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ORIGINAL PAPER

Microbial abundance and community structure in a melting alpine snowpack

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Abstract Snowmelt is a crucial period for alpine soil ecosystems, as it is related to inputs of nutrients, particulate matter and microorganisms to the underlying soil. Although snow-inhabiting microbial communities represent an important inoculum for soils, they have thus far received little attention. The distribution and structure of these microorganisms in the snowpack may be linked to the physical properties of the snowpack at snowmelt. Snow samples were taken from snow profiles at four sites (1930–2519 m a.s.l.) in the catchment of the *Tiefengletscher*, Canton Uri, Switzerland. Microbial (Archaea, Bacteria and Fungi) communities were investigated through T-RFLP profiling of the 16S and 18S rRNA genes, respectively. In parallel, we assessed physical and chemical parameters relevant to the understanding of melting processes. Along the snow profiles, density increased with depth due to compaction, while other physico-chemical parameters, such as temperature and concentrations of DOC and soluble ions, remained in the same range (e.g. <2 mg DOC L^{-1}, 5–30 μg NH_4^+-N L^{-1}) in all samples at all sites. Along the snow profiles, no major change was observed either in cell abundance or in bacterial and fungal diversity. No Archaea could be detected in the snow. Microbial communities, however, differed significantly between sites. Our results show that meltwater rearranges soluble ions and microbial communities in the snowpack.

Keywords Snow bacteria · Snow fungi · T-RFLP · 16S rRNA gene · Snow physics

Introduction

In the European Alps, seasonal climatic shifts are very pronounced. A typical year includes snow-covered winters and springs, warm summers and wet, cold autumns. Plant and animal communities inhabiting alpine environments are adapted to such conditions, by showing, for example, short vegetative seasons, and physiological adaptations (Körner 1999; Lütz 2010). Microbial communities in alpine systems also display a wide range of physiological flexibility to changing environmental conditions, such as modifications of the membrane structures and cell metabolism (Mayr et al. 1999; Meyer et al. 2004). Moreover, extreme cold habitats seem to favour survival strategies such as spore and cyst formation or dormancy (Bauer et al. 2002; Remias et al. 2010; Harding et al. 2011).

The snow-covered seasons are of particular interest. Snow below 0 °C is a two-phase system consisting of ice grains and air. At every precipitation event during winter, new snow layers pose on the previous ones, producing a stratified structure. Each snow layer has unique properties (i.e. density, water content and permeability), and undergoes rapid structural changes due to metamorphism of ice grains and settling (Waldner et al. 2004; Pinzer et al. 2012). During snow accumulation in winter, the forming

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snowpack also includes particles (Meyer and Wania 2008; Steltzer et al. 2009). The winter snowpack thus serves as a reservoir for ions, organic carbon and pollutants deposited from the atmosphere (e.g. organic particles, mineral dust, soot; cf. Grannas et al. 2007).

At the timepoint of snowmelt, in late spring, higher daily temperatures cause the formation of meltwater. Snow therefore changes to a three-phase system with a variable content of liquid water (1–13 %; Waldner et al. 2004; Techel et al. 2011). Meltwater may infiltrate to deeper snow layers, refreeze at temperatures of <0 °C and produce ice lenses (Bowman 1992; Pomeroy and Brun 2001). Stratification may be gradually lost with the increasing flushes of meltwater in the melting season. The meltwater will initially flush through the snowpack along preferential flow paths, and will eventually reach the underlying soil surface (Pomeroy and Brun 2001).

Physical characteristics of the snowpack (e.g. ice layers; Feng et al. 2001; Lee et al. 2008) and the chemical properties of the deposited compounds, determine their retention or release, as soluble compounds are more mobile in snow. In terrestrial ecosystems, initial snowmelt plays a crucial role in solute transport through the snowpack to the soil, as the first meltwater contains high concentrations of soluble species, which are released as a ionic pulse (Bales et al. 1990; Williams et al. 2009; Björkman et al. 2014).

Snow is a habitat for various types of microorganisms ranging from psychrotolerant and psychrophilic bacteria to fungi and microeukaryotes (Margesin and Schinner 1994; Larose et al. 2013). Most of the taxa found in snow correspond to either microorganisms with specific adaptive strategies (i.e. presence of pigments for UV protection, spore formation), or to opportunists. For example, Bacteroidetes, Firmicutes and Proteobacteria seem to be predominant phyla inhabiting the snowpack (Amato et al. 2007; Møller et al. 2013). In particular, the highly versatile class Betaproteobacteria (e.g. *Variovorax*, *Janthinobacterium* and *Polaromonas*) has been often found in snow (Segawa et al. 2005; Liu et al. 2009; Hell et al. 2013). In snow meltwater from Svalbard, also Sphingobacteria and Flavobacteria were commonly detected (Larose et al. 2010), but different communities were found at different seasons (early vs. late snowmelt). The photoautotrophic Cyanobacteria were found dominant only on pristine snow in North Pole ice floes (Hauptmann et al. 2014).

As snow is a highly dynamic system, data on snow microbiology are hard to be interpreted if the snowpack properties are not sufficiently investigated. Many studies provide solid and valuable data on microbial communities, but limited information on snow chemistry, physics and transport processes. These factors are essential for an ecological understanding of the snow environment.

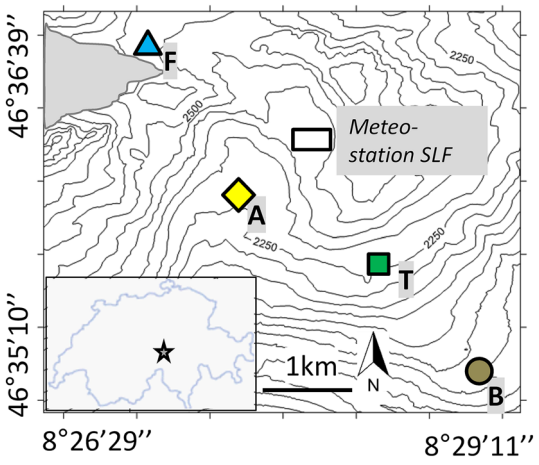

Fig. 1 Map of sampling locations in Canton Uri, Switzerland. *B* Börtli, *T* Tätsch, *A* Älpetli, *F* Forefield of the *Tiefengletscher*. In *grey*, the glacier

Currently, for example, little is known about microbial community distribution in the different snowpack layers. Furthermore, the fate of microbial cells once deposited on the snowpack surface is largely unknown. Some of the taxa not adapted to the extreme conditions of the snow habitat may die, and only the survivors will be detected in the snow. Moreover, with snowmelt and layer compaction, the different communities may be homogenised along the depth profile, and not represent single deposition events.

