Reviewer 2:

Thank you much for the review of our manuscript. We have addressed all the comments. Please see below for our point-by-point responses to the reviewers (in blue and preceded by "REPLY:").

The present manuscript explores the morphological and taxonomic variability of red snow algae in different elevational gradients and substrate types (soil-based or ice-based) across Alaskan glacier and its forefield, using molecular as well as microscopic approaches. The study provides valuable insights into the composition of snow algae in various habitats, as well as information on variations in algal communities responsible for red snow across elevation and substrate gradients. I also value the comparison and discussion on different approaches and their outputs. The manuscript is well written, with only minor language inconsistencies that are easy to correct.

REPLY: We sincerely thank the reviewer for the positive and encouraging assessment of our manuscript. We appreciate the reviewer's recognition of the value of combining microscopic and molecular approaches to examine the morphological and taxonomic variability of red snow algae across elevational and substrate gradients. We are also pleased that the comparison of these approaches and their respective outputs was considered informative.

My main concern lies in the analysis of soluble nutrients, which are evaluated throughout the study. The approach used to collect soluble nutrients, especially phosphorus, is not commonly used, so validation of this method for these types of samples is needed or needs to be referred in the study. This concern stopped me from further review of the data and results connected to soluble nutrients. Also, when evaluating surface and subsurface data, I miss further information on radiation conditions from the sampling campaigns, as cells of snow algae can migrate within the snow layer. I believe that such data could contribute substantially to a better evaluation of the results. I describe all my comments in point-by-point below.

I must also stress that I am not an algologist by specialization. I recommend this paper to be seen by a specialist in snow and glacier algae, as I am not fully available to review the algal information and data in detail.

REPLY: We thank the reviewer for identifying these important concerns. The comments regarding the collection and preservation of samples for soluble nutrient analysis,

particularly phosphorus, and the potential influence of radiation conditions on the vertical distribution of snow algal cells were highly valuable. In response, we have clarified and revised the relevant methodological descriptions and added further information and discussion where appropriate.

Abstract

L16: the sentence, as currently written, is not fully consistent with results which state that (e.g., L195) mean concentration of spherical cells between environments were similar. Please revise it so that its meaning is clear and consistent with the results.

REPLY: The original sentence referred to differences in morphotype composition rather than differences in the absolute concentration of spherical cells. We have revised the sentence to clarify that it describes the relative proportions of spherical and non-spherical cells. Now you can read "Microscopic observations revealed that the ice-based snowpack was characterized by a greater proportion of spherical cells, whereas the soil-based snowpack was characterized by a greater proportion of non-spherical cells and larger spherical cells." in Lines 16–18.

Methods

L110 + Sect. 2.3: For the chemical analysis of PO_4^{3-}, acid-prewashed bottles are commonly used to prevent adsorption of P to the vessel surface, for example, and further bias in analyses. Were your vessels acid-washed before sampling?

REPLY: The sample tubes were not acid-washed before use. However, before collection of the final aliquot, each tube was rinsed twice with the corresponding filtered sample. In addition, Milli-Q water blanks analyzed together with the samples showed no detectable PO_4^{3-} peak. We have added these sample-handling details to the revised Methods. Now you can read "For chemical analysis, the meltwater was filtered immediately after melting through a 0.45 μm ion-free disposable filter designed for ion-chromatographic sample preparation (25AI ChromatoDisk, GL Sciences, Japan) to minimize the potential influence of particulate material on the dissolved solute measurements, following procedures used in previous studies of snow and glacier samples (Terashima et al., 2017; Takeuchi et al., 2019; Román et al., 2026). In addition, samples were also transferred into 6 ml tubes for molecular analysis at selected sites located (S1, S4, S7, S9, S10, S14, S22, S25, and S30). Samples for chemical and molecular analyses were transported frozen and stored in a freezer (−20 °C) at Chiba University and thawed immediately before analysis whereas samples for microscopic observations were stored at room temperature." in Lines 123–130.

