RC1: 'Comment on egusphere-2025-4903', Anonymous Referee #1, 09 Feb 2026

1) *This manuscript investigates microbial communities in surface and subsurface snow as well as the geochemistry of these snow zones in the Swiss Alps. The study presents seasonal data from ~1800-2600 m a.s.l. The study of subsurface and surface snow in relation to seasonal changes addresses key uncertainties in snow microbial ecology and biogeochemical cycling. Few studies have investigated surface and subsurface snow, particularly in this altitude range, seasonally. Differences between snow layers are a key variable that is difficult to constrain across locations (even those in close proximity) and seasons.*

   We thank the reviewer for thorough and productive review of our manuscript, and for acknowledging the value of examining surface and subsurface layers within the seasonal snowpack along an altitudinal gradient. We have prepared a revised version that addresses the reviewers' comments.

2) *Some broader comments:*

   I am curious about the use of "layers" throughout. It appears that you sampled at specific depths regardless of any apparent structure in the snow. But snow layers have a specific definition — they are distinct horizons in the snow based on water events. And typically have characteristics specific to a snowfall event. Please update the manuscript accordingly to either specifically state how you identified layers in the snow pack or update the wording to better reflect that you sampled specific depths regardless of snow layers or structure. I have similar comments below about "surface" vs. "bulk" snow. Both samples are "bulk".

   We thank the reviewer for this comment. We have replaced "layer" throughout the manuscript with specific sampling depths (independent of snow stratigraphy) to reflect better our sampling strategy (0-2 cm: surface and 10-15 cm: subsurface) (Lines 12, 81, 99-100, 183-185, 189, 191, 205, 210, 220-225, 240-244, 252-258, 305, 326-328, 355, 363, 369-371, 433, 435). Figure legends now also indicate depth directly as well (Figures 2, 3, 4, 5; Table 2).

3) *Standard reporting of geochemical analsyes would include supplying a table of the actual data. I would encourage the results and discussion sections to more explicitly address the concentrations. As such, the reader has no context for how these samples might compare to other snow and ice studies. The results and discussion address only patterns and do not integrate with other studies on snow chemistry, limiting the potential utility of the data set.*

   The reviewer is absolute correct. We have now included a table (Table 2) with the summary of ion chromatography results along with the limits of quantification. In addition, we included a supplementary table (Table S1) that has the full data of ion and organic acid concentrations for each sample. In addition, this data is also available as part of the metadata table deposited in GitHub (a-kosolapova/alpine-snow-microbiome-2023) and Zenodo (10.5281/zenodo.17249708).

Table 2:

	Ion	Surface (0-2 cm) Mean (Min-Max), mg/L	Subsurface (10-15 cm) Mean (Min-Max), mg/L	Limit of quantification, mg/L
Cations	NH_4^+	0.237 (0.054-0.530)	0.191 (0.018-0.498)	0.01

	Ca^{2+}	0.412 (<0.01-6.231)	0.470 (<0.01-5.526)	0.01
	Mg^{2+}	0.011 (<0.01-0.075)	0.022 (<0.01-0.068)	0.01
	K^{+}	0.020 (<0.01-0.050)	0.032 (<0.01-0.070)	0.01
	Na^{+}	0.044 (0.007-0.255)	0.089 (0.012-0.163)	0.0025
Anions	Br^{-}	0.000 (<0.001-0.003)	0.002 (<0.001-0.005)	0.001
	Cl^{-}	0.097 (0.023-0.489)	0.170 (0.022-0.433)	0.02
	F^{-}	0.001 (<0.001-0.006)	0.002 (<0.001-0.005)	0.001
	NO_3^{-}	0.579 (0.084-1.301)	0.346 (<0.05-0.859)	0.05
	NO_2^{-}	0.004 (<0.001-0.016)	0.002 (<0.001-0.010)	0.001
	PO_4^{3-}	0.006 (<0.01-0.021)	0.009 (<0.01-0.025)	0.01
	SO_4^{2-}	0.178 (0.027-0.416)	0.223 (<0.02-1.839)	0.02
Organic acids	$C_3H_7COO^{-}$	0.002 (<0.001-0.009)	0.002 (<0.001-0.009)	0.001
	$HCOO^{-}$	0.134 (<0.001-0.290)	0.211 (<0.001-0.640)	0.001
	$CH_3CH(OH)COO^{-}$	0.002 (<0.001-0.024)	0.015 (<0.001-0.091)	0.001
	$C_4H_4O_5^{2-}$	0.014 (<0.001-0.034)	0.022 (0.002-0.055)	0.001
	$C_2O_4^{2-}$	0.013 (<0.001-0.041)	0.025 (<0.001-0.070)	0.001

