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 manuscript by Ono et al., presents interesting data on the vertical distribution and dispersal of algae and microscopic invertebrates in the snow patches. Snow ecosystems are poorly known in general, therefore each piece of data on the algae and their consumers are crucial. Although interesting, manuscript cannot be accepted in the present form. Manuscript require corrections and changes of the tone in the interpretation of the results. I like the idea of MASS and in my opinion the idea makes sense, at least for algae and invertebrates. However, without some clarifications (e.g. sampling) the evaluation of the data robustness is difficult.

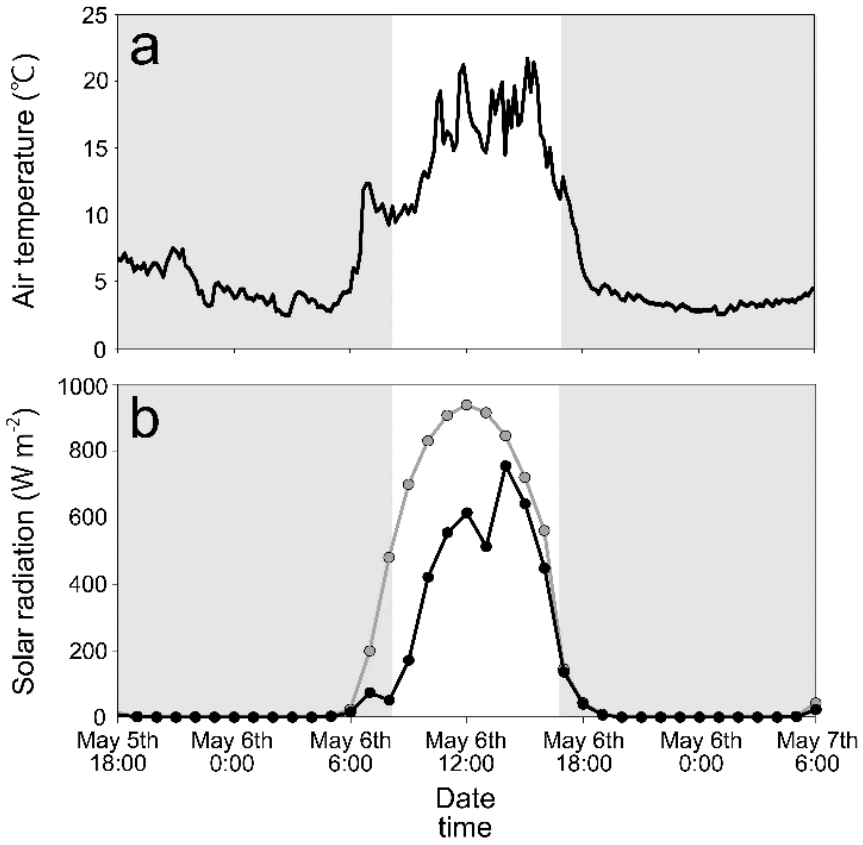
REPLY: As the reviewer pointed out, we revised the method to include more details especially in sampling.

- I would mute the tone of the results and discussion. The studies were conducted in the Japanese forests, on the mountain slope in the specific insular climate, it is hard to extrapolate these data to other snow ecosystems. It will be better to highlight in many places that results are valid for Japan. Therefore, I support the importance of the findings but some sentences are overstated.

REPLY: In the title, discussion, and conclusion, we would like to state that the findings of this study are only applicable to Japan and need further studies to confirm the presence of MASS layers in other countries. We added the words "in northern Japan" in Title and Line 411. Now you can read "The diel vertical migration of microbes within snowpacks in northern Japan driven by solar radiation and nutrients", and "The layers above this depth, MASS layer, are likely to store and circulate carbon and nitrogen produced by snow-ice microbes; thus, they play an important role in snowpack ecosystems in northern Japan.", respectively. We also revised the sentences "Further studies on microbial DVM and MASS layers in snowpacks are necessary to understand the biogeochemical roles of seasonal snow cover in alpine and polar environments." in Line 388-390 to "Further studies on microbial DVM and MASS layers in snowpacks are necessary. These studies should first confirm the presence of the MASS layer in snow not only in northern Japan but also in other countries, and then aim to understand the biogeochemical roles of seasonal snow cover in alpine and polar environments.".

