Reviewer 1 (Daniel Remias):

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:").

This is a fascinating study about seasonal dynamics of snow microbes during the melting season at a location below canopy. Despite much research has been performed with blooms in snowpacks above timerline or on glaciers, the special situation of snow algae blooms under decidous trees in Japan still deserves more attention. The authors did an outstanding work in repeatatly sampling the same site throughout the season, developing a picture about temporal changes and vertical shifts within the community. The data are about snow algae, microinvertebrates and snow fungi.The new insight of this work is that birch buds fertilize snow and support phototrophlic microbes. Bacterial abundances were not assessed because no molecular protocols were applied to describe the communty by metagenomics. Snow chemistry was evalated well, but changes in the snow-water-content, which are expected to influence vitality and migragion capacity of organisms within the snow, were not recorded. Please regard this comment as a suggestion for future studies.

REPLY: We thank the reviewer for these constructive comments. While DNA data are not included in the present manuscript, they are currently being analyzed and will be presented in a separate manuscript in preparation by our group. Regarding the water content, we did not measure it in this study, but we fully agree with the reviewer that it is an important parameter. We will make efforts to include water content measurements in our future fieldworks.

The results are very detailled and sufficient figures and literature is provided. The manuscript sometimes has repetitive elements in argumentation or conclusion and the text could be condensed here and there without effecting the scientific message. I suggest to alter the title in a way like "**Temporal and vertical changes in microbial snow communities during the melting season below canopy in Northern Japan**"

REPLY: We will revise the title in consideration of the reviewer's comment. The revised title will be "Temporal and vertical changes in snow microbial communities during the melting season below canopy in Northern Japan"

Line 10, start sentence with "During snowmelt, diverse ...

REPLY: We will correct suggested. It will read “During snowmelt, diverse cold-tolerant microbes thrive within snowpacks.”.

Line 23, delete "for the snowpack layers".

REPLY: We will correct as suggested. It will read “Analyses of snow pits and cores revealed that the active layers of microbes were distinct between snow algae/fungi (surface layer) and microinvertebrates (subsurface layers), probably because of their preferable conditions.”.

Line 32, algae bloom "in" snowpacks, not only "on"

REPLY: We will correct as suggested. It will read “During the snowmelt season, snow algae often bloom in snowpacks and change the color of the snow surface to green, red, yellow, or orange (Tanabe et al., 2011; Remias et al., 2013; Procházková et al., 2019b; Hoham and Remias, 2020; Procházková et al., 2021; Matsuzaki et al., 2022; Raymond et al., 2022).”.

Line 38, delete "during the melting season". Moreover, nutrients are not only deposited by snowfall and rain, but also airborne by winds.

REPLY: We will correct and change the sentence as suggested. It will read “In snowpack ecosystems, materials such as carbon, nitrogen, and phosphate are circulated through the metabolism of various microbes. These nutrients are deposited by snowfall, rain, airborne dusts, fire ash, and then transformed through freeze–thaw cycles, microbial processes, and hydrological movements within the snowpack (Jones, 1999).”.

Line 45, 46: Here, a very important reference is not cited (though later in the discussion): Hoham et al. 2008, who described in detail that balsam fir litter supports the growth of snow algae. Tuhs, in general, it has already been known that leaches from vascular plants support algae blooms.

REPLY: We will cite a paper and changed the sentence. It will read “In forested areas, leachate from organic matter (e.g., balsam fir leaf) deposited by trees during the late snowmelt season contains nutrients for algal growth (Jones, 1991, 1999; Hoham et al., 2008).”.

Line 55: delete "to the snow surface", because it was never observed that flagellates swim actively to the surface, they stopp their migration earlier in time to reach a favourable layer below.

REPLY: We will delete the words. It will read “For example, snow algae germinate at the soil surface or firns below snowpacks through the supply of meltwater and migrate upward (Jones, 1991; Hoham and Duval, 2001; Matsumoto et al., 2024; Rea and Dial, 2024).”.

Line 56, insert "sometimes" before "concentrated", because this situation described by Hoham 1975b does occur all the times.

REPLY: We will correct as suggested. It will read “Snow algae are sometimes concentrated above the subsurface ice layers formed by refrozen meltwater within snowpacks (Hoham, 1975b).”.