In this study, we investigated how cell abundance and diversity is distributed in the snowpack at the time of snowmelt. In addition, we evaluated whether alpine snowpack at different altitudes would harbour different microbial community structures. At snowmelt (May–June 2013), four different depth profiles from the snowpack of an alpine glacier catchment (*Tiefengletscher*, Canton UR, Switzerland, latitude 46°36′N, longitude 8°28′E) were characterized in terms of their physical–chemical and microbiological properties. Microbial communities were assessed through T-RFLP profiling of rRNA genes and flow cytometry.

Materials and methods

Study sites and meteorological data

Four different locations in the catchment of the *Tiefengletscher*, Canton UR, Switzerland, were selected: Börtli (B), Tätsch (T), Älpetli (A) and the forefield of *Tiefengletscher* (F) (Fig. 1). The locations are situated within an area of approximately 5 km^2 at different altitudes (Table 1). Site A, B, T are alpine meadows, while site F is a scarcely vegetated glacier forefield. A and T were at similar

Table 1 Sampling locations and description

Location name	Code	N	E	Altitude (m a.s.l.)	Sampling date	Height of snowpack (cm)	Melting rate (cm/h)[b]
Forefield of *Tiefengletscher*[a]	F	46°36′33.8″	08°27′08.1″	2519	18 June 2013	123	1.15 ± 0.14
Älpetli	A	46°36′01.1″	08°27′35.4″	2269	13 June 2013	105	0.98 ± 0.12
Tätsch	T	46°35′19.7″	08°28′16.5″	2244	12 June 2013	105	0.82 ± 0.11
Börtli	B	46°35′19.7″	08°29′01.7″	1930	21 May 2013	93	0

[a] The forefield of *Tiefengletscher* has been described by Lazzaro et al. (2009) and Meola et al. (2014)

[b] Melting rate was measured at each location by placing wooden sticks in the snowpack, and measuring the exposed sections between 11:00 and 16:00. Values represent average melting rates ± standard deviation ($n = 5$)

altitudes, but A was located in a basin and T on a wind-exposed ridge.

At the time of sampling, the height of the snowpack at the four locations varied between 93 and 123 cm. The weather at each sampling date (between May and June 2013) was sunny and clear except for Börtli where light snowfall was observed. Meteorological data was collected from a meteostation located close by (Fig. 1). The meteostation included a non-heated gauge. In parallel, we performed manual estimations of snowmelt rates in the field.

Sampling of snow

At each location, a 3-m-long trench was dug in the snowpack, from the snow surface to the soil surface (defined as 0 cm height). The trench was divided into 3 adjacent sections to allow replication of the sampling. As snow layers were not clearly distinguishable at all sites, samples were taken at regular height intervals of 20 cm. The in situ snow temperature was measured with a Testo 925 temperature sensor (Testo AG, Mönchaltdorf, Switzerland), and snow density was estimated gravimetrically, starting from a knife-cut snow wedge. Triplicate snow samples were taken directly with 50-mL sterile Falcon tube for microbial community analysis (T-RFLP and flow cytometry). Additional 50-mL sterile Falcon tubes were used for sampling snow to be used for chemical characterization. At the snowpack surface, and at intermediate levels a shovel (115 × 5.8 × 24 cm) was used to collect approximately 1 L of snow for particulate matter (PM) characterization. The snow for PM characterization was melted on the spot and syringe-filtered through a 1.6-μm glass fibre filter (VWR, Leuven, The Netherlands).

Physical and chemical characterization of snow

For pH, conductivity and chemical properties, the snow (triplicates for each location and snow height) was allowed to slowly melt and equilibrate at room temperature. pH and conductivity were measured with a Multi 340i Multimeter (Cole-Palmer, Weilheim, Germany). For DOC measurements, the melted snow was immediately syringe-filtered through a 0.45-μm PES filter (VWR). Ten mL of the filtrate was acidified with 40 μL of 37 % HCl prior to measurement with a Shimadzu TOC analyser (Shimadzu GmbH, Reinach, Switzerland).

Soluble anions (NO_3^-, SO_4^{2-}, PO_4^{3-}) were measured with a Dionex D-320 Ion Chromatography system and Chromeleon package (Dionex, Sunnyvale, CA), while NH_4^+ was estimated with the colorimetric method of Mulvaney (1996).

For PM characterization, the glass fibre filters were allowed to dry and stored in boxes at room temperature until analysis. The filters were weighed before and after filtering. The difference in weight expressed the amount of PM contained in the snow, was recorded. The PM weight of each of the filters was corrected according to the original snow sample volume.

Microbial cell counts

An aliquot (50 mL) of the snow samples was fixed with glutaraldehyde (Sigma-Aldrich, Buchs, Switzerland), and used for biomass estimations. Two hundred μL of each sample were stained with 2 μL of Sybr Green (Life Technologies, Zug, Switzerland) and incubated for 10 min at 37 °C. Cell numbers were subsequently estimated on a BD Accuri C6 Flow cytometer (BD Biosciences, San Jose, CA).The measurements were performed on all the triplicate samples (biological replicates).

