms in subarctic lake ice. Arctic 1970;23:201. doi:10.14430/arctic3174.

[23] Larose C, Prestat E, Cecillon S, Berger S, Malandain C, Lyon D, et al. Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. PLoS One 2013;8:e79972. doi:10.1371/journal.pone.0079972.

[24] Prosser JI. Dispersing misconceptions and identifying opportunities for the use of "omics" in soil microbial ecology. Nat Rev Microbiol 2015;13:439–46. doi:10.1038/nrmicro3468.

[25] Pomeroy JW, Brun E. Physical Properties of Snow. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW, editors. Snow Ecol. An interdisciplinary examination of snow-covered ecosystems, Cambridge University Press; 2001, p. 45–126.

[26] Schmidt SK, Lipson D a. Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil 2004;259:1–7. doi:10.1023/B:PLSO.0000020933.32473.7e.

[27] Ewert M, Carpenter SD, Colangelo-Lillis J, Deming JW. Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. J Geophys Res Ocean 2013;118:726–35. doi:10.1002/jgrc.20055.

[28] Methé B a, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, et al. The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci U S A 2005;102:10913–8. doi:10.1073/pnas.0504766102.

[29] Deming JW. Sea Ice Bacteria and Viruses. In: Thomas DN, Dieckmann GS, editors. Sea Ice An Introd. to Its Physics, Chemistry, Biology, Oxford, UK: Wiley-Blackwell; 2009, p. 247–82. doi:10.1002/9781444317145.

[30] Barber DG, Ehn JK, Pućko M, Rysgaard S, Deming JW, Bowman JS, et al. Frost flowers on young Arctic sea ice: The climatic, chemical, and microbial significance of an emerging ice type. J Geophys Res Atmos 2014;119:11,593–11,612. doi:10.1002/2014JD021736.

[31] Ewert M. Microbial Challenges and Solutions to Inhabiting the Dynamic Architecture of Saline Ice Formations.PhD thesis, University of Washington, Seattle, 2013.

[32] Bakermans C, Ayala-del-Río HL, Ponder M a, Vishnivetskaya T, Gilichinsky D, Thomashow MF, et al. *Psychrobacter cryohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. Int J Syst Evol Microbiol 2006;56:1285–91. doi:10.1099/ijs.0.64043-0.

[33] Bowman JP, Nichols DS, McKeekin TA. *Psychrobacter glacincolia* sp. nov., a halotolerant, psychrophilic bacterium isolated from antarctic sea ice. Syst Appl Microbiol 1997;20:209–15.

[34] Rodrigues DF, da C Jesus E, Ayala-Del-Río HL, Pellizari VH, Gilichinsky D, Sepulveda-Torres L, et al. Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*. ISME J 2009;3:658–65. doi:10.1038/ismej.2009.25.

[35] Fierz C, Armstrong RL, Durand Y, Etchevers P, Greene E, McClung DM, et al. The International Classification for Seasonnal Snow on the Ground. IHP-VII Te. UNESCO-IHP, Paris; 2009.

[36] Langlois A, Mundy CJ, Barber DG. On the winter evolution of snow thermophysical properties over land-fast first-year sea ice. Hydrological processes 2007;716:705–16. doi:10.1002/hyp.

[37] Collins RE, Deming JW. An inter-order horizontal gene transfer event enables the catabolism of compatible solutes by *Colwellia psychrerythraea* 34H. Extremophiles 2013;17:601–10. doi:10.1007/s00792-013-0543-7.

[38] Techel F, Pielmeier C. Point observations of liquid water content in wet snow - Investigating methodical, spatial and temporal aspects. Cryosphere 2011;5:405–18. doi:10.5194/tc-5-405-2011.

[39] Stibal M, Elster J, Sabacká M, Kastovská K. Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivali*s (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. FEMS Microbiol Ecol 2007;59:265–73. doi:10.1111/j.1574-6941.2006.00264.x.

[40] Sourjik V. Receptor clustering and signal processing in E. coli chemotaxis. Trends Microbiol 2004;12:569–76. doi:10.1016/j.tim.2004.10.003.

[41] Armitage JP. Bacterial tactic responses. Adv. Microb. Physiol., vol. 41, 1999, p. 229–89. doi:10.1016/S0065-2911(08)60168-X.

[42] Alexandre G, Greer-Phillips S, Zhulin IB. Ecological role of energy taxis in microorganisms. FEMS Microbiol Rev 2004;28:113–26. doi:10.1016/j.femsre.2003.10.003.

[43] Lacal J, García-Fontana C, Muñoz-Martínez F, Ramos J-L, Krell T. Sensing of environmental signals: classification of chemoreceptors according to the size of their ligand binding regions. Environ Microbiol 2010;12:2873–84. doi:10.1111/j.1462-2920.2010.02325.x.

[44] Kohls K, Abed RMM, Polerecky L, Weber M, de Beer D. Halotaxis of cyanobacteria in an intertidal hypersaline microbial mat. Environ Microbiol 2010;12:567–75. doi:10.1111/j.1462-2920.2009.02095.x.

[45] Takada Y, Hayashinaka N, Hagihara-Nukui E, Fukunaga N. Chemotaxis in a psychrophilic marine bacterium, *Vibrio* sp. strain ABE-1. J Gen Appl Microbiol 1993;39:371–9. doi:10.2323/jgam.39.371.

[46] Junge K, Eicken H, Deming JW. Motility of *Colwellia psychrerythraea* Strain 34H at Subzero Temperatures. Appl Environ Microbiol 2003;69:4282–4. doi:10.1128/AEM.69.7.4282-4284.2003.

[47] Li C, Boileau AJ, Kung C, Adler J. Osmotaxis in *Escherichia coli*. Proc Natl Acad Sci 1988;85:9451–5. doi:10.1073/pnas.85.24.9451.

[48] Hoham RW, Duval B. Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: Jones HG, Pomeroy J, Walker DA, Hoham R., editors. Snow Ecol., 2001.

[49] Gradinger R, Nurnberg D. Snow algal communities on Arctic pack ice floes dominated by Chlamydomonas nivalis (Bauer) Wille 1996:35–43.

[50] Remias D, Wastian H, Lütz C, Leya T. Insights into the biology and phylogeny of *Chloromonas polyptera* (Chlorophyta), an alga causing orange snow in Maritime Antarctica. Antarct Sci 2013;25:648–56. doi:10.1017/S0954102013000060.

[51] Lu N, Wei D, Jiang X-L, Chen F, Yang S-T. Regulation of lipid metabolism in the snow alga *Chlamydomonas nivalis* in response to NaCl stress: An integrated analysis by cytomic and lipidomic approaches. Process Biochem 2012;47:1163–70. doi:10.1016/j.procbio.2012.04.011.

[52] Lu N, Wei D, Jiang X-L, Chen F, Yang S-T. Fatty Acids Profiling and Biomarker Identification in Snow Alga *Chlamydomonas Nivali*s by NaCl Stress Using GC/MS and Multivariate Statistical Analysis. Anal Lett 2012;45:1172–83. doi:10.1080/00032719.2012.673094.

[53] Gorton HL, Vogelmann TC. Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. Photochem Photobiol 2003;77:608–15.

[54] Remias D, Lütz-Meindl U, Lütz C. Photosynthesis, pigments and ultrastructure of the alpine snow alga *Chlamydomonas nivalis*. Eur J Phycol 2005;40:259–68. doi:10.1080/09670260500202148.

[55] Rezanka T, Nedbalová L, Sigler K, Cepák V. Identification of astaxanthin diglucoside diesters from snow alga *Chlamydomonas nivalis* by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Phytochemistry 2008;69:479–90. doi:10.1016/j.phytochem.2007.06.025.

[56] Remias D, Albert a., Lütz C. Effects of realistically simulated, elevated UV irradiation on photosynthesis and pigment composition of the alpine snow alga *Chlamydomonas nivalis* and the arctic soil alga *Tetracystis* sp. (Chlorophyceae). Photosynthetica 2010;48:269–77. doi:10.1007/s11099-010-0033-4.

[57] Badania P. Ecology of snow algae 2 . General characteristics of snow algae 3 . Environmental factors with respect to the development of snow algae , adaptations , interrelationships . 1986:407–15.

[58] Jékely G. Evolution of phototaxis. Philos Trans R Soc Lond B Biol Sci 2009;364:2795–808. doi:10.1098/rstb.2009.0072.

[59] Hirschberg R, Stavis R. Phototaxis mutants of Phototaxis Mutants of *Chlamydomonas reinhardtii* 1977;129.

[60] Kröger P, Hegemann P. Photophobic responses and phototaxis in *Chlamydomonas* are triggered by a single rhodopsin photoreceptor. FEBS Lett 1994;341:5–9.

[61] Hou S-Y, Govorunova EG, Ntefidou M, Lane CE, Spudich EN, Sineshchekov OA, et al. Diversity of *Chlamydomonas* channelrhodopsins. Photochem Photobiol 2012;88:119–28. doi:10.1111/j.1751-1097.2011.01027.x.

[62] Okita N, Isogai N, Hirono M, Kamiya R, Yoshimura K. Phototactic activity in *Chlamydomonas* "non-phototactic" mutants deficient in Ca2+-dependent control of flagellar dominance or in inner-arm dynein. J Cell Sci 2005;118:529–37. doi:10.1242/jcs.01633.

[63] Halldal P. Factors Affecting Light Response in Phototactic Algae. Physiol Plant 1959;12:742–52. doi:10.1111/j.1399-3054.1959.tb08909.x.

[64] Nultsch W. Effect of external factors on phototaxis of *Chlamydomonas reinhardtii*. Arch Microbiol 1977;112:179–85. doi:10.1007/BF00429333.

[65] Buchheim MA, Buchheim JA, Carlson T, Kugrens P. phylogeny of lobocharacium (chlorophyceae) and allies: a study of 18s and 26S rDNA. J Phycol 2002;38:376–83. doi:10.1046/j.1529-8817.2002.01145.x.

[66] Okita N. Phototactic activity in *Chlamydomonas* "non-phototactic" mutants deficient in Ca2+-dependent control of flagellar dominance or in inner-arm dynein. J Cell Sci 2005;118:529–37. doi:10.1242/jcs.01633.

Concluding Remarks

While the snowpack appears to be a critical component of the cryosphere, it is disappearing. However, the perturbation of biological processes within the snowpack are rarely included in cryospheric climate change biological models, because snow as biological entity is largely underestimated. During the last few decades, snow microbial inhabitants have received an increasing interest and the view of snowpack as a biologically inert biosphere compartment has been challenged. However, our knowledge about the microbial community ecology within the snowpack remains limited and the purpose of this thesis was to determine whether the snow is a functional ecosystem with four major purposes addressed.

The first objective was to determine how variable is the snow microbial community and if this variability is related to changes in environmental conditions. Literature analyses in the first chapter revealed that as common to all frozen water environments, the snow microbial community is highly variable in terms of abundance and composition between different types of snowpack, from different location and even in the same place with depth and seasons. This variability was also observed in the results of studies presented in this thesis. Moreover, the correlation between microbial functions and environmental conditions; fluctuations along spring season in Chapter 2 and vertical distribution in Chapter 3, supports the hypothesis that microbial community is interacting with the fluctuating abiotic characteristics of their habitat. Preliminary results in the first section of Chapter 4 also showed that a strong variability exists between present and active microorganisms within snowpack. Although technical and conceptual issues remain, RNA-based high throughput sequencing is an encouraging tool to evaluate short-term responses of microbial communities to environmental fluctuations.

Despite high variability, if snow is an ecosystem, then the microbial community inhabiting it should have features related to their adaptation to the specific conditions of this environment. The second question of this thesis work addressed this concept. As described in literature (Chapter 1), specific microbial adaptations to stringent conditions of frozen habitat have been identified in microbes isolated from snow and ice, although some remain to be described for snow microbes. Comparison of snow metagenomes with other polar or non-polar environments in Chapter 2 supports the concept that the snow community is specific to the arctic snow environment. Although well-described cold adaptations are critical to life in the cold, other physical and chemical parameters, such as photochemical reactions are crucial to defining the Arctic snowpack microbial structure. At a more local scale in Chapter 3, the snowpack community is also unique and significantly different from connected environments. Snow microbial community specificity implicates a selective process. As in the atmosphere/snow/sea ice model depicted in Chapter 3, the snow microbial community is largely influenced by, but differs from its seeding sources in response to specific environmental conditions. Preliminary results in the first section of Chapter 4 also showed that a strong variability exists between present and actively reacting microorganisms is also high within snowpack.

The results presented from field campaigns include observations of microbial communities over time and space in relation to environmental conditions specific to the snow conditions at the time of observation. The monitored genomic and transcriptional response of snow microbial community to controlled external perturbation within snow microcosms will help to determine the dynamics of microorganisms within their fluctuating environment. Moreover C-labeled microcosms would help to decipher (or confirm) which part of the microbial community is metabolically active within the snowpack.

The development of mechanistic approaches described in the second section of Chapter 4 will help to determine colonization processes within snowpack. As reviewed in Chapter 1, few data are available to determine microbial interactions, such as succession, predation, competition and synergy within snowpack. Integration of complex community interactions would be a further step to understanding the snow microbial processes, slow individually but likely not negligible on a global scale.

Supplemental Figures and Tables

Chapter 1: Bibliography - frozen water habitats and inhabitants

Table S1: Molecular adaptation in cold-adapted bacteria (modified from Casanueva et al., 2010)

Adaptation	Role	Mechanisms
Membrane fluidity	Increase of membrane fluidity	Unsaturated fatty acid synthesis genes, desaturases, dioxygen lipid desaturases
Freeze-protection	Reduction of freezing point of cytoplasm and stabilisation of macromolecules	Genes for synthesis of compatible solutes, membrane transporters, antifreeze proteins and ice-binding proteins
Cold-shock and -acclimation response	Cellular response to lowering temperature	Signal transduction proteins, RNA-binding proteins and helicases, heat-shock proteins, compatible solutes synthesis proteins
Proteins activity	Maintain catalytic efficiency	Increased flexibility and activity decreased thermostability (see Figure S1)
Genome plasticity	Adaptation ability	Transposases, prophages, plasmids

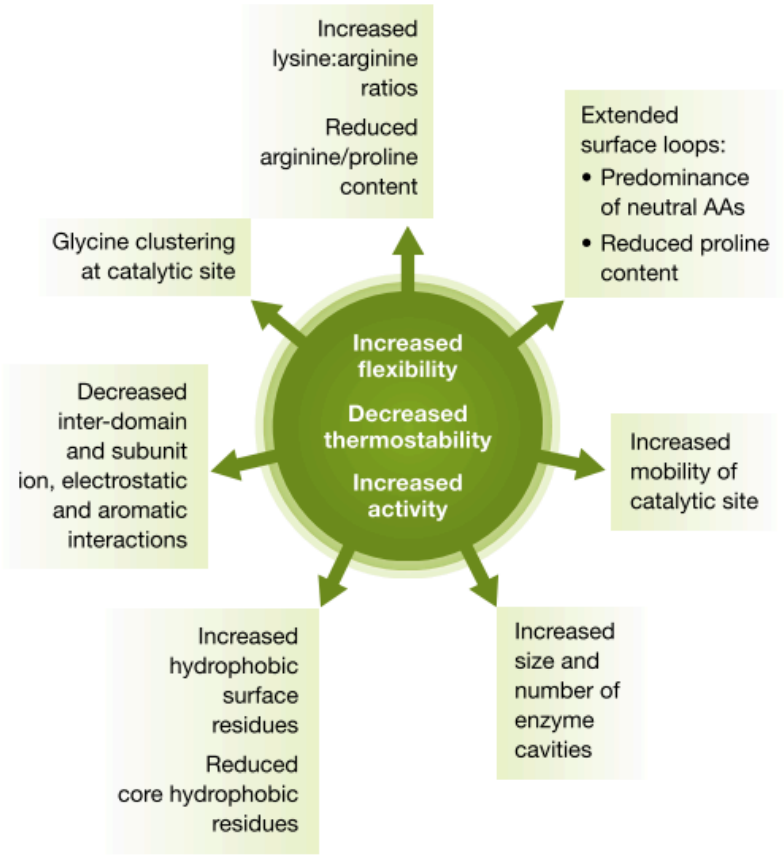

Figure S1: Common structural modifications of psychrophilic enzymes resulting in decreased thermostability, increased flexibility and increased specific activity from crystallographic and structural studies (from Maayer et al., 2014)

Table S1: Publically available metagenomic datasets used for environmental comparison, deposited in MG-RAST sofawre (Meyer et al., 2008)