Additionally, we have now contextualised our chemical composition measurements by comparing them to previous alpine snow measurements in the Discussion section (Lines 343-368).

Discussion, Lines 343-368: "In the broader context, our measurements of the major ion concentrations fell into the expected ranges defined by previous studies on precipitation and snowpack chemical composition in the European Alps, although enrichment was particular for the certain ions. While nitrate concentrations fell into the expected range, ammonium concentrations were elevated compared to the previously reported full-column snowpack measurements across Alps, including multi-year means from the Austrian Alps (Greilinger et al., 2016) and 2005-2006 season snowpack measurements from the Aosta Valley, Italy (Filippa et al., 2010). As our measurements reflect recently deposited surface and subsurface snow rather than whole-snowpack composition, the elevated ammonium concentrations may indicate enhanced ammonium deposition at our sites during spring, likely driven by the fertilizer application and agricultural ammonium emissions from the adjacent Rhône valley (Ammann and Valach, 2024; Filippa et al., 2010; Greilinger et al., 2016; Novak et al., 2025). In contrast, both nitrogen species concentrations far exceeded values observed in remote Arctic

snowpacks, highlighting the anthropogenic influence at our sampling sites (Krnavek et al., 2012; Nickus, 2003).
Comparison between surface and underlying 10-15 cm deep snow revealed significant dissimilarities in chemical composition, which likely originated from the differences in chemical composition of various snowfall events characterised by distinct air mass sources. Surface snow, with mean nitrate and ammonium concentrations of 0.58 and 0.24 mg/L respectively, is consistent with air masses coming from Central Europe's industrialised and densely populated areas (Supp. Fig. S3), explaining the higher concentrations of nitrogen species, which correlated positively with sulphate, commonly associated with anthropogenic pollution (Filippa et al., 2010; Greilinger et al., 2016; Novak et al., 2025). Subsurface snow showed higher levels of chloride and sodium (0.17 and 0.089 mg/L, respectively), potentially indicative of a preceding precipitation event with stronger Atlantic influence, though no firm attribution to a specific snowfall can be made. These concentrations nonetheless fall within the range previously reported for other Alpine sites, and are substantially lower than those observed at Arctic sites dominated by marine aerosol input. This variability at both sampling depths may originate not only from differences in the initial chemical composition of each snowfall event, but also from processes occurring after deposition. For example, the lower TIN concentrations in the 10-15 cm deep snow may reflect losses of nitrogen species through photolysis and volatilisation caused by prolonged UV exposure (Jacobi and Hilker, 2007; Trachsel et al., 2019), as well as biological uptake of TIN. The strong positive correlation between major ions and organic acids in the deeper snow may reflect post-depositional redistribution processes occurring in the snowpack (Raben and Theakstone, 1994)."

4) *The surface layer may be quite different, physically, from surface snow. Water content, physical properties of the snow, and potential abundance of particles (and particle-associated microbes). Particles also carry nutrients. Was this investigated as part of the study, or what is the justification for only focusing on aqueous measurements? The surface particles likely control the chemistry below, and the findings that Ca2+ is important or related to community structure strongly point to the need to assess the full chemistry of the snow, which would include dust, sediments, and other surface constituents.*

We thank the reviewer for this observation. To maintain a direct link between chemical and microbiological analyses (while managing the limited sample volume imposed by a campaign covering 19 sites (114 samples) within a two-day window), samples were processed as filtered snowmelt water. Chemical analysis was therefore performed on the dissolved fraction (filtrate), while the retentate (particulate) fraction was used for microbiological analysis and therefore not included in chemical analysis. However, as DNA was extracted from the filter membranes (0.22 µm pore size standard for microbiological analysis), microbial communities associated with particles are captured in our microbiological analysis, ensuring that particle-associated microbes are not excluded from the community profiling. We prioritised this approach to ensure sufficient DNA yield for community characterisation across all 19 sites. We acknowledge the exclusion of the particulate fraction as a limitation of our chemical characterisation, particularly given the likely role of dust and mineral particles as carriers of both microbes and nutrients, and have added a note in the Discussion (Lines 420-421).

