https://doi.org/10.5194/egusphere-2025-2617
Preprint. Discussion started: 23 June 2025

Temporal and vertical changes in biological communities within snowpacks during melting season in Northern Japan

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Abstract. During the snowmelt season, diverse cold-tolerant microbes thrive within snowpacks. Snow conditions in forested areas change temporally with air temperature and budburst of trees. However, their effects on relevant biological communities are not well documented. Based on periodic sampling throughout the snowmelt season (March–May, 2021), this study describes the temporal and vertical changes in biological communities, including snow algae, microinvertebrates, and snow fungi, within snowpacks in Northern Japan. The melting season was divided into three periods: when the daily minimum air temperature was below the freezing point (Period A), when it was above the freezing point and before the budburst of beech trees (Period B), and after the budburst over the snow surface (Period C). During Period A, two types of algae and one of fungus were ubiquitously observed in the snowpack. During Period B, the abundance of microbes increased in the surface layer and green algal blooms visibly emerged. Later in this period, nutrients (NO_3^-, NH_4^+, and PO_4^{3-}) depleted, likely inhibiting algal growth and consequently restricting the microinvertebrate population. Surface layer nutrient concentrations increased again during Period C, thereby increasing the abundance of algae and microinvertebrates. This increase in nutrients was likely due to the rainwater and tree-derived litter deposited on the snowpack. 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 for the snowpack layers. This study highlights potentially important patterns in the dynamic interactions between microbial communities and environmental changes within snowpacks, revealing how tree phenology and snowmelt conditions jointly shape the vertical distribution and seasonal succession of snow-ice microbes.

1 Introduction

Despite the threat of its disappearance (Brown et al., 2017), seasonal snow provides a habitat for various cold-tolerant microbes. The microbes, referred to as snow-ice microbes, include snow algae (Hoham and Duval, 2001; Hoham and Remias, 2020), microinvertebrates (e.g., tardigrades and rotifers; Hanzelová et al., 2018; Ono et al., 2021, 2022; Yakimovich et al., 2020), invertebrates (e.g., springtails and winter stoneflies; Negoro, 2009; Hao et al., 2020), fungi (Irwin et al., 2021; Matsuzaki

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et al., 2021; Nakanishi et al., 2023), and bacteria (Segawa et al., 2005; Amato et al., 2007). During the snowmelt season, snow algae often bloom on 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). Active interspecies interactions such as microinvertebrate predation and fungal parasitism have been observed in colored snow (Ono et al., 2021; Nakanishi et al., 2023). Thus, seasonal snowpacks can be regarded as unique ecosystems (Domine, 2019).

In snowpack ecosystems, materials such as carbon, nitrogen, and phosphate are circulated through the metabolism of various microbes during the melting season. These nutrients are deposited by snowfall and rain and then transformed through freeze–thaw cycles, microbial processes, and hydrological movements within the snowpack (Jones, 1999). Photosynthetic microbes, such as snow algae and cyanobacteria, produce organic matter from inorganic carbon, nitrogen, and phosphorus sources. Especially in relation to nitrogen, studies from the Arctic have shown that the concentration of NO_3^- in snowpacks increased through nitrification, and subsequent denitrification or nitrification released NO into the atmosphere (Amoroso et al., 2010). In spring, when snow begins to melt, ammonification and assimilation of NH_4^+ dominate the snowpack (Larose et al., 2013). Through predation and parasitic relationships reported previously (Ono et al., 2021; Nakanishi et al., 2023), the nitrogen and carbon contained in algal cells are transferred to other organisms. In forested areas, leachate from organic matter deposited by trees during the late snowmelt season contains nutrients for algal growth (Jones, 1991, 1999). However, many uncertainties remain regarding when and where these biological activities begin within snowpacks and for how long they persist, and the factors influencing their distribution remain poorly understood.