Line 61, snow "particles", I guess you rather mean "crystals"?
Line 63, maybe you prefer to cite a much newer and well fitting reference about the swimming activity of snow algae instead? See: Détain, A., Suzuki, H., Wijffels, R. H., Leborgne-Castel, N., & Hulatt, C. J. (2025). Snow algae exhibit diverse motile behaviors and thermal responses. *io*, *16*(5), e02954-24. https://doi.org/10.1128/mbio.02954-24

REPLY: We thank the reviewer for suggesting the inclusion of recent studies, and we will cite the paper as suggested. After carefully reviewing the International Association for Cryospheric Sciences (IACS) definition, we concluded that “grains” is more appropriate than “crystals” in this context, and will revise the text accordingly. It will read “Snow grains metamorphose into granular structures, and meltwater percolates through the snow layers, allowing microbes to migrate within the snowpacks via these meltwater (Hoham and Duval, 2001; Cruaud et al., 2020; Détain et al., 2025).”.

Line 66, add to sentence "during the season."

REPLY: We will correct as suggested. It will read “However, there is a lack of high-frequency, continuous observations linking these environmental changes to microbial dynamics within snowpacks during the season.”.

Line 97, start sentence probably with "First surface green algal blooms ..."

REPLY: We will correct as suggested. It will read “First surface green algal blooms appeared on April 28.”.

Line 101, start sentence probably with "The remaining snow depth ..."

REPLY: We will correct as suggested. It will read “The remaining snow depth was 6 cm on May 20.”.

Line 106, so the two snow surfaces sampled adjacent to the blooms were your controls? If yes, state them as "white snow" control or similar.

REPLY: We decided to keep the word “adjacent”. The snow surfaces adjacent to the blooms did not show visible algal coloration, but at the same time they were not “pristine white snow.”

Figure 1: Were all the photos taken at the same time of the day?

REPLY: All photos were taken in the morning (between 5:00 and 10:00), but the exact shooting times were not standardized. We will therefore add the time period to the figure captions. It will read “Figure 1 : Temporal changes at the study site during the study period. All the photos were taken in the morning (between 5:00 and 10:00).”.

Line 112, please state the brand/type of ice auger

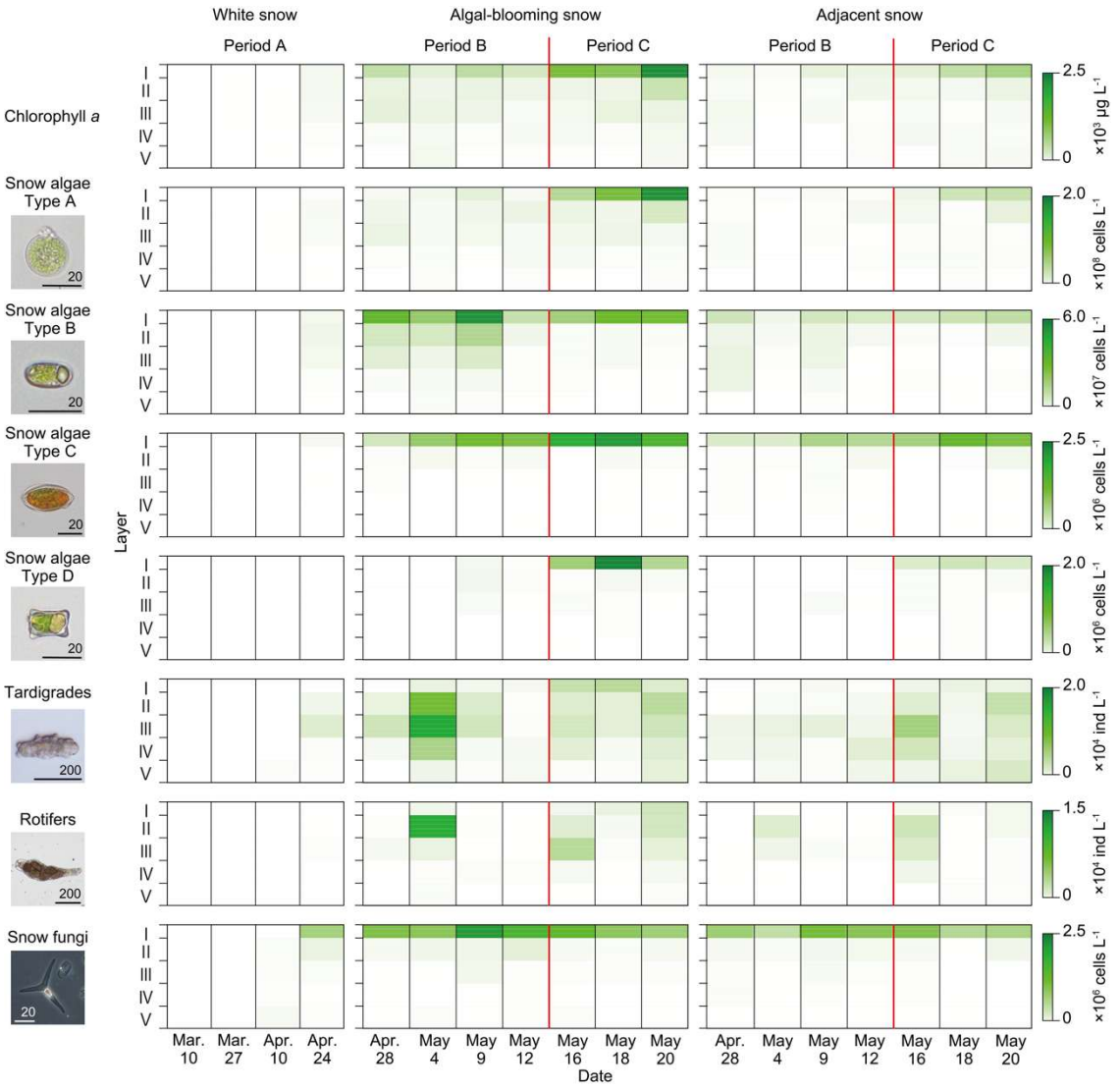
REPLY: We will add the information of the auger. It will read “To determine the vertical distribution of microbes at the bottom of the snowpack, snow cores were collected from the surface to the bottom of the snowpack using a hand auger manufactured by the Institute of Low Temperature Science, Hokkaido University, Japan (length: 1 m, diameter: 90 mm).”.