Discussion, Lines 420-421: "The high number of OTUs positively associated with calcium may reflect the diversity of bacteria that were deposited together with the dust particles, though the particulate fraction was not assessed and calcium concentration measurements reflect dissolved fraction only."

5) *Do you have any evidence that these microbial communities are active in either location? And how much movement of cells is expected? Presumably, more water later in a melt season could move cells (dead or alive) into the subsurface — this would not be evidence that those microbes are important to active processes, merely that their biomass is mobile. The study would be strengthened by microscopy to visualize intact cells (and by a biomass measure to relate recovered DNA and 16S rRNA sequences). Indeed, the discussion invokes movement of solutes — cells too I assume. In reviewing the paper, it isn't clear if the point is cell movement through the snow or distinct communities expected throughout the snowpack.*

   Thank you for raising this important point. We agree that our data cannot provide direct evidence of microbial activity in the sampled snowpack, as the study is based on 16S rRNA gene sequencing and thus cannot distinguish among active, dormant, and dead bacterial cells, or environmental DNA. We also agree that passive redistribution of biomass may have contributed to the observed differences in community structure, although the present data do not allow us to either confirm or rule out this possibility. We have revised the Discussion accordingly (Lines 369–381) to clarify our interpretation of these patterns and to more explicitly acknowledge the limitations of the current dataset.

   Discussion, Lines 369-381: "Bacterial community structure differed consistently between snow depths. Surface snow contained twice as many bacterial genera in the core community as subsurface snow, and nearly all subsurface core genera were also present in surface snow. Surface snow also showed higher diversity and greater cross-site similarity. The observed depth-related differences possibly reflect a combination of differences in the initial communities deposited to the snowpack and subsequent post-depositional processes. Surface and subsurface layers likely originated from different deposition events, resulting in distinct initial community compositions, although it was not possible to assign the samples to specific precipitation events. Post-depositional selection may then have further differentiated these communities over time, reducing local diversity in subsurface snow while increasing between-site variability through selection for taxa adapted to local conditions. Similar selection toward snow-specific communities has been documented in Greenland snow-ice communities and was accompanied by changes in functional profiles (Maccario et al., 2019). Physical redistribution within the snowpack may also have occurred under late-spring Alpine conditions, leading to vertical movement of solutes and possibly microbial cells and environmental DNA (Lazzaro et al., 2015). However, with the available data, it is difficult to disentangle the relative importance of these processes or to determine the extent to which they occurred in the sampled snowpack."

6) *If allowed by The Cryosphere, I suggest combining the results and discussion. The Discussion is short and not well integrated with the data presented. For example, deposition could be discussed when the observed patterns are discussed (along with a section on what the actual concentrations are and how they relate to other studies). I mentioned this above, but there is no justification or context provided for the different valleys but this is also part of the discussion.*

   Thank you for this suggestion. We appreciate the reviewer's recommendation to more closely integrate the interpretation with the presented results. While we chose in this case to retain separate Results and Discussion sections, we revised the manuscript throughout to improve coherence between the Introduction, Methods, Results, and Discussion. This included revising the Introduction to provide stronger context for the role of topography in shaping snow microbial variability (Lines 67-76), clarifying the sampling design in the Methods (Lines 94-102), expanding the Results to better address geographical and topographic patterns (Lines 259-278), and revising the Discussion to more directly engage with the observed depth-related and topography-related patterns (Lines 369-407).