Metagenome	Environment	Metagenome Name	bp Count	Sequence Count	Biome	References
4451073.3	snow	svn8	1.11E+07	2.91E+04	Snow	This Study
4451070.3	snow	svn7	1.49E+07	4.30E+04	Snow	This Study
4451071.3	snow	svn65	4.05E+06	1.22E+04	Snow	This Study
4451072.3	snow	svn64	6.86E+06	2.16E+04	Snow	This Study
4451069.3	snow	svn56	7.77E+06	2.55E+04	Snow	This Study
4451076.3	snow	svn40	8.04E+06	2.37E+04	Snow	This Study
4451065.3	snow	svn35	1.17E+07	3.48E+04	Snow	This Study
4451075.3	snow	svn18	1.10E+07	3.84E+04	Snow	This Study
4451068.3	snow	svn48	7.76E+06	2.43E+04	Snow	This Study
4446892.3	soil	RothE1	3.84E+08	1.05E+06	Grassland_Soil	(Delmont et al. 2011)
4446896.3	soil	RothE41	5.02E+08	1.23E+06	Grassland_Soil	(Delmont et al. 2011)
4446895.3	soil	RothE4B	3.62E+08	1.02E+06	Grassland_Soil	(Delmont et al. 2011)
4446894.3	soil	RothE4A	5.19E+08	1.22E+06	Grassland_Soil	(Delmont et al. 2011)
4446902.3	soil	RothF47	5.51E+08	1.24E+06	Grassland_Soil	(Delmont et al. 2011)
4446903.3	soil	RothF48	4.36E+08	1.06E+06	Grassland_Soil	(Delmont et al. 2011)
4446904.3	soil	RothL01	3.50E+08	8.60E+05	Grassland_Soil	(Delmont et al. 2011)
4446153.3	soil	Puerto_Rico_Soil	3.22E+08	7.82E+05	Forest_Soil	(DeAngelis et al. 2011)
4443697.3	open_ocean	open_ocean_P1	6.81E+07	2.93E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4443725.3	open_ocean	open_ocean_NA1	5.90E+07	2.57E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4443729.3	open_ocean	open_ocean_NA2	6.74E+07	2.89E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4443713.3	coast_ocean	coast_ocean_MB1	5.30E+07	2.22E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4443714.3	coast_ocean	coast_ocean_MB2	4.50E+07	1.89E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4443716.3	coast_ocean	coast_ocean_MB3	5.30E+07	2.23E+05	Marine_Habitat	MgRast (Meyer et al. 2008)
4445126.3	Polar_Microbial_Mat	Polar_Microbial_Mat_MIS	5.34E+07	2.57E+05	Polar Micorboial Mats	(Varin et al. 2012b)
4445129.3	Polar_Microbial_Mat	Polar_Microbial_Mat_WHI	6.17E+07	3.36E+05	Polar Micorboial Mats	(Varin et al. 2012b)
4445845.3	Polar_Microbial_Mat	Polar_Microbial_Mat_MM	3.10E+07	8.33E+04	Polar Micorboial Mats	(Varin et al. 2012b)

Table S2: Functions more represented in snow and polar mat ecosystems than in other ecosystems (level 2)

level 1	level 2	p-values	Polar Microbial Mat	coast ocean	open ocean	soil	snow
Regulation and Cell signaling	Two-component regulatory systems in Campylobacter	2.77E-06	0.120	0.013	0.009	0.078	0.174
Phages, Prophages, Transposable elements, Plasmids	Transposable elements	8.33E-04	0.159	0.052	0.050	0.133	0.198
Clustering-based subsystems	Biosynthesis of galactoglycans and related lipopolysacharides	1.26E-03	0.606	0.161	0.211	0.538	0.762
Regulation and Cell signaling	Regulation of virulence	1.29E-03	0.188	0.182	0.146	0.150	0.307
Cofactors, Vitamins, Prosthetic Groups, Pigments	NAD and NADP	1.72E-03	0.507	0.424	0.467	0.490	0.745
Iron acquisition and metabolism	Iron acquisition in Vibrio	2.29E-03	0.279	0.294	0.229	0.191	0.679
Membrane Transport	Transport of Manganese	2.66E-03	0.074	0.041	0.035	0.080	0.089
Clustering-based subsystems	CBSS-176280.1.peg.1561	5.00E-03	0.107	0.072	0.047	0.094	0.148
Clustering-based subsystems	Shikimate kinase containing cluster	1.41E-02	0.036	0.046	0.046	0.032	0.078
Clustering-based subsystems	CBSS-235.1.peg.567	2.13E-02	0.303	0.314	0.270	0.215	0.331
Clustering-based subsystems	CBSS-288681.3.peg.1039	2.57E-02	0.062	0.003	0.005	0.028	0.072
Clustering-based subsystems	CBSS-316273.3.peg.2709	2.57E-02	0.062	0.003	0.005	0.028	0.072
Carbohydrates	Monosaccharides	2.62E-02	1.089	0.825	0.830	1.307	1.384
Respiration	Soluble cytochromes and functionally related electron carriers	3.28E-02	0.197	0.094	0.121	0.165	0.264
Regulation and Cell signaling	Programmed Cell Death and Toxin-antitoxin Systems	3.84E-02	0.107	0.015	0.013	0.067	0.157
Carbohydrates	Aminosugars	3.88E-02	0.172	0.103	0.090	0.180	0.239
Clustering-based subsystems	Staphylococcus aureus hypothetical repetitive gene loci	3.91E-02	0.069	0.051	0.053	0.067	0.106
Potassium metabolism	Potassium homeostasis	3.93E-02	0.257	0.182	0.137	0.373	0.403
Fatty Acids, Lipids, and Isoprenoids	Phospholipids	4.92E-02	0.475	0.413	0.437	0.454	0.579
Carbohydrates	Glycoside hydrolases	4.93E-02	0.036	0.014	0.011	0.043	0.065
Stress Response	Bacterial hemoglobins	5.64E-02	0.054	0.004	0.003	0.047	0.114
Iron acquisition and metabolism	Transport of Iron	6.61E-02	0.091	0.118	0.131	0.071	0.144
Cell Wall and Capsule	Gram-Positive cell wall components	7.18E-02	0.094	0.069	0.076	0.106	0.206
Iron acquisition and metabolism	Iron(III) dicitrate transport system Fec	7.49E-02	0.006	0.015	0.002	0.002	0.027
Membrane Transport	Ton and Tol transport systems	8.09E-02	0.364	0.381	0.283	0.307	0.633
Stress Response	SigmaB stress responce regulation	1.43E-01	0.046	0.004	0.010	0.044	0.053
Clustering-based subsystems	Spore Coat	1.44E-01	0.016	0.007	0.007	0.023	0.052
Potassium metabolism	Glutathione-regulated potassium-efflux system and associated functions	1.51E-01	0.054	0.029	0.024	0.030	0.067
Sulfur Metabolism	Inorganic sulfur assimilation	1.59E-01	0.384	0.270	0.259	0.364	0.430
Cofactors, Vitamins, Prosthetic Groups, Pigments	Coenzyme M	1.71E-01	0.008	0.014	0.015	0.011	0.025
Regulation and Cell signaling	Proteolytic pathway	1.85E-01	0.001	0.000	0.003	0.006	0.053
Photosynthesis	Electron transport and photophosphorylation	1.98E-01	0.244	0.030	0.199	0.002	0.306

Clustering-based subsystems	CBSS-316273.3.peg.2378	1.45E-05	0.046	0.004	0.004	0.005	0.008
DNA Metabolism	Type I Restriction-Modification	9.56E-05	0.356	0.032	0.026	0.102	0.245
DNA Metabolism	Restriction-Modification System	1.11E-04	0.391	0.035	0.035	0.113	0.272
Photosynthesis	Light-harvesting complexes	4.38E-04	0.109	0.003	0.026	0.000	0.028
Cell Division and Cell Cycle	Cyanobacterial Circadian Clock	1.05E-03	0.129	0.003	0.037	0.032	0.127
Clustering-based subsystems	CBSS-196620.1.peg.2477	1.33E-03	0.291	0.103	0.045	0.176	0.215
Iron acquisition and metabolism	Campylobacter Iron Metabolism	2.17E-02	0.121	0.109	0.099	0.062	0.115
Nitrogen Metabolism	Nitrate and nitrite ammonification	2.62E-02	0.180	0.022	0.014	0.135	0.162
Clustering-based subsystems	LMPTP YfkJ cluster	5.04E-02	0.042	0.011	0.005	0.035	0.040
Stress Response	Acid stress	5.72E-02	0.066	0.009	0.021	0.044	0.059
Regulation and Cell signaling	Oxygen and light sensor PpaA-PpsR	6.32E-02	0.059	0.006	0.008	0.019	0.040
Amino Acids and Derivatives	Arginine; urea cycle, polyamines	7.53E-02	1.537	0.973	1.055	1.219	1.308
Clustering-based subsystems	CRISPRs and associated hypotheticals	9.52E-02	0.021	0.000	0.000	0.008	0.012
Clustering-based subsystems	CBSS-393131.3.peg.612	1.17E-01	0.026	0.005	0.003	0.013	0.022
DNA Metabolism	CRISPs	1.20E-01	0.051	0.001	0.001	0.020	0.039
Clustering-based subsystems	Possible Ammonia conversion cluster	1.39E-01	0.091	0.073	0.080	0.050	0.089

Table S3: Relative abundance of annotated reads among known cold adaptation related mechanisms, given as mean relative frequency (%). P-values are calculated with Anova analysis between cryospheric environments (Polar Microbial Mat and Snow) and mesophilic (Soil, Open ocean and Coastal ocean). Parenthetic values are standard deviation

Cold adptation related subsystems	p-values	Cryospheric		Mesophylic		Polar mat	coast ocean	open ocean	snow	soil
AceE (pyruvate dehydrogenase E1 component)	1.01E-02	(9.66)	6.71	15.02	(1.50)	14.06	14.74	14.24	8.19	15.41
AceF (dihydrolipoamide acetyltransferase)	4.82E-01	(6.95)	4.70	5.97	(1.38)	6.25	5.72	4.44	7.19	6.64
Chaperone DnaK and DnaJ	3.16E-01	(6.20)	2.81	5.35	(0.86)	7.68	4.24	6.09	5.70	5.50
Choline and betaine uptake, betaine biosynthesis	9.16E-01	(8.57)	16.92	8.05	(4.59)	3.34	13.93	12.47	10.31	4.19
DnaA (replication initiator protein)	6.97E-01	(3.97)	2.86	4.30	(0.71)	3.60	4.44	3.58	4.09	4.51
Exopolysaccharide biosynthesis	3.21E-01	(0.73)	2.20	0.11	(0.11)	0.24	0.00	0.12	0.89	0.14
Fatty acid desaturases	4.66E-02	(3.34)	2.56	1.78	(0.71)	2.87	0.85	1.15	3.50	2.37
Glutamate biosynthesis	2.62E-01	(24.28)	10.10	27.67	(3.00)	26.81	23.68	26.83	23.44	29.48
Glycine biosynthesis	8.93E-02	(7.02)	4.29	4.87	(0.79)	4.12	4.84	3.66	7.99	5.34
GyrA (DNA gyrase A)	3.72E-01	(9.63)	7.63	11.60	(1.46)	14.66	12.38	12.92	7.96	10.82
HU-β (DNA supercoiling)	2.48E-01	(0.55)	0.96	0.23	(0.14)	0.18	0.16	0.33	0.68	0.22
OstA (trehalose phosphate synthase)	4.31E-01	(0.68)	0.88	0.94	(0.74)	0.83	0.05	0.23	0.62	1.54
Peptidyl-prolyl cis-trans isomerase	2.15E-01	(0.85)	0.94	0.51	(0.22)	1.17	0.45	0.34	0.74	0.59
Purine nucleoside phosphorylase (PNP)	1.11E-01	(1.34)	1.74	0.53	(0.24)	0.68	0.57	0.16	1.56	0.65
RecA (recombination factor A)	6.57E-01	(12.98)	13.13	11.33	(1.47)	10.36	11.64	11.78	13.86	11.05
tRNA dihydrouridine synthase	6.01E-02	(3.24)	2.69	1.74	(0.47)	3.14	2.31	1.66	3.28	1.55

Table S1: Approximation of the number of cells per bacterial taxon. The number of bacterial cells potentially affiliated to a taxon was approximate using the percentage of reads associated with the taxon and the potential number of bacterial cell sequenced (cells concentration, as determined by microscopy, multiplied by volume of filtration for each sample). The highest value are shown in red whereas the lowest are in green. Unclassified means that reads were not annotated at a deeper taxonomic level.

NB: This analysis relies on three major assumptions:

1. The genome size is of the same order for all bacterial cells, as is the DNA extraction efficiency
2. 1 ng used for sequencing is representative of the entire community
3. The probability of annotation is the same for each taxon.

	SL0		SL1		SL2		SL3		BR		SW	
	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd
Alteromonadales	1.4E+05	7.3E+04	5.7E+04	1.1E+04	3.7E+05	1.4E+05	3.8E+07	1.7E+06	4.6E+06	2.9E+06	1.3E+06	3.0E+04
Rhizobiales	2.7E+05	8.0E+04	2.2E+05	8.4E+04	1.3E+05	3.1E+04	6.7E+05	8.4E+04	9.6E+05	1.0E+05	1.3E+06	2.4E+05
Actinomycetales	1.3E+05	6.6E+04	1.2E+05	6.7E+04	1.4E+05	5.2E+04	6.3E+05	9.3E+04	8.2E+06	1.3E+06	1.4E+06	2.0E+05
Acidobacteriales	4.4E+04	4.3E+03	1.0E+05	6.9E+04	1.0E+05	3.6E+04	5.4E+05	3.4E+04	1.4E+04	1.5E+03	3.7E+04	1.4E+03
Oceanospirillales	1.1E+04	5.1E+03	9.2E+03	5.3E+03	5.6E+03	3.1E+03	1.0E+06	1.6E+05	1.5E+06	1.0E+06	7.7E+05	2.2E+04
Nostocales	4.9E+04	2.6E+04	8.1E+04	8.0E+04	5.3E+04	5.6E+04	7.0E+04	2.7E+04	1.5E+04	1.2E+03	3.0E+04	1.9E+03
Rhodobacterales	4.4E+04	6.0E+03	3.4E+04	2.1E+04	1.3E+04	3.9E+03	6.0E+05	8.7E+04	2.3E+07	2.8E+06	9.2E+06	8.2E+05
Rhodospirillales	2.1E+04	6.2E+03	2.2E+04	1.2E+04	3.4E+04	1.3E+04	3.1E+05	6.4E+04	1.4E+05	1.5E+04	9.4E+05	8.8E+04
Ktedonobacterales	2.2E+04	1.5E+03	2.6E+04	1.4E+04	4.4E+04	1.0E+04	2.1E+05	5.6E+03	3.0E+03	1.5E+03	7.1E+03	2.6E+03
Sphingobacteriales	1.8E+04	3.7E+03	3.5E+04	1.6E+04	4.2E+04	1.5E+04	5.2E+04	9.6E+03	1.2E+05	1.1E+04	3.9E+05	2.1E+04
Cytophagales	1.5E+04	3.9E+03	2.4E+04	8.3E+03	3.3E+04	7.9E+03	1.2E+05	8.5E+03	1.4E+05	1.9E+04	5.3E+05	1.6E+04
Burkholderiales	1.4E+04	3.3E+03	2.0E+04	1.0E+04	2.6E+04	8.9E+03	2.0E+05	4.3E+04	1.5E+06	1.4E+05	4.1E+05	2.3E+04
Oscillatoriales	9.0E+03	3.1E+03	2.7E+04	2.6E+04	4.9E+04	6.4E+04	3.4E+04	1.1E+04	2.7E+04	3.4E+03	5.4E+04	5.2E+03
Pseudomonadales	1.9E+04	1.4E+04	4.0E+04	2.5E+04	9.3E+03	5.9E+03	9.5E+04	1.7E+04	6.2E+05	7.0E+04	5.5E+05	2.5E+04
Sphingomonadales	1.3E+04	7.7E+03	1.8E+04	7.2E+03	2.4E+04	1.0E+04	1.4E+05	3.1E+04	1.3E+05	4.4E+04	1.6E+05	8.4E+03
unclassified Gammaproteobacteria	3.1E+04	2.4E+04	1.1E+04	4.9E+03	4.5E+03	3.7E+02	1.7E+05	2.7E+04	6.5E+06	8.7E+05	1.2E+07	8.1E+05
Flavobacteriales	2.2E+04	5.9E+03	1.9E+04	7.1E+03	7.3E+03	1.5E+03	1.1E+05	1.4E+04	4.0E+06	6.4E+05	4.2E+06	1.8E+05
Planctomycetales	1.1E+04	3.2E+03	1.4E+04	8.1E+03	1.7E+04	6.8E+03	1.1E+05	1.3E+04	1.0E+05	2.6E+04	2.4E+06	2.5E+05
unclassified Alphaproteobacteria	3.5E+04	2.3E+04	1.6E+04	6.1E+03	4.4E+03	1.3E+03	2.3E+04	1.4E+03	2.3E+06	6.4E+05	1.6E+07	1.0E+06
unclassified Betaproteobacteria	9.5E+03	2.7E+03	1.6E+04	1.5E+04	3.9E+03	1.4E+03	1.2E+05	6.2E+03	1.6E+07	9.1E+05	1.1E+05	2.0E+04

Table S2: Functions more abundant in snow (Level 3 of SEED database annotations; relative abundance of sequences associated with each functions in percentage of total annotated sequences; functions organized in decreasing order of abundance)