- Could you compare radiation in the forests and open landscape? I'm not convinced that UV could be explanation of the migration in the forests (even without leafes trees offer sort of protection).

REPLY: We do not have the data of UV while measured solar radiation outside the forest. We added the data of solar radiation outside the forest in Figure 3b, and revised its caption "Figure 3: Meteorological conditions recorded during the study period. (a) air temperature, (b) solar radiation. Solar radiation recorded inside the forest is shown as a black line while that recorded outside the forest is shown as a gray line. The period of nighttime is shown as gray zones.".



Revised Figure 3

The data showed that the intensities of solar radiation were lower inside the forest. We added the description and data regarding solar radiation recorded outside the forest in Line 100-102, 177-180, and 315. Now you can read "The intensity of solar radiation inside and outside the forest was recorded every 10s using a pyranometer (ML-020VM, EKO, Japan) with a data logger (LR5091, HIOKI, Japan) at a height of 5 cm from the snow surface by setting it on a small pedestal.", "Solar radiation ranged from 0 to 755 W

m^{-2} inside the forest, and 1 to 938 W m^{-2} outside the forest (Fig. 3b). The intensity of solar radiation started to increase at 6:00, reached its peak at 14:00 inside the forest (755 W m^{-2}), 12:00 outside the forest (938 W m^{-2}), respectively, and then decreased continuously until 20:00, while that of outside the forest. In this study, daytime was defined as the period from 9:00 to 16:00 when the intensity exceeded 150 W m^{-2} inside the forest, and nighttime was defined as the remaining period of the day.", and "After the maximum intensity of solar radiation at 14:00 (755 W m^{-2}) inside the forest, the position of the maximum population changed again to the upper layer (layer I), suggesting that the algae moved upward over time.", respectively.

We concluded that even these intensities were too strong for microbes. The results we cited in the discussion (Line 324-327) were much lower than the intensities observed in this study. We also added a sentence "The daytime intensity of solar radiation recorded in this study, both inside and outside the forest was higher than that shown in previous studies." in Line 327. Now you can read "For example, light irradiation experiments have shown that an irradiation intensity of 95 μmol PAR m^{-2} s^{-1} (equivalent to 19 W m^{-2}) was most suitable for the sexual reproduction of snow algae (Hoham et al., 1998), and photoinhibition occurred at radiation intensities stronger than 200 μmol PAR m^{-2} s^{-1} (equivalent to 40 W m^{-2}) (Procházková et al., 2019a). The daytime intensity of solar radiation recorded in this study, both inside and outside the forest was higher than that shown in previous studies. Another study calculated that the snow depth (approximately 2 m), where snow algae were observed, was the layer through which 0.1% of the solar radiation passed through wet snow (Curl et al., 1972).".

- I would be happy to see discussion on another option/reason of migration between snow layers. I would expect that predators (e.g. springtails) could impact on the migration; preys avoid predators (birds and ice worms on the North American glaciers are a good example of such relation).

REPLY: We agreed that the presence of predators could be affect the microbial vertical distribution. We addressed this in the discussion by citing papers which described several microinvertebrates in the snowpack (Hanzelová et al., 2018; Yakimovich et al., 2020) and added the sentences "In this study, other predators, such as springtails which have observed in previous study (Hanzelová et al., 2018; Yakimovich et al., 2020), were not counted. Therefore, there remains a possibility that the observed behavior is a response to predation pressure. Future research should also consider the impact of organisms that prey on microinvertebrates." in Line 368.

- it is not clear, how many cores were collected at each spot? One core = one sample. What about subsamples = layers? what about replicates = sampling of the same layers?

REPLY: As describes in the method in Line 120-122, one core was collected (at 5:00 on the May 7th). Three different surfaces (locations) were collected at each sampling time as described in Line 112. In each surface, we collected the samples from an area of 5 × 5 cm in five layers across snow depths as described in Line 110-112.