Introduction, Lines 67-76: "Mountain topography strongly influences the spatial distribution and properties of alpine snowpacks (Helbig et al., 2024; Mott et al., 2018). At large scales, orographic lifting of air masses enhances snowfall with increasing elevation, making altitude a major factor of snow accumulation in mountainous terrain (Helbig et al., 2024; Mott et al., 2018). At smaller ridge and slope scales, interactions between wind flow, terrain, and snow particles generate heterogeneous deposition patterns through processes such as preferential snowfall deposition, which enhances accumulation on leeward slopes and reduces it on windward slopes (Helbig et al., 2024; Lehning et al., 2008, 2011). These topographically driven processes also influence snow chemistry by shaping precipitation pathways, aerosol scavenging, and local dust inputs, leading to spatial variability in ion concentrations and deposition loads across altitudinal gradients (Lafrenière and Sinclair, 2011; Rogora et al., 2006). Such spatial variability in snow chemistry may in turn influence the composition of bacterial communities colonising the snowpack."

Methods, Lines 94-102: "A total of 114 samples were collected at 19 sampling sites. Sampling sites were distributed across three Alpine valleys and included several peripheral sites (Table 1), were selected to cover a compact geographic area (within ~25 km) but spanning a substantial elevational gradient. Representative photographs of the sampling sites are provided in supplementary (Fig. S1). Snow surface conditions were visually assessed at each site. No visible algal blooms or dust deposition were observed, except for site 17, which displayed a visibly darker and more granular snow surface. At each sampling site, snow samples were collected in triplicate from two fixed depth ranges using an ethanol-sterilised scoop: 0–2 cm (referred to as surface) and 10–15 cm (referred to as subsurface). The fixed-depth sampling design was chosen over the stratigraphic layer identification to ensure standardised and rapid sampling of all 19 sites within a 2-days sampling campaign. The depths were selected to capture the differences between snow samples of potentially different ages."

Results, Lines 259-278: "Geographic distance exhibited only a weak correlation with community dissimilarity in surface snow (Bray-Curtis distance: $r = 0.14$, $p = 0.009$), and showed no significant link with subsurface snow phylogenetic composition (weighted UniFrac distance: $r = 0.05$, $p = 0.26$), although a signal persisted for subsurface Bray-Curtis distances ($r = 0.16$, $p = 0.005$) (Table S2). Resistance distance (distance accounting for terrain barriers) yielded similar results in surface snow ($r = 0.14$, $p = 0.006$) but demonstrated a stronger association in deeper snow ($r = 0.21$, $p = 0.002$), remaining significant after controlling for elevation (partial Mantel test, $r = 0.15$, $p = 0.002$) (Table S2). Slope difference between sites showed an independent association with surface snow community dissimilarity after controlling for both elevation and geographic distance (partial Mantel tests: $r = 0.08$, $p = 0.046$ and $r = 0.09$, $p = 0.037$) but showed no significant association in deeper snow (all $p > 0.05$) (Table S2). Aspect showed no consistent association with community dissimilarity across metrics or sample depths (Tables S2).

Elevation difference between sites was the strongest of investigated geographical predictors of community dissimilarity at both depths, maintaining its signal after controlling for geographic distance (partial Mantel test; surface: $r = 0.23$, $p < 0.001$; subsurface: $r = 0.20$, $p = 0.002$) (Table S2). In contrast, geographic distance signals in surface snow largely disappeared after controlling for elevation ($r = 0.07$, $p = 0.069$) (Table S2). NMDS ordination coloured by elevation further indicated an elevational gradient in community composition, particularly in surface snow (Fig. 3bc).

At the within-sample level, community evenness increased with elevation in both surface ($r = 0.35$, $p = 0.008$) and subsurface snow ($r = 0.34$, $p = 0.010$), whereas Shannon diversity and Faith's phylogenetic diversity showed no significant trends with elevation. Slope and aspect showed no significant correlation with alpha-diversity metrics at either sampling depth (all p-values > 0.05). Moran's I revealed no significant spatial autocorrelation in alpha-diversity across sites (all p-values > 0.1)."