My main concern is freezing samples for chemical analysis. Even though the samples were filtered, samples for P analysis are commonly stored in the refrigerator, not frozen, as freezing might break down fine particles, for example, and affect the final P concentration in the analysed water. Has your approach been validated for this type of sample? If so, providing a reference to the particular validation or detailed justification for the particular approach would help to increase the reliability of your results.

REPLY: Although we did not conduct a direct comparison between refrigerated and frozen aliquots of the samples analyzed in this study, comparable filtration and storage procedures have been used in previous chemical analyses of snow and glacier samples. Takeuchi et al. (2019; "Variations in Phototroph Communities on the Ablating Bare-Ice Surface of Glaciers on Brøggerhalvøya, Svalbard", Frontiers in Earth Science, 7:4, doi:10.3389/feart.2019.00004) used a 0.45 µm ion-free disposable ChromatoDisk to filter glacier meltwater before measuring soluble ions, including PO_4^{3-}, by ion chromatography. Terashima et al. (2017; "Microbial Community Analysis of Colored Snow from an Alpine Snowfield in Northern Japan Reveals the Prevalence of Betaproteobacteria with Snow Algae", Frontiers in Microbiology, 8:1481, doi:10.3389/fmicb.2017.01481) filtered colored-snow samples and stored them at −20 °C before chemical analyses that included total phosphate. More directly comparable to the present study, Román et al. (2026; "Unveiling the Large Coverage of Red Snow Algae Blooms in Antarctic Coastal Snowfields", Communications Earth & Environment, 7:53, doi:10.1038/s43247-025-03156-6) filtered red-snow samples, stored the filtrates frozen in PE tubes, and subsequently measured PO_4^{3-}. No artificial increase in PO_4^{3-} attributable to frozen storage was reported in these studies. We have therefore added the filter specifications, tube-rinsing procedure, storage and thawing conditions information, and the relevant references to the revised Methods. Nevertheless, we acknowledge that these published applications provide methodological precedents rather than a direct refrigerated-versus-frozen validation of the specific samples analyzed in the present study. As this concern overlaps with the preceding comment, the corresponding revisions to the Methods are the same as those described in our response above.

L136: I might have overlooked it, but it is not clear to me at which section the quantitative comparison of morphotype composition with molecular taxonomic composition by calculating volumetric biomass for each morphotype is present.

REPLY: The morphotype composition shown in Fig. 6d was calculated on the basis of relative volumetric biomass and was compared with the molecular taxonomic composition shown in Fig. 6c. We have revised the Methods to provide the calculation explicitly.

Now you can read “The average cell counts and the volume of sample examined were used to calculate the concentration of each morphotype per unit volume of snow meltwater (cells mL^{-1}). For each morphotype, volumetric biomass ($\mu L\ L^{-1}$) was calculated by multiplying its cell concentration by its mean cell volume. For samples subjected to molecular analysis, morphotype composition based on relative volumetric biomass was compared with molecular taxonomic composition based on relative sequence abundance.” in Lines 161–165.

L177: As discussed further, all morphotypes can vary in their species composition, also some snow algae species revealed cryptic diversity (i.e. Remias et al. 2023). How was the pooling of all non-spherical cells for further analyses and evaluation justified? They might differ in their metabolic/environmental requirements and pooling such data into a single category may limit the explanatory power of further analyses and evaluation of results.

