

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 timeline or on glaciers, the special situation of snow algae blooms under deciduous trees in Japan still deserves more attention. The authors did an outstanding work in repeatedly 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 phototrophic microbes. Bacterial abundances were not assessed because no molecular protocols were applied to describe the community by metagenomics. Snow chemistry was evaluated well, but changes in the snow-water-content, which are expected to influence vitality and migration 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 detailed 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 revised the title in consideration of the reviewer's comment. The revised title is "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 corrected suggested. Now you can read “During snowmelt, diverse cold-tolerant microbes thrive within snowpacks.” in Line 10.

Line 23, delete "for the snowpack layers".

REPLY: We corrected as suggested. Now you can 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.” in Line 21-23.

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

REPLY: We corrected as suggested. Now you can 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).” in Line 31-34.

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

REPLY: We corrected and changed the sentence as suggested. Now you can 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).” in Line 37-39.

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 cited a paper and changed the sentence. Now you can 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).” in Line 45-46.

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 deleted the words. Now you can 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).” in Line 54-56.

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

REPLY: We corrected as suggested. Now you can read “Snow algae are sometimes concentrated above the subsurface ice layers formed by refrozen meltwater within snowpacks (Hoham, 1975b).” in Line 56-57.

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 cited 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 revised the text accordingly. Now you can 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).” in Line 61-63.

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

REPLY: We corrected as suggested. Now you can read “However, there is a lack of high-frequency, continuous observations linking these environmental changes to microbial dynamics within snowpacks during the season.” in Line 65-67.

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

REPLY: We corrected as suggested. Now you can read “First surface green algal blooms appeared on April 28.” in Line 98.

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

REPLY: We corrected as suggested. Now you can read “The remaining snow depth was 6 cm on May 20.” in Line 182-183.

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 therefore added the time period to the figure captions. Now you can read “Figure 1: Temporal changes at the study site during the study period. Temporal changes at the study site during the study period. The mean canopy height of beech forests in this region is 12.6 m (Shoji and Yoshimura, 2025). All the photos were taken in the morning (between 5:00 and 10:00).” in Line 108-110.

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

REPLY: We added the information about the auger. Now you can 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).” in Line 111-113.