Discussion, Lines 369-407: “Bacterial community structure differed consistently between snow depths. Surface snow contained twice as many bacterial genera in the core community as subsurface snow, and nearly all subsurface core genera were also present in surface snow. Surface snow also showed higher diversity and greater cross-site similarity. The observed depth-related differences possibly reflect a combination of differences in the initial communities deposited to the snowpack and subsequent post-depositional processes. Surface and subsurface layers likely originated from different deposition events, resulting in distinct initial community compositions, although it was not possible to assign the samples to specific precipitation events. Post-depositional selection may then have further differentiated these communities over time, reducing local diversity in subsurface snow while increasing between-site variability through selection for taxa adapted to local conditions. Similar selection toward snow-specific communities has been documented in Greenland snow-ice communities and was accompanied by changes in functional profiles (Maccario et al., 2019). Physical redistribution within the snowpack may also have occurred under late-spring Alpine conditions, leading to vertical movement of solutes and possibly microbial cells and environmental DNA (Lazzaro et al., 2015). However, with the available data, it is difficult to disentangle the relative importance of these processes or to determine the extent to which they occurred in the sampled snowpack.

Similar to the chemical composition patterns, the valley of origin showed no clear association with the bacterial community composition. Consistent with the lack of valley effect, neither geographical nor terrain-corrected distance remained significantly associated with community composition after controlling for elevation, highlighting that spatial separation and terrain barriers among sites had little influence on community composition at the local scale (<25 km). As for the snowpack chemistry, these findings suggest the predominant influence of the large-scale atmospheric transport, with bacterial communities across sites reflecting a shared atmospheric microbial pool rather than local or valley-specific inputs.

Topographic variables such as slope and aspect showed little to no association with the community composition. These results are likely connected, as the ecological relevance of aspect depends strongly on slope angle. On near-flat terrain, north- and south-facing surfaces receive similar solar irradiance regardless of their orientation, and strong microclimatic contrasts between aspects emerge only as slopes steepen (Barry, 1992). With slopes ranging from gentle to moderate (0.8°–20°), aspect-driven radiation contrasts across our sites were likely too subtle to drive microbial differentiation. At steeper slopes, where snow redistribution and differential melt between aspects are more pronounced, such contrasts could become sufficient to structure microbial communities (Pomeroy et al., 2004). Aspect-driven effects on snowpack communities may also require sustained differential solar exposure to accumulate over time, through processes such as UV-mediated selection, melt-freeze cycling, and differential nutrient dynamics (Sanchez-Cid et al., 2023). The relatively short snow residence time of studied snow depth may therefore have been insufficient for such signals to emerge.

Among the topographic variables examined, elevation most strongly structured bacterial community composition, with its signal persisting at both sampling depths. Community evenness increased with elevation at both depths while Shannon diversity and Faith's phylogenetic diversity showed no such trend, indicating that elevation reshapes dominance patterns rather than overall diversity. Consistent with this pattern, the largest cluster of co-occurring OTUs was negatively correlated with elevation and enriched in lichen-associated genera, suggesting stronger terrestrial inputs at lower elevation sites closer to vegetated terrain. More broadly, higher evenness at greater elevations may reflect limited local terrestrial inputs and reduced bacterial growth, with communities more closely mirroring the regional atmospheric pool, while at lower elevations lichen-associated deposition and warmer conditions likely favour dominance of fewer taxa. Without direct activity measurements, however, we cannot distinguish whether this pattern results from active ecological processes or differences in depositional sources.”

7) *Line 9: Particles that are transported in the atmosphere. Semantic, maybe, but these do not originate in the atmosphere.*

   Changed from "atmospheric particles" to "atmospherically transported particles" (Line 9), thank you.

8) *Line 20: Putative? Or is spore formation monophyletic in these groups?*

   Revised to avoid assigning spore-formation to the full genera "Genera containing spore-forming taxa (*Neobacillus, Niallia, Sporosarcina*)" (Line 21).

9) *Line 21-23: These aren't really enriched (you did not add nutrients). Perhaps link these observations to what you did measure - N and Ca concentrations.*

   We thank the reviewer for letting us clarify this point. Here, we used the word "enriched" in terms of "statistically enriched" to indicate statistically significant association of certain taxa with snow calcium concentration. To avoid ambiguity, we revised the wording.