Line 122, which mesh size had the sieve?

REPLY: We used a sieve with a mesh size of US mesh #40. We will add this information in the sentence. It will read “After melting, the snow samples were separated as follows: 5 mL for chlorophyll *a*, 5 mL for chemical analysis after passing through a sieve (US mesh #40) to remove plant litter, 10 mL for the dry weight of total insoluble particles, and the remainder for microinvertebrates.”.

Lines 138 - 140: It is a flaw that the classes of snow algae (A-D) are not described here! Ok, the authors perform no morphological identification based on frozen and rethawn material, which is difficult, but still, which would have allowed a tendative taxonomical addressing by LM even without marker sequencing. In this context, it is wrong to say that vegetative cells could be determined from the material, because Matsuzaki describred his key using laboratorial strain material. Contrary, with field material, the identification of non-vegetative cysts or spores would have been easier, even from frozen material. The reader does not need the information about exact species ID to understand the reported communty dynamics, but stillt the 4 morphological classes (in fact stages, because it does not mean 4 different species!) should be briefly presented.

REPLY: We thank the reviewer for pointing out the importance of presenting the morphological classes of snow algae. In the revised manuscript, we will clarify in the Methods section that species-level identification was not attempted because morphological keys based on vegetative cells are not directly applicable to frozen samples. Instead, we categorized the observed snow algae into four morphological classes (A–D). In the Results section, we will briefly describe these four classes and add photographs of each microbes in Figure 4 as shown below. It will read " Particularly in snow algae, identification at the species level was not performed in this study because species identification based on the morphology of frozen samples is difficult. Therefore, the observed algae were classified into morphological types." in Methods, and " Microscopic observations of snow samples revealed various microbes, as some overlap with Ono and Takeuchi (2025). They were morphologically identified using microscopy as snow algae Types A, B, C, and D; tardigrades (*Hypsibius nivalis*, *Hypsibius* sp.); rotifers (Philodinidae gen. sp.); and snow fungi (*Chionaster* (*Chi.*) *nivalis*). Snow algae types A, B, and C are the same as those described in Ono and Takeuchi (2025), and they represent various species of the genus *Chloromonas*. Type A is characterized by a spherical shape, Type B by an oval shape, and Type C by an oval shape with ribbed walls. In addition, Type D was observed in this study, which is barrel-shaped with projections at the corners. The length of this alga ranged from 14.7 to 21.3 μm (18.3 ± 1.4 μm), and the width from 10.3 to 15.0 μm (12.9 ± 1.1 μm). This corresponds to the zygote stage of *Oocystis lacustris* f. *nivalis* reported in previous studies (Fukushima, 1963; Matsuzaki et al., 2022)." in Results section.



Revised Figure4

Line 149, elution "from" tree-derived litter?

REPLY: We will correct as suggested. It will read “2.3 Elution from tree-derived litter”.

Line 177, delete last word "season"?

REPLY: We will correct as suggested. It will read “3.1 Meteorological conditions at the study site during snowmelt”.

Lines 195.196, here again, it is not acceptable just to cite a reference to describe which organisms were present. At least the supplement should provide a brief description of the snow algae stages A, B, C and D, ideally with a microphotograph. The tardigrada and the snow fungi information is clear.