L3	p-value	ATM	SL0	SL1	SL2	SL3	BR	SW
Protein_chaperones	1.92E-07	0.51	0.77	0.89	0.82	0.76	0.76	0.71
Coenzyme_B12_biosynthesis	6.93E-07	0.56	0.81	0.71	0.51	0.21	0.50	0.50
Oxidative_stress	5.66E-08	0.43	0.52	0.81	0.59	0.39	0.35	0.22
Maltose_and_Maltodextrin_Utilization	9.49E-09	0.29	0.68	0.64	0.57	0.43	0.17	0.09
DNA_Repair_Base_Excision	2.32E-02	0.54	0.57	0.57	0.71	0.49	0.52	0.56
linker_unit-arabinogalactan_synthesis	1.98E-20	0.47	0.44	0.64	0.55	0.18	0.35	0.35
Pentose_phosphate_pathway	2.08E-02	0.33	0.62	0.52	0.50	0.52	0.50	0.55
Rhamnose_containing_glycans	8.23E-19	0.43	0.42	0.66	0.54	0.20	0.37	0.37
Glycogen_metabolism	1.07E-14	0.32	0.52	0.59	0.49	0.41	0.12	0.04
Trehalose_Biosynthesis	4.11E-13	0.29	0.41	0.57	0.53	0.20	0.07	0.02
NAD_and_NADP_cofactor_biosynthesis_global	3.20E-04	0.33	0.59	0.40	0.43	0.33	0.38	0.36
Respiratory_dehydrogenases_1	1.56E-03	0.17	0.54	0.38	0.40	0.28	0.27	0.26
CO2_uptake,_carboxysome	3.00E-04	0.13	0.50	0.52	0.25	0.05	0.07	0.07
Cobalamin_synthesis	8.35E-05	0.35	0.47	0.47	0.32	0.15	0.29	0.30
Ubiquinone_Biosynthesis	6.71E-03	0.23	0.53	0.32	0.34	0.26	0.31	0.40
dTDP-rhamnose_synthesis	1.96E-14	0.26	0.25	0.47	0.36	0.08	0.25	0.24
Carotenoids	6.32E-08	0.19	0.28	0.38	0.35	0.06	0.11	0.14
Carboxysome	1.64E-04	0.16	0.29	0.38	0.29	0.13	0.07	0.06
Urea_decomposition	2.40E-07	0.25	0.34	0.32	0.30	0.09	0.46	0.13
Retron-type reverse transcriptase	8.79E-06	0.13	0.29	0.40	0.26	0.14	0.08	0.05
cAMP-binding proteins - catabolite gene activator and regulatory subunit of cAMP-dependent protein kinases	2.30E-03	0.16	0.44	0.21	0.15	0.02	0.02	0.00
Chlorophyll_Biosynthesis	2.83E-07	0.04	0.17	0.29	0.29	0.10	0.04	0.01
LOS_core_oligosaccharide_biosynthesis	2.61E-02	0.20	0.19	0.23	0.28	0.21	0.13	0.13
Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)	1.55E-05	0.12	0.26	0.18	0.22	0.22	0.05	0.00
Aromatic_amino_acid_degradation	2.69E-04	0.11	0.26	0.22	0.16	0.11	0.17	0.12
NAD_regulation	3.03E-02	0.17	0.31	0.12	0.21	0.18	0.17	0.16
Circadian input kinase A	6.02E-06	0.01	0.15	0.27	0.18	0.04	0.02	0.00
Capsular_heptose_biosynthesis	3.49E-02	0.08	0.27	0.12	0.19	0.06	0.11	0.11
Xylose_utilization	1.48E-07	0.07	0.13	0.21	0.21	0.06	0.13	0.07
Chromosome partition protein smc	6.42E-04	0.04	0.23	0.19	0.10	0.05	0.14	0.17
Colanic_acid_biosynthesis	4.27E-03	0.05	0.26	0.12	0.14	0.07	0.06	0.05
Lipid_A-Ara4N_pathway_(_Polymyxin_resistance_)	7.90E-06	0.15	0.19	0.13	0.19	0.14	0.10	0.12
Chitin_and_N-acetylglucosamine_utilization	1.59E-04	0.07	0.17	0.13	0.19	0.06	0.13	0.10
Selenocysteine_metabolism	3.64E-02	0.03	0.32	0.07	0.09	0.16	0.08	0.08
Predicted_carbohydrate_hydrolases	3.95E-03	0.08	0.10	0.16	0.17	0.06	0.08	0.06
Glutathione:_Redox_cycle	8.31E-03	0.05	0.17	0.14	0.09	0.13	0.08	0.07
Protocatechuate_branch_of_beta-ketoadipate_pathway	5.94E-03	0.07	0.11	0.15	0.14	0.13	0.17	0.08
Teichuronic_acid_biosynthesis	2.56E-03	0.08	0.14	0.11	0.13	0.10	0.08	0.08
Signal recognition particle, subunit Ffh SRP54 (TC 3.A.5.1.1)	5.58E-04	0.10	0.26	0.06	0.04	0.08	0.09	0.12

Table S3: Summary of functions examined for osmotic and photochemical stress response (from SEED database or specific gene database marked with *)

	Enzymes / Genes
Photochemical stress response	
Enzymatic response to oxidative stress	Catalase - Superoxide dismutase - Rubredoxin - Glutaredoxin
DNA repair	Exonuclease - Endonuclease - Base excision repair -UVrABC system -
Non-enzymatic sunscreens and antioxidants (biosynthesis)	Carotenoids - Glutathione - Tocopherol Scytonemin* - Melanin* - MAA* - Manitol*
Alternative pathway	Fumarase - Aconitase - Cytochrome C oxidase
Osmotic stress response	
Compatible solutes (biosynthesis - uptake)	Sarcosine oxidase - Choline dehydrogenase - High affinity choline uptake protein BetT - Betaine aldehyde dehydrogenase - Choline sulfatase - L-ectoine synthase
Antiporter	Na+/H+-dicarboxylate symporters - Na+/H+ antiporter

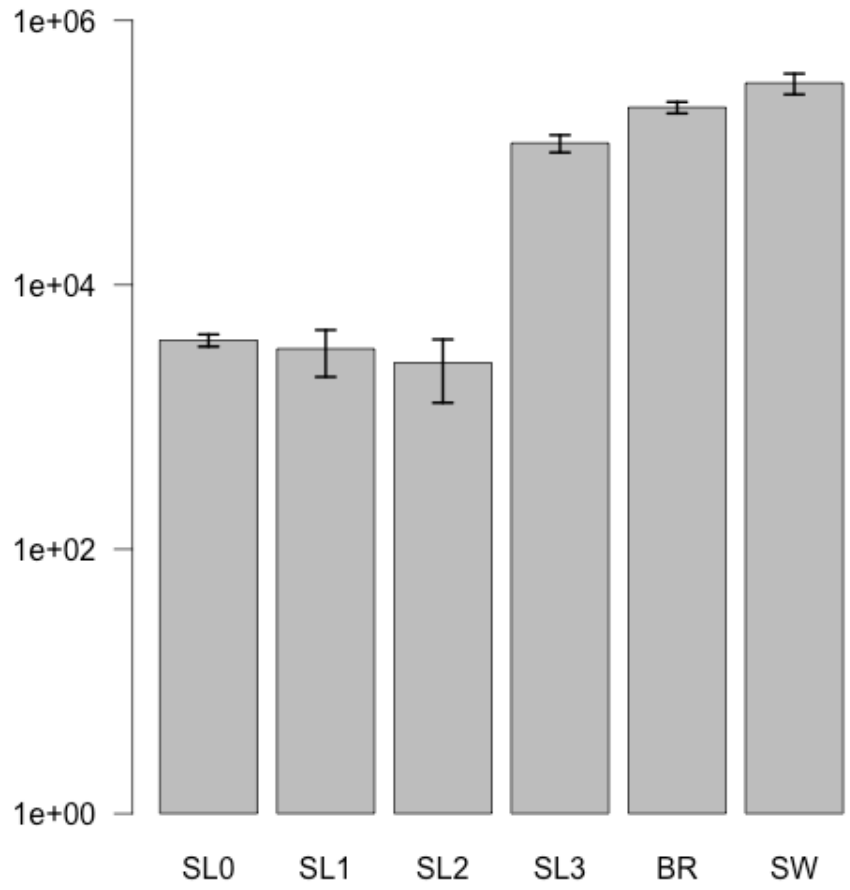

Figure S1: Microbial cells count in the different layers of the sea ice gradient (Microscopy - DAPI staining) as a number of cells ml-1. Counts for snow samples (SL0, SL1, SL2 and SL3) are scaled to millimeter of melted snow

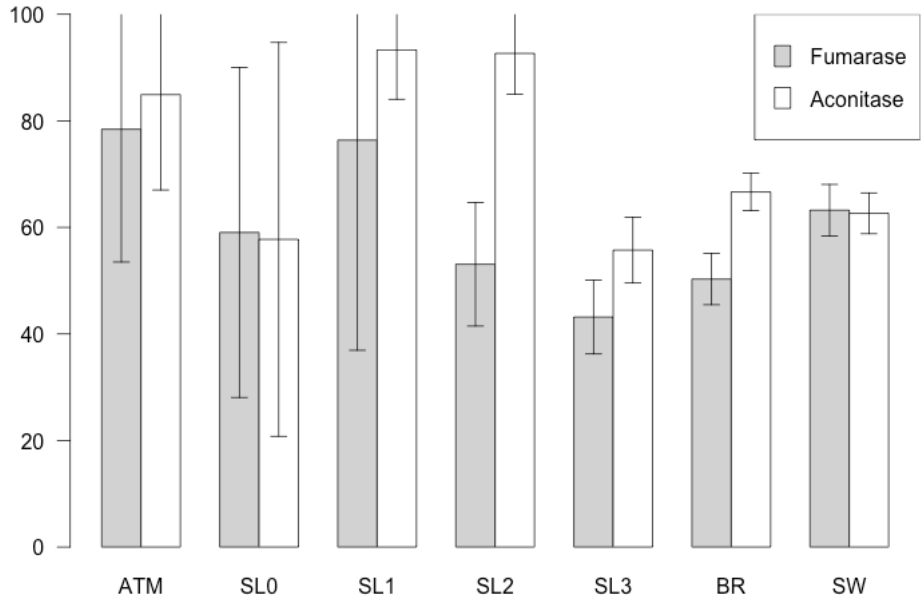

Figure S2: Oxidative resistant isozymes Fumarase CII (EC 4.2.1.2) and Aconitase A (EC 4.2.1.3). Proportion of reads associated with oxidative resistant isoform of enzymes as a percentage of to reads associated with all isoenzymes (in percentage).

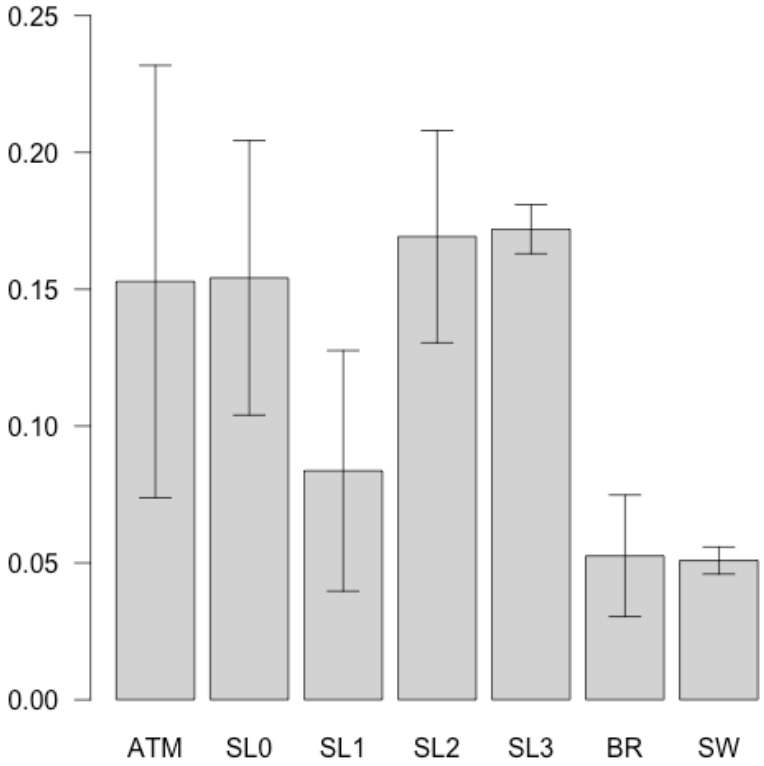

Figure S3: Relative abundance (percentage) of reads associated with genes coding Antiporters (Na+/H+-dicarboxylate symporters, Formate hydrogenlyase subunit 3/Multisubunit Na+/H+ antiporter, MnhD subunit, Na+/H+ antiporter, Na+/H+ antiporter NhaB) (in percentage of all annotated sequences).

Chapter 4: Assessing the activity of snowpack microbial communities

Section 1: The use of meta-omics to explore the active part of snow microbial communities

Table S1: Homemade RNA later like buffer recipe (from De Wit et al., 2012)- The Simple Fool's Guide to Population Genomics via RNA-Seq: An Introduction to High-Throughput Sequencing Data Analysis

	For 1.5 Liters (adjust pH to 5.2)
Autoclaved MilliQ water	935 ml
Ammonium Sulfate (stir until dissolved)	700 g
1M Sodium Citrate	25 ml
0.5 M EDTA	40 ml

Bibliography

Achberger, A. M., Brox, T. I., Skidmore, M. L., and Christner, B. C. (2011). Expression and partial characterization of an ice-binding protein from a bacterium isolated at a depth of 3,519 m in the Vostok ice core, Antarctica. *Front. Microbiol.* 2, 1–8. doi:10.3389/fmicb.2011.00255.

Alexandre, G., Greer-Phillips, S., and Zhulin, I. B. (2004). Ecological role of energy taxis in microorganisms. *FEMS Microbiol. Rev.* 28, 113–26. doi:10.1016/j.femsre.2003.10.003.

Allewell, N. M., and Sama, A. (1974). The effect of ammonium sulfate on the activity of ribonuclease A. *Biochim. Biophys. Acta - Enzymol.* 341, 484–488. doi:10.1016/0005-2744(74)90240-X.

Alonso-Sáez, L., Sánchez, O., Gasol, J. M., Balagué, V., and Pedrós-Alio, C. (2008). Winter-to-summer changes in the composition and single-cell activity of near-surface Arctic prokaryotes. *Environ. Microbiol.* 10, 2444–54. doi:10.1111/j.1462-2920.2008.01674.x.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.

Alvarez-Martinez, C. E., Lourenço, R. F., Baldini, R. L., Laub, M. T., and Gomes, S. L. (2007). The ECF sigma factor sigma(T) is involved in osmotic and oxidative stress responses in *Caulobacter crescentus*. *Mol. Microbiol.* 66, 1240–55. doi:10.1111/j.1365-2958.2007.06005.x.

Amato, P., Hennebelle, R., Magand, O., Sancelme, M., Delort, A.-M., Barbante, C., Boutron, C., and Ferrari, C. (2007). Bacterial characterization of the snow cover at Spitzberg, Svalbard. *FEMS Microbiol. Ecol.* 59, 255–64. doi:10.1111/j.1574-6941.2006.00198.x.

Amoroso, a, Domine, F., Esposito, G., Morin, S., Savarino, J., Nardino, M., Montagnoli, M., Bonneville, J.-M., Clement, J.-C., Ianniello, a, et al. (2010). Microorganisms in dry polar snow are involved in the exchanges of reactive nitrogen species with the atmosphere. *Environ. Sci. Technol.* 44, 714–9. doi:10.1021/es9027309.

Anastasio, C., Hoffmann, M., Klán, P., and Sodeau, J. (2013). "Photochemistry in Terrestrial Ices," in *The Science of Solar System Ices* Astrophysics and Space Science Library., eds. M. S. Gudipati and J. Castillo-Rogez (New York, NY: Springer New York). doi:10.1007/978-1-4614-3076-6.

Andresen, P. A., Kaasen, I., Styrvold, O. B., Boulnois, G., and Strøm, A. R. (1988). Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. *J. Gen. Microbiol.* 134, 1737–46.

Anesio, A. M., and Bellas, C. M. (2011). Are low temperature habitats hot spots of microbial evolution driven by viruses? *Trends Microbiol.* 19, 52–7. doi:10.1016/j.tim.2010.11.002.

Anesio, A. M., Hodson, A. J., Fritz, A., Psenner, R., and Sattler, B. (2009). High microbial activity on glaciers: importance to the global carbon cycle. *Glob. Chang. Biol.* 15, 955–960. doi:10.1111/j.1365-2486.2008.01758.x.

Anesio, A. M., and Laybourn-Parry, J. (2012). Glaciers and ice sheets as a biome. *Trends Ecol. Evol.* 27, 219–25. doi:10.1016/j.tree.2011.09.012.

Anesio, A. M., Mindl, B., Laybourn-Parry, J., Hodson, A. J., and Sattler, B. (2007). Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). *J. Geophys. Res.* 112, G04S31. doi:10.1029/2006JG000350.

Antoniades, D., Veillette, J., Martineau, M.-J., Belzile, C., Tomkins, J., Pienitz, R., Lamoureux, S., and Vincent, W. F. (2009). Bacterial dominance of phototrophic communities in a High Arctic lake and its implications for paleoclimate analysis. *Polar Sci.* 3, 147–161. doi:10.1016/j.polar.2009.05.002.

Antony, R., Mahalinganathan, K., Krishnan, K. P., and Thamban, M. (2012). Microbial preference for different size classes of organic carbon: a study from Antarctic snow. *Environ. Monit. Assess.* 184, 5929–43. doi:10.1007/s10661-011-2391-1.

Armitage, J. P. (1999). "Bacterial Tactic Responses," in *Advances in Microbial Physiology*, 229–289. doi:10.1016/S0065-2911(08)60168-X.

Arrigo, K. R. (2014). Sea ice ecosystems. *Ann. Rev. Mar. Sci.* 6, 439–67. doi:10.1146/annurev-marine-010213-135103.

Aslam, S. N., Cresswell-Maynard, T., Thomas, D. N., and Underwood, G. J. C. (2012). Production and Characterization of the Intra- and Extracellular Carbohydrates and Polymeric Substances (EPS) of Three Sea ice Diatom Species, and Evidence for a Cryoprotective Role for EPS. *J. Phycol.* 48, 1494–1509. doi:10.1111/jpy.12004.

Azevedo, J. S. N., Correia, A., and Henriques, S. (2013). Molecular analysis of the diversity of genus Psychrobacter present within a temperate estuary. *FEMS Microbiol. Ecol.* 84, 451–460.

Bachy, C., López-García, P., Vereshchaka, A., and Moreira, D. (2011a). Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the North Pole at the end of the polar night. *Front. Microbiol.* 2, 1–12. doi:10.3389/fmicb.2011.00106.

Bachy, C., López-García, P., Vereshchaka, A., and Moreira, D. (2011b). Diversity and vertical distribution of microbial eukaryotes in the snow, sea ice and seawater near the north pole at the end of the polar night. *Front. Microbiol.* 2, 106. doi:10.3389/fmicb.2011.00106.

Badania, P. (1986). Ecology of snow algae 2 . General characteristics of snow algae 3 . Environmental factors with respect to the development of snow algae , adaptations , interrelationships . 407–415.