   Abstract, Lines 21-33: "Genera containing spore-forming taxa (*Neobacillus, Niallia, Sporosarcina*) were significantly associated with higher nitrogen concentration, while *Brevundimonas, Cryobacterium*, and *Polaromonas* were associated with higher-calcium concentrations."

10) *Line 65: Is it necessary to say "In contrast to previous studies"? Please cite them if so and be specific about how this study differs.*

    We revised as suggested (Lines 77).

11) *Line 70: According to the methods, you sample the 10-15 cm zone. Please update.*

    Depth indication is updated to match Methods (Line 81).

12) *Line 74: specific gradients of chemistry in the snow? I would not know what chemical snow is let alone gradients in this.*

    Rephrased as "variation in snowpack chemistry" (Line 84).

13) *Line 85: How long were samples stored? What impact did freeze-thaw have on downstream chemical analyses? Also provide specific methods for how samples were collected. And pictures of the field samples that could be included? How did you choose sites to sample? What was visible on the snow surface?*

    Methods section 2.1 was expanded to clarify sampling procedure and samples storage (Lines 94-105, see above response to Comment #6). Photos of the sampling sites are added to the supplementary materials (Supp. Fig. S1).

Supplementary Fig. S1. Photos of the sampling sites. Photos were not taken at the Site 5 and Site 8.

14) *Line 88: Please discuss detection limits and field blanks.*

Limits of quantification for each measured ion are added to the Table 2 (Comment #3). Methods Section 2.2 is expanded to include details of chemical analysis (Lines 107-113).

Methods, Lines 107-113: "Before chemical analysis, ~150 mL of snowmelt water samples (N=113) were gradually thawed at 4°C and filtered with Sterivex 0.22 µm filter units (EMD Millipore Corporation, USA), alongside a MQ water blank processed under identical conditions as a negative control. Filters were subsequently stored at -20°C for DNA extraction. The anion (F^-, Cl^-, NO_2^-, NO_3^-, Br^-, SO_4^{2-}, PO_4^{3-}) and cation (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}), as well as concentrations of several organic acids (formate, malate, lactate, butyrate, oxalate), were estimated with the use of ion chromatography (Dionex Integrion HPIC System, Thermo Scientific, USA) in the Central Environmental Laboratory (EPFL, Sion). Limits of quantification for each ion are provided in Table 2 and Table S1. Concentrations below the limit of quantification were set to zero for subsequent analyses."

15) *Line 94: Any negative controls to discuss? Low DNA concentrations? Did you assess the amount of biomass in each sample type to see if that might impact the recovered sequences? Similarly, presumably smaller volumes were sampled for the snow surface? Or did you collect the same volume and if so, how do account for that potential bias? How much snow was filtered? How were samples store, etc etc. Many methods missing here.*

We have extensively revised the Methods to include which negative controls we used, the DNA concentration information, filtered volumes, and sample storage details (Lines 107-108, 116-125). Biomass was not measured and is therefore not available for this dataset. Sampling targeted the same surface area for the surface and the matching underlying subsurface sample, though we acknowledge that the bias could be introduces.

Methods, Lines 107-108: "Filters were subsequently stored at -20°C for DNA extraction."