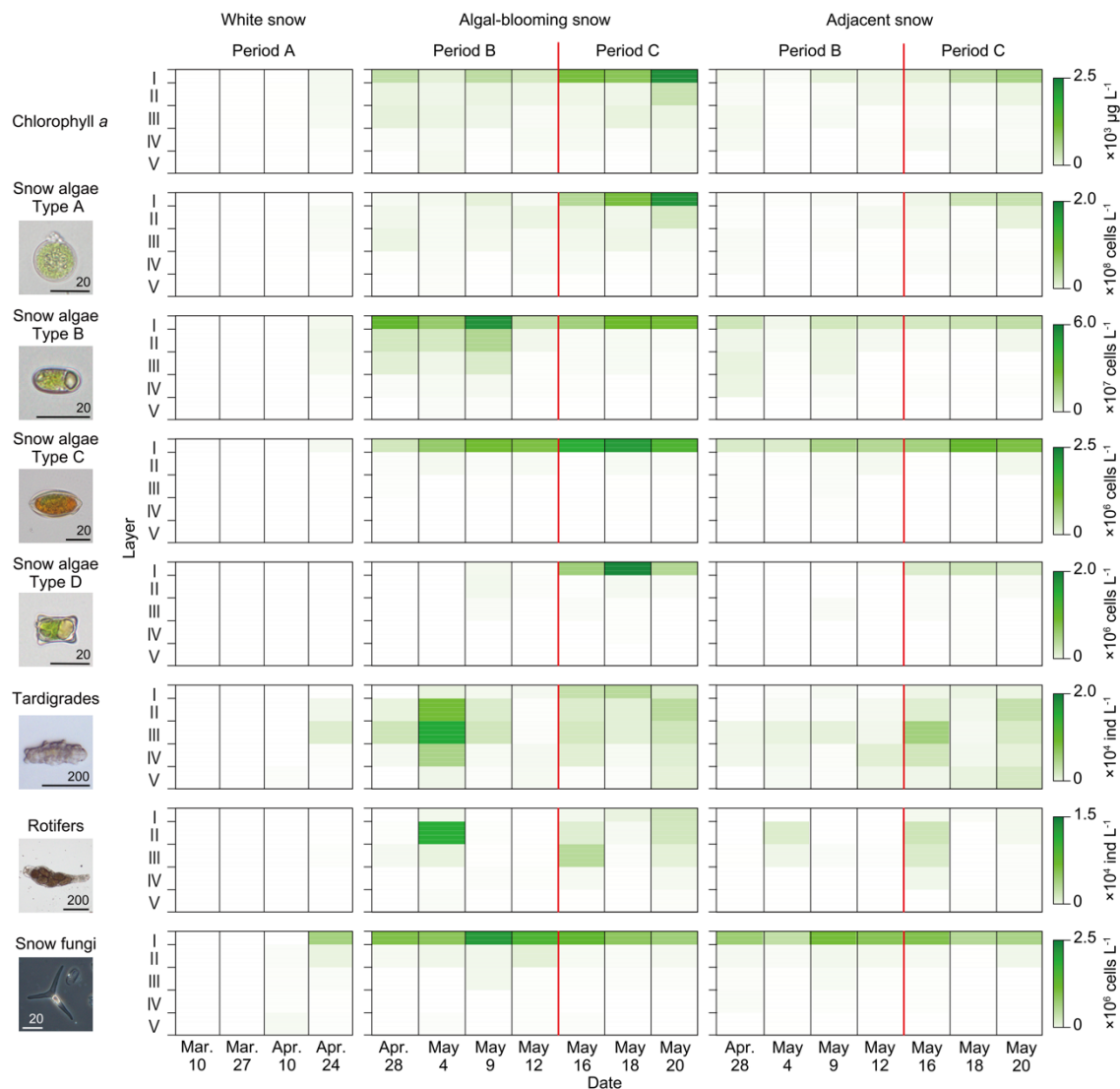
Line 122, which mesh size had the sieve?

REPLY: We used a sieve with a mesh size of US mesh #40. We added this information to the sentence. Now you can 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.” in Line 123-125.

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 tentative 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 described 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

community dynamics, but still 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 clarified 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 briefly described these four classes and add photographs of each microbes in Figure 4 as shown below. Now you can 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 (Line 140-142), 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 ($18.3 \pm 1.4 \mu\text{m}$), and the width from 10.3 to 15.0 μm ($12.9 \pm 1.1 \mu\text{m}$). This corresponds to the zygote stage of *Oocystis lacustris* f. *nivalis* reported in previous studies (Fukushima, 1963; Matsuzaki et al., 2022)." in Results section (Line 201-208).



Revised Figure4

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

REPLY: We corrected as suggested. Now you can read “2.3 Elution from tree-derived litter” in Line 151.

Line 177, delete last word "season"?

REPLY: We corrected as suggested. Now you can read “3.1 Meteorological conditions at the study site during snowmelt” in Line 179.

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 addressed these points consistently with our response to Lines 139–141.

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 corrected as suggested. Now you can read “Chlorophyll *a* was detected in the subsurface layers (III and V) for the first time (March 10, Period A).” in Line 211.

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

REPLY: We corrected as suggested. Now you can read “The red line indicates the time from which on the forest trees sprouted.” in Line 225-226.

Line 252, delete "chemical" in headline?

REPLY: We corrected as suggested. Now you can read “3.6 Temporal and vertical changes in solutes within the snowpack” in Line 263.

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

REPLY: Our original wording was misleading. We revised the sentence to clarify what we want to say. Now you can 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.” in Line 317-319.

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 revised the manuscript accordingly and added the reference. Now you can 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).” in Line 325-327.

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

REPLY: We corrected as suggested. Now you can 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.” in Line 329-331.

Line 322, the "upper" snowpack?

REPLY: We corrected as suggested. Now you can 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.” in Line 333-334.