REPLY: We agree that pooling the non-spherical morphotypes was not intended to imply that they were taxonomically or ecologically equivalent. In our samples, each individual non-spherical morphotype occurred only sporadically and at low abundance. Their separate site-level proportions could therefore not be estimated reliably, and retaining each morphotype as an independent category would have produced several highly sparse categories. We pooled them only to provide a broad compositional comparison between the dominant spherical cells and the less abundant non-spherical cells in Fig. 6d. The pooled category was not used to infer that its constituent morphotypes shared the same metabolic or environmental requirements. We have revised the Methods to clarify the limited purpose of this pooling and have added a statement to the Discussion acknowledging that it may obscure taxon-specific ecological patterns, particularly given the potential cryptic diversity of snow algae (Remias et al., 2023). Now you can read “Because each non-spherical morphotype occurred only sporadically and at low abundance, their separate site-level biomass proportions could not be estimated reliably. They were therefore pooled into a descriptive category (“non-spherical cells”) only for the broad comparison with spherical cells. This pooling does not imply taxonomic or ecological equivalence among the constituent morphotypes.” in Lines 212–215, and “The individual non-spherical morphotypes occurred at low abundance and were therefore pooled for the broad compositional comparison. However, this pooled category should not be interpreted as a single taxonomic or functional group. Morphologically similar snow algae may comprise genetically distinct lineages with different ecological characteristics (Remias et al., 2023), and pooling the non-spherical morphotypes may therefore obscure taxon-specific distribution patterns.” in Lines 341–345.

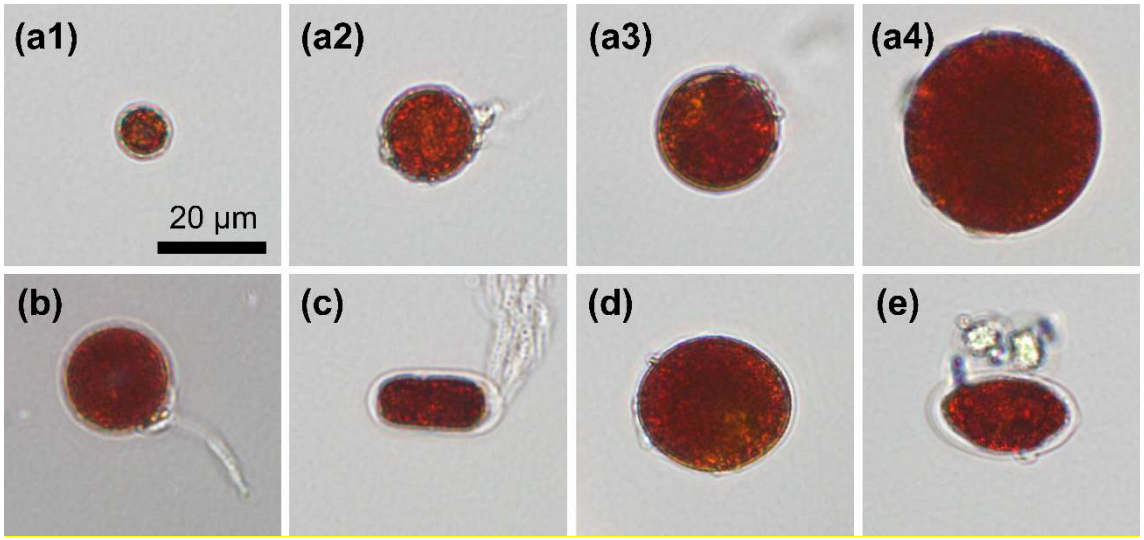
Results

3.1. I suggest calling the section “Red snow algae morphotypes in snow samples” as the section refers solely to morphotypes.

REPLY: Corrected as suggested.

L190: I would recommend including the scale bar size directly in the picture to help with the orienting in the figure.

REPLY: We added a scale bar size in the figure as suggested, and deleted the sentence “A scale bar is 20 µm.” from the caption. Now you can see the modified Figure 4 below.



Sect. 3.3. While reading this section, I am wondering why subsurface samples weren’t taken and analysed also for soil-based samples if the reported lowest depth was 4 cm in all sites and subsurface samples were taken approx. 3–5 cm deep? I cannot find any justification for this decision as such results might be very valuable for the study.