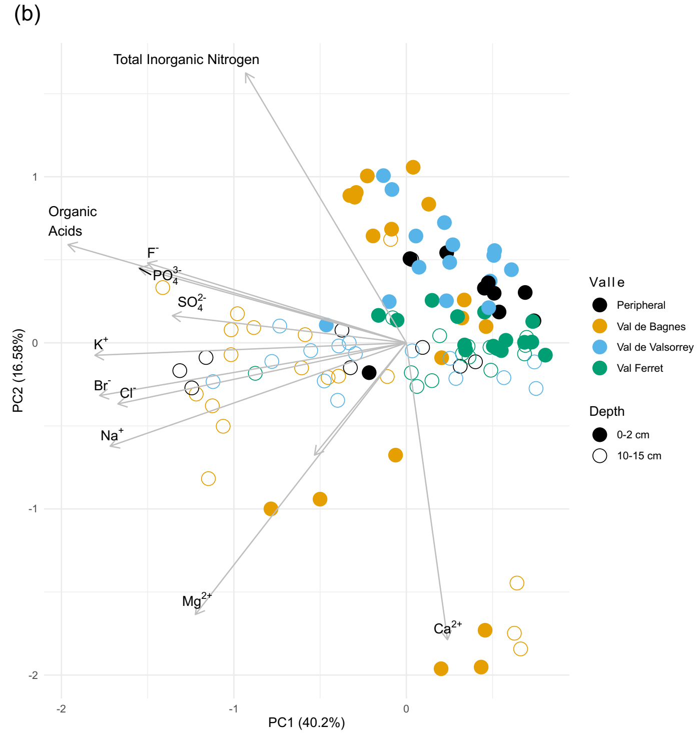
Methods, Lines 116-125: "Extraction blanks were included in each batch of DNA extractions (negative controls), amplified and sequenced alongside samples to control for contamination. DNA concentrations were assessed using the Qubit HS DNA kit (Thermo Fisher Scientific, USA). Concentrations were generally low, with many samples below the detection limit (0.1 ng/µL), and median of 0.175 ng/µL in samples with detectable DNA. For amplification of the full-length 16S rRNA gene, we used primer set 27f (AGAGTTTGATCMTGGCTCAG) and 1427r (CGGTTACCTTGTTACGACTT) (Zorz et al., 2023). The target sequence was amplified with KAPA HiFi HotStart ReadyMix polymerase (Roche, Switzerland). Successful amplification was confirmed by agarose gel electrophoresis, and extraction blanks showed no amplification. PCR products were cleaned up with AMPure beads (Beckman Coulter, USA) according to the manufacturer's protocol. DNA concentrations of PCR products after cleanup were estimated with the Qubit HS DNA kit."

16) *Line 144: Please provide the actual concentration data in a table.*

Per-depth summary table with mean ions concentrations, ranges and limits of quantifications are included as Table 2 (see above, response to Comment #3). Detailed per-sample concentration data is available as Supplementary Table 1.

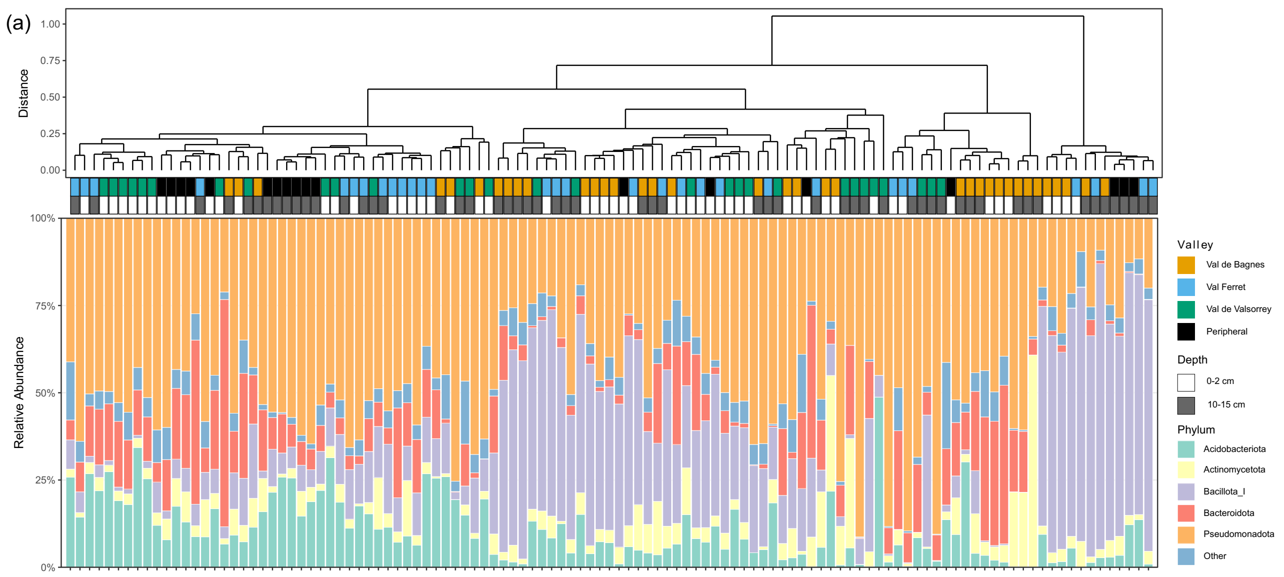
17) *Figure 2: The PCA is too small to read. This figure also includes regions or valleys that were not introduced anywhere before this figure. Please provide sample site context and relate this to the data if that is a key part of the study. Here you also use bulk and surface to describe the snow samples. Is surface not also a "bulk" sample? I'm not understanding how these labels are informative.*