To prevent confusing, we deleted a word "subsample" in Line 132, 141, and 147, and modified a sentence about the snow core sampling in Line 120-122. Now you can read "The samples for snow algae and fungi were stored in 10 mL plastic tubes with 3% formaldehyde.", "Twenty microliters of the sample were transferred from the sample onto a glass slide.", "5–200 µL from the samples were injected into a filter holder equipped with a 0.45 µm PTFE membrane filter (JHWP01300, Merck Millipore, Germany), then filtered using a pump (Linicon LV-125, Nitto Kohki, Japan).", and "The snow core sample was collected at 5:00 a.m., immediately after sunrise on May 7th. The core, with a total length of 113 cm, was cut horizontally every 10 cm using a snowsaw and preserved in Whirl–Pak bags.", respectively.

- How many cores? How many pits?

REPLY: Snow pits were collected on three surfaces for each time period, and a snow core collected only once (5:00 on May 7th).

- Why algae were not identified to genus or family level? Even though many snow algae are morphological species complexes, still their identification at higher taxonomic level is possible.

REPLY: We agreed reviewer's point. We identified the genus level (*Chloromonas*), then described at the genus level in Line 186. Now you can read "Three morphological types of *Chloromonas* snow algae were dominant in the snowpack (snow algae types A–C) (Fig. 4a–d).". We have already described about classification of algae in Line 144-145, then added the words "species level". Now you can read "Algal species were not identified species level in this study, because morphological taxa are not always represented as phylogenetic species of snow algae".

- Why authors did not use the best predictor (e.g. by PCA approach), then focus on the analysis involving the most important variables.

REPLY: We have conducted PCA and CCA approaches but the results were unclear, and we believe our current description is clearer.

- Im not sure that tardigrades are migrating between layers. It seems they are splitting from one big group into upper and lower groups. It could be accidental?

REPLY: The reviewer is correct in pointing out that Figure 5 shows a separation between the upper and lower groups. However, looking at the vertical distribution of tardigrades at 8:00 in Supplementary Figure S2, only a few individuals are concentrated on the snow surface. This suggests that tardigrades also moved vertically. The high proportion of observations on the surface at 11:00 and 14:00 is based on the fact that a few individuals were found only on the snow surface, which is reflected in the percentages.

- Finally, I miss basic data on the general dispersal features of algae and micorinvertebrates. Do we know what is the avarege distance the target organisms can disperse withing specific time? At least any basic data could help in the convincing reviewers to observed dispersal.

REPLY: For examples, the average swimming speed of *Chloromonas* algae (*Chloromonas reinhardtii*) was 106 μm s^{-1} (Liu et al., 2020) and the average walking speed of *Hypsibius* tardigrade (*Hypsibius exemplaris*) was 163 μm s^{-1} (Nirody et al., 2021). We added this information in discussion "These microbes were presumed to move tens of centimeters in the snow within a few hours. For example, *Chloromonas reinhardtii*, a species similar to snow algae, has been shown to move at an average speed of 106 μm s^{-1} (Liu et al., 2020), and *Hypsibius exemplaris*, a genus related to tardigrades found in the snow, has been shown to move at an average speed of 163 μm s^{-1} (Nirody et al., 2021)." between Line 287 and 288.

Abstract:

I suggest to higlight that percolation do not impact the results.

REPLY: We added a sentence "without washed out by percolated meltwater" in Line 13. Now you can read "Other microbes, including algal spores and fungi, remained on the surface layer throughout the day without washed out by percolated meltwater.".

Introduction:

Line 26: i.a. tardigrades and rotifers

REPLY: We corrected as reviewer suggested in the revised manuscript. Now you can read "These animals include i.a. tardigrades, rotifers (Hanzelová et al., 2018; Yakimovich et al., 2020; Ono et al., 2021, 2022), springtails (Hao et al., 2020), and winter stoneflies (Negoro, 2009).".

Line 27: add reference

REPLY: We added a reference (Ono et al., 2021), then now you can read “They feed on algae and redistribute organic matter as they migrate through the snowpack (Ono et al., 2021).”.

Line 30: tropically? typo

REPLY: We meant “trophically”. We corrected it, then now you can read “Because these organisms are trophically associated with each other (Brown et al., 2015; Ono et al., 2021), snowpacks can be acknowledged as unique ecosystems (Domine, 2019).”.