Microbial community diversity

For community analyses through terminal restriction fragment length polymorphism (T-RFLP), 100 mL of snow from each of the triplicates was allowed to melt under cold sterile conditions and then filtered using a sterile Millipore 0.2 μm filter unit system (Merck Millipore, Darmstadt,

Germany). DNA was extracted from the filters using the MOBIO PowerWater DNA extraction kit (MOBIO Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. A negative control, represented by a clean filter, was extracted in parallel. Aliquots of the extracted DNA (2–5 ng) were then PCR-amplified with primers targeting the Archaeal (primer pair 21F-Uni-b Rev; Reysenbach et al. 2000) and bacterial (primer pair 27F-1406 Rev; Winsley et al. 2012) 16S rRNA gene, and the fungal (primer pair 813F-1357 Rev; Borneman and Hardin 2000) 18S rRNA gene. All of the forward primers used were FAM-labelled at the 5′ end. PCR reactions included 1× DreamTaq PCR buffer, 0.5 μM of each primer, 0.2 mM dNTPs, 2 U DreamTaq polymerase, all in a 25-μL end volume. All reagents were supplied by Fermentas (Wohlen, Switzerland). PCR consisted of a first step of 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 1 min. The reaction was terminated by a final elongation step of 72 °C for 4 min. Positive PCR products were validated through agar gel electrophoresis and GelRed staining (Invitrogen, Life Technologies, Zug, Switzerland). The PCR products were then digested with equal volumes of the restriction enzyme AluI in 1 % Y+ Tango buffer (Fermentas). Finally, 3 μL of digestions were mixed with 10 μL of HIDI Formamide (Applied Biosystems ABI, LifeTechnologies) and 0.1 μL of ROX 1000 standard (Bioventures Inc., Murfreesboro, TN), denatured for 2 min at 95 °C and immediately placed on ice.

Terminal restriction patterns were examined by capillary electrophoresis on a ABI 3130XL sequencer (ABI) and analysed with Genemapper software version 3.7 (ABI).

Physiological profiles

BIOLOG incubations of surface samples or of bulk snow (taken at approximately 40 cm height in the snowpack) were performed in triplicates by inoculating 200 μL of melted snow samples in each Biolog™ ECOplate well (BIOLOG, Hayward, CA) and incubating at 0 °C. Colour development was assessed daily by measuring the optical density (OD) of the samples at 595 nm with a Biotek plate reader (Bio-TeK, Winooski, VT). The time point of maximum average well colour development (AWCD) was defined as lag time (Stelmach et al. 2012) and was observed after 30–31 days of incubation. AWCD for each timepoint was calculated according to the formula:

$$\text{AWCD} = \Sigma\ (\text{Abs sample} - \text{Abs water})/n \quad (1)$$

where $n = 31$, corresponding to the number of substrates in the plate.

Carbon source utilization by communities was illustrated by the ratio:

$$\text{C source utilisation} = (\text{Abs sample} - \text{Abs water})/\text{AWCD}. \quad (2)$$

Infiltration experiment

To test the behaviour of microbial cells in a melting snowpack, we performed a bacterial infiltration experiment in the field at the F location. As cell inoculum, we used a liquid culture of *Paenibacillus sabinae*. In preliminary experiments, the culture was tested for its resistance at 0 °C and in snow for several hours. At the field site, 10 mL of liquid culture (approximately 10^8 cells) were mixed with 2 μL 5 mM SYTO®9 green fluorescent nucleic acid stain (Molecular Probes, Life Technologies, Zug, Switzerland) and diluted in 500 mL of water containing 50 mg L^{-1} of brilliant blue. The resulting solution was immediately sprinkled evenly on a surface of 0.45 m^2. After allowing the solution to percolate for 15 min, we took triplicate samples with sterile 50-mL Falcon tubes. Samples were taken at three different depths of the snowpack, allowing a lag time of 15 min to permit the solution to percolate. The replicates were taken at regular distances and included both, preferential flow paths visualized by brilliant blue, and unstained snow. After sampling, the samples were immediately spiked with 200 μL of glutaraldehyde to fix the cells.

In the laboratory, the snow was allowed to slowly melt under cold conditions. The concentration of brilliant blue which was percolating in the snow samples was calculated from absorption at 595 nm with a UV–Vis spectrophotometer (Varian Inc., Palo Alto, USA). The concentration of the SYTO®9 *Paenibacillus* cells was measured by flow cytometry as described before. The resulting cell number was related to brilliant blue concentrations.

Statistical analysis

Significances of the means of all measured parameters were estimated with two-way ANOVA analysis of variance, by testing the factors "height" (between all samples and within samples from a single site) and "site" (between all samples). All analyses were performed with Systat V.12.

Ward's hierarchical clustering with Euclidean distances was applied to each replicate T-RFLP profile in order to visualize heterogeneity between samples. In order to obtain a representative image of the microbial communities at each height, each electropherogram was first analysed separately, and then merged with the other two replicates to obtain an averaged T-RFLP profile for each sampling height at each location. In brief, the individual raw T-RFLP electropherograms were converted into relative abundance profiles by relating each fluorescence peak height to the total fluorescence detected in the profile. Richness, Evenness, Simpson, Inverse Simpson, and Shannon Diversity indices were calculated on the basis of the presence/absence and relative abundance of each peak as described before (Blackwood et al. 2007). Bray–Curtis Dissimilarity

was estimated pairwise for each combination of two T-RFLP profiles and plotted as a heatmap. Finally, the constrained ordination method RDA was applied by combining the T-RFLP data with major log-transformed environmental variables (snow parameters). A reduced model with 100 permutations was performed to visualize significant effects of environmental variables. All analyses were performed with the "vegan" package R (Oksanen et al. 2005).

Results

Physical and chemical characteristics of the snowpack

The information collected from the WSL meteostation on snow height (supplementary Fig. 2) showed a decline in snowpack height of approximately 200 cm in 5 weeks (melting rate 4 cm day^{-1}), indicating that all sampling dates fell at snowmelt. Our own measurements performed at the specific sampling dates (Table 1) showed that the melting rate on a sunny day could rise up to approximately 10 cm day^{-1}.

Snow density ranged between 470 and 580 kg m^{-3} in all the depth profiles, and tended to increase with depth (Fig. 2a). Both pH and density values showed site-specific significant differences (Table S1). pH showed a highly significant variability along each transect (Table S1; Fig. 2b). The pH values measured were generally slightly acidic. The strongest pH variation in relation to depth was measured in the samples from T, where pH rose from 6 to 7.5 and then decreased again to approximately 5.5 in the lowest levels of the snowpack.