REPLY: As also explained in our response to Reviewer 1, we revised the Methods to clarify the rationale for collecting subsurface samples only from the ice-based snowpack. Although snow depth at the soil-based sites was at least 4 cm, these snowpacks were generally shallow and close to the underlying soil. Collecting deeper samples therefore posed a greater risk of contamination by soil particles and soil-associated organisms. For this reason, sampling at the soil-based sites was restricted to the surface layer. We have added this explanation to the revised Methods and clarified the sampling depths and definitions of surface and subsurface samples. Now you can read “Red snow was collected from a 10 × 10 cm area using a stainless-steel scoop. The upper 2 cm was defined as the

surface layer because visible red-snow blooms are generally concentrated in the uppermost few centimetres of the snowpack, and a 0–2 cm layer has commonly been used to quantify surface snow algal communities (Onuma et al., 2022). However, recent studies have shown that snow algae can also occur below the immediate surface, including within deeper snow layers (Schuler and Mikucki, 2023). We therefore additionally collected the underlying 2–5 cm layer as a subsurface sample at the ice-based sites. Subsurface sampling was not conducted at soil-based sites because the snowpack was generally shallow and deeper sampling could have introduced soil particles and soil-associated organisms (Fig. 2)" in Lines 115–121.

Fig. 6 + 7. The orientation in the figures while reading the results is rather difficult. For the better orientation, I recommend adding a heading along the y-axis describing the data origin (e.g., 18S rRNA, potentially red pigmented, etc.).

REPLY: As recommended, we changed Figures 6 and 7 to indicate the origin and category of the data, including 18S rRNA data and potentially red-pigmented algal taxa. Because this information is now shown directly in the figures, we removed the corresponding explanatory text from the figure captions to avoid redundancy. Now you can see modified figures 6 and 7 below.

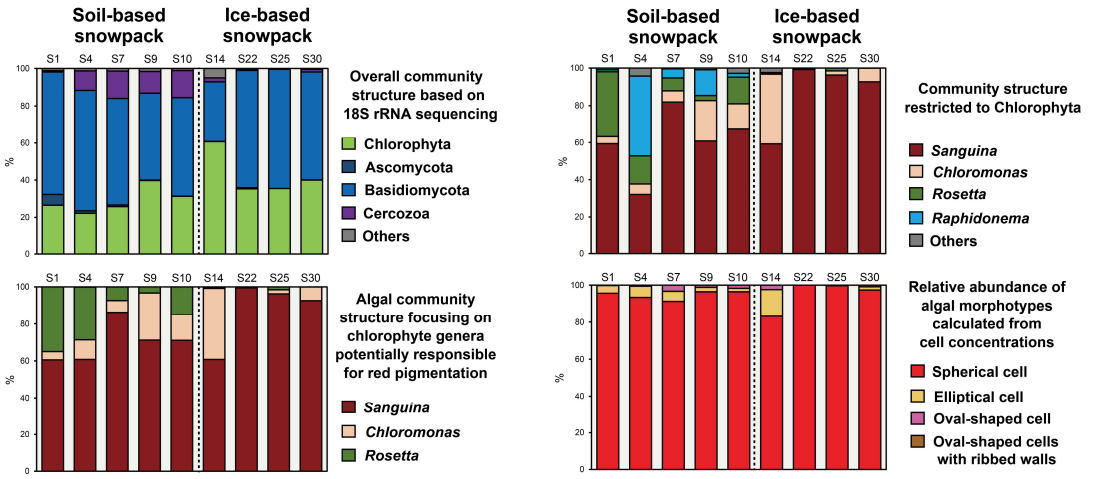


Figure 6: Microbial community structure on the snow surface based on 18S rRNA gene sequencing and morphological observations. Community structure at each elevation is presented as stacked bar charts normalized to 100%. For the molecular dataset, only phyla or genera accounting for >5% of the total reads were included in the analysis.