Bakermans, C., Ayala-del-Río, H. L., Ponder, M. a, Vishnivetskaya, T., Gilichinsky, D., Thomashow, M. F., and Tiedje, J. M. (2006). *Psychrobacter cryohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. *Int. J. Syst. Evol. Microbiol.* 56, 1285–91. doi:10.1099/ijs.0.64043-0.

Ball, M. M., Gómez, W., Magallanes, X., Rosales, R., Melfo, A., and Yarzábal, L. A. (2014). Bacteria recovered from a high-altitude, tropical glacier in Venezuelan Andes. *World J. Microbiol. Biotechnol.* 30, 931–941. doi:10.1007/s11274-013-1511-1.

Balskus, E. P., and Walsh, C. T. (2010). The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science* 329, 1653–6. doi:10.1126/science.1193637.

Bano, N., and Hollibaugh, J. T. (2002). Phylogenetic Composition of Bacterioplankton Assemblages from the Arctic Ocean. *Appl. Environ. Microbiol.* 68, 505–518. doi:10.1128/AEM.68.2.505-518.2002.

Barber, D. G., Ehn, J. K., Pućko, M., Rysgaard, S., Deming, J. W., Bowman, J. S., Papakyriakou, T., Galley, R. J., and Søgaard, D. H. (2014). Frost flowers on young Arctic sea ice: The climatic, chemical, and microbial significance of an emerging ice type. *J. Geophys. Res. Atmos.* 119, 11,593–11,612. doi:10.1002/2014JD021736.

Barletta, R. E., Priscu, J. C., Mader, H. M., Jones, W. L., and Roe, C. H. (2012). Chemical analysis of ice vein microenvironments: II. Analysis of glacial samples from Greenland and Antarctica. *J. Glaciol.* 58, 1109–1118. doi:10.3189/2012JoG12J112.

Barrie, L. A. (1991). “Snow Formation and Processes in the Atmosphere that Influence its Chemical Composition,” in *Seasonal Snowpacks SE - 1* NATO ASI Series., eds. T. D. Davies, M. Tranter, and H. G. Jones (Springer Berlin Heidelberg), 1–20. doi:10.1007/978-3-642-75112-7_1.

Barry, R. G., Jania, J., and Birkenmajer, K. (2011). Review article “A. B. Dobrowolski – the first cryospheric scientist – and the subsequent development of cryospheric science.” *Hist. Geo. Space Sci.* 2, 75–79. doi:10.5194/hgss-2-75-2011.

Barsdate, R. J., and Alexander, V. (1970). Photosynthetic Organisms in Subarctic Lake Ice. *ARCTIC* 23, 201. doi:10.14430/arctic3174.

Bauer, H., Kasper-giebl, A., and Lo, M. (2002). The contribution of bacteria and fungal spores to the organic carbon content of cloud water , precipitation and aerosols. 64, 109–119.

Bellas, C. M., and Anesio, A. M. (2013). High diversity and potential origins of T4-type bacteriophages on the surface of Arctic glaciers. *Extremophiles* 17, 861–70. doi:10.1007/s00792-013-0569-x.

Bellas, C. M., Anesio, A. M., Telling, J., Stibal, M., Tranter, M., and Davis, S. (2013). Viral impacts on bacterial communities in Arctic cryoconite. *Environ. Res. Lett.* 8, 045021. doi:10.1088/1748-9326/8/4/045021.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Wheeler, D. L. (2005). GenBank. *Nucleic Acids Res.* 33, D34–8. doi:10.1093/nar/gki063.

Bonner, F. T., Hughes, M. N., Poole, R. K., and Scott, R. I. (1991). Kinetics of the reactions of trioxodinitrate and nitrite ions with cytochrome d in *Escherichia coli*. *Biochim. Biophys. Acta - Bioenerg.* 1056, 133–138. doi:10.1016/S0005-2728(05)80279-8.

Booth, B. C., and Horner, R. A. (1997). Microalgae on the arctic ocean section, 1994: species abundance and biomass. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 44, 1607–1622. doi:10.1016/S0967-0645(97)00057-X.

Boutron, C. F., Candelone, J.-P., and Hong, S. (1994). Past and recent changes in the large-scale tropospheric cycles of lead and other heavy metals as documented in Antarctic and Greenland snow and ice: A review. *Geochim. Cosmochim. Acta* 58, 3217–3225. doi:10.1016/0016-7037(94)90049-3.

Bowers, R. M., Lauber, C. L., Wiedinmyer, C., Hamady, M., Hallar, A. G., Fall, R., Knight, R., and Fierer, N. (2009). Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. *Appl. Environ. Microbiol.* 75, 5121–30. doi:10.1128/AEM.00447-09.

Bowman, J. P., McCammon, S. a, Brown, M. V, Nichols, D. S., and McMeekin, T. a (1997a). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl. Environ. Microbiol.* 63, 3068–78.

BOWMAN, J. P., McCAMMON, S. A., BROWN, J. L., and McMEEKIN, T. A. (1998). *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea ice habitats. *Int. J. Syst. Bacteriol.* 48, 1213–1222. doi:10.1099/00207713-48-4-1213.

Bowman, J. P., Nichols, D. S., and McKeekin, T. A. (1997b). *Psychrobacter glacincolia* sp. nov., a Halotolerant, Psychrophilic Bacterium Isolated from Antarctic Sea Ice. *Syst. Appl. Microbiol.* 20, 209–215.

Bowman, J. S., Berthiaume, C. T., Armbrust, E. V., and Deming, J. W. (2014). The genetic potential for key biogeochemical processes in Arctic frost flowers and young sea ice revealed by metagenomic analysis. *FEMS Microbiol. Ecol.* 89, 376–87. doi:10.1111/1574-6941.12331.

Bowman, J. S., Larose, C., Vogel, T. M., and Deming, J. W. (2013). Selective occurrence of Rhizobiales in frost flowers on the surface of young sea ice near Barrow, Alaska and distribution in the polar marine rare biosphere. *Environ. Microbiol. Rep.* 5, 575–82. doi:10.1111/1758-2229.12047.

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., and Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME J.* 6, 11–20. doi:10.1038/ismej.2011.76.

Boyd, E. S., Hamilton, T. L., Havig, J. R., Skidmore, M. L., and Shock, E. L. (2014). Chemolithotrophic primary production in a subglacial ecosystem. *Appl. Environ. Microbiol.* 80, 6146–53. doi:10.1128/AEM.01956-14.

Bramucci, A., Han, S., Beckers, J., Haas, C., and Lanoil, B. (2013). Composition, Diversity, and Stability of Microbial Assemblages in Seasonal Lake Ice, Miquelon Lake, Central Alberta. *Biology (Basel).* 2, 514–532. doi:10.3390/biology2020514.

Brinkmeyer, R., Knittel, K., Jürgens, J., Amann, R., Helmke, E., Ju, J., and Weyland, H. (2003a). Diversity and Structure of Bacterial Communities in Arctic versus Antarctic Pack Ice Diversity. doi:10.1128/AEM.69.11.6610.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., and Helmke, E. (2003b). Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl. Environ. Microbiol.* 69, 6610–9.

Brock, T. D. (1985). Life at high temperatures. *Science* 230, 132–8. doi:10.1126/science.230.4722.132.

Brown, A. D. (1978). Compatible solutes and extreme water stress in eukaryotic micro-organisms. *Adv. Microb. Physiol.* 17, 181–242.

Brown, J. R., Seymour, J. D., Brox, T. I., Skidmore, M. L., Wang, C., Christner, B. C., Luo, B. H., and Codd, S. L. (2014). Recrystallization inhibition in ice due to ice binding protein activity detected by nuclear magnetic resonance. *Biotechnol. Reports* 3, 60–64. doi:10.1016/j.btre.2014.06.005.

Brown, M. V., and Bowman, J. P. (2001). A molecular phylogenetic survey of sea ice microbial communities (SIMCO). *FEMS Microbiol. Ecol.* 35, 267–275.

Brown, T. a., and Belt, S. T. (2012). Identification of the sea ice diatom biomarker IP25 in Arctic benthic macrofauna: Direct evidence for a sea ice diatom diet in Arctic heterotrophs. *Polar Biol.* 35, 131–137. doi:10.1007/s00300-011-1045-7.

Buchheim, M. A., Buchheim, J. A., Carlson, T., and Kugrens, P. (2002). phylogeny of *lobocharacium* (chlorophyceae) and allies: a study of 18s and 26s rDNA data1. *J. Phycol.* 38, 376–383. doi:10.1046/j.1529-8817.2002.01145.x.

Burke, C. M., and Burton, H. R. (1988). The ecology of photosynthetic bacteria in Burton Lake, Vestfold Hills, Antarctica. *Hydrobiologia* 165, 1–11. doi:10.1007/BF00025569.

Burrows, S. M., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., and Lawrence, M. G. (2009). Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems. *Atmos. Chem. Phys.* 9, 9281–9297. doi:10.5194/acp-9-9281-2009.

Cabiscol, E., Tamarit, J., and Ros, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int. Microbiol.* 3, 3–8.

Cameron, K. a, Hagedorn, B., Dieser, M., Christner, B. C., Choquette, K., Sletten, R., Crump, B., Kellogg, C., and Junge, K. (2014). Diversity and potential sources of microbiota associated with snow on western portions of the Greenland Ice Sheet. *Environ. Microbiol.* doi:10.1111/1462-2920.12446.

Cameron, K. a, Hodson, A. J., and Osborn, a M. (2012). Structure and diversity of bacterial, eukaryotic and archaeal communities in glacial cryoconite holes from the Arctic and the Antarctic. *FEMS Microbiol. Ecol.* 82, 254–67. doi:10.1111/j.1574-6941.2011.01277.x.

Cappa, F., Suciu, N., Trevisan, M., Ferrari, S., Puglisi, E., and Sandro, P. (2014). Science of the Total Environment Bacterial diversity in a contaminated Alpine glacier as determined by culture-based and molecular approaches. *Sci. Total Environ.* 497-498, 50–59. doi:10.1016/j.scitotenv.2014.07.094.

Carlson, R. (1977). A trophic state index for lakes. *Limnol. Oceanogr.* 22, 361–369.

Carpenter, E. J., Lin, S., and Capone, D. G. (2000a). Bacterial Activity in South Pole Snow Bacterial Activity in South Pole Snow. 66. doi:10.1128/AEM.66.10.4514-4517.2000.Updated.

Casanueva, A., Tuffin, M., Cary, C., and Cowan, D. a (2010). Molecular adaptations to psychrophily: the impact of "omic" technologies. *Trends Microbiol.* 18, 374–81. doi:10.1016/j.tim.2010.05.002.

Choi, S. C., Chase, T., and Bartha, R. (1994). Enzymatic catalysis of mercury methylation by Desulfovibrio desulfuricans LS. *Appl. Environ. Microbiol.* 60, 1342–6.

Christner, B. C., Royston-Bishop, G., Foreman, C. M., Arnold, B. R., Tranter, M., Welch, K. a., Lyons, W. B., Tsapin, A. I., Studinger, M., and Priscu, J. C. (2006). Limnological conditions in Subglacial Lake Vostok, Antarctica. *Limnol. Oceanogr.* 51, 2485–2501. doi:10.4319/lo.2006.51.6.2485.

Christner, B. C., Skidmore, M. L., Priscu, J. C., Tranter, M., and Foreman, C. M. (2008). Bacteria in Subglacial Environments. 51–71.

Chu, V. W. (2013). *Greenland ice sheet hydrology: A review*. doi:10.1177/0309133313507075.

Chuvochina, M. S., Alekhina, I. a., Normand, P., Petit, J.-R., and Bulat, S. a. (2011a). Three events of Saharan dust deposition on the Mont Blanc glacier associated with different snow-colonizing bacterial phylotypes. *Microbiology* 80, 125–131. doi:10.1134/S0026261711010061.

Chuvochina, M. S., Marie, D., Chevaillier, S., Petit, J.-R., Normand, P., Alekhina, I. a., and Bulat, S. a. (2011b). Community Variability of Bacteria in Alpine Snow (Mont Blanc) Containing Saharan Dust Deposition and Their Snow Colonisation Potential. *Microbes Environ.* 26, 237–247. doi:10.1264/jsme2.ME11116.

Cloutier, J., Prévost, D., Nadeau, P., and Antoun, H. (1992). Heat and cold shock protein synthesis in arctic and temperate strains of rhizobia. *Appl. Environ. Microbiol.* 58, 2846–53.

Cockell, C. S., Rettberg, P., Horneck, G., Wynn-Williams, D. D., Scherer, K., and Gugg-Helminger, A. (2002). Influence of ice and snow covers on the UV exposure of terrestrial microbial communities: dosimetric studies. *J. Photochem. Photobiol. B Biol.* 68, 23–32. doi:10.1016/S1011-1344(02)00327-5.

Colbeck, S. C. (1982). An overview of seasonal snow metamorphism. *Rev. Geophys.* 20, 45. doi:10.1029/RG020i001p00045.

Collins, R. E., Carpenter, S. D., and Deming, J. W. (2008). Spatial heterogeneity and temporal dynamics of particles, bacteria, and pEPS in Arctic winter sea ice. *J. Mar. Syst.* 74, 902–917. doi:10.1016/j.jmarsys.2007.09.005.

Collins, R. E., and Deming, J. W. (2011a). Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. *Polar Biol.* 34, 1819–1830. doi:10.1007/s00300-011-1041-y.

Collins, R. E., and Deming, J. W. (2011b). Abundant dissolved genetic material in Arctic sea ice Part I: Extracellular DNA. *Polar Biol.* 34, 1819–1830. doi:10.1007/s00300-011-1041-y.

Collins, R. E., and Deming, J. W. (2013). An inter-order horizontal gene transfer event enables the catabolism of compatible solutes by *Colwellia psychrerythraea 34H*. *Extremophiles* 17, 601–10. doi:10.1007/s00792-013-0543-7.

Collins, R. E., Rocap, G., and Deming, J. W. (2010). Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. *Environ. Microbiol.* 12, 1828–1841. doi:10.1111/j.1462-2920.2010.02179.x.

Cowan, D. a, and Tow, L. A. (2004). Endangered antarctic environments. *Annu. Rev. Microbiol.* 58, 649–90. doi:10.1146/annurev.micro.57.030502.090811.

Cowan, D. A., Chown, S. L., Convey, P., Tuffin, M., Hughes, K., Pointing, S., and Vincent, W. F. (2011). Non-indigenous microorganisms in the Antarctic: assessing the risks. *Trends Microbiol.* 19, 540–8. doi:10.1016/j.tim.2011.07.008.

Cowie, R. O. M., Maas, E. W., and Ryan, K. G. (2011). Archaeal diversity revealed in Antarctic sea ice. *Antarct. Sci.* 23, 531–536. doi:10.1017/S0954102011000368.

Daly, G. L., and Wania, F. (2004). Simulating the influence of snow on the fate of organic compounds. *Environ. Sci. Technol.* 38, 4176–86.

Dani, K. G. S., Mader, H. M., Wolff, E. W., and Wadham, J. L. (2012). Modelling the liquid-water vein system within polar ice sheets as a potential microbial habitat. *Earth Planet. Sci. Lett.* 333-334, 238–249. doi:10.1016/j.epsl.2012.04.009.

DeAngelis, K. M., Allgaier, M., Chavarria, Y., Fortney, J. L., Hugenholtz, P., Simmons, B., Sublette, K., Silver, W. L., and Hazen, T. C. (2011). Characterization of trapped lignin-degrading microbes in tropical forest soil. *PLoS One* 6, e19306. doi:10.1371/journal.pone.0019306.

DeLeon-Rodriguez, N., Lathem, T. L., Rodriguez-R, L. M., Barazesh, J. M., Anderson, B. E., Beyersdorf, A. J., Ziemba, L. D., Bergin, M., Nenes, A., and Konstantinidis, K. T. (2013). Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proc. Natl. Acad. Sci. U. S. A.* 110, 2575–80. doi:10.1073/pnas.1212089110.

Delmont, T. O., Malandain, C., Prestat, E., Larose, C., Monier, J.-M., Simonet, P., and Vogel, T. M. (2011a). Metagenomic mining for microbiologists. *ISME J.* 5, 1837–43. doi:10.1038/ismej.2011.61.

Delmont, T. O., Robe, P., Clark, I., Simonet, P., and Vogel, T. M. (2011b). Metagenomic comparison of direct and indirect soil DNA extraction approaches. *J. Microbiol. Methods* 86, 397–400. doi:10.1016/j.mimet.2011.06.013.

DeLong, E. F. (2005). Microbial community genomics in the ocean. *Nat. Rev. Microbiol.* 3, 459–69. doi:10.1038/nrmicro1158.

Delort, A.-M., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., Laj, P., and Deguillaume, L. (2010). A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmos. Res.* 98, 249–260. doi:10.1016/j.atmosres.2010.07.004.

Deming, J. W. (2002). Psychrophiles and polar regions. *Curr. Opin. Microbiol.* 5, 301–9.

Deming, J. W. (2009). "Sea Ice Bacteria and Viruses," in *Sea Ice: An Introduction to Its Physics, Chem- istry, Biology, and Geology*, eds. D. N. Thomas and G. S. Dieckmann (Oxford, UK: Wiley-Blackwell), 247–282. doi:10.1002/9781444317145.

Derksen, C., and Brown, R. (2013). Arctic Report Card - Snow -.

Dinsdale, E. a, Edwards, R. a, Bailey, B. a, Tuba, I., Akhter, S., McNair, K., Schmieder, R., Apkarian, N., Creek, M., Guan, E., et al. (2013). Multivariate analysis of functional metagenomes. *Front. Genet.* 4, 41. doi:10.3389/fgene.2013.00041.

Dinsdale, E. a, Edwards, R. a, Hall, D., Angly, F., Breitbart, M., Brulc, J. M., Furlan, M., Desnues, C., Haynes, M., Li, L., et al. (2008). Functional metagenomic profiling of nine biomes. *Nature* 452, 629–32. doi:10.1038/nature06810.

