RC2: 'Comment on egusphere-2025-4903', Anonymous Referee #2, 12 Feb 2026

Reviewer #2

This study explores the chemical characteristics, bacterial diversity, and composition of snow in the Swiss Alps, comparing these features across three sampling sites and two distinct snow layers: surface snow and bulk snow (15 cm depth). The results indicate that these layers differ in both chemical properties and bacterial diversity and composition, with surface snow exhibiting greater diversity than bulk snow. In surface snow, Total Inorganic Nitrogen (TIN) concentrations were higher than in bulk snow, whereas organic acids, Ca^{2+}, PO_4^{3-}, Mg^{2+}, Na^{+}, *and* K^{+} *were more abundant in the bulk layer. Regarding potential relationships between environmental variables and bacterial genera, the authors report a positive and statistically significant correlation between various spore-forming genera and TIN concentrations in surface snow, as well as between cold-adapted bacteria and calcium concentrations in bulk snow.*

General comments:

1) *This study involves a substantial number of snow samples, and the effort is commendable. However, certain aspects, particularly the introduction and discussion, could be improved.*

   Thank you for acknowledging the extent of our work and for your constructive feedback. A revised manuscript has been prepared addressing all comments below.

2) *The introduction places considerable emphasis on the role of airborne bacterial dispersal and post-depositional processes in microbial selection. While this is a relevant aspect of snow microbial ecology, it does not align well with the study objectives outlined therein. For instance, the authors highlight topographic differences as a key feature of their study, given the sampling of three distinct Swiss Alpine valleys. The introduction would benefit from incorporating more background on how topography may influence snow microbial composition and chemical characteristics, thereby providing better context to frame the study. Furthermore, the altitudinal gradient is mentioned but barely developed, with no substantial relevant results presented.*

   Thank you for this insightful comment and suggestion. In the revised version of the manuscript, we have now included the paragraph introducing the effect of the mountain topography on the alpine snowpack properties at both large scales (orographic effects along altitudinal gradients) and smaller scales (wind-driven preferential deposition across ridges and slopes), and how this relates to spatial variability in snow ion concentrations (Lines 67-76).

   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.”

3) *In the Materials and Methods section, the rationale for selecting the snow sampling depths (surface vs. 15 cm) is not specified, despite its apparent relevance to the paper's framing. The authors propose that the sampled snow originated from two distinct snowfall events (three days*

and one day prior to collection), yet they provide no justification for these depth choices or assurance that the deeper snow layer corresponds to the earlier event, which they discuss later. Moreover, most comparisons in the results focus on snow depth rather than topographic variables, which misaligns with the initially stated objectives of investigating topography. Reframing the Materials and Methods to better address the introduction's questions would strengthen the study.

We thank reviewer for the comment and acknowledge that the sampling rational might have been insufficiently explained. Our study covered 19 sites, with three replicates collected at each depth, all within a two-day window. The two depth ranges were chosen to sample snow of potentially different ages: surface 0-2 cm snow being more recently deposited and 10-15 cm deep snow representing older accumulation. We clarify this in the Methods (Lines 94-102). Given the large spatial scale of the campaign and the limited time we had to spend at each individual site, we chose to focus on the fixed-depth sampling protocol to ensure sampling standardisation across all sites. We admit that the manuscript would benefit greatly from the evaluation of the snow stratigraphy and subsequent sampling of the defined snow layers, but it was unfeasible given the time and logistical constrains, and we have now added this caveat in the Discussion (Lines 359-361, 373-374). Given the sampling design, we recognise that we cannot attribute each samples depth to a specific snowfall event, and we have ensured this uncertainty is not overstated in the manuscript.

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. SXX). 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."

Discussion, Lines 359-361: "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."

Discussion, Lines 373-374: "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."

4) *The results provide detailed descriptions of differences between snow layers but fail to present findings for comparisons between sampling sites or emphasize the altitudinal gradient. This applies to both chemical and bacterial composition analyses. Most comparisons focus on depth rather than topography, overlooking these initial objectives.*

We agree that comparisons between sampling sites and the altitudinal gradient were not sufficiently emphasized in the original results. We also thank the reviewer, as addressing this comment has greatly improved our manuscript and led us to identify patterns that we had previously missed.

To address this, we have performed additional statistical analyses and expanded the Results section to address the effect of topography on the bacterial community composition (Lines

259-378). Specifically, we added a series of Mantel tests examining the relationship between community dissimilarity and multiple geographical predictors, including geographic distance, resistance distance (geographical distance accounting for the physical barriers between sampling sites), elevation, slope, and aspect (Lines 259-371; Table S2). Elevation difference between sites emerged as the strongest geographical predictor of community composition at both depths, maintaining its signal after controlling for geographic distance (added new figure: Fig 3bc). In contrast, geographic and resistance distance signals largely disappeared after controlling for elevation, indicating that the elevational gradient is the primary geographic driver (Lines 271-272). Slope showed an independent though weak association with surface snow community dissimilarity after controlling for elevation and geographic distance, while aspect showed no consistent signal (Lines 264-267). At the alpha-diversity level, community evenness increased with elevation in both depth layers (Lines 274-276).