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 made it clear by adding the words the reviewer suggested, and citing references which reported chemical composition of rain in both Northern Japan, and Yamagata. Now you can 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).” in Line 338-341.

Line 345, "was" instead of "is"

Line 346, which period? The study period?

REPLY: We corrected and made it clear. Now you can 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).” in Line 356-358.

Line 360, delete "forest" in the headline?

REPLY: We corrected as suggested. Now you can read “4.3 Enhancement of microbial growth by nutrients from canopy (Period C)” in Line 372.

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 detailed the reference you suggested, and revised the sentence. Now you can 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.” in Line 387-393.

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

REPLY: We corrected as suggested. Now you can 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.” in Line 394-396.

Line 378: here I disagree 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 revised 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 therefore revised the sentences accordingly, and also referred to algal cell concentration instead of chlorophyll *a* as an indicator of algal growth. Now you can 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.” in Line 394-396.

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.

Line 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, particularly for chlorophyll b.

REPLY: Our response here is the same as that provided for Line 378. Now you can 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).” in Line 399-401.

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

REPLY: We corrected as suggested. Now you can 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.” in Line 427-428.

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

REPLY: We corrected as suggested. Now you can read “Additionally, phenological shifts due to rising temperatures, such as the earlier budburst of beech trees, may support earlier snow algal blooms.” in Line 438-439.

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

REPLY: We revised the paragraph to focus on detailed activity (biochemical cycles), and added the sentences as another reviewer suggested. Now you can 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.” in Line 447-461.

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:").

This is a review of "Temporal and vertical changes in biological communities within snowpacks during melting season in Northern Japan". First of all I wanted to note I really enjoyed reading this papers. I don't deal with green deciduous canopies over snow, so it's a fun problem to think about. For the authors, please note I am reviewing this as a snow hydrologist, and so that's my bias. My first comment is that I think the title needs to be tightened up. Perhaps something like "Changes in biological communities within snowpacks during ablation in Northern Japan"?

REPLY: We changed the title as another reviewer suggested.

My main concern is that the recent advances in understanding the complex snowpack-solute processes do not seem to be considered. Specifically, the ion exclusion that occurs can cause the first melt pulse to have substantially more than subsequent pulses, e.g., Costa, et al 2018, 2019. My concern is then that this complicates some of the correlative metrics used herein. I am hoping that the authors can better describe their framework within the context of these snowpack processes.

REPLY: Similar ion pulses derived from snowpacks have been reported in Japan, particularly for nitrate (Ohte et al., 2004; Osaka et al., 2016), typically occurring between March and April. In our study, concentrations of solutes decreased between April 10 and April 24, likely reflecting such a melt-induced ion pulse. Importantly, our correlation analyses included concentrations both before and after the presumed melt pulse. The subsequent decrease in concentrations is conceptually equivalent to the nutrient depletion discussed in relation to biological activity. Therefore, we believe that our main conclusions regarding correlations between nutrient availability and biological activity are not substantially affected by this process.

Nevertheless, we acknowledge that considering the effects of melt pulses is an important perspective that we had not previously incorporated. To address this, we added sentences in the Discussion highlighting that such snowpack physical processes can influence nutrient availability and community dynamics, and that future work should construct and test models to evaluate these impacts, as suggested by Costa et al. (2018, 2019). Now you

can 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 help clarify how physical and biological processes jointly shape snowpack dynamics, nutrient availability, and biogeochemical cycling.” in Line 447-461.

Secondly, the authors allude to air temperature during the melt, but the radiative processes are not noted. For example, the darkening of the snow decreases the albedo and increases the solar shortwave absorption. As well, as the trees heat up, they emit longwave radiation to the snowpack. The trees can be well above air temperature, even in sub-zero conditions. I realize this is not a melt dynamics paper, however these processes should be noted as the feedback is substantial, e.g.,

Aubry - Wake, C., Bertoncini, A. & Pomeroy, J. W. Fire and Ice: The Impact of Wild-fire - Affected Albedo and Irradiance on Glacier Melt. *Earth's Futur.* **10**, (2022).