Domine, F., Albert, M., Huthwelker, T., Jacobi, H.-W., Kokhanovsky, A. A., Lehning, M., Picard, G., and Simpson, W. R. (2008b). Snow physics as relevant to snow photochemistry. *Atmos. Chem. Phys.* 8, 171–208. doi:10.5194/acp-8-171-2008.

Dominé, F., Lauzier, T., Cabanes, A., Legagneux, L., Kuhs, W. F., Techmer, K., and Heinrichs, T. (2003). Snow metamorphism as revealed by scanning electron microscopy. *Microsc. Res. Tech.* 62, 33–48. doi:10.1002/jemt.10384.

Dominé, F., and Shepson, P. B. (2002). Air-snow interactions and atmospheric chemistry. *Science* 297, 1506–10. doi:10.1126/science.1074610.

Dommergue, A., Larose, C., Faïn, X., Clarisse, O., Hintelmann, H., Schneider, D., and Ferrari, C. P. (2010). Deposition of mercury species in the Ny-Alesund area (79 degrees N) and their transfer during snowmelt. *Environ. Sci. Technol.* 44, 901–7. doi:10.1021/es902579m.

Dowdeswell, J. a., and Siegert, M. J. (2003). The physiography of modern Antarctic subglacial lakes. *Glob. Planet. Change* 35, 221–236. doi:10.1016/S0921-8181(02)00128-5.

Doyle, S., Montross, S., Skidmore, M., and Christner, B. (2013). Characterizing Microbial Diversity and the Potential for Metabolic Function at −15 °C in the Basal Ice of Taylor Glacier, Antarctica. *Biology (Basel).* 2, 1034–1053. doi:10.3390/biology2031034.

Duval, B., Shetty, K., and Thomas, W. H. (2000). Phenolic compounds and antioxidant properties in the snow alga Chlamydomonas nivalis after exposure to UV light. 559–566.

Dziewit, L., Grzesiak, J., Ciok, A., Nieckarz, M., Zdanowski, M. K., and Bartosik, D. (2013). Sequence determination and analysis of three plasmids of *Pseudomonas s*p. GLE121, a psychrophile isolated from surface ice of Ecology Glacier (Antarctica). *Plasmid* 70, 254–262. doi:10.1016/j.plasmid.2013.05.007.

Eddie, B., Krembs, C., and Neuer, S. (2008). Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. *Mar. Ecol. Prog. Ser.* 354, 107–117. doi:10.3354/meps07243.

Edwards, A. C., Scalenghe, R., and Freppaz, M. (2007). Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. *Quat. Int.* 162-163, 172–181. doi:10.1016/j.quaint.2006.10.027.

Edwards, A., Pachebat, J. a, Swain, M., Hegarty, M., Hodson, A. J., Irvine-Fynn, T. D. L., Rassner, S. M. E., and Sattler, B. (2013). A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environ. Res. Lett.* 8, 035003. doi:10.1088/1748-9326/8/3/035003.

Eisenman, H. C., and Casadevall, A. (2012). Synthesis and assembly of fungal melanin. *Appl. Microbiol. Biotechnol.* 93, 931–40. doi:10.1007/s00253-011-3777-2.

Eric Collins, R., Rocap, G., and Deming, J. W. (2010). Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. *Environ. Microbiol.* 12, 1828–1841. doi:10.1111/j.1462-2920.2010.02179.x.

Euzebi, J. P. (1997). List of Bacterial Names with Standing in Nomenclature: a Folder Available on the Internet. *Int. J. Syst. Bacteriol.* 47, 590–592. doi:10.1099/00207713-47-2-590.

Ewert, M. (2013). Microbial Challenges and Solutions to Inhabiting the Dynamic Architecture of Saline Ice Formations.

Ewert, M., Carpenter, S. D., Colangelo-Lillis, J., and Deming, J. W. (2013a). Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. *J. Geophys. Res. Ocean.* 118, 726–735. doi:10.1002/jgrc.20055.

Ewert, M., Carpenter, S. D., Colangelo-Lillis, J., and Deming, J. W. (2013b). Bacterial and extracellular polysaccharide content of brine-wetted snow over Arctic winter first-year sea ice. *J. Geophys. Res. Ocean.* 118, 726–735. doi:10.1002/jgrc.20055.

Ewert, M., and Deming, J. (2013). Sea Ice Microorganisms: Environmental Constraints and Extracellular Responses. *Biology (Basel).* 2, 603–628. doi:10.3390/biology2020603.

Ewert, M., and Deming, J. W. (2014). Bacterial responses to fluctuations and extremes in temperature and brine salinity at the surface of Arctic winter sea ice. *FEMS Microbiol. Ecol.* 89, 476–89. doi:10.1111/1574-6941.12363.

Ewert, M., and Deming, J. W. (2011). Selective retention in saline ice of extracellular polysaccharides produced by the cold-adapted marine bacterium *Colwellia psychrerythraea* strain 34H. 52, 111–117.

Falkowski, P. G., and LaRoche, J. (1991). Acclimation to spectral irradiance in algae. *J. Phycol.* 27, 8–14. doi:10.1111/j.0022-3646.1991.00008.x.

Felip, M., Sattler, B., Psenner, R., and Catalan, J. (1995). Highly active microbial communities in the ice and snow cover of high mountain lakes. *Appl. Environ. Microbiol.* 61, 2394–401.

Feller, G., and Gerday, C. (2003). Psychrophilic enzymes: hot topics in cold adaptation. *Nat. Rev. Microbiol.* 1, 200–208. doi:10.1038/nrmicro773.

Ferreira, A., O'Byrne, C. P., and Boor, K. J. (2001). Role of sigma(B) in heat, ethanol, acid, and oxidative stress resistance and during carbon starvation in *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 67, 4454–7.

Fiala, M., Kuosa, H., Kopczyńska, E. E., Oriol, L., and Delille, D. (2006). Spatial and seasonal heterogeneity of sea ice microbial communities in the first-year ice of Terre Adélie area (Antarctica). *Aquat. Microb. Ecol.* 43, 95–106. doi:10.3354/ame043095.

Fierer, N., and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* 103, 626–31. doi:10.1073/pnas.0507535103.

Fierz, C., Armstrong, R. L., Durand, Y., Etchevers, P., Greene, E., McClung, D. M., Nishimura, K., and Satyawali, P.K. and Sokratov, S. A. (2009). *The International Classification for Seasonnal Snow on the Ground*. IHP-VII Te. UNESCO-IHP, Paris.

Flint, D. H. (1994). Initial kinetic and mechanistic characterization of Escherichia coli fumarase A. *Arch. Biochem. Biophys.* 311, 509–16. doi:10.1006/abbi.1994.1269.

Foreman, C. M., Cory, R. M., Morris, C. E., SanClements, M. D., Smith, H. J., Lisle, J. T., Miller, P. L., Chin, Y., and Mcknight, D. M. (2013). Microbial growth under humic-free conditions in a supraglacial stream system on the Cotton Glacier , Antarctica. *Environ. Res. Lett.* 8, 1–11. doi:10.1088/1748-9326/8/3/035022.

Foreman, C. M., Dieser, M., Greenwood, M., Cory, R. M., Laybourn-Parry, J., Lisle, J. T., Jaros, C., Miller, P. L., Chin, Y.-P., and McKnight, D. M. (2011). When a habitat freezes solid: microorganisms over-winter within the ice column of a coastal Antarctic lake. *FEMS Microbiol. Ecol.* 76, 401–12. doi:10.1111/j.1574-6941.2011.01061.x.

Frey, A. D., Farre, J., Bollinger, C. J. T., and Kallio, P. T. (2002). Bacterial Hemoglobins and Flavohemoglobins for Alleviation of Nitrosative Stress in *Escherichia coli*. 68, 4835–4840. doi:10.1128/AEM.68.10.4835.

Fritsen, C. H., and Priscu, J. C. (1998). Cyanobacterial assemblages in permanent ice covers on antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. *J. Phycol.* 34, 587–597. doi:10.1046/j.1529-8817.1998.340587.x.

Frost, L. S., Leplae, R., Summers, A. O., and Toussaint, A. (2005). Mobile genetic elements: the agents of open source evolution. *Nat. Rev. Microbiol.* 3, 722–32. doi:10.1038/nrmicro1235.

Galtier, N., Tourasse, N., and Gouy, M. (1999). A non hyperthermophilic common ancestor to extant life forms. *Science* 283, 220–1.

Gandolfi, I., Bertolini, V., Ambrosini, R., Bestetti, G., and Franzetti, A. (2013). Unravelling the bacterial diversity in the atmosphere. *Appl. Microbiol. Biotechnol.* 97, 4727–36. doi:10.1007/s00253-013-4901-2.

Gessler, N. N., Egorova, A. S., and Belozerskaya, T. A. (2014). Melanin pigments of fungi under extreme environmental conditions (Review). *Appl. Biochem. Microbiol.* 50, 105–113. doi:10.1134/S0003683814020094.

Gilbert, J. a, Field, D., Huang, Y., Edwards, R., Li, W., Gilna, P., and Joint, I. (2008). Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. *PLoS One* 3, e3042. doi:10.1371/journal.pone.0003042.

Gilbert, J. A., Davies, P. L., and Laybourn-Parry, J. (2005). A hyperactive, Ca2+-dependent antifreeze protein in an Antarctic bacterium. *FEMS Microbiol. Lett.* 245, 67–72. doi:10.1016/j.femsle.2005.02.022.

GLIMS, and National Snow and Ice Data Center. (2012). *GLIMS Glacier Database.*, Boulder, Colorado USA: National Snow and Ice Data . doi:http://dx.doi.org/10.7265/N5V98602.

Gomelsky, M., and Hoff, W. D. (2011). Light helps bacteria make important lifestyle decisions. *Trends Microbiol.* 19, 441–8. doi:10.1016/j.tim.2011.05.002.

González-Toril, E., Amils, R., Delmas, R. J., Petit, J.-R., Komárek, J., and Elster, J. (2008). Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glacial areas (Antarctica, Alps and Andes). *Biogeosciences Discuss.* 5, 1607–1630. doi:10.5194/bgd-5-1607-2008.

Goodman, D. (1971). Ecological Investigations of Ice Worms on Casement Glacier, Southeastern Alaska.

Goretski, J., Zafiriou, O. C., and Hollocher, T. C. (1990). Steady-state nitric oxide concentrations during denitrification. *J. Biol. Chem.* 265, 11535–8.

Gorton, H. L., and Vogelmann, T. C. (2003). Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. *Photochem. Photobiol.* 77, 608–15.

Gosink, J. J., Herwig, R. P., and Staley, J. T. (1997). *Octadecabacter arcticus* gen. nov., sp. nov., and *O. antarcticus*, sp. nov., Nonpigmented, Psychrophilic Gas Vacuolate Bacteria from Polar Sea Ice and Water. *Syst. Appl. Microbiol.* 20, 356–365. doi:10.1016/S0723-2020(97)80003-3.

Gosink, J., and Staley, J. (1995). Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. *Appl. Envir. Microbiol.* 61, 3486–3489.

Gradinger, R., Friedrich, C., and Spindler, M. (1999). Abundance, biomass and composition of the sea ice biota of the Greenland Sea pack ice. *Deep. Res. Part II Top. Stud. Oceanogr.* 46, 1457–1472. doi:10.1016/S0967-0645(99)00030-2.

Gradinger, R., and Nurnberg, D. (1996). Snow algal communities on Arctic pack ice floes dominated by Chlamydomonas nivalis (Bauer) Wille. 35–43.

Grannas, A. M., Jones, A. E., Dibb, J., Ammann, M., Anastasio, C., Beine, H. J., Bergin, M., and Bottenheim, J. (2007). An overview of snow photochemistry : evidence , mechanisms and impacts. 4329–4373.

Grannas, A. M., Shepson, P. B., and Filley, T. R. (2004). Photochemistry and nature of organic matter in Arctic and Antarctic snow. *Global Biogeochem. Cycles* 18, n/a–n/a. doi:10.1029/2003GB002133.

Granskog, M. A., and Kaartokallio, H. (2004). An Estimation of the Potential Fluxes of Nitrogen, Phosphorus, Cadmium and Lead from Sea Ice and Snow in the Northern Baltic Sea. *Water, Air, Soil Pollut.* 154, 331–347. doi:10.1023/B:WATE.0000022975.74321.27.

Grant, W. D. (2004). Life at low water activity. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 359, 1249–66; discussion 1266–7. doi:10.1098/rstb.2004.1502.

Grossmann S, Dieckmann GS. (1994). Bacterial Standing Stock, Activity, and Carbon Production during Formation and Growth of Sea Ice in the Weddell Sea, Antarctica. Appl. Environ. Microbiol. 60:2746–53.

Groudieva, T., Kambourova, M., Yusef, H., Royter, M., Grote, R., Trinks, H., and Antranikian, G. (2004). Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. *Extremophiles* 8, 475–88. doi:10.1007/s00792-004-0409-0.

Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J. C., Diderichsen, B., and Plemenitaš, a. (2003). Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. *Phys. Chem. Earth, Parts A/B/C* 28, 1273–1278. doi:10.1016/j.pce.2003.08.056.

Halldal, P. (1959). Factors Affecting Light Response in Phototactic Algae. *Physiol. Plant.* 12, 742–752. doi:10.1111/j.1399-3054.1959.tb08909.x.

Harding, T., Jungblut, A. D., Lovejoy, C., and Vincent, W. F. (2011). Microbes in high arctic snow and implications for the cold biosphere. *Appl. Environ. Microbiol.* 77, 3234–43. doi:10.1128/AEM.02611-10.

Hartzell, P. L., Nghiem, J. V, Richio, K. J., and Shain, D. H. (2005). Distribution and phylogeny of glacier ice worms (Mesenchytraeus solifugus and Mesenchytraeus solifugus rainierensis). *Can. J. Zool.* 83, 1206–1213. doi:10.1139/z05-116.

Hauptmann, A. L., Stibal, M., Bælum, J., Sicheritz-Pontén, T., Brunak, S., Bowman, J. S., Hansen, L. H., Jacobsen, C. S., and Blom, N. (2014). Bacterial diversity in snow on North Pole ice floes. *Extremophiles*. doi:10.1007/s00792-014-0660-y.

Hell, K., Edwards, A., Zarsky, J., Podmirseg, S. M., Girdwood, S., Pachebat, J. a, Insam, H., and Sattler, B. (2013). The dynamic bacterial communities of a melting High Arctic glacier snowpack. *ISME J.* 7, 1814–26. doi:10.1038/ismej.2013.51.

Hinkler, J., Hansen, B. U., Tamstorf, M. P., Sigsgaard, C., and Petersen, D. (2008). Snow and Snow-Cover in Central Northeast Greenland. *Adv. Ecol. Res.* 40, 175–195. doi:10.1016/S0065-2504(07)00008-6.

Hirschberg, R., and Stavis, R. (1977). Phototaxis mutants of Phototaxis Mutants of *Chlamydomonas reinhardtii*. 129.

Hodson, A., Anesio, A. M., Tranter, M., Fountain, A., Osborn, M., Priscu, J. C., Laybourn-Parry, J., and Sattler, B. (2008). Glacial Ecosystems. *Ecol. Monogr.* 78, 41–67.

Hoham, R. W., and Duval, B. (2001). "Microbial Ecology of Snow and Freshwater Ice with Emphasis on Snow Algae," in *Snow Ecology*, eds. H. G. Jones, J. Pomeroy, D. A. Walker, and R. . Hoham.

Hou, S.-Y., Govorunova, E. G., Ntefidou, M., Lane, C. E., Spudich, E. N., Sineshchekov, O. A., and Spudich, J. L. (2012). Diversity of *Chlamydomonas* channelrhodopsins. *Photochem. Photobiol.* 88, 119–28. doi:10.1111/j.1751-1097.2011.01027.x.

Van Houdt, R., Deghorain, M., Vermeersch, M., Provoost, A., Lo Giudice, A., Leys, N., Perez-Morga, D., Van Melderen, L., and Michaud, L. (2013). Characterization of culturable *Paenibacillus* spp. from the snow surface on the high Antarctic Plateau (DOME C) and their dissemination in the Concordia research station. *Extremophiles* 17, 565–73. doi:10.1007/s00792-013-0539-3.

Hünken, M., Harder, J., and Kirst, G. O. (2008). Epiphytic bacteria on the Antarctic ice diatom Amphiprora kufferathii Manguin cleave hydrogen peroxide produced during algal photosynthesis. *Plant Biol.* 10, 519–526. doi:10.1111/j.1438-8677.2008.00040.x.

Huson, D. H., Auch, A. F., Qi, J., and Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Res.* 17, 377–86. doi:10.1101/gr.5969107.

Huson, D. H., Mitra, S., Ruscheweyh, H.-J., Weber, N., and Schuster, S. C. (2011). Integrative analysis of environmental sequences using MEGAN4. *Genome Res.* 21, 1552–60. doi:10.1101/gr.120618.111.

Ihaka, R., and Gentleman, R. (1996). R: A Language for Data Analysis and Graphics. *J. Comput. Graph. Stat.* 5, 299–314. doi:10.1080/10618600.1996.10474713.

Irvine-Fynn, T. D. L., Edwards, a, Newton, S., Langford, H., Rassner, S. M., Telling, J., Anesio, a M., and Hodson, a J. (2012). Microbial cell budgets of an Arctic glacier surface quantified using flow cytometry. *Environ. Microbiol.* 14, 2998–3012. doi:10.1111/j.1462-2920.2012.02876.x.

Irvine-fynn, T. D. L., Hodson, A. J., Moorman, B. J., Vatne, G., and Hubbard, A. L. (2011). Polythermal glacier hydrology : A REVIEW. *Rev. Geophys.* 49, 1–37. doi:10.1029/2010RG000350.1.