Temperature and conductivity of each snowpack was fairly constant at all heights (data not shown). Temperature was approximately -0.25 ± 0.01 °C and conductivity was generally below 0.2 µS cm^{-1}. Staining of the snowpack with erioglaucine ("Brilliant Blue"; Schneebeli 1995) showed ice lenses in the first decimeters of the snowpack (Fig. 3), and preferential flow paths.

Particulate matter on the surface and in the snowpack

The amount of PM trapped in the snowpack ranged from 0.1 to 0.6 mg/L in the bulk snow taken at different heights of the snowpack (Fig. 4; Table S2). On the surface snow, the dry weight was highly variable. For example, in the three replicate surface samples from B, the measured dry masses of PM were 7.8, 21.6 and 43.7 mg L^{-1}. On the glass fibre filters from the replicate surface samples from T and A, the PM (Table S1) dry weight averaged 34.2 ± 7.9 and 25.5 ± 19.9 mg, respectively, while the PM in the surface samples from F was slightly lower (17.1 ± 6.9 mg).

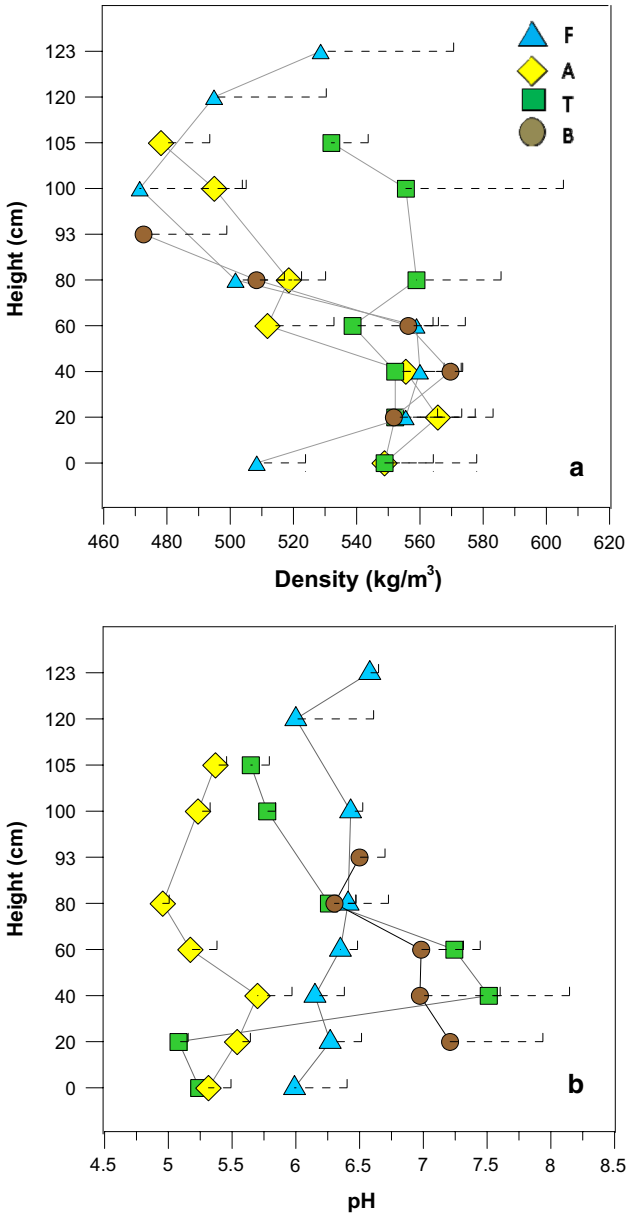

Fig. 2 Snow density and pH. Values are average ± standard deviation ($n = 3$). For clarity of presentation, only *positive error bars* are visualized

DOC and soluble nutrients in the snowpack

DOC remained below 2 mg L^{-1} in all the samples, and showed little variability with height within each depth profile (Fig. 5a). SO_4^{2-}–S was below 0.4 mg L^{-1} in the snowpack from B, T and A. It was significantly higher, however, in the surface and top layers of the F snowpack (Fig. 5b). NH_4^+-N was also constantly low through the snow depth profiles, with some significant variability at different heights of the B depth profile (Table S1; Fig. 5c). NO_3^--N tended to increase significantly in the upper layers of the

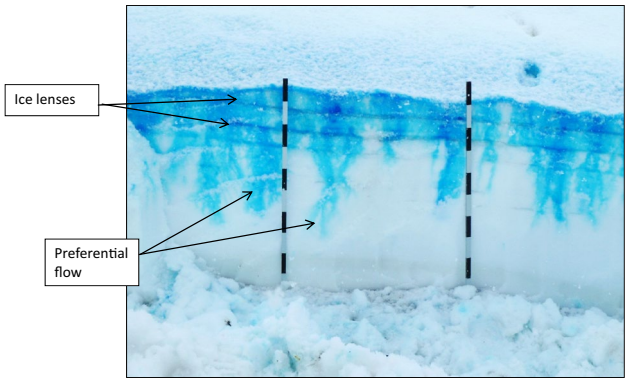

Fig. 3 Snow profile (picture taken from location T) visualized after application of a solution of 0.5 mg L^{-1} of Brilliant Blue dye (erioglaucine) to the snow surface. The dye was allowed to percolate for 45 min before taking the picture. Preferential flow paths and extensive ice layers are clearly visible. *Black-white bars* mark each 10-cm interval