Conway, H., Gades, A. & Raymond, C. F. Albedo of dirty snow during conditions of melt. *Water Resour Res.* **32**, 1713–1718 (1996).

I think the inclusion of these feedback is notable due to feedbacks with the biological communities themselves. E.g., the more the communities expand, the faster they can melt the snowpack, etc.

REPLY: We thank the reviewer for highlighting the potential role of radiative processes,

including changes in albedo and radiation from trees, on snowmelt and microbial communities in snowpacks. We agreed that such processes could influence the activity of snow-ice microbes, particularly near tree canopies. However, in our study, we did not measure longwave or shortwave radiation, nor spectral changes in the snow surface, so we cannot discuss this feedback in detail. Nevertheless, we acknowledge that this is an important perspective. We added a statement in the Discussion noting that future studies should consider radiative effects and related feedback between snowpack physical processes and biological communities, alongside other factors such as melt-induced ion pulses. We added the sentences “In this study, changes in radiation associated with tree leaf-out, such as increased longwave radiation as shown in previous study (Goodfellow and Barkham, 1974) to the snowpack or changes in shortwave absorption due to albedo reduction were not explicitly considered. These processes may also affect snow-ice microbes and should be examined in future studies.” in Line 414-416. We also added the sentence in Line 447-461 as shown in previous answer to the comment.

Otherwise this is a quite interesting study. Specific comments are below.

References:

Costa, D., Pomeroy, J. & Wheeler, H. A numerical model for the simulation of snowpack solute dynamics to capture runoff ionic pulses during snowmelt: the PULSE model. *_Advances in Water Resources_* (2018) doi:10.1016/j.advwatres.2018.09.008.

Costa, D., Baulch, H., Elliott, J., Pomeroy, J. & Wheeler, H. Modelling nutrient dynamics in cold agricultural catchments: A review. *_Environ Modell Softw_* ****124****, 104586 (2019).

REPLY: We cited the papers in Discussion.

Specific comments:

L27 "its disappearance" this reference is fine, but perhaps adding a more regional specific citation would help with local context. Is this area seeing this disappearance faster or slower than global average?

REPLY: In Japan, the changes in snow cover vary considerably with elevation and region, so it is not straightforward to compare them directly with the global average. Nevertheless, we agreed with your point, and we added references (Kawase et al., 2020; Kawase et al.,

2023) on snow cover changes in Japan to provide regional context. Now you can read “Despite the threat of its disappearance (Brown et al., 2017; Kawase et al., 2020, 2023), seasonal snow provides a habitat for various cold-tolerant microbes.” in Line 27-28.

L50 I assume dust and forest fire ash are important and should be added too.

REPLY: We agreed that dust is an important source of nutrients, and we added this point as suggested in other sentence (“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).” in Line 38-39).

L50 "flushed down" I suggest a different expression, e.g., percolating

REPLY: We revised the sentence as suggested. Now you can read “They are not percolated into deeper layers by meltwater, but remain at the surface layer of the snowpacks even if surface melting occurs during the daytime (Grinde, 1983).” in Line 50-52.

L61 "gradually deform" This description of snow crystal metamorphism can be tightened up and using terms more common in that literature

REPLY: We replaced “gradually deform” by “metamorphose”. Now you can read “Snow grains metamorphose into granular structures, and meltwater percolates through the snow layers, allowing microbes to migrate within the snowpacks via meltwater (Hoham and Duval, 2001; Cruaud et al., 2020; Détain et al., 2025).” in Line 61-63.

L63 "allow microbes to migrated within..." because of the increased pore space? or because of entrainment in advection of melt water?

REPLY: The migration of algae in the snowpack is mainly due to entrainment in meltwater flow, rather than increased pore space. We clarified this in the text by adding the words “via meltwater”. Now you can 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).” in Line 61-63.

L75 forest fire ash?

REPLY: Fire ash is indeed a possible factor, so we added it in our response to L50. However, no fire ash was observed in the surrounding area during this study, and therefore it is not addressed in the discussion.

L83 "information regarding..." Is this the source for all the information below? It is not

clear

REPLY: We removed the relevant sentence as it was potentially misleading.