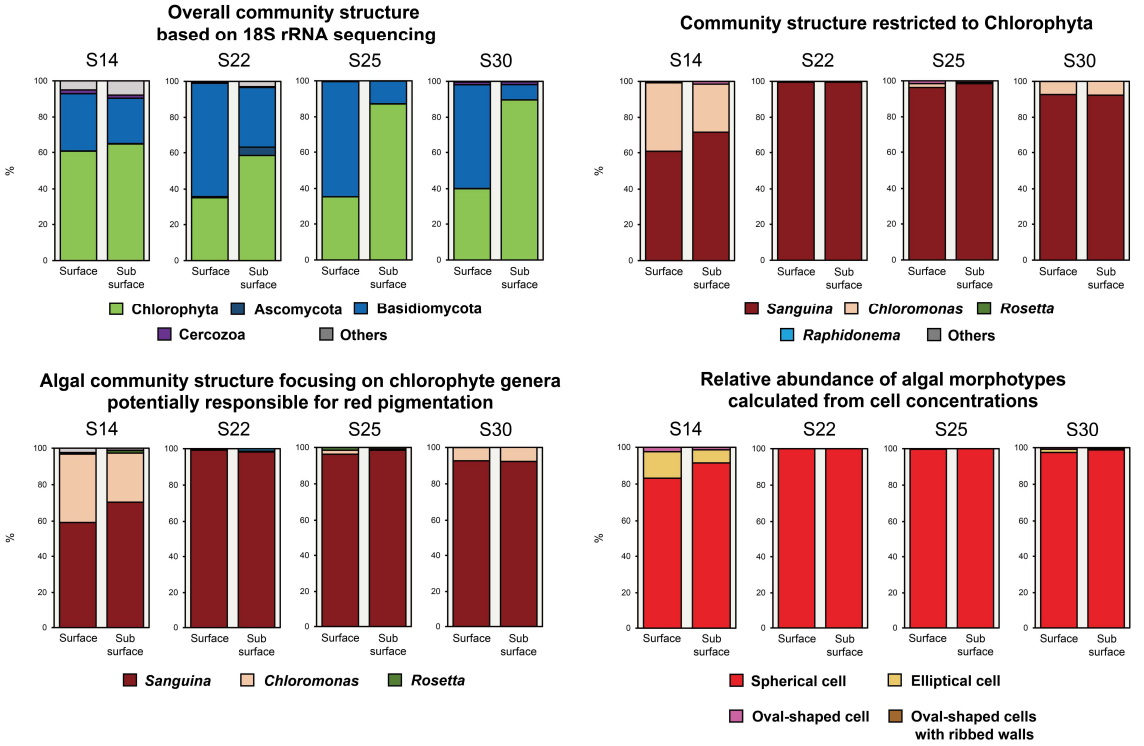


Figure 7: Microbial community structure on the surface and subsurface in ice-based snowpack. Community structure is presented as stacked bar charts normalized to 100% for each site, comparing the snow surface (left) and snow subsurface (right) across four sites. For the molecular dataset, only phyla or genera accounting for >5% of the total reads were included.

Section: 3.5, as highlighted above. I would like to see evidence that the methodological approach used in this study has been validated for this type of samples as higher P than N in snow samples is at least unexpected.

REPLY: As detailed in our responses to the two comments above, the sample tubes were rinsed twice with the corresponding filtered sample, and no detectable PO_4^{3-} peak was observed in the Milli-Q water blanks. Moreover, comparable filtration and frozen-storage procedures have been used for phosphate measurements in previous studies of snow and red-snow samples, without a reported increase in PO_4^{3-} attributable to freezing. We have added these methodological details and supporting references to the revised Methods.

Figures in overall: The study uses similar colours for different variables in various figures. For example, Fig. 5 and Fig. 6 use the same red colour which make the orientation harder for reader. I would suggest choosing colour-schemes to be unified for the same variables to make the orientation in figures easier.

REPLY: As shown in the revised figures described above, we changed the color schemes in Figures 5 and 6 to avoid using similar colors for different variables and to improve

readability. In contrast, the colors used in Figures 3 and 4 represent the same sampling-site categories and are already applied consistently across both figures; therefore, these color assignments were retained.

Discussion

L274–275: The sentence needs to be reworded slightly to make the meaning clearer. I would also recommend dividing it into shorter sentences to make it easier to read. I am not sure what information the authors wanted to convey to the reader, as both the soil-based site and S14 had a higher proportion of non-spherical cells and Chloromonas.