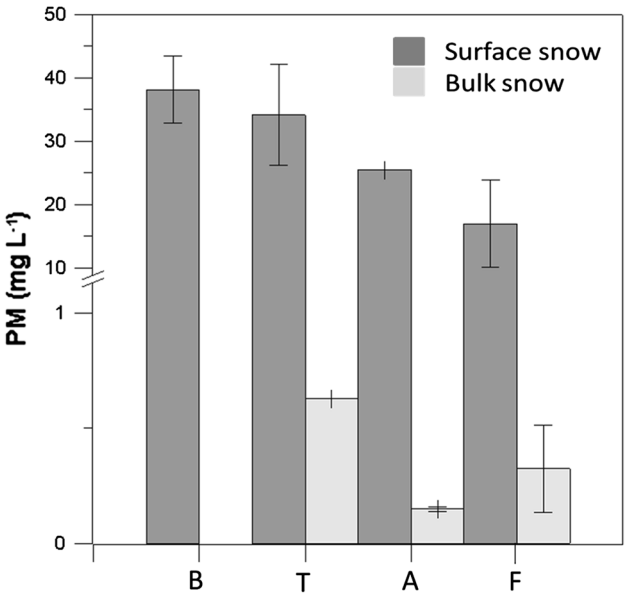

Fig. 4 Particulate matter (PM) measured from bulk and surface snow at the 4 different locations. No data for bulk snow in B are available

snowpack from A and from F, while it remained constant in samples from B and T (Fig. 5d). Site-related variation was highly significant ($p < 0.01$) for all the chemical parameters measured.

Microbial biomass

Flow cytometry measured a typical range of 10–40 cells μL^{-1} snow in all samples (Fig. 6). Generally, a higher number of cells were found in the bottommost snowpack layer (adjacent to the soil). At this level (level 0 cm), recorded cell counts were ~140 cells μL $snow^{-1}$ in the samples from A.

T-RFLP profiling of bacterial communities

The T-RFLP profiles of bacterial communities indicated a high richness and diversity at all heights of each depth profiles (Table S3). Richness appeared lower in the B snowpack. The highest richness observed was in the T-RFLP profiles from A, where OTU numbers were in a range between 34 and 81. Evenness followed an opposite trend, reaching its highest levels in the snowpack from B (0.3–0.4) and its lowest in the surface samples of T (0.1). In the samples from F, the highest bacterial richness and diversity were detected in the top centimetres (57–60 OTUs detected) and at the bottom of the snowpack (51 OTUs detected).

The Shannon diversity index showed a tendency ($p > 0.05$) of a higher bacterial community diversity in the surface layers B (3.1), A (2.7) and F (2.6). Cluster analysis (Fig. S2) and Bray–Curtis dissimilarity (Fig. 7a) showed that the bacterial communities from B and F clustered apart from those from T and A (Fig. 7a). In the heatmap (Fig. 7a), the surface sample from B (B93) was distinguished from the rest of the B samples. On the contrary, the surface samples from F did not show any particular difference from the rest of the F samples. The A and T surface samples (A120 and T120) appeared to cluster together. RDA analysis (Fig. 8; Table S4) explained 23 % of the total variability, and showed a significant clustering of the samples from F in relation to NO_3^{-1} concentrations, and a significant clustering of the samples from B in relation to NH_4^+ concentrations. Also pH appeared to be significantly ($p < 0.05$) related to the B samples.

T-RFLP profiling of fungal and archaeal communities

Fungal richness appeared lower than bacterial richness, with a general OTU richness range between 21 and 51 in all locations (Table S5). No significant variation of richness with height within each snowpack was observed. The Shannon index also followed the same pattern. In the samples from F, it was not possible to detect any fungal OTU.

Cluster analysis (Fig. S3) and Bray–Curtis dissimilarity (Fig. 7b) showed that the fungal T-RFLP profiles for a snowpack within each location clustered apart from each other (Fig. 7b). No particular separation of the surface samples or trend with snow height could be noticed.

The constrained proportion of variance explained by RDA (Fig. S4) was 29.2 %, and density, pH, SO_4^{2-} and site significantly correlated with the T-RFLP profiles.