L83 "heavy" in rate? density? amount? be specific

REPLY: We revised the sentence. Now you can read “Because of the monsoon, large amounts of snow accumulate during winter and snow persists until early summer (Kariya, 2002, 2005).” in Line 83-84.

L85 "met conditions..." in the basin?

REPLY: We revised the sentence. Now you can read “Meteorological conditions, including air temperature and solar radiation, were monitored at the snow surface within the basin during the spring season of 2021.” in Line 86-87.

L88 "help of a probe" is this a ventilated thermocouple?

REPLY: No, it was a simple probe. We attached the data loggers directly to the probe itself, not using a ventilated thermocouple.

L89 "every 10min after" why the change?

REPLY: The change in interval was due to maintenance. Before April 25, fieldworks were conducted only every 1–2 weeks, so we used longer intervals to avoid battery depletion. After April 25, with daily maintenance, we increased the observation frequency to every 10 minutes.

L90 "this data logger" word choice

REPLY: We replaced “this” by “the”. Now you can read “The data logger was placed in areas with and without trees above the snow surface (referred to as tree-covered and -free areas, respectively) and held at a height of 5 cm above the snow surface with the help of a small pedestal.” in Line 92-94.

L93 "the hourly means" move this closer to the 10s sampling statement on L 89

REPLY: We moved the sentence next to the sentence as suggested. Now you can read “The intensity of the solar radiation was recorded every 10 s from May 5 to May 17 using a pyranometer (ML-020VM; EKO, Japan) equipped with a data logger (LR5091; HIOKI, Japan). The hourly means of air temperature and solar radiation were obtained from the observations.” in Line 90-91.

L93 "records" observations?

REPLY: We corrected as suggested. Now you can read “The hourly means of air temperature and solar radiation were obtained from the observations.” in Line 91-92.

L97 "Green algal [...]" on ward there are a lot of results mixed in with the methodology. I would suggest splitting this up as much as a possible into methodology and results that are separate. I understand some of the results informed the on going observations, but the way it's written it ends up being a bit unclear what is the result and what is the methodology.

REPLY: We agreed the reviewer. We moved the result of snow depth in the first section of the results (3.1 Meteorological conditions at the study site during snowmelt in Line 180-183). Now you can read “First surface green algal blooms appeared on April 28. The budburst of beech trees in the study area occurred between May 12 and 15 (Figures 1, 2). Rainfall occurred on April 25, 29, May 2, 10, 17, and 19.” in Line 98-100. These observations were not measured variables but field records, so we decided to keep them in the Methods.

L101 "rainfall" was there much rain on snow?

REPLY: Rain fell directly onto the snow surface. We did not measure precipitation in the present study; however, during the fieldwork conducted this year, daily precipitation ranged from 0.7 to 15.0 mm day⁻¹.

L102 It is a new pit each time, correct? Make this clear

REPLY: We made it clear as suggested. Now you can read “Snow samples were collected from five layers across snow depths with a new pit for each sampling (Figure 2): an area of 10 × 10 cm² with depths of 0–3 cm (Layer I), 3–8 cm (Layer II), 8–13 cm (Layer III), 13–18 cm (Layer IV), and 18–23 cm (Layer V).” in Line 100-102.

L110 Figure 1. Great figure. The canopy over snow is very striking! Add something to help the reader know what direction is being viewed. A spatial scale would be excellent if possible

REPLY: The photographs were all taken in the same direction (approximately toward the west), and we have now indicated this in the new Figure 1 as shown below. Unfortunately, no spatial scale is available for these photos. Nevertheless, previous studies indicate that the mean canopy height of beech forests in this region is 12.6 m (Shoji and Yoshimura, 2025). We added this information to the figure caption accordingly. Now you can read

“Temporal changes at the study site during the study period. Temporal changes at the study site during the study period. The mean canopy height of beech forests in this region is 12.6 m (Shoji and Yoshimura, 2025). All the photos were taken in the morning (between 5:00 and 10:00).” in Line 108-110.