REPLY: We will address these points consistently with our response to Lines 138–140.

Question: were any snow ciliates observed during the study?

REPLY: Yes. As we have reported in other studies (Ono et al. (2021), Scientific reports https://doi.org/10.1038/s41598-021-85462-5), Abundant ciliates were also observed under microscopic observations.

Line 200, maybe you mean "at the first time of sampling" instead of "for the first time ..."

REPLY: We will correct as suggested. It will read “Chlorophyll *a* was detected in the subsurface layers (III and V) for the first time (March 10, Period A).”.

Line 215, maybe you mean "from which on" instead of "at which"?

REPLY: We will correct as suggested. It will read “The red line indicates the time from which on the forest trees sprouted.”.

Line 252, delite "chemical" in headline?

REPLY: We will correct as suggested. It will read “3.6 Temporal and vertical changes in solutes within the snowpack”.

Line 306, microbial growth: of microinvertebrates? Because algae thrive already in the first month of snowmelt?

REPLY: Our original wording was misleading. We will revise the sentence to clarify what we want to say. It will read “These findings suggest that, although microbes may already be present, their abundance does not increase substantially during the first month after snowmelt onset, and visible algal blooms do not yet occur.”.

Line 315, "fatty acids and in the form of cysts", I guess you mean "fatty acids and other compounds like osmolytes, which increase during cyst formation" ? Another reference describes that the cyst morphology itself supports freezing tolerance: Ezzedine, J. A., Uwizeye, C., Si Larbi, G., Villain, G., Louwagie, M., Schilling, M., ... & Maréchal, E. (2023). Adaptive traits of cysts of the snow alga Sanguina nivaloides unveiled by 3D subcellular imaging. *Nature Communications*, *14*(1), 7500.

REPLY: We agreed with the reviewer’s comment. We will revise the manuscript accordingly and add the reference. It will read “A large proportion of vegetative cells in snow and ice algae are known to not recover after exposure to freezing (Hoham, 1975a); however, they can enhance freeze tolerance by accumulating polyunsaturated fatty acids and

other compounds like osmolytes, which increase during cyst formation (Prochazkova et al., 2018; Procházková et al., 2019a; Ezzedine et al., 2023).".

Line 318, maybe insert "in the upper snow layer" after "growth"? The deeper snow pack is hardly influenced by air temperatures.

REPLY: We will correct as suggested. It will read "Compared with previous research, our findings indicate that Period A corresponds to a phase in which microbes are active but their growth in the upper snow layer is limited by the freezing air temperatures.".

Line 322, the "upper" snowpack?

REPLY: We will correct as suggested. It will read "The temporal changes in snow algae and chemical solutes observed during Period B suggest that the nitrate (NO_3^-) present in the upper snowpack was utilized for the growth and blooming of snow algae.".

Line 326 - 329: this needs more explanation, are you talking about the local climatic conditions and composition of rainfall, which is here influenced by oceanic conditions? If yes, make this more clear to the reader and insert at line 328 "in this region" after "growth" and maybe give another reference about precipitation in Northern Japan?

REPLY: We will make it clear by adding the words the reviewer suggested, and citing references which reported chemical composition of rain in both Northern Japan, and Yamagata. It will read "The high concentration of NO_3^- in rainwater, the relationship of Type B algae with NO_3^- from the CCA analysis (Figure S4), and the relationship of NO_3^- with sea salt-derived components, such as Na^+ and Cl^-, also indicate that the nitrogen used by algal growth **in this region** could be of atmospheric origin (Fukuzaki et al., 1999; Shinomiya et al., 2018).".

Line 345, "was" instead of "is"
Line 346, which period? The study period?

REPLY: We will correct and make it clear. It will read "A previous study showed that snow algal growth reaches its maximum at a nitrate concentration that was more than 56 times higher than the concentration observed during Period B in this study (Broadwell et al., 2023).".

Line 360, delete "forest" in the headline?

REPLY: We will correct as suggested. It will read "4.3 Enhancement of microbial growth by nutrients from canopy (Period C)".

Line 375, 376: please discuss reference Hoham et al 2008 in more detail and compare your results. In their study, it was needle trees which fertilized, in your case buds of broad-leaf trees. This reference should already be mentioned in the introduction.

REPLY: We will detail the reference you suggested, and revise the sentence. It will read "Especially in Hoham (2008) reported from culture experiments using leachates of balsam fir needles that strain identity and leachate concentration affected the algal populations but did not alter the shape of population growth trajectories over time. In contrast, our study suggests that a large amount of nutrients may have leached from impurities (bud scales) derived from beech forests, potentially triggering a rapid algal growth. This indicates that the impacts on microbial communities can differ depending on plant species. Such effects could be further evaluated by analyzing the chemical composition of leachates from impurities originating from different tree species and by conducting algal culture experiments under these conditions.".