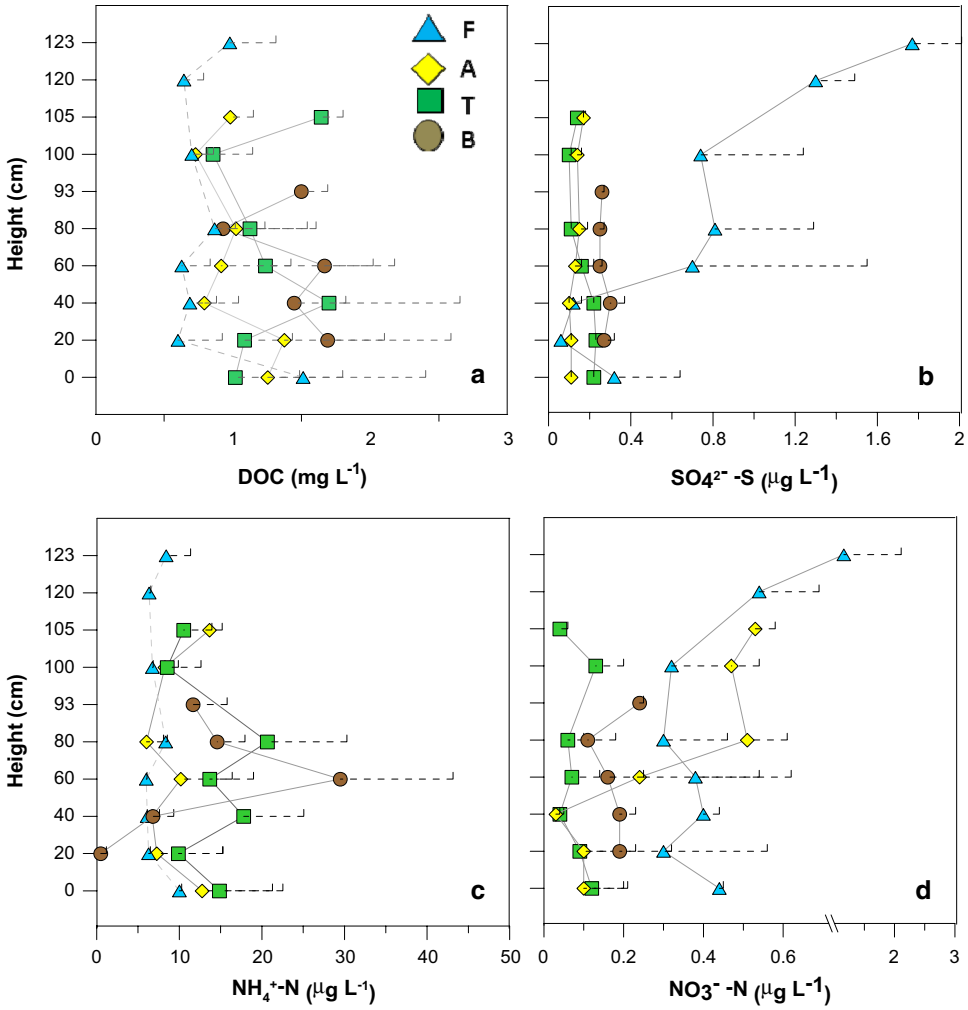

Fig. 5 Soluble nutrients measured in the different depth profiles. Values are averages ± standard deviation ($n = 3$). For clarity of presentation, only *positive error bars* are visualized

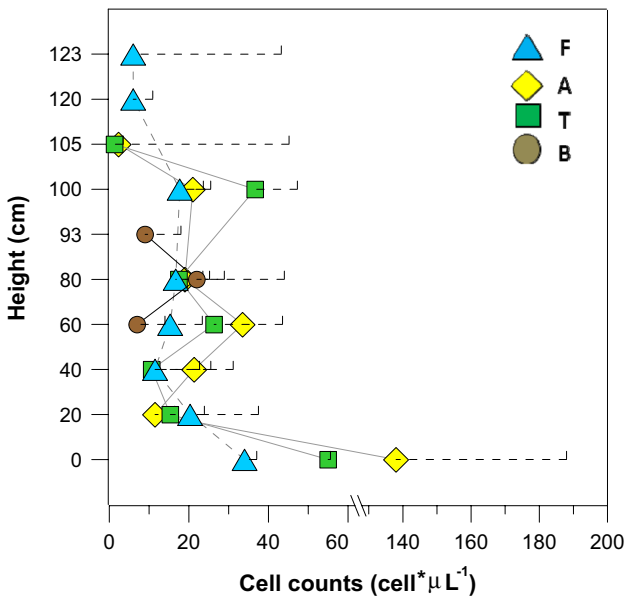

Fig. 6 Cell counts derived from flow cytometry of snow samples. Values are averages ± standard deviation ($n = 3$). For B, no 0 and 93 cm samples were available

Archaeal communities could not be analysed with T-RFLP profiling as the 16S rRNA gene could not be amplified.

Microbial community physiological profiles

The BIOLOG incubations showed that the physiological profiles were similar in all the surface and bulk snow samples (Table S6). In particular, growth at 0 °C was observed on polymers (i.e. Tween 40, Tween 80, α-cyclodextrin and glycogen), and on phenolic compounds (2-hydrobenzoic acid, 4-hydrobenzoic acid).

Microbial infiltration in the snow

Through the infiltration experiment, we could successfully detect the stained cells in the snow through flow cytometry. Due to the dilution of the cells in the solution, and the sprinkling effect, only a fraction of the inoculated cells could be sampled. However, linear regression (Fig. 9) showed a correlation ($R^2 = 0.68$) between concentration of brilliant blue and detected cells, indicating that a large fraction of cells followed the preferential flow paths.

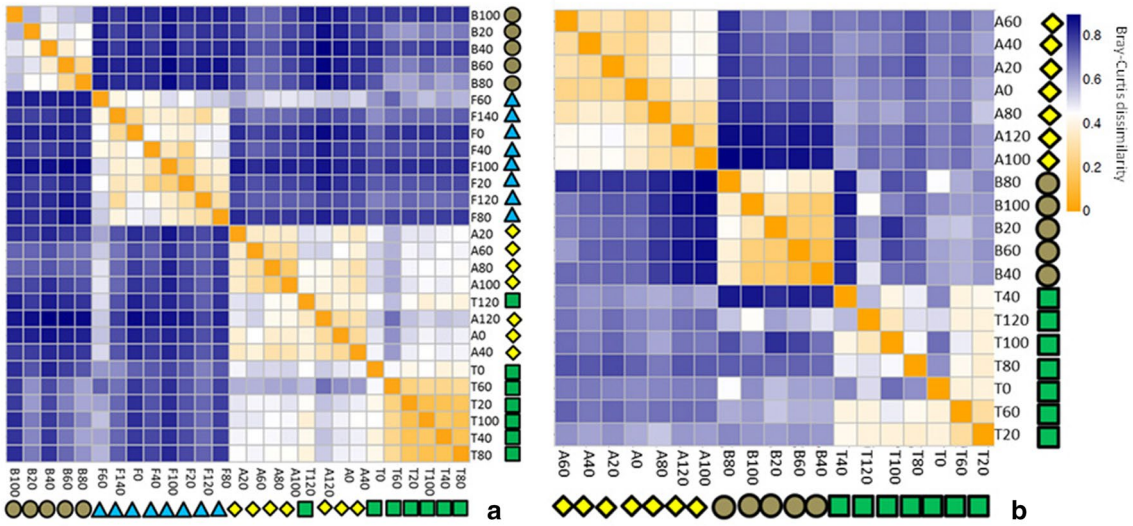

Fig. 7 Comparison of microbial T-RFLP profiles. Heatmaps are based on pairwise Bray–Curtis dissimilarity (0 = total; 1 = total dissimilarity similarity between two T-RFLP profiles) calculated from averaged T-RFLP profiles ($n = 3$) derived from each sampling height. **a** Bacterial 16S rRNA gene profiles, **b** fungal 18S rRNA gene profiles