Jékely, G. (2009). Evolution of phototaxis. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364, 2795–808. doi:10.1098/rstb.2009.0072.

Jones, H. G. (1999). The ecology of snow-covered systems: a brief overview of nutrient cycling and life in the cold. *Hydrol. Process.* 13, 2135–2147. doi:10.1002/(sici)1099-1085(199910)13:14/15<2135::aid-hyp862>3.3.co;2-p.

Jungblut, A. D., Vincent, W. F., and Lovejoy, C. (2012). Eukaryotes in Arctic and Antarctic cyanobacterial mats. *FEMS Microbiol. Ecol.* 82, 416–428. doi:10.1111/j.1574-6941.2012.01418.x.

Junge, K., Eicken, H., and Deming, J. W. (2003). Motility of *Colwellia psychrerythraea* Strain 34H at Subzero Temperatures. *Appl. Environ. Microbiol.* 69, 4282–4284. doi:10.1128/AEM.69.7.4282-4284.2003.

Junge, K., Eicken, H., and Deming, J. W. (2004). Wintertime Sea Ice Bacterial Activity at -2 to -20 ° C in Arctic Wintertime Sea Ice. 70. doi:10.1128/AEM.70.1.550.

Junge, K., Imhoff, F., Staley, T., and Deming, J. W. (2002). Phylogenetic diversity of numerically important Arctic sea ice bacteria cultured at subzero temperature. *Microb. Ecol.* 43, 315–28. doi:10.1007/s00248-001-1026-4.

Junge K, Krembs C, Deming J, Stierle A, Eicken H. (2001). A microscopic approach to investigate bacteria under in situ conditions in sea-ice samples. Ann. Glaciol. 33:304–310.

Karl, D. ., Bird, D. F., Björkman, K., Houlihan, T., Shackelford, R., and Tupas, L. (1999). Microorganisms in the Accreted Ice of Lake Vostok , Antarctica. *Science (80-.).* 286, 2144–2147.

Kashino, Y., Fujimoto, k., Akamatsu, a., Koike, h., Satoh, k., and Kudoh, s. (1998). Photosynthetic pigment composition of ice algal and phytoplankton assemblages in early spring in saroma Ko lagoon, Hokkaido, Japan (19th Symposium on Polar Biology). *Proc. NIPR Symp. Polar Biol.* 11, 22–32.

Kepner, R. L., Wharton, R. a, and Suttle, C. a (1998). Viruses in Antarctic lakes. *Limnol. Oceanogr.* 43, 1754–1761. doi:10.4319/lo.1998.43.7.1754.

Keskitalo, J., Leppäranta, M., and Arvola, L. (2013). First records of primary producers of epiglacial and supraglacial lakes in western Dronning Maud Land, Antarctica. *Polar Biol.* 36, 1441–1450. doi:10.1007/s00300-013-1362-0.

King, M. D., France, J. L., Fisher, F. N., and Beine, H. J. (2005). Measurement and modelling of UV radiation penetration and photolysis rates of nitrate and hydrogen peroxide in Antarctic sea ice: An estimate of the production rate of hydroxyl radicals in first-year sea ice. *J. Photochem. Photobiol. A Chem.* 176, 39–49. doi:10.1016/j.jphotochem.2005.08.032.

King, M. D., and Simpson, W. R. (2001). Extinction of UV radiation in Arctic snow at Alert, Canada (82°N). *J. Geophys. Res.* 106, 12499. doi:10.1029/2001JD900006.

Kirst, G. O. (1998). "Osmotic Adjustment in Phytoplankton and MacroAlgae," in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds | Springer*, ed. P. T. Keller, M.D., Kiene, R.P., Kirst, G.O., Visscher (Plenum Press), 121–129.

Koh, E. Y., Martin, A. R., McMinn, A., and Ryan, K. G. (2012). Recent Advances and Future Perspectives in Microbial Phototrophy in Antarctic Sea Ice. *Biology (Basel).* 1, 542–556. doi:10.3390/biology1030542.

Kohls, K., Abed, R. M. M., Polerecky, L., Weber, M., and de Beer, D. (2010). Halotaxis of cyanobacteria in an intertidal hypersaline microbial mat. *Environ. Microbiol.* 12, 567–75. doi:10.1111/j.1462-2920.2009.02095.x.

Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S., and Johnson, C. H. (1993). Circadian rhythms in prokaryotes: luciferase as a reporter of circadian gene expression in cyanobacteria. *Proc. Natl. Acad. Sci. U. S. A.* 90, 5672–6.

Konopka, A. (2009). What is microbial community ecology? *ISME J.* 3, 1223–30. doi:10.1038/ismej.2009.88.

Krapp, A. R., Humbert, M. V., and Carrillo, N. (2011). The soxRS response of Escherichia coli can be induced in the absence of oxidative stress and oxygen by modulation of NADPH content. *Microbiology* 157, 957–65. doi:10.1099/mic.0.039461-0.

Krembs, C., Eicken, H., and Deming, J. W. (2011). Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3653–8. doi:10.1073/pnas.1100701108.

Krembs, C., Gradinger, R., and Spindler, M. (2000). Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. *J. Exp. Mar. Bio. Ecol.* 243, 55–80. doi:10.1016/S0022-0981(99)00111-2.

Kröger, P., and Hegemann, P. (1994). Photophobic responses and phototaxis in *Chlamydomonas* are triggered by a single rhodopsin photoreceptor. *FEBS Lett.* 341, 5–9.

Krulwich, T. A., Hicks, D. B., and Ito, M. (2009). Cation/proton antiporter complements of bacteria: why so large and diverse? *Mol. Microbiol.* 74, 257–60. doi:10.1111/j.1365-2958.2009.06842.x.

Kumar, G. S., Jagannadham, M. V, and Ray, M. K. (2002). Low-temperature-induced changes in composition and fluidity of lipopolysaccharides in the antarctic psychrotrophic bacterium *Pseudomonas syringae*. *J. Bacteriol.* 184, 6746–9. doi:10.1128/JB.184.23.6746-6749.2002.

Kyrke-Smith, T. M., Katz, R. F., and Fowler, A. C. (2014). Subglacial hydrology and the formation of ice streams. *Proc. R. Soc. A* 470, 20130494. doi:10.1098/rspa.2013.0494.

Lacal, J., García-Fontana, C., Muñoz-Martínez, F., Ramos, J.-L., and Krell, T. (2010). Sensing of environmental signals: classification of chemoreceptors according to the size of their ligand binding regions. *Environ. Microbiol.* 12, 2873–84. doi:10.1111/j.1462-2920.2010.02325.x.

Lader, E. S. (2003). Methods and reagents for preserving RNA in cell and tissue samples.

Langford, H. J., Irvine-Fynn, T. D. L., Edwards, a., Banwart, S. a., and Hodson, a. J. (2014). A spatial investigation of the environmental controls over cryoconite aggregation on Longyearbreen glacier, Svalbard. *Biogeosciences Discuss.* 11, 3423–3463. doi:10.5194/bgd-11-3423-2014.

Langlois, A., Mundy, C. J., and Barber, D. G. (2007). On the winter evolution of snow thermophysical properties over land-fast first-year sea ice. 716, 705–716. doi:10.1002/hyp.

Larose, C., Berger, S., Ferrari, C., Navarro, E., Dommergue, A., Schneider, D., and Vogel, T. M. (2010a). Microbial sequences retrieved from environmental samples from seasonal arctic snow and meltwater from Svalbard, Norway. *Extremophiles* 14, 205–12. doi:10.1007/s00792-009-0299-2.

Larose, C., Dommergue, A., De Angelis, M., Cossa, D., Averty, B., Marusczak, N., Soumis, N., Schneider, D., and Ferrari, C. (2010b). Springtime changes in snow chemistry lead to new insights into mercury methylation in the Arctic. *Geochim. Cosmochim. Acta* 74, 6263–6275. doi:10.1016/j.gca.2010.08.043.

Larose, C., Dommergue, A., and Vogel, T. (2013a). The Dynamic Arctic Snow Pack: An Unexplored Environment for Microbial Diversity and Activity. *Biology (Basel).* 2, 317–330. doi:10.3390/biology2010317.

Larose, C., Dommergue, A., and Vogel, T. M. (2013b). Microbial nitrogen cycling in Arctic snowpacks. *Environ. Res. Lett.* 8, 035004. doi:10.1088/1748-9326/8/3/035004.

Larose, C., Prestat, E., Cecillon, S., Berger, S., Malandain, C., Lyon, D., Ferrari, C., Schneider, D., Dommergue, A., and Vogel, T. M. (2013c). Interactions between snow chemistry, mercury inputs and microbial population dynamics in an Arctic snowpack. *PLoS One* 8, e79972. doi:10.1371/journal.pone.0079972.

Laybourn-Parry, J., Marshall, W. a., and Madan, N. J. (2007). Viral dynamics and patterns of lysogeny in saline Antarctic lakes. *Polar Biol.* 30, 351–358. doi:10.1007/s00300-006-0191-9.

Lazzaro, A., Wismer, A., Schneebeli, M., Erny, I., and Zeyer, J. (2015). Microbial abundance and community structure in a melting alpine snowpack. *Extremophiles*. doi:10.1007/s00792-015-0744-3.

Leeson, A. A., Shepherd, A., Briggs, K., Howat, I., Fettweis, X., Morlighem, M., and Rignot, E. (2015). Supraglacial lakes on the Greenland ice sheet advance inland under warming climate. *Nat. Clim. Chang.* 5, 51–55. doi:10.1038/NCLIMATE2463.

Lehman, N. (2013). Origin of life: Cold-hearted RNA heats up life. *Nat. Chem.* 5, 987–9. doi:10.1038/nchem.1811.

Lemoine, Y., and Schoefs, B. (2010). Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. *Photosynth. Res.* 106, 155–77. doi:10.1007/s11120-010-9583-3.

Leonardo, M. R. (2013). Review of Polar Microbiology: Life in a Deep Freeze. *J. Microbiol. Biol. Educ.* 14, 283–284. doi:10.1128/jmbe.v14i2.666.

Leya, T., Rahn, A., Lütz, C., and Remias, D. (2009). Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. *FEMS Microbiol. Ecol.* 67, 432–43. doi:10.1111/j.1574-6941.2008.00641.x.

Li, C., Boileau, A. J., Kung, C., and Adler, J. (1988). Osmotaxis in *Escherichia coli*. *Proc. Natl. Acad. Sci.* 85, 9451–9455. doi:10.1073/pnas.85.24.9451.

Lionard, M., Péquin, B., Lovejoy, C., and Vincent, W. F. (2012). Benthic cyanobacterial mats in the high arctic: multi-layer structure and fluorescence responses to osmotic stress. *Front. Microbiol.* 3, 140. doi:10.3389/fmicb.2012.00140.

Liu, S.-B., Chen, X.-L., He, H.-L., Zhang, X.-Y., Xie, B.-B., Yu, Y., Chen, B., Zhou, B.-C., and Zhang, Y.-Z. (2012). Structure and Ecological Roles of a Novel Exopolysaccharide from the Arctic Sea Ice Bacterium *Pseudoalteromonas* sp. Strain SM20310. *Appl. Environ. Microbiol.* 79, 224–230. doi:10.1128/AEM.01801-12.

Liu, Y., Yao, T., Jiao, N., Kang, S., Xu, B., Zeng, Y., Huang, S., and Liu, X. (2009). Bacterial diversity in the snow over Tibetan Plateau Glaciers. *Extremophiles* 13, 411–23. doi:10.1007/s00792-009-0227-5.

Llubes, M., Lanseau, C., and Rémy, F. (2006). Relations between basal condition, subglacial hydrological networks and geothermal flux in Antarctica. *Earth Planet. Sci. Lett.* 241, 655–662. doi:10.1016/j.epsl.2005.10.040.

Lopatina, A., Krylenkov, V., and Severinov, K. (2013). Activity and bacterial diversity of snow around Russian Antarctic stations. *Res. Microbiol.* 164, 949–58. doi:10.1016/j.resmic.2013.08.005.

López-Bueno, A., Tamames, J., Velázquez, D., Moya, A., Quesada, A., and Alcamí, A. (2009). High diversity of the viral community from an Antarctic lake. *Science* 326, 858–61. doi:10.1126/science.1179287.

Lorenz, M. G., Aardema, B. W., and Krumbein, W. E. (1981). Interaction of marine sediment with DNA and DNA availability to nucleases. *Mar. Biol.* 64, 225–230. doi:10.1007/BF00397113.

Lu, N., Wei, D., Jiang, X.-L., Chen, F., and Yang, S.-T. (2012a). Fatty Acids Profiling and Biomarker Identification in Snow Alga *Chlamydomonas Nivalis* by NaCl Stress Using GC/MS and Multivariate Statistical Analysis. *Anal. Lett.* 45, 1172–1183. doi:10.1080/00032719.2012.673094.

Lu, N., Wei, D., Jiang, X.-L., Chen, F., and Yang, S.-T. (2012b). Regulation of lipid metabolism in the snow alga *Chlamydomonas nivalis* in response to NaCl stress: An integrated analysis by cytomic and lipidomic approaches. *Process Biochem.* 47, 1163–1170. doi:10.1016/j.procbio.2012.04.011.

Luhtanen, A.-M., Eronen-Rasimus, E., Kaartokallio, H., Rintala, J.-M., Autio, R., and Roine, E. (2014). Isolation and characterization of phage-host systems from the Baltic Sea ice. *Extremophiles* 18, 121–30. doi:10.1007/s00792-013-0604-y.

Lund, B. O., Miller, D. M., and Woods, J. S. (1993). Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochem. Pharmacol.* 45, 2017–24.

Lutz, S., Anesio, A. M., Jorge Villar, S. E., and Benning, L. G. (2014). Variations of algal communities cause darkening of a Greenland glacier. *FEMS Microbiol. Ecol.* 89, 402–14. doi:10.1111/1574-6941.12351.

Ma, L.-J., Rogers, S. O., Catranis, C. M., and Starmer, W. T. (2000). Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* 92, 286–295. doi:10.2307/3761562.

De Maayer, P., Anderson, D., Cary, C., and Cowan, D. A. (2014). Some like it cold: understanding the survival strategies of psychrophiles. *EMBO Rep.* 15, 508–17. doi:10.1002/embr.201338170.

Maccario, L., Vogel, T. M., and Larose, C. (2014). Potential drivers of microbial community structure and function in Arctic spring snow. *Front. Microbiol.* 5, 413. doi:10.3389/fmicb.2014.00413.

MacDonell, S., and Fitzsimons, S. (2008). The formation and hydrological significance of cryoconite holes. *Prog. Phys. Geogr.* 32, 595–610. doi:10.1177/0309133308101382.

Mader, H. M., Pettitt, M. E., Wadham, J. L., Wolff, E. W., and Parkes, R. J. (2006). Subsurface ice as a microbial habitat. *Geology* 34, 169. doi:10.1130/G22096.1.

Maeda, H., Sakuragi, Y., Bryant, D. A., and Dellapenna, D. (2005). Tocopherols protect *Synechocystis* sp. strain PCC 6803 from lipid peroxidation. *Plant Physiol.* 138, 1422–35. doi:10.1104/pp.105.061135.

Mancuso Nichols, C. a., Garon, S., Bowman, J. P., Raguénès, G., and Guézennec, J. (2004). Production of exopolysaccharides by Antarctic marine bacterial isolates. *J. Appl. Microbiol.* 96, 1057–1066. doi:10.1111/j.1365-2672.2004.02216.x.

Maranger, R., Bird, D. F., and Juniper, S. K. (1994). Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. *Mar. Ecol. Prog. Ser.* 111, 121–128. doi:10.3354/meps111121.

Margesin, R., and Miteva, V. (2011). Diversity and ecology of psychrophilic microorganisms. *Res. Microbiol.* 162, 346–61. doi:10.1016/j.resmic.2010.12.004.

Margesin, R., Zacke, G., and Schinner, F. (2002). Characterization of Heterotrophic Microorganisms in Alpine Glacier Cryoconite. *Arctic, Antarct. Alp. Res.* 34, 88–93.

Marquardt, M., Kramer, M., Carnat, G., and Werner, I. (2011). Vertical distribution of sympagic meiofauna in sea ice in the Canadian Beaufort Sea. *Polar Biol.* 34, 1887–1900. doi:10.1007/s00300-011-1078-y.

Marshall, S. J. (2011). *The cryosphere*. Princeton University Press.

Martin, A., Hall, J., and Ryan, K. (2009). Low salinity and high-level UV-B radiation reduce single-cell activity in antarctic sea ice bacteria. *Appl. Environ. Microbiol.* 75, 7570–3. doi:10.1128/AEM.00829-09.

Matsumura, S., and Yamazaki, K. (2012). Eurasian Subarctic Summer Climate in Response to Anomalous Snow Cover. *J. Clim.* 25, 1305–1317. doi:10.1175/2011JCLI4116.1.

McMillan, M., Nienow, P., Shepherd, A., Benham, T., and Sole, A. (2007). Seasonal evolution of supra-glacial lakes on the Greenland Ice Sheet. *Earth Planet. Sci. Lett.* 262, 484–492. doi:10.1016/j.epsl.2007.08.002.

Menor-Salván, C., and Marín-Yaseli, M. R. (2012). Prebiotic chemistry in eutectic solutions at the water-ice matrix. *Chem. Soc. Rev.* 41, 5404–15. doi:10.1039/c2cs35060b.

Methé, B. a, Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X., Moult, J., Madupu, R., Nelson, W. C., Dodson, R. J., et al. (2005). The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10913–8. doi:10.1073/pnas.0504766102.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., et al. (2008). The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9, 386. doi:10.1186/1471-2105-9-386.

Michaud, L., Lo Giudice, A., Mysara, M., Monsieurs, P., Raffa, C., Leys, N., Amalfitano, S., and Van Houdt, R. (2014). Snow Surface Microbiome on the High Antarctic Plateau (DOME C). *PLoS One* 9, e104505. doi:10.1371/journal.pone.0104505.