Line 37: use one unit (C or K)

REPLY: We corrected as reviewer suggested by using ℃. Now you can read “The climate model projected that the timing of snowmelt in the mountainous areas of central Japan would begin half a month earlier than the present climate when the global air temperature is 2℃ warmer than that in the pre-industrial period and that the snowpack would disappear two months earlier when it is 4℃ warmer (Kawase et al., 2020).”.

Lines 45: can author say mating in term considered to flagellate algae including various species s of algae?

REPLY: There is nothing specific that can be described about mating in this study. However, the term "algal growth" used in the discussion (in Line 341 and 404) including their mating. We added the words “, including their mating” in Line 341. Now you can read “This result implies that snow algae migrate upward to the surface layer at night, which is the preferred layer in terms of nutrients for algal growth, including their mating.”.

Line 64: reference is missing

REPLY: We added references (Engstrom et al., 2020; Ono et al., 2021), then you can read “Tardigrades and rotifers have often been observed feeding on snow algal cells in snowpacks (Ono et al., 2021) and may affect the distribution of snow algae, although there have been few reports on the association between snow algae and consumers (Engstrom et al., 2020; Ono et al., 2021).”.

Results:

Line 193: not genera. Phyla.

REPLY: We corrected as reviewer suggested. Now you can read “Two phyla (Tardigrada and Rotifera) of microinvertebrates and snow fungi were also

frequently observed in the snowpack (Fig. 4e–g).”.

Line 195: specimens instead of species

REPLY: We corrected as reviewer suggested. Now you can read “Identifying the rotifer specimens was difficult because of the absence of live species; however, Philodinidae dominated (Fig. 4f).”.

Figure 4: add names of higher taxonomic levels before names of species, for example Tardigrade Hypsibius spp.

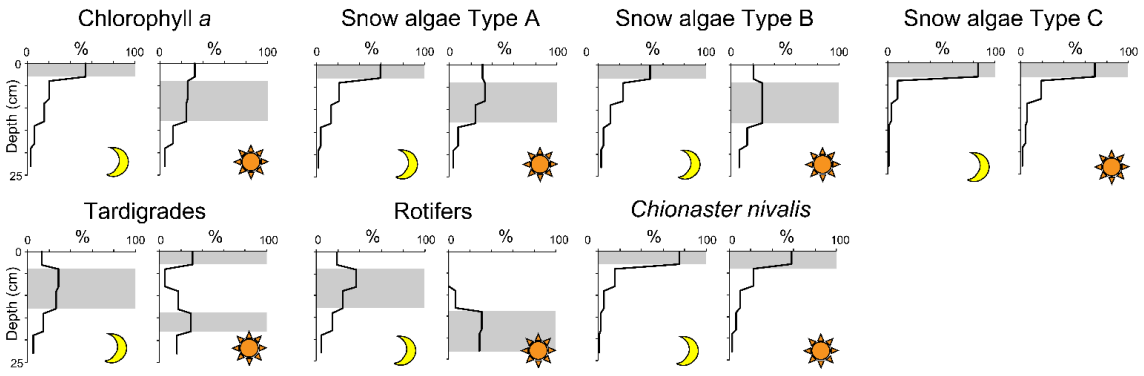
REPLY: We added higher taxonomic levels for tardigrade, rotifer, and fungi. Now you can read “Figure 4: Snow-ice microbes inhabit the snow. (a), (b) Snow algae Type A (LM), arrowheads indicate flagella, (c) Snow algae Type B (LM), (d) Snow algae Type C (LM, dormant state), (e) Tardigrade *Hypsibius* spp. (LM), (e1) Skin of *Hypsibius nivalis* (PCM), (e2) Skin of *Hypsibius* sp. (PCM), (f) Rotifer Philodinidae gen. sp. (LM), (g) Fungi Chionaster nivalis (PCM). All scale bars are in micrometers.”.

Line 219: 26:00??

REPLY: In the methods, a sentence “In this study, the period from 0:00 on May 6th to 0:00 on May 8th was represented as 0:00 to 48:00.” was described in Line 113-114 in original manuscript. If this description is misleading, we can correct in the revised manuscript.

Figure 5: For tardigrades, rotifers and fungi the names of axis are missing.

REPLY: We added “%”at the axis as reviewer suggested. The revised Figure 5 was shown below.



Revised Figure 5