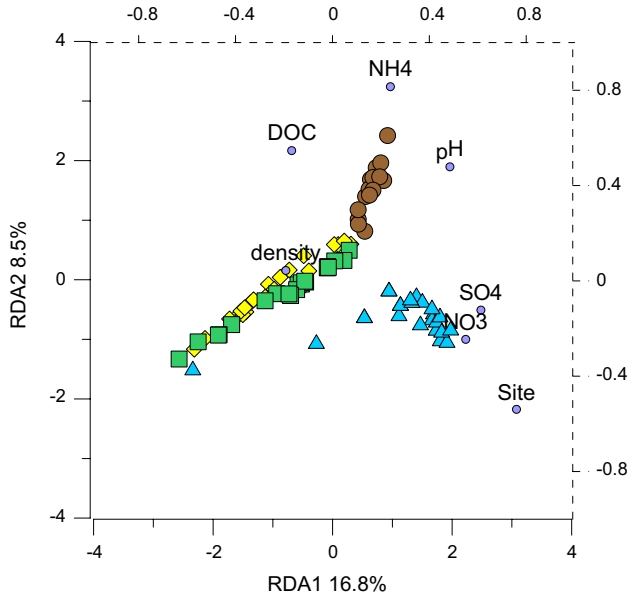

Fig. 8 Redundancy analysis (RDA). RDA plot based on bacterial T-RFLP profiles. *Dotted axes* are related to environmental variables for clarity of presentation

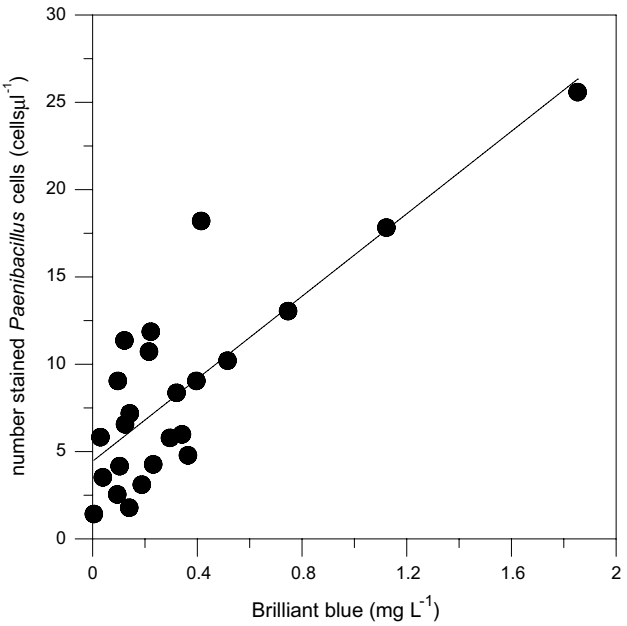

Fig. 9 Linear regression of SYTO®9-stained *Paenibacillus sabinae* cells and Brilliant blue concentrations retrieved in the snow after the snow infiltration experiment. Equation $y = 11.81x + 4.4$. Coefficient of determination, $R^2 = 0.68$

Discussion

General characteristics of the snowpack

The sampling at the field sites took place in May and June at snowmelt (Fig. 3). Our snow density values are in agreement with published data from this phase (Pomeroy and Brun 2001; Waldner et al. 2004). Moreover, we observed the presence of several ice layers in the first few decimeters of the snowpack (Fig. 3), although the remaining bulk snow appeared poorly structured. This suggested that homogenisation of the snow profile and release of soluble species from the snowpack with the meltwater flow had already begun.

Generally, the rate at which chemical compounds are released at snowmelt depends on the way a chemical is incorporated in the growing snowflake (Pomeroy et al. 2005). Important microbial nutrients such as NO_3^- and SO_4^{2-}, for example, are not incorporated into the snow grain structure, and are released earlier (Tranter et al. 1986). Despite the low concentrations measured (e.g. <0.2 μg L^{-1} NO_3^--N in B and T, <0.4 μg L^{-1} SO_4^{2-}–S in B, T, A) suggesting that a large fraction of these compounds already flushed out (Bowman 1992), we could trace other relevant ions in the snowpack. For example, the higher levels of NH_4^+ measured (up to 30 μg L^{-1} NH_4^+-N) may be related to a stronger adsorption onto snow.

DOC in the snowpack may also be important to microbial communities since it may serve as a C source. The DOC concentrations measured in this study were similar to those measured in other snow systems in Colorado (Mladenov et al. 2012), but they are one order of magnitude larger than data collected from systems in the French Alps and Greenland (Legrand et al. 2013). It is not possible to explain the origin of the snow DOC in our samples, although it is likely it will be a mixture of atmospheric deposition and in situ transformation of biogenic organic material (McNeill et al. 2012; Runa et al. 2014).

The concentrations of soluble species presented in this study are expressed in μg or mg L^{-1} and thus related to the total snow volume, without taking into account the actual liquid water content. However, if such values would be extrapolated to mL liquid water, they would give significantly higher amounts (approximately 20 times larger). Lautenschlager et al. (2013) showed that concentrations as low as 10 μg L^{-1} of assimilable organic carbon (AOC) are enough to stimulate growth of microorganisms. It is therefore likely that at least a fraction of the DOC measured could permit the growth of snow-inhabiting microorganisms.

Assessment of snow microbial communities

In our samples, we could assess the presence of bacterial and fungal communities, but not of Archaea. So far, only one study reports the presence of Archaea in early season snow (Maccario et al. 2014). Therefore, it is possible that either Archaea were below detection limit for T-RFLP analysis, or that they were flushed down the snowpack with the first snowmelt. Further evidence has to be collected in order to understand the possible reasons for the lack of archaeal detection in the snowpack.

Although with our DNA-based profiling we could not assess the viability of the microbial communities, our cell number estimations (averaging approximately 20 cells μL^{-1}) fall in the range of cell numbers detected in other snow samples (2×10^4 cells mL^{-1}; Amato et al. 2007; Bauer et al. 2002; 1×10^7 DNA containing-particles L^{-1}; Christner et al. 2008), and show a high diversity in fungal and bacterial communities at all heights.

In addition, we could also assess the potential physiological properties of microorganisms living in the snowpack. As the BIOLOG profiles suggested, the snow system might be dominated by psychrophilic microorganisms, potentially able to degrade polymers, phenolic compounds and biopolymers as carbon sources. Such microorganisms have been reported in cold aquatic systems (Tamaki et al. 2003) and in glacier ice (Simon et al. 2009; Stibal et al. 2012). In addition, the high affinity that we observed for polymeric substrates (i.e. Tween) might be related to the positive effect on enzymatic activities, as previously reported in other systems (Kamande et al. 2000), or to the presence of cold-adapted lipolytic bacteria, yeasts and fungi (Lo Giudice et al. 2006; Kim et al. 2010; Joseph et al. 2008).