Revised Figure1

L113 "ice auger" I assume the authors mean a snow sampler liked an? were they weighed for density?

REPLY: No, the instrument used was a hand auger manufactured by the Institute of Low Temperature Science, Hokkaido University, Japan. The length of the hand auger is 1 m, and diameter is 90 mm). The collected samples were not weighed, so density was not determined.

L120 "all the samples" including rain samples?

REPLY: Yes.

L121 "slowly melted" why?

REPLY: We slowly melted the samples to prevent changes in microbial activity and chemical composition that could occur with rapid thawing. This helped preserve the original state of the samples. We added information, and Now you can read “The samples were slowly melted in a refrigerator (5°C) prior to analysis for minimizing changes in microbial activity and chemical composition.” in Line 123-124.

L135, how were the cell counts done? manually? automatic?

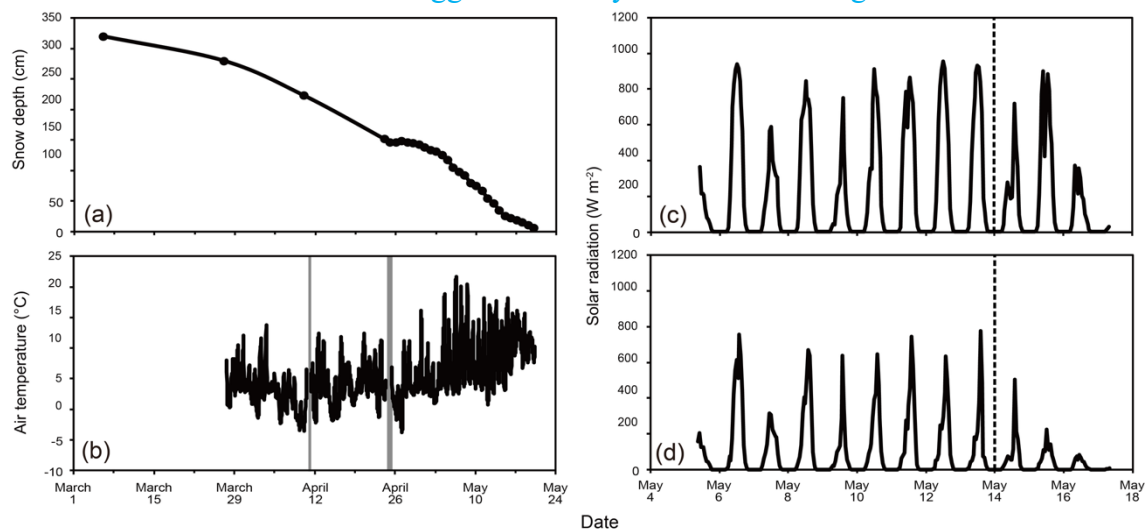
REPLY: We counted manually. We added information, and you can read “Cell counts were performed manually three times, and the mean cell number and sample volume used for filtration were used to calculate the cell concentration per water equivalent of the snow sample (cells L⁻¹).” in Line 137-139.

L163 "1-2 days" why the variance and did it impact the results?

REPLY: The drying time of 1–2 days at 60 °C varied depending on the sample’s water content and size; samples with higher moisture required longer to reach a constant weight. This variation did not affect the results, as all samples were dried until they reached a stable weight before analysis. We added information, now you can read “They were then dried at 60°C for 1–2 days until they reached a constant weight, and weighed.” in Line 164-165.