Miezcan, T., Górniak, D., Swiatecki, A., Zdanowski, M., Tarkowska-Kukuryk, M., and Adamczuk, M. (2013). Vertical microzonation of ciliates in cryoconite holes in Ecology Glacier, King George Island. *Polish Polar Res.* 34, 201–212. doi:10.2478/popore.

Missall, T. A., Lodge, J. K., and Mcewen, J. E. (2004). Mechanisms of Resistance to Oxidative and Nitrosative Stress : Implications for Fungal Survival in Mammalian Hosts. 3. doi:10.1128/EC.3.4.835.

Miteva, V. (2008). "Bacteria in Snow and Glacier Ice," in *Psychrophiles: from Biodiversity to Biotechnology*, eds. F. Margesin, RosaSchinner, J.-C. Marx, and C. Gerday.

Miteva, V., Burlingame, C., Sowers, T., and Brenchley, J. (2014a). Comparative evaluation of the indigenous microbial diversity vs. drilling fluid contaminants in the NEEM Greenland ice core. *FEMS Microbiol. Ecol.*, 1–19. doi:10.1111/1574-6941.12286.

Miteva, V. I., and Brenchley, J. E. (2005). Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. *Appl. Environ. Microbiol.* 71, 7806–18. doi:10.1128/AEM.71.12.7806-7818.2005.

Miteva, V. I., Sheridan, P. P., and Brenchley, J. E. (2004). Phylogenetic and Physiological Diversity of Microorganisms Isolated from a Deep Greenland Glacier Ice Core. *Appl. Environ. Microbiol.* 70, 202–213. doi:10.1128/AEM.70.1.202.

Miteva, V., Sowers, T., and Brenchley, J. (2013). Penetration of fluorescent microspheres into the NEEM (North Eemian) Greenland ice core to assess the probability of microbial contamination. *Polar Biol.* 37, 47–59. doi:10.1007/s00300-013-1409-2.

Miteva, V., Teacher, C., Sowers, T., and Brenchley, J. (2009). Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates. *Environ. Microbiol.* 11, 640–56. doi:10.1111/j.1462-2920.2008.01835.x.

Miyakawa, S., Cleaves, H. J., and Miller, S. L. (2002). The cold origin of life : hydrogen cyanide and formamide. *Orig. Life Evol. Biosph.* 32, 195–208.

Moan, J., and Peak, M. J. (1989). Effects of UV radiation on cells. *J. Photochem. Photobiol. B Biol.* 4, 21–34. doi:10.1016/1011-1344(89)80099-5.

Møller, A. K., Barkay, T., Abu Al-Soud, W., Sørensen, S. J., Skov, H., and Kroer, N. (2011). Diversity and characterization of mercury-resistant bacteria in snow, freshwater and sea ice brine from the High Arctic. *FEMS Microbiol. Ecol.* 75, 390–401. doi:10.1111/j.1574-6941.2010.01016.x.

Moller, A. K., Barkay, T., Hansen, M. a., Norman, A., Hansen, L. H., Sorensen, S. J., Boyd, E. S., and Kroer, N. (2014). Mercuric reductase genes (merA) and mercury resistance plasmids in High Arctic snow, freshwater and sea ice brine. *FEMS Microbiol. Ecol.* 87, 52–63. doi:10.1111/1574-6941.12189.

Møller, A. K., Søborg, D. A., Al-soud, W. A., Sørensen, S. J., and Kroer, N. (2013). Bacterial community structure in High-Arctic snow and freshwater as revealed by pyrosequencing of 16S rRNA genes and cultivation. *Polar Res.* 1, 1–11.

Moosvi, S. A., Pacheco, C. C., McDonald, I. R., De Marco, P., Pearce, D. A., Kelly, D. P., and Wood, A. P. (2005). Isolation and properties of methanesulfonate-degrading *Afipia felis* from Antarctica and comparison with other strains of *A. feli*s. *Environ. Microbiol.* 7, 22–33. doi:10.1111/j.1462-2920.2004.00661.x.

Moran, M. A., Satinsky, B., Gifford, S. M., Luo, H., Rivers, A., Chan, L.-K., Meng, J., Durham, B. P., Shen, C., Varaljay, V. a, et al. (2013). Sizing up metatranscriptomics. *ISME J.* 7, 237–43. doi:10.1038/ismej.2012.94.

Morgan-kiss, R. M., Priscu, J. C., Pocock, T., Gudynaite-savitch, L., and Huner, N. P. A. (2006). Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently Cold Environments. *Microbiolohy Mol. Biol. Rev.* 70, 222–252. doi:10.1128/MMBR.70.1.222.

Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol. Rev.* 39, 144–67.

Mueller, D. R., Vincent, W. F., Bonilla, S., and Laurion, I. (2005). Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol. Ecol.* 53, 73–87. doi:10.1016/j.femsec.2004.11.001.

Mundy, C. J., Gosselin, M., Ehn, J. K., Belzile, C., Poulin, M., Alou, E., Roy, S., Hop, H., Lessard, S., Papakyriakou, T. N., et al. (2011). Characteristics of two distinct high-light acclimated algal communities during advanced stages of sea ice melt. *Polar Biol.* 34, 1869–1886. doi:10.1007/s00300-011-0998-x.

Murakami, T., Segawa, T., Bodington, D., and Dial, R. (2015). Census of bacterial microbiota associated with the glacier ice worm Mesenchytraeus solifugus. *FEMS Microbiol. Ecol.*, 1–28.

Mykytczuk, N. C. S., Foote, S. J., Omelon, C. R., Southam, G., Greer, C. W., and Whyte, L. G. (2013). Bacterial growth at -15 °C; molecular insights from the permafrost bacterium *Planococcus halocryophilus* Or1. *ISME J.* 7, 1211–26. doi:10.1038/ismej.2013.8.

Naff, C. S., Darcy, J. L., and Schmidt, S. K. (2013). Phylogeny and biogeography of an uncultured clade of snow chytrids. *Environ. Microbiol.* doi:10.1111/1462-2920.12116.

Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E. (1987). *Escherichia coli* and *Salmonella typhimurium - Cellular and molecular biology*. , eds. F. C. Neidhardt, K. B. Ingraham, J. L.;Low, B. Magasanik, M. Schaechter, and H. E. Umbarger American Society for Microbiology.

Ng, K. W., Pointing, S. B., and Dvornyk, V. (2013). Patterns of nucleotide diversity of the ldpA circadian gene in closely related species of cyanobacteria from extreme cold deserts. *Appl. Environ. Microbiol.* 79, 1516–22. doi:10.1128/AEM.03439-12.

Nielsen, K. M., Johnsen, P. J., Bensasson, D., and Daffonchio, D. Release and persistence of extracellular DNA in the environment. *Environ. Biosafety Res.* 6, 37–53. doi:10.1051/ebr:2007031.

Nies, D. H. (1992). Resistance to cadmium, cobalt, zinc, and nickel in microbes. *Plasmid* 27, 17–28.

Nultsch, W. (1977). Effect of external factors on phototaxis of *Chlamydomonas reinhardtii*. *Arch. Microbiol.* 112, 179–185. doi:10.1007/BF00429333.

Nyyssola, A., Kerovuo, J., Kaukinen, P., von Weymarn, N., and Reinikainen, T. (2000). Extreme halophiles synthesize betaine from glycine by methylation. *J. Biol. Chem.* 275, 22196–201. doi:10.1074/jbc.M910111199.

Okita, N. (2005). Phototactic activity in *Chlamydomonas* "non-phototactic" mutants deficient in Ca2+-dependent control of flagellar dominance or in inner-arm dynein. *J. Cell Sci.* 118, 529–537. doi:10.1242/jcs.01633.

Okita, N., Isogai, N., Hirono, M., Kamiya, R., and Yoshimura, K. (2005). Phototactic activity in *Chlamydomonas* "non-phototactic" mutants deficient in Ca2+-dependent control of flagellar dominance or in inner-arm dynein. *J. Cell Sci.* 118, 529–37. doi:10.1242/jcs.01633.

Overbeek, R., Begley, T., Butler, R. M., Choudhuri, J. V, Chuang, H.-Y., Cohoon, M., de Crécy-Lagard, V., Diaz, N., Disz, T., Edwards, R., et al. (2005). The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res.* 33, 5691–702. doi:10.1093/nar/gki866.

Paget, E., Monrozier, L. J., and Simonet, P. (1992). Adsorption of DNA on clay minerals: protection against DNaseI and influence on gene transfer. *FEMS Microbiol. Lett.* 97, 31–39. doi:10.1111/j.1574-6968.1992.tb05435.x.

Paget, M. S., Kang, J. G., Roe, J. H., and Buttner, M. J. (1998). sigmaR, an RNA polymerase sigma factor that modulates expression of the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor* A3(2). *EMBO J.* 17, 5776–82. doi:10.1093/emboj/17.19.5776.

Panikov, N. S., Flanagan, P. W., Oechel, W. C., Mastepanov, M. a., and Christensen, T. R. (2006). Microbial activity in soils frozen to below -39°C. *Soil Biol. Biochem.* 38, 785–794. doi:10.1016/j.soilbio.2005.07.004.

Parks, D. H., and Beiko, R. G. (2010). Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* 26, 715–21. doi:10.1093/bioinformatics/btq041.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian, Y., Brown, S. D., Brandt, C. C., et al. (2013). The genetic basis for bacterial mercury methylation. *Science* 339, 1332–5. doi:10.1126/science.1230667.

Peel, M. C., Finlayson, B. L., and McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* 11, 1633–1644. doi:10.5194/hess-11-1633-2007.

Perovich, D. . (2002a). "Ultraviolet Radiation and the Optical Properties of Sea Ice and Snow," in *UV Radiation and Arctic Ecosystems* Ecological Studies., ed. D. O. Hessen (Berlin, Heidelberg: Springer Berlin Heidelberg), 73–89. doi:10.1007/978-3-642-56075-0.

Perovich, D. K. (2002b). "UV and the optocal Properties of Sea Ice and Snow," in *UV Radiation and Arctic Ecosystems*, ed. D. Hessen (Springer), 73–87.

Petri, R., and Imhoff, J. F. (2014). Genetic analysis of sea ice bacterial communities of the Western Baltic Sea using an improved double gradient method. *Polar Biol.* 24, 252–257. doi:10.1007/s003000000205.

Polz, M. F., Alm, E. J., and Hanage, W. P. (2013). Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends Genet.* 29, 170–175.

Pomeroy, J. W., and Brun, E. (2001). "Physical Properties of Snow," in *Snow Ecology : An Interdisciplinary Examination of Snow-Covered Ecosystems*, eds. H. G. Jones, J. W. Pomeroy, D. A. Walker, and R. W. Hoham (Cambridge University Press), 45–126.

Poretsky, R. S., Hewson, I., Sun, S., Allen, A. E., Zehr, J. P., and Moran, M. A. (2009). Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. *Environ. Microbiol.* 11, 1358–75. doi:10.1111/j.1462-2920.2008.01863.x.

Possmayer, M., Berardi, G., Beall, B. F. N., Trick, C. G., Hüner, N. P. a, and Maxwell, D. P. (2011). Plasticity of the psychrophilic green alga *chlamydomonas raudensis* (UWO 241) (Chlorophyta) to supraoptimal temperature stress. *J. Phycol.* 47, 1098–1109. doi:10.1111/j.1529-8817.2011.01047.x.

Preyer, J. M., and Oliver, J. D. (1993). Starvation-Induced Thermal Tolerance as a Survival Mechanism in a Psychrophilic Marine Bacterium. *Appl. Envir. Microbiol.* 59, 2653–2656.

Price, P. B. (2000). A habitat for psychrophiles in deep Antarctic ice. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1247–51.

Price, P. B. (2009). Microbial genesis, life and death in glacial ice. *Can. J. Microbiol.* 55, 1–11. doi:10.1139/W08-117.

Price, P. B. (2007). Microbial life in glacial ice and implications for a cold origin of life. *FEMS Microbiol. Ecol.* 59, 217–231. doi:10.1111/j.1574-6941.2006.00234.x.

Price, P. B., and Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4631–6. doi:10.1073/pnas.0400522101.

Priscu, J. C., Adams, E. E., Lyons, W. B., Voytek, M. A., Mogk, D. W., Brown, R. L., Mckay, C. P., Takacs, C. D., Welch, K. A., Wolf, C. F., et al. (1999). Geomicrobiology of Subglacial Ice Above Lake Vostok , Antarctica. *Science (80-.).* 286, 2141–2144.

Priscu, J. C., and Christner, B. C. (2004). "earth's icy biosphere," in *Microbial Diversity and Bioprospecting*, ed. A. T. Bull (American Society for Microbiology, Washington, D.C), 130–145.

Prosser, J. I. (2015). Dispersing misconceptions and identifying opportunities for the use of "omics" in soil microbial ecology. *Nat. Rev. Microbiol.* 13, 439–46. doi:10.1038/nrmicro3468.

Ralph, P. J., McMinn, A., Ryan, K. G., and Ashworth, C. (2005). Short-term effect of temperature on the photokinetics of microalgae from the surface layers of antarctic pack ice. *J. Phycol.* 41, 763–769. doi:10.1111/j.1529-8817.2005.00106.x.

Ravanat, J. L., Douki, T., and Cadet, J. (2001). Direct and indirect effects of UV radiation on DNA and its components. *J. Photochem. Photobiol. B.* 63, 88–102.

Raymond, J. a. (2014). The ice-binding proteins of a snow alga, *Chloromonas brevispina*: probable acquisition by horizontal gene transfer. *Extremophiles*, 987–994. doi:10.1007/s00792-014-0668-3.

Raymond, J. a., Christner, B. C., and Schuster, S. C. (2008). A bacterial ice-binding protein from the Vostok ice core. *Extremophiles* 12, 713–717. doi:10.1007/s00792-008-0178-2.

Raymond, J. a., and Kim, H. J. (2012). Possible role of horizontal gene transfer in the colonization of sea ice by Algae. *PLoS One* 7. doi:10.1371/journal.pone.0035968.

Reddy, T. B. K., Thomas, A. D., Stamatis, D., Bertsch, J., Isbandi, M., Jansson, J., Mallajosyula, J., Pagani, I., Lobos, E. A., and Kyrpides, N. C. (2014). The Genomes OnLine Database (GOLD) v.5: a metadata management system based on a four level (meta)genome project classification. *Nucleic Acids Res.* 43, D1099–1106. doi:10.1093/nar/gku950.

Remias, D., Albert, a., and Lütz, C. (2010). Effects of realistically simulated, elevated UV irradiation on photosynthesis and pigment composition of the alpine snow alga *Chlamydomonas nivalis* and the arctic soil alga *Tetracystis sp*. (Chlorophyceae). *Photosynthetica* 48, 269–277. doi:10.1007/s11099-010-0033-4.

Remias, D., Holzinger, A., Aigner, S., and Lütz, C. (2012). Ecophysiology and ultrastructure of *Ancylonema nordenskiöldii* (Zygnematales, Streptophyta), causing brown ice on glaciers in Svalbard (high arctic). *Polar Biol.* 35, 899–908. doi:10.1007/s00300-011-1135-6.

Remias, D., Lütz-Meindl, U., and Lütz, C. (2005a). Photosynthesis, pigments and ultrastructure of the alpine snow alga *Chlamydomonas nivalis*. *Eur. J. Phycol.* 40, 259–268. doi:10.1080/09670260500202148.

Remias, D., Schwaiger, S., Aigner, S., Leya, T., Stuppner, H., and Lütz, C. (2011). Characterization of an UV- and VIS-absorbing, purpurogallin-derived secondary pigment new to algae and highly abundant in *Mesotaenium berggrenii* (Zygnematophyceae, Chlorophyta), an extremophyte living on glaciers. *FEMS Microbiol. Ecol.* 79, 638–648. doi:10.1111/j.1574-6941.2011.01245.x.

Remias, D., Wastian, H., Lütz, C., and Leya, T. (2013). Insights into the biology and phylogeny of *Chloromonas polyptera* (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarct. Sci.* 25, 648–656. doi:10.1017/S0954102013000060.

Rezanka, T., Nedbalová, L., Sigler, K., and Cepák, V. (2008). Identification of astaxanthin diglucoside diesters from snow alga *Chlamydomonas nivalis* by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Phytochemistry* 69, 479–90. doi:10.1016/j.phytochem.2007.06.025.

Roberts, M. F. (2005). Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline Systems* 1, 5. doi:10.1186/1746-1448-1-5.

Rodrigues, D. F., da C Jesus, E., Ayala-Del-Río, H. L., Pellizari, V. H., Gilichinsky, D., Sepulveda-Torres, L., and Tiedje, J. M. (2009). Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*. *ISME J.* 3, 658–65. doi:10.1038/ismej.2009.25.

Rodrigues, D. F., and Tiedje, J. M. (2008). Coping with our cold planet. *Appl. Environ. Microbiol.* 74, 1677–86. doi:10.1128/AEM.02000-07.

Rohde, R. A., and Price, P. B. (2007). Diffusion-controlled metabolism for long-term survival of single isolated microorganisms trapped within ice crystals. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16592–7. doi:10.1073/pnas.0708183104.

Roos, J. C., and Vincent, W. F. (1998). Temperature dependence of uv radiation effects on antarctic cyanobacteria. *J. Phycol.* 34, 118–125. doi:10.1046/j.1529-8817.1998.340118.x.

Rosa, L. H., Vaz, A. B. M., Caligiorne, R. B., Campolina, S., and Rosa, C. A. (2008). Endophytic fungi associated with the Antarctic grass Deschampsia antarctica Desv. (Poaceae). *Polar Biol.* 32, 161–167. doi:10.1007/s00300-008-0515-z.