Physical and chemical differences between the snowpack surface and the underlying bulk snow

In our study, considering softness and heterogeneity of the snowpack surface, in order to obtain a homogeneous sampling we operationally defined a surface snow layer as the first 20 cm from top. Such region of the snowpack might hold different characteristics from the underlying bulk snow, and thus affect the inhabiting microbial communities.

The surface snow is in direct contact with the surrounding environment, and is a sink for deposited particles and microorganisms (Xiang et al. 2009; Margesin and Miteva 2011; Polymenakou 2012). The concentration, chemistry and distribution of the deposited particles (particulate, hydrophobic and hydrophilic compounds) may depend on several different factors such as slope, surface heterogeneity, and winds. Along with the transported mineral and organic particles, microorganisms may also be deposited as free cells or associated with particle material (Burrows et al. 2009; Womack et al. 2010). We measured a high variability in cell numbers in our surface samples, indicating a heterogeneous mechanism of deposition.

When associated with PM, hydrophobic substances tend to accumulate at the surface of the melting snowpack and are released only at the end of the snowmelting phase (Björkman et al. 2014). We observed an accumulation of PM in the first few centimetres of the snowpack, and no penetration into the deeper layers. The measured PM values correspond to an average PM deposition between 4.75 (F) and 9.5 kg ha^{-1} (T), which is similar to the atmospheric load estimated by Brankatschk et al. (2010) at various alpine sites.

The snowpack below the surface layer represents older snow, which has been progressively compacted and undergone metamorphism. The presence of ice layers in the snowpack, for example plays a central role in trapping cells

(Jones 2001), and has been related to variability in cell numbers and in concentrations of major ions (Björkman et al. 2014). We also observed the presence of ice layers at our sampling sites. Due to the dynamic properties of snow, however, with our experimental setup it was not possible to associate defined microstructures in the snowpack to neither soluble ion concentrations nor to cell numbers.

Redistribution of microbial communities in the snowpack

With the infiltration experiment, we could show that microorganisms tend to behave as soluble particles, and are therefore strongly related to meltwater flushes. In fact, neither microbial richness nor diversity from our T-RFLP profiles appeared to show any peculiar trend in any of the bulk snow layers, suggesting that microorganisms were homogenised in the snowpack. Horizontal and upward movement has been shown to occur in wet snow (Walter et al. 2013). In addition, microbial properties such as the presence of flagella, might contribute to the distribution of microbial cells in the bulk snow (Jones 2001). Our results do not, however, reflect those from a recent study by Björkman et al. (2014) who showed that in a melting Arctic snowpack the elution of microbial cells resembled that of hydrophobic substances, and that microbial cells were strongly associated with particulate material. Such snowpack, however, appeared strongly stratified and rich in ice layers, features which influence the retention of cells and their subsequent elution. In our study, we have neither evidence of association of microbial cells with PM, nor of an evident snowpack stratigraphy, and this may have caused the contrasting observations.

Finally, depending on the infiltration capacity of the snow-covered soil, meltwater can form a slush layer at the soil–snow interface at the bottom of the snowpack, creating a transit for microbial cells (Horton 1935). A thorough comparison of the T-RFLP profiles visualizing shared OTUs between surface snow/underlying soil and bottom snow/underlying soil (data not shown), however, suggested that only <36 % of all bacterial OTUs, and <18 % of all fungal OTUs identified is shared between snow and soil samples. Therefore, the exchange between soil microbial communities and the snow system is minimal.

Site-related differences in snow microbial communities

Chemical composition of snow may show an extreme spatial variability (Tranter et al. 1986), reflecting the presence of pollution sources and vegetation-related effects. Our T-RFLP profiles highlighted that also the microbiology of the snowpack showed a certain medium-scale (hundreds of m to km) spatial variability. RDA analysis based on the bacterial T-RFLP profiles highlighted in particular the similarities between snow communities at A and T, which are geographically very close to each other, in contrast with those from F and B. Site, SO_4^{2-} and NO_3^- were correlated with the F cluster, while NH_4^+ was significantly correlated with the B cluster. Indeed, both NO_3^- and SO_4^{2-} were present in larger concentrations all through the F snow profile. In B, pH appeared slightly more alkaline than in the other locations sampled. Snow pH is highly dependent on particles (i.e. NH_4^+) transported along with precipitation, which may in turn vary with different altitudes (Lovett and Kinsman 1990; Thimonier et al. 2005).

Our results highlight the importance of the N pools in snow. Total (dry and wet) deposition of N calculated for the year 2010 in our sampling locations (Rhim, personal communication), for example, ranged between 5.9 (B) and 8.7 (F) kg N ha year^{-1}. Considering an average N content in the snowpack ranging from 11.6 (F) to 12.6 (A) μg L^{-1}, and estimating an average flush of 50 L m^2 day^{-1} of meltwater during 38 days of snowmelt (daily snowmelt data collected at the field sites), we can estimate that the snowpack is able to provide approximately 2.75–3.73 % of the annual wet and dry N deposition to the underlying soil. Such estimation falls in the lower ranges reported in a previous study performed on dry alpine meadows in Colorado (Bowman 1992). Therefore, the snowpack of the Tiefen catchment may be considered as a winter N sink and a spring N source for plant communities.

Conclusions

A snowpack at snowmelt is characterized by faint stratigraphy, and increasing flushes of meltwater. In this study, we showed that meltwater flow enhances the vertical transport of soluble ions and microbial cells. However, particulate matter remains retained at the surface of the snowpack and will be released only at the end of snowmelt. As a consequence, snowpack microbial communities tended to be homogeneously distributed through the whole depth of the snowpack. A comparison of the snowpack communities at 4 different altitudes within the same glacier catchment showed pronounced site-specific variability, which is probably due to different atmospheric deposition sources and regimes.

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