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 will change 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 form 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 will add 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). 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 help clarify how physical and biological processes jointly shape snowpack dynamics, nutrient availability, and biogeochemical cycling." in the last paragraph of "4.4. Possible effects of changes in climate and forest phenology on microbial communities within snowpacks".

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 Wildfire - 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 will add 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 will add 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." as the last paragraph in "4.3 Enhancement of microbial growth by nutrients from canopy (Period C)". We will also add the sentence in the last paragraph of "4.4. Possible effects of changes in climate and forest phenology on microbial communities within snowpacks" as shown in previous answer to the comment.

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

References:

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

REPLY: We will cite 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 will add references (Kawase et al., 2020; Kawase et al., 2023) on snow cover changes in Japan to provide regional context. It will 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."

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 will add 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).").

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

REPLY: We will revise the sentence as suggested. It will 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).".

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

REPLY: We will replace "gradually deform" by "metamorphose". It will 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).".

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 will clarify this in the text by adding the words "via meltwater". 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)."

L75 forest fire ash?

REPLY: Fire ash is indeed a possible factor, so we will add 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 will remove the relevant sentence as it was potentially misleading.

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

REPLY: We will revise the sentence. It will read "Because of the monsoon, large amounts of snow accumulate during winter and snow persists until early summer (Kariya, 2002, 2005).".

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

REPLY: We will revise the sentence. It will read "Meteorological conditions, including air temperature and solar radiation, were monitored at the snow surface within the basin during the spring season of 2021.".

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 will replace "this" by "the". It will 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.".

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

REPLY: We will move the sentence next to the sentence as suggested. It will 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."

L93 "records" observations?

REPLY: We will correct as suggested. It will read "The hourly means of air temperature and solar radiation were obtained from the observations.".

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 will move the result of snow depth in the first section of the results (3.1 Meteorological conditions at the study site during snowmelt). It will 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.". 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 will make it clear as suggested. It will 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)."

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 will add this information to the figure caption accordingly. It will read "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)."



Revised Figure 1

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 100 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 will add information, and it will read "The samples were slowly melted in a refrigerator (5°C) prior to analysis for minimizing changes in microbial activity and chemical composition."

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

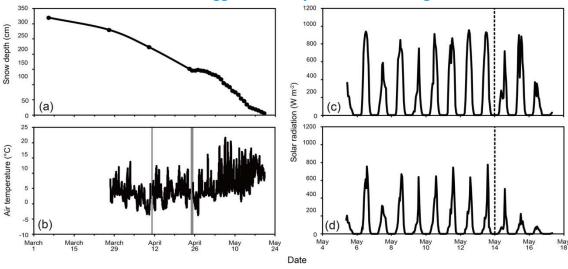
REPLY: We counted manually. We will add information, and you can read "Cell counts were performed manually three times, and the mean cell number and sample volume used for filtration were used to calculate the cell concentration per water equivalent of the snow sample (cells L⁻¹).".

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 will add information, It will read "They were then dried at 60°C for 1–2 days until they reached a constant weight, and weighed."

L190 Figure needs axis labels and titles

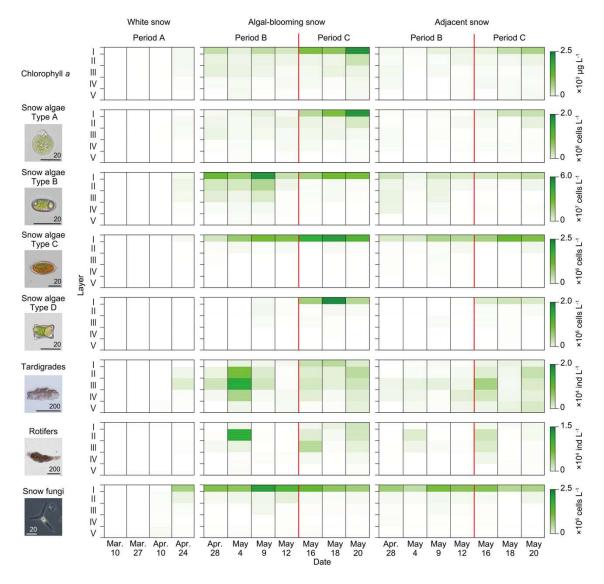
REPLY: We added as reviewer suggested. Now you can see new Figure 3 as shown below.



Revised Figure3

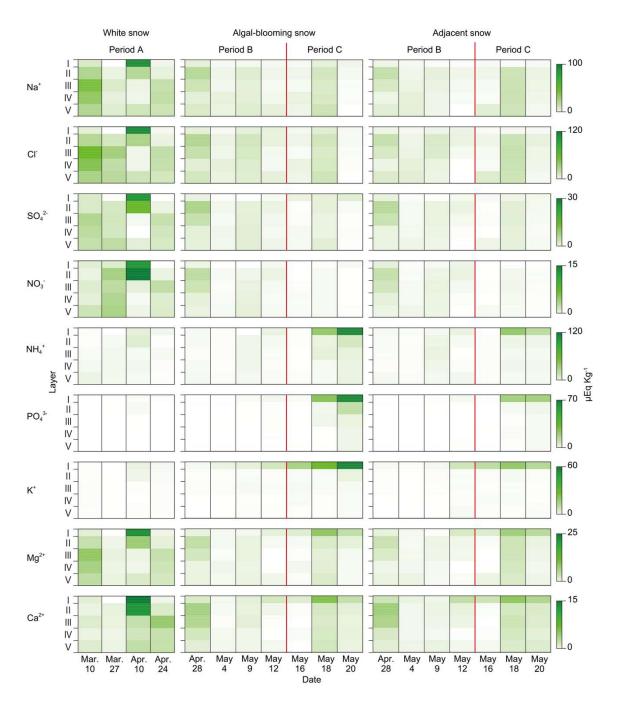
L215 Figure 4, legends are impossible to read

REPLY: We will revise as reviewer suggested. You will 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 will revise as reviewer suggested. You will see new Figure 6 as shown below.



Revised Figure6

L300 "temperature fluctuations" air temp?

REPLY: We will correct as suggested. It will 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.".