Rothschild, L. J., and Mancinelli, R. L. (2001). Life in extreme environments. *Nature* 409, 1092–101. doi:10.1038/35059215.

Ryan, K. G., McMinn, A., Mitchell, K. A., and Trenerry, L. (2002). Mycosporine-like amino acids in Antarctic sea ice algae, and their response to UVB radiation. *Z. Naturforsch. C.* 57, 471–7.

Saenz, B. T., and Arrigo, K. R. (2012). Simulation of a sea ice ecosystem using a hybrid model for slush layer desalination. *J. Geophys. Res.* 117, C05007. doi:10.1029/2011JC007544.

Sanguino, L., Franqueville, L., Vogel, T. M., and Larose, C. (2015). Linking environmental prokaryotic viruses and their host through CRISPRs. *FEMS Microbiol. Ecol.* doi:10.1093/femsec/fiv046.

Santos, A. L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N. C. M., Almeida, A., Correia, A., and Cunha, Â. (2013). Wavelength dependence of biological damage induced by UV radiation on bacteria. *Arch. Microbiol.* 195, 63–74. doi:10.1007/s00203-012-0847-5.

Satomi, M., and Fujii, T. (2014). "The Family Oceanospirillaceae," in *The Prokaryotes - Gammaproteobacteria*, eds. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (Berlin, Heidelberg: Springer Berlin Heidelberg), 491–527. doi:10.1007/978-3-642-38922-1.

Sattler, B., Puxbaum, H., and Psenner, R. (2001). Bacterial growth in supercooled cloud droplets. *Geophys. Res. Lett.* 28, 239–242. doi:10.1029/2000GL011684.

Saum, S. H., Pfeiffer, F., Palm, P., Rampp, M., Schuster, S. C., Müller, V., and Oesterhelt, D. (2013). Chloride and organic osmolytes: a hybrid strategy to cope with elevated salinities by the moderately halophilic, chloride-dependent bacterium halobacillus halophilus. *Environ. Microbiol.* 15, 1619–33. doi:10.1111/j.1462-2920.2012.02770.x.

Säwström, C., Granéli, W., Laybourn-Parry, J., and Anesio, A. M. (2007). High viral infection rates in Antarctic and Arctic bacterioplankton. *Environ. Microbiol.* 9, 250–5. doi:10.1111/j.1462-2920.2006.01135.x.

Schimel, J. P., Bilbrough, C., and Welker, J. M. (2004). Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biol. Biochem.* 36, 217–227.

Schmidt, S. K., and Lipson, D. a. (2004). Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. *Plant Soil* 259, 1–7. doi:10.1023/B:PLSO.0000020933.32473.7e.

Schmitz, O. (2000). CikA, a Bacteriophytochrome That Resets the Cyanobacterial Circadian Clock. *Science (80-.).* 289, 765–768. doi:10.1126/science.289.5480.765.

Schroeder, W. H., Anlauf, K. G., Barrie, L. A., Lu, J. Y., Steffen, A., Schneeberger, D. R., and Berg, T. (1998). Arctic springtime depletion of mercury. 394, 331–332. doi:10.1038/28530.

Seeberg, E., Eide, L., and Bjørås, M. (1995). The base excision repair pathway. *Trends Biochem. Sci.* 20, 391–397. doi:10.1016/S0968-0004(00)89086-6.

Segawa, T., Miyamoto, K., Ushida, K., Agata, K., Okada, N., and Kohshima, S. (2005). Seasonal Change in Bacterial Flora and Biomass in Mountain Snow from the Tateyama Mountains , Japan , Analyzed by 16S rRNA Gene Sequencing and Real-Time PCR. 71, 123–130. doi:10.1128/AEM.71.1.123.

Selbmann, L., Isola, D., Zucconi, L., and Onofri, S. (2011). Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. *Fungal Biol.* 115, 937–44. doi:10.1016/j.funbio.2011.02.016.

Senčilo, A., Luhtanen, A.-M., Saarijärvi, M., Bamford, D. H., and Roine, E. (2014). Cold-active bacteriophages from the Baltic Sea ice have diverse genomes and virus-host interactions. *Environ. Microbiol.* doi:10.1111/1462-2920.12611.

Shain, D. H., Mason, T. a., Farrell, A. H., and Michalewicz, L. a. (2001). Distribution and behavior of ice worms (*Mesenchytraeus solifugus*) in south-central Alaska. *Can. J. Zool.* 79, 1813–1821. doi:10.1139/cjz-79-10-1813.

Shtarkman, Y. M., Koçer, Z. a, Edgar, R., Veerapaneni, R. S., D'Elia, T., Morris, P. F., and Rogers, S. O. (2013). Subglacial Lake Vostok (Antarctica) accretion ice contains a diverse set of sequences from aquatic, marine and sediment-inhabiting bacteria and eukarya. *PLoS One* 8, e67221. doi:10.1371/journal.pone.0067221.

Simon, C., and Daniel, R. (2011). Metagenomic analyses: past and future trends. *Appl. Environ. Microbiol.* 77, 1153–61. doi:10.1128/AEM.02345-10.

Simon, C., Wiezer, A., Strittmatter, A. W., and Daniel, R. (2009). Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. *Appl. Environ. Microbiol.* 75, 7519–26. doi:10.1128/AEM.00946-09.

Singh, P., Hanada, Y., Singh, S. M., and Tsuda, S. (2013). Antifreeze protein activity in Arctic cryoconite bacteria. *FEMS Microbiol. Lett.*, 1–9. doi:10.1111/1574-6968.12345.

Sinha, R. P., and Häder, D. P. (2002). UV-induced DNA damage and repair: a review. *Photochem. Photobiol. Sci.* 1, 225–36.

Skidmore, M., Anderson, S. P., Sharp, M., Foght, J., and Lanoil, B. D. (2005). Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl. Environ. Microbiol.* 71, 6986–97. doi:10.1128/AEM.71.11.6986-6997.2005.

Smith, J. J., Howington, J. P., and McFeters, G. A. (1994). Survival, physiological response and recovery of enteric bacteria exposed to a polar marine environment. *Appl. Environ. Microbiol.* 60, 2977–84.

Smith, L. C., Chu, V. W., Yang, K., Gleason, C. J., Pitcher, L. H., and Rennermalm, A. K. (2015). Efficient meltwater drainage through supraglacial streams and rivers on the southwest Greenland ice sheet. 1–6. doi:10.1073/pnas.1413024112.

Smith, T., Pitts, K., McGarvey, J. A., and Summers, A. O. (1998). Bacterial oxidation of mercury metal vapor, Hg(0). *Appl. Environ. Microbiol.* 64, 1328–32.

Solomon, P. S., Waters, O. D. C., and Oliver, R. P. (2007). Decoding the mannitol enigma in filamentous fungi. *Trends Microbiol.* 15, 257–62. doi:10.1016/j.tim.2007.04.002.

Souchez, R., Petit, J. R., Tison, J. L., Jouzel, J., and Verbeke, V. (2000). Ice formation in subglacial Lake Vostok, Central Antarctica. *Earth Planet. Sci. Lett.* 181, 529–538. doi:10.1016/S0012-821X(00)00228-4.

Sourjik, V. (2004). Receptor clustering and signal processing in E. coli chemotaxis. *Trends Microbiol.* 12, 569–76. doi:10.1016/j.tim.2004.10.003.

Stewart, F. J., and Fritsen, C. H. (2004). Bacteria-algae relationships in Antarctic sea ice. *Antarct. Sci.* 16, 143–156. doi:10.1017/S0954102004001889.

Stewart, F. J., Ottesen, E. a, and DeLong, E. F. (2010). Development and quantitative analyses of a universal rRNA-subtraction protocol for microbial metatranscriptomics. *ISME J.* 4, 896–907. doi:10.1038/ismej.2010.18.

Stibal, M., Bælum, J., Holben, W. E., Sørensen, S. R., Jensen, A., and Jacobsen, C. S. (2012a). Microbial degradation of 2,4-dichlorophenoxyacetic acid on the Greenland ice sheet. *Appl. Environ. Microbiol.* 78, 5070–6. doi:10.1128/AEM.00400-12.

Stibal, M., Elster, J., Sabacká, M., and Kastovská, K. (2007). Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. *FEMS Microbiol. Ecol.* 59, 265–73. doi:10.1111/j.1574-6941.2006.00264.x.

Stibal, M., Hasan, F., Wadham, J. L., Sharp, M. J., and Anesio, A. M. (2012b). Prokaryotic diversity in sediments beneath two polar glaciers with contrasting organic carbon substrates. *Extremophiles* 16, 255–265. doi:10.1007/s00792-011-0426-8.

Stibal, M., Šabacká, M., and Žárský, J. (2012c). Biological processes on glacier and ice sheet surfaces. *Nat. Geosci.* 5, 771–774. doi:10.1038/NGEO1611.

Stibal, M., Telling, J., Cook, J., Mak, K. M., Hodson, A., and Anesio, A. M. (2012d). Environmental controls on microbial abundance and activity on the greenland ice sheet: a multivariate analysis approach. *Microb. Ecol.* 63, 74–84. doi:10.1007/s00248-011-9935-3.

Takada, Y., Hayashinaka, N., Hagihara-Nukui, E., and Fukunaga, N. (1993). Chemotaxis in a psychrophilic marine bacterium, *Vibrio* sp. strain ABE-1. *J. Gen. Appl. Microbiol.* 39, 371–379. doi:10.2323/jgam.39.371.

Takeuchi, N. (2001). Structure, formation, and darkening process of albedo-reducing material cryoconite on a Himalayan glacier: a granular algal mat growing on the glacier. *Arctic, Antarct. Alp. Res.* 33, 115–122.

Tansley, A. . (1935a). The use and abuse of vegetational concepts and terms. *Ecology* 16, 284–307.

Tansley, A. G. (1935b). The Use and Abuse of Vegetational Concepts and Terms. *Ecology* 16, 284. doi:10.2307/1930070.

Techel, F., and Pielmeier, C. (2011). Point observations of liquid water content in wet snow - Investigating methodical, spatial and temporal aspects. *Cryosphere* 5, 405–418. doi:10.5194/tc-5-405-2011.

Thomas, D. N., and Papadimitriou, S. (2008). "Biogeochemistry of Sea Ice," in *Sea Ice: An Introduction to its Physics, Chemistry, Biology and Geology*, eds. D. N. Thomas and G. S. Dieckmann (Blackwell publishing), 267–302.

Thomas, W. H., and Duval, B. (1995). Sierra Nevada , California , UeSeA , Snow Algae : Snow Albedo Changes , Algal-Bacterial Interrelationships , and Ultraviolet Radiation Effects. *Arct. Alp. Res.* 27, 389–399.

Trinks, H., Schröder, W., and Biebricher, C. K. (2005). Ice and the origin of life. *Orig. Life Evol. Biosph.* 35, 429–445. doi:10.1007/s11084-005-5009-1.

Tung, H. C., Bramall, N. E., and Price, P. B. (2005). Microbial origin of excess methane in glacial ice and implications for life on Mars. *Proc. Natl. Acad. Sci. U. S. A.* 102, 18292–6. doi:10.1073/pnas.0507601102.

Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., and Schuster, S. C. (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS One* 3, e2527. doi:10.1371/journal.pone.0002527.

Uusikivi, J., Vähätalo, A. V, Granskog, M. A., and Sommaruga, R. (2010). Contribution of mycosporine-like amino acids and colored dissolved and particulate matter to sea ice optical properties and ultraviolet attenuation. *Limnol. Oceanogr.* 55, 703–713.

Vaïtilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., Amato, P., Chaumerliac, N., and Delort, A.-M. (2013). Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. *Proc. Natl. Acad. Sci. U. S. A.* 110, 559–64. doi:10.1073/pnas.1205743110.

Vancoppenolle M, Meiners KM, Michel C, Bopp L, Brabant F, Carnat G, et al. (2013). Role of sea ice in global biogeochemical cycles: emerging views and challenges. Quat. Sci. Rev. 79:207–230.

Varghese, S., Tang, Y., and Imlay, J. A. (2003). Contrasting sensitivities of *Escherichia coli* aconitases A and B to oxidation and iron depletion. *J. Bacteriol.* 185, 221–30.

Varin, T., Lovejoy, C., Jungblut, A. D., Vincent, W. F., and Corbeil, J. (2012). Metagenomic analysis of stress genes in microbial mat communities from Antarctica and the High Arctic. *Appl. Environ. Microbiol.* 78, 549–59. doi:10.1128/AEM.06354-11.

Vincent, W. F., Mueller, D. R., and Bonilla, S. (2004). Ecosystems on ice: The microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology* 48, 103–112. doi:10.1016/j.cryobiol.2004.01.006.

Völker, U., Maul, B., and Hecker, M. (1999). Expression of the sigmaB-dependent general stress regulon confers multiple stress resistance in *Bacillus subtilis*. *J. Bacteriol.* 181, 3942–8.

Vollmers, J., Voget, S., Dietrich, S., Gollnow, K., Smits, M., Meyer, K., Brinkhoff, T., Simon, M., and Daniel, R. (2013). Poles apart: Arctic and Antarctic *Octadecabacter* strains share high genome plasticity and a new type of xanthorhodopsin. *PLoS One* 8, e63422. doi:10.1371/journal.pone.0063422.

Wang, G., and Maier, R. J. (2004). An NADPH Quinone Reductase of *Helicobacter pylori* Plays an Important Role in Oxidative Stress Resistance and Host Colonization. *Infect. Immun.* 72, 1391–1396. doi:10.1128/IAI.72.3.1391-1396.2004.

Warren, S. G., and Hudson, S. R. (2003). Bacterial Activity in South Pole Snow Is Questionable Letters to the Editor Bacterial Activity in South Pole Snow Is Questionable. 69, 6340–6342. doi:10.1128/AEM.69.10.6340.

Wells, L. E., and Deming, J. W. (2006). Brief report Modelled and measured dynamics of viruses in Arctic winter sea ice brines. *Environ. Microbiol.* 8, 1115–1121. doi:10.1111/j.1462-2920.2005.00984.x.

Werner, I., Ikävalko, J., and Schünemann, H. (2007). Sea ice algae in Arctic pack ice during late winter. *Polar Biol.* 30, 1493–1504. doi:10.1007/s00300-007-0310-2.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Perspective Prokaryotes : The unseen majority. 95, 6578–6583.

Wilhelm, S. W., and Suttle, C. A. (1999). Viruses and Nutrient Cycles in the Sea aquatic food webs. *Bioscience* 49, 781–788.

Williams, W. E., Gorton, H. L., and Vogelmann, T. C. (2003). Surface gas-exchange processes of snow algae. *Proc. Natl. Acad. Sci. U. S. A.* 100, 562–6. doi:10.1073/pnas.0235560100.

De Wit, P., Pespeni, M. H., Ladner, J. T., Barshis, D. J., Seneca, F., Jaris, H., Therkildsen, N. O., Morikawa, M., and Palumbi, S. R. (2012). The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. *Mol. Ecol. Resour.* 12, 1058–67. doi:10.1111/1755-0998.12003.

Wu, Y., Wu, M., Zhang, Y., Li, W., Gao, Y., Li, Z., Wang, Z., Lubec, G., and Zhang, C. (2012). Lyophilization is suitable for storage and shipment of fresh tissue samples without altering RNA and protein levels stored at room temperature. *Amino Acids* 43, 1383–8. doi:10.1007/s00726-011-1212-8.

Yallop, M. L., Anesio, A. M., Perkins, R. G., Cook, J., Telling, J., Fagan, D., MacFarlane, J., Stibal, M., Barker, G., Bellas, C., et al. (2012). Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. *ISME J.* 6, 2302–13. doi:10.1038/ismej.2012.107.

Zarsky, J. D., Stibal, M., Hodson, A., Sattler, B., Schostag, M., Hansen, L. H., Jacobsen, C. S., and Psenner, R. (2013). Large cryoconite aggregates on a Svalbard glacier support a diverse microbial community including ammonia-oxidizing archaea. *Environ. Res. Lett.* 8, 035044. doi:10.1088/1748-9326/8/3/035044.

Zawar-Reza, P., Argüello-Astorga, G. R., Kraberger, S., Julian, L., Stainton, D., Broady, P. a, and Varsani, A. (2014). Diverse small circular single-stranded DNA viruses identified in a freshwater pond on the McMurdo Ice Shelf (Antarctica). *Infect. Genet. Evol.* 26, 132–8. doi:10.1016/j.meegid.2014.05.018.

Zawierucha, K., Kolicka, M., Takeuchi, N., and Kaczmarek, Ł. (2014). What animals can live in cryoconite holes ? A faunal review. *J. Zool.*, 1–11. doi:10.1111/jzo.12195.

Zeng, Y. X., Yan, M., Yu, Y., Li, H. R., He, J. F., Sun, K., and Zhang, F. (2013). Diversity of bacteria in surface ice of Austre Lovénbreen glacier, Svalbard. *Arch. Microbiol.* 195, 313–322. doi:10.1007/s00203-013-0880-z.

Zhang, S., Yang, G., Wang, Y., and Hou, S. (2010). Abundance and community of snow bacteria from three glaciers in the Tibetan Plateau. *J. Environ. Sci.* 22, 1418–1424. doi:10.1016/S1001-0742(09)60269-2.

Zhang, X. F., Yao, T. D., Tian, L. D., Xu, S. J., and An, L. Z. (2008). Phylogenetic and physiological diversity of bacteria isolated from Puruogangri ice core. *Microb. Ecol.* 55, 476–488. doi:10.1007/s00248-007-9293-3.

Ziegelhoffer, E. C., and Donohue, T. J. (2009). Bacterial responses to photo-oxidative stress. 7, 856–863. doi:10.1038/nrmicro2237.Bacterial.

Ziegler, C., Bremer, E., and Krämer, R. (2010). The BCCT family of carriers: from physiology to crystal structure. *Mol. Microbiol.* 78, 13–34. doi:10.1111/j.1365-2958.2010.07332.x.