L190 Figure needs axis labels and titles

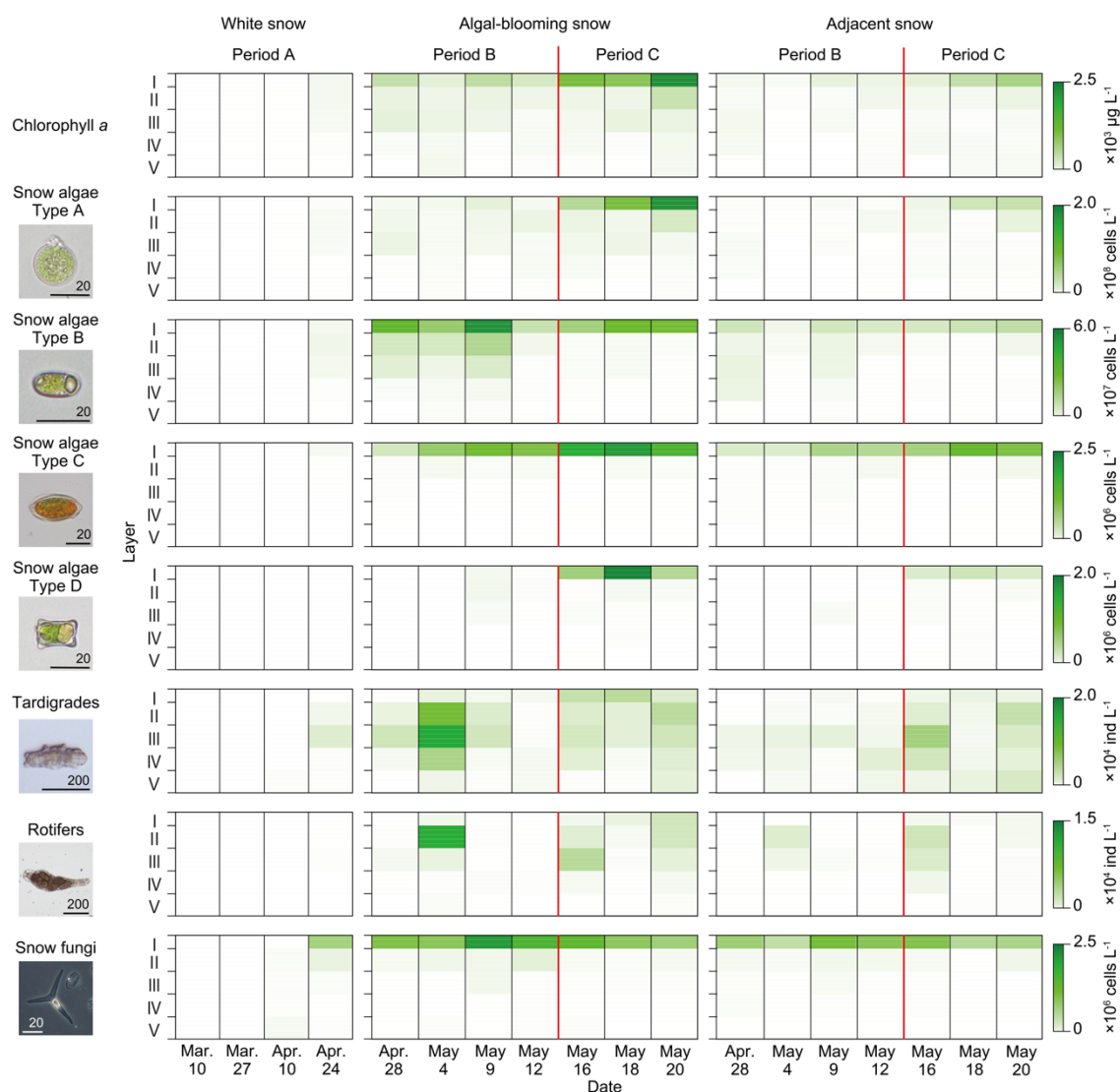
REPLY: We added as reviewer suggested. Now you can see new Figure 3 as shown below.



Revised Figure3

L215 Figure 4, legends are impossible to read

REPLY: We revised as reviewer suggested. You can see new Figure 4 as shown below.



Revised Figure4

L280 Figure 6, red line is hard to see make it thicker. legend is impossible to read clearly

REPLY: We revised as reviewer suggested. You can see new Figure 6 as shown below.



Revised Figure6

L300 "temperature fluctuations" air temp?

REPLY: We corrected as suggested. Now you can read “Despite the insulating properties of snow, studies have shown that air temperature fluctuations can affect snow temperatures down to depths of 20–30 cm, especially during frequent melt–freeze cycles.” in Line 311-312.