REPLY: We have divided the sentence into shorter statements and clarified that these sites showed both higher relative abundances of Chloromonas and Rosetta and a higher proportion of non-spherical cells, whereas the upper ice-based sites were characterized by Sanguina dominance and a greater proportion of spherical cells. Now you can read "Across the glacier valley, *Chloromonas* and *Rosetta* were relatively abundant at the soil-based site and the lower ice-based site, including S14. These sites also showed a higher proportion of non-spherical cells in the morphotype data. In contrast, *Sanguina* dominated at the upper ice-based sites, where spherical cells accounted for a greater proportion of the algal community (Fig. 6c, d)." in Lines 326–329.

L279: "Previous studies support this explanation." I think this sentence is unnecessary, particularly given the paragraph's logic. The authors discuss the fact that the spherical morphotype is taxonomically heterogeneous, yet they continue to refer to studies supporting the spherical cell type for Sanguina.

REPLY: We deleted it from the revised manuscript as suggested.

L284: "Therefore, morphology alone can merge taxonomically distinct algae into the same category, especially for spherical cells." In my opinion this sentence is redundant with L278.

REPLY: We agree that this sentence was redundant with the preceding discussion. We have therefore deleted it from the revised manuscript to avoid repetition.

L322: I miss the reference for the sentence on modulation of local growth.

REPLY: We have added a reference (Almela et al., 2024, "Laboratory Experiments Suggest a Limited Impact of Increased Nitrogen Deposition on Snow Algae Blooms," Environmental Microbiology Reports, 16, doi:10.1111/1758-2229.70052) supporting the potential influence of nutrient availability on snow algal growth. Because direct evidence

that nutrients regulate life-stage transitions in red snow algae remains limited, we also revised the sentence to avoid making this broader claim. Now you can read “ However, nutrient availability may still influence local algal growth once cells reach the snow surface (Almela et al., 2024).” in Lines 386-387.

Sect. 4.4. As radiation can affect the migration of algal cells, I would like to see more information and data on light conditions during sampling, as this information could substantially contribute to evaluating the results. This is particularly important when samples are taken within four days at which the light conditions could potentially differ.

REPLY: Radiation conditions were not measured during the sampling , and we therefore cannot directly evaluate their effects on the vertical distribution of algal cells. However, the red-pigmented stages that typically dominate red-snow blooms are generally non-motile cysts or resting spores rather than actively swimming cells. This applies particularly to *Sanguina* and to the mature red cyst stages of many snow-inhabiting *Chloromonas* species (Procházková et al., 2019; Hoham and Remias, 2020). Therefore, short-term active migration of the abundant red cells observed in this study in response to radiation conditions during sampling was likely limited. Nevertheless, some snow algae possess motile stages during their life cycles. Moreover, molecular data do not allow us to determine the life stage of each detected taxon. We therefore acknowledge that radiation conditions before and during sampling could have influenced the distribution of some algal stages. We have added this limitation and revised the Discussion to avoid attributing the observed vertical patterns directly to active migration. Now you can read " The taxonomic composition also differed between the surface and subsurface layers. Although Sanguina was detected in both layers, the relative abundance of Chloromonas was higher at the surface. This pattern indicates taxonomic differentiation between layers but does not identify the mechanism responsible for the observed distribution. The red spherical cells associated with Sanguina represent non-motile cyst stages (Procházková et al., 2019; Hoham and Remias, 2020), making short-term active migration during sampling unlikely. However, motile stages of other taxa, passive redistribution with meltwater, and environmental conditions preceding sampling may have contributed to the vertical distributions. Although solar radiation, a critical factor shaping vertical microhabitats, was not directly measured during the sampling period and should therefore be considered when interpreting the vertical patterns, our findings suggest that vertical positioning is influenced by both physical transport and taxon-specific traits.

Overall, these results underscore that the subsurface snowpack serves as an essential, persistent seed bank that feeds surface blooms as snowmelt progresses. Because surface-

only observations risk underestimating total algal biomass and missing early-stage dynamics, incorporating subsurface biomass reservoirs into spatial models will be crucial for accurately predicting future red snow blooms and their feedback on glacier albedo." in Lines 438–450.