Line 377: rather "below" instead of "in" the tree covered-area?

REPLY: We will correct as suggested. It will read "The timing of the decrease in solar radiation below the tree-covered area coincided with the budburst of beech trees and an increase in snow algae, suggesting that the budburst-associated reduced solar radiation could also contribute to algal growth.".

Line 378: here I dissagree with the authors. Why the increased shading in course of budburst should enhance algal growth due to decreased irradiation? In a low light scenario, photosynthesis is light limited. Flagellates have to overcome this by migration to the surface.

REPLY: We agreed that reduced solar radiation alone does not directly enhance nutrient uptake or photosynthesis, and we will revise the sentences to clarify our argument. Our intention was to emphasize that excessive solar radiation on the snow surface can impose strong stress on snow algae. The onset of beech budburst reduces this stress, thereby allowing algal cells to remain active at the snow surface, where nutrients are abundant. In such conditions, algae can grow without being strongly limited by either nutrients or light. We will therefore revise the sentences accordingly, and also refer to algal cell concentration instead of chlorophyll *a* as an indicator of algal growth. It will read "The timing of the decrease in solar radiation below the tree canopy coincided with the budburst of beech trees and an increase in snow algae, suggesting that the budburst-associated reduction in solar radiation mitigates excessive light stress, enabling algae to remain active at the nutrient-rich snow surface.".

Line 381: But snow algae cope with high-light stress by the formation of astaxanthin and the bloom turns red. This does not happen below canopy?

REPLY: In fact, during the study period in 2021 and earlier years (2018–2020), we observed orange snow but did not find red snow or red algal cells. However, since 2022, red snow has also been observed within the forest. This was a new finding for us, and we are currently continuing investigations into this phenomenon.

Linie 382: *I do not understand the argumentation.* Wow can reduced light intensity contribute to the uptake of nutrients? In terms of physiology? Furthermore, increase in intracellular chlorophyll content is a side effect of long-term low light conditions, particuallary for chlorophyll b.

REPLY: Our response here is the same as that provided for Line 378. It will read “Under these conditions, algal growth—as indicated by both increases in algal cell concentration—may be facilitated by the simultaneous availability of sufficient nutrients and moderate light levels (Suzuki and Takeuchi, 2023; Ono and Takeuchi, 2025).”.

Line 407: insert "or from soil" after "layers"?

REPLY: We will correct as suggested. It will read “One of such factors is snow depth (including maximum snow depth and air temperature), which determines the period during which microbes can migrate from the lower layers or from soil.”.

Line 417, use "support" instead of "have triggered"?

REPLY: We will correct as suggested. It will read “Additionally, phenological shifts due to rising temperatures, such as the earlier budburst of beech trees, may support earlier snow algal blooms.”.

Line 426: too general sentence. Which activity should be monitored in future?

REPLY: We will revise the paragraph to focus on detailed activity (biochemical cycles), and add the sentences as another reviewer suggested. It will read “Because these conditions vary across regions, future studies should examine not only algal growth and microbial biomass, but also their roles in driving biogeochemical processes such as carbon and nitrogen cycling within the snowpack. Considering the projected reductions in maximum snow depth and earlier dates of snow disappearance due to climate change, along with the expected changes in phenology, it is likely that the durations of Periods A and B will shorten. In contrast, the onset of Period C may occur earlier in response to an earlier budburst; however, its duration is expected to remain relatively unchanged. Changes in

the durations of these periods and their interactions with the surrounding environment are expected not only to affect the abundance of organisms themselves, but also to influence biogeochemical processes driven by their biological activity. In future studies, it will be important to consider processes such as ion export during snowmelt and radiative feedback, including albedo changes. For example, as observed in Japanese snowpacks (Ohte et al., 2004; Osaka et al., 2016), melt-induced ion pulses could substantially influence nutrient availability and community dynamics. Future studies should consider constructing and testing models to evaluate the impacts of these snowpack physical processes on biological activity, as suggested by Costa et al. (2018, 2020). In addition to the effects of melt-induced ion pulses, future research should also take into account radiative feedback between trees, snow surface albedo, and snow-ice microbes (Conway et al., 1996; Aubry‐Wake et al., 2022). Understanding these interactions will clarify how physical and biological processes jointly shape snowpack dynamics, nutrient availability, and biogeochemical cycling.”.