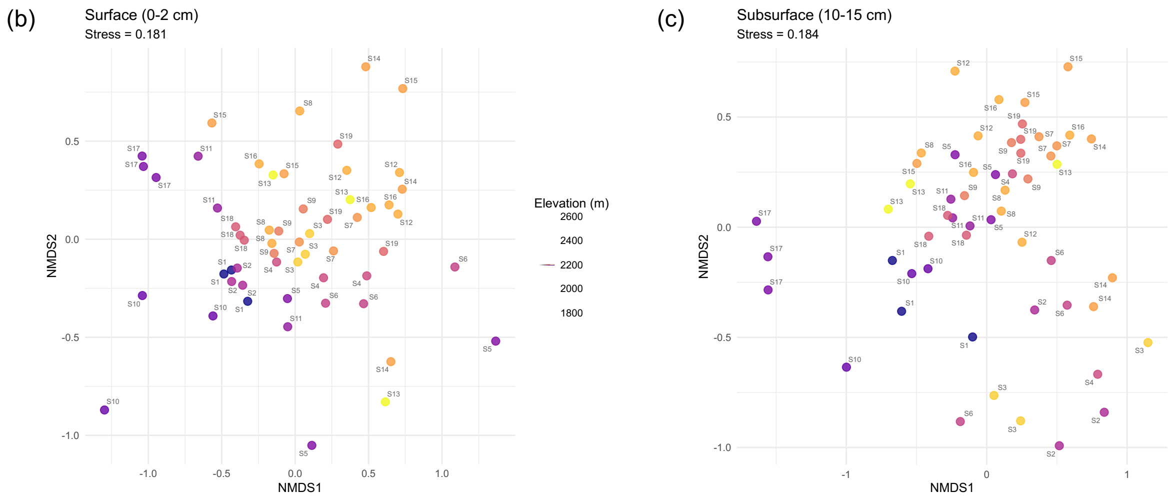
These results corroborated our previous analysis, with no distinct clustering by valley detected, consistent with the weak influence of geographical distance on community composition. Elevation, being the strongest geographical driver of bacterial community dissimilarity, was further investigated through dbRDA analysis and WGCNA, where the module of OTUs negatively correlation with elevation was identified. At the chemical composition level, ion concentrations were similarly correlated with elevation, with compound-specific responses observed across depths (Section 3.1).

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).”

Figure 3b:



5) *In the discussion, the data presented are interesting but challenging to interpret meaningfully, as the methodological details and linkage to the introduction are insufficiently explained. Consequently, drawing robust conclusions is difficult.*

I suggest that the authors reconsider the paper's objectives as presented in the introduction, potentially shifting focus to snow layers, and provide stronger justification. Specifically, what criteria determined the depth cutoff? Were differences observed in snow characteristics, and how were they assessed? Adding site comparison results or reframing the introduction, methods, and discussion to ensure coherence would greatly strengthen the manuscript.

We thank the reviewer for the thorough and constructive evaluation – we agree that the Discussion lacked coherence with the Introduction. We have revised the manuscript, and the discussion now reflects the expanded geographical and topographic analysis (Lines 382-407). To address the previous misalignment between the original framing of Introduction and the corresponding Results and Discussion sections, we have also edited the Introduction (see response to Comment #2), expanded the Results (Comment #4) and Discussion (Lines 382-407) sections to address impact of topography on bacterial community. Additionally, we have expanded on the Methods to clarify sampling design (Lines 94-102; see response to Comment #3).

Discussion, Lines 382-409: "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."

Specific comments:

6) *Line 83: specify the volume of snow collected.*

   Line 89: specify the volume of melted snow filtered for chemical analyses

   The volume is added (Line 103, 107): the snow was collected into the bags of 450 mL, resulting in ~150 mL of meltwater.

7) *Line 109: There appears to be an error here, referring to ASV instead of OTUs.*

   Corrected, thank you.

8) *Line 143: Please provide a table with the results of the chemical characterization.*

   We now included a table (Table 2) summarising the results of chemical characterisation per sampling depth. In addition, the full per-sample measurements are available in supplementary (Table S1).

   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

9) *Lines 166-170: This result contributes little to the study and is not discussed further. Additionally, no context is provided to justify its inclusion.*

   We agree that the original version of the paragraph summarising these results lacked the connection to the broader manuscript. In the revised version, we highlighted the elevation-related pattern in snow chemistry and discussed them as a topographic driver of chemical composition (Lines 214-215).

   Results, Lines 214-215: "Elevation was positively correlated with TIN, organic acids, and SO_4^{2-} at both sampling depths. In contrast, Ca^{2+} showed a negative correlation with elevation in subsurface snow, indicating compound-specific response to the elevation."

10) *Line 187: The rationale for conducting the core microorganisms analysis is not contextualized.*

   We added the rationale for conducting the core microbiome analysis at the beginning of the section (Lines 232-234).

   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%."

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