The PCA plot was edited for readability (Figure 2b). The assignment of each sampling site to a valley is indicated in Table 1.
The usage of "bulk" and "surface" was addressed as part of the broader revision above (Comment #2). The terminology was revised throughout the manuscript: "bulk" and "surface" were replaced with "surface (0–2 cm)" and "subsurface (10–15 cm)" to refer explicitly the two fixed sampling depths.



18) *Figure 3: Why are the valleys denoted with a different color palette than in Figure 2? This is very confusing. It's also nearly impossible to discern between the shades.*

We agree with this point completely. The colour-coding is now changed to match Figure 2. Additionally, to increase readability of the plot we now have a separate colour for each valley and depth is now indicated separately (Figure 3a).



19) Lines 187-194: Is this a Core analysis? Or an abundance analysis? Where are the methods and justification for these cutoffs?

We have expanded Methods to describe the core microbiome analysis and to include justification of the applied prevalence (≥50% of samples) and relative abundance (≥0.5%) thresholds (Lines 154-157). We added the rationale for conducting the core microbiome analysis at the beginning of the section (Lines 232-234).

Methods, Lines 154-157: “A core microbiome analysis was performed at the genus level; core genera were defined as those present in ≥50% of samples with a minimum relative abundance of 0.5%. This prevalence-based threshold was used to identify taxa consistently occurring across samples while accounting for expected spatial variability in snow microbial communities.”

Results, Lines 232-234: “To characterise bacterial genera consistently present across sampling sites, we performed core microbiome analysis at the genus level. Core genera were defined as those present in at least 50% of samples with a minimum relative abundance of 0.5%.”

20) Line 191: Ecological adaptations of individual microbes cannot be investigated with 16S rRNA gene sequences.

We have revised the manuscript to clarify that these genera include taxa with known ecological adaptations, based on previous studies, rather than inferring traits directly from 16S rRNA gene sequences (Line 236).

21) Line 196: were not significant

We revised as suggested (Line 245).

22) Figure 3: What are the color codes on the x-axis of e for? Is the reader supposed to know these models? I guess we get them in the text but looking at the figure and then back to the text is quite confusing.

We have revised the Figure 3e legend to clarify the WGCNA module colour labels and the heatmap colour scale. In WGCNA, modules are automatically assigned arbitrary colour names as identifiers that serve as unique labels to track modules across analyses.

Figure 3e: (e) Weighted gene co-expression network analysis (WGCNA) heatmap showing correlations between microbial modules (y-axis) and environmental factors (x-axis), values show Pearson correlation coefficients with p-values in parentheses. Colour intensity reflects the direction and strength of the correlation, with positive correlations shown in red and negative correlations shown in blue. Module names are arbitrary colour-based identifiers automatically assigned by the WGCNA package.

23) *Figure 3e and Table 2 are the same data? Is there a reason to have both?*

Figure 3e shows correlations between microbial modules and environmental variables, whereas Table 2 (Table 3 after revision) reports taxa that are statistically enriched within those modules, thus presenting complementary information.

24) *Line 240: I'm struggling to see these as gradients. They are distinct samples.*

Changed from "chemical gradients" to "variation in snowpack chemistry" (Line 308), thank you.

25) *Line 280: What are the actual concentrations? How do these relate to deposition rates, or marine sources, etc. Please provide specific details as well as the actual data.*

The actual concentrations are added as Table 2 (summary per sampling depth) and Supplementary Table 1 (per-sample concentrations), and Discussion was revised to include ion concentrations and better contextualise them against other alpine studies (Line 343-365). For the details, refer above to the response to Comment #3.

References:

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