Previous studies have only partially investigated the distribution of microbes in snowpacks and the factors that determine their distribution. For instance, snow algae bloom on nutrient-rich snowpack surfaces (Jones, 1991). They are not flushed down to the lower layers by percolating meltwater, but remain at the surface layer of the snowpacks even if surface melting occurs during the daytime (Grinde, 1983). Heterotrophic organisms such as microinvertebrates are also concentrated on the snow surface because they prefer to eat snow algae, which are abundant in the surface layer (Ono et al., 2021). The heterogeneous distribution of meltwater within snowpacks may influence the vertical distribution of microbes. For example, snow algae germinate at the soil surface or firns below snowpacks through the supply of meltwater and migrate upward to the snow surface (Jones, 1991; Hoham and Duval, 2001; Matsumoto et al., 2024; Rea and Dial, 2024). Snow algae are concentrated above the subsurface ice layers formed by refrozen meltwater within snowpacks (Hoham, 1975b). The vertical distribution of snow algae and microinvertebrates within snowpacks may vary depending on the intensity of solar radiation (Ono and Takeuchi, 2025).

The environmental conditions that influence snow-ice microbes change dynamically throughout the snowmelt season, thereby affecting their distribution and activity. During the transition from winter to spring, rising temperatures increase the water content of snowpacks, thereby promoting microbial mobility (Felip et al., 1995). Snow particles gradually deform into granular structures, and meltwater percolates through the snow layers, allowing microbes to migrate within the snowpacks (Hoham and Duval, 2001; Cruaud et al., 2020). Variations in snow depth and density further influence microbial habitats and determine their exposure to light and nutrient availability (Curl et al., 1972; Ono and Takeuchi, 2025). In forested areas, tree

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budburst can lead to a decrease in solar radiation. However, there is a lack of high-frequency, continuous observations linking these environmental changes to microbial dynamics within snowpacks.

Diverse snow-ice microbes have been observed in snowpacks in the mountainous areas of Japan, where heavy snowfall occurs every winter due to Asian monsoons (Fukushima, 1963). Continuous sampling of surface snow during the snowmelt season has shown that the abundance of snow algae increases exponentially until snowpack disappearance (Onuma et al., 2016). Another study showed that algal abundance increases after the budburst of trees, with an increase in nutrient concentrations on the snowpack surface (Suzuki and Takeuchi, 2023). A recent study revealed that snow ice microbes actively inhabit the subsurface layers within snowpacks, referred to as the microbially active snow surface layer (MASS layer, 30 cm in depth, Ono and Takeuchi, 2025). In this study, temporal and vertical changes in microbial communities and chemical conditions of snowpacks in forested areas of Japan throughout the melting season were described to understand the environmental factors driving microbial activities within snowpacks.

2 Study site and Methods

2.1 Study site and sample collection

Sampling was conducted at Yumiharidaira Park (38° 30′ N, 140° 00′ E; 770 m above sea level (a.s.l.)) on Mt. Gassan, Yamagata Prefecture, Japan (Figure 1). This site is suitable for investigating the relationship between tree phenology and snow-ice microbial communities as it hosts a diverse range of microbes (Tanabe et al., 2011; Ono et al., 2021, 2022; Suzuki and Takeuchi, 2023; Ono and Takeuchi, 2025) and retains snow after mid-May, when beech trees begin to sprout leaves (Suzuki and Takeuchi, 2023). Information regarding Mt. Gassan was provided by Ono et al. (2021, 2022). Because of the monsoon, heavy snowfall occurs during winter and snow persists until early summer (Kariya, 2002, 2005). The vegetation on Mt. Gassan changes from forest to alpine plants as the elevation increases. Broad-leaved and coniferous trees dominate below the forest line (1400–1500 m a.s.l.), followed by dwarf forests above 1500 m a.s.l. (Kariya, 2005). Meteorological conditions, including air temperature and solar radiation, were monitored during the spring season of 2021. The air temperature was recorded from March 27 to May 19 using a temperature/humidity data logger (TR-72nw; T&D Corporation, Japan). This data logger was suspended 50 cm above the snow surface with the help of a probe. It recorded every hour from March 27 to April 24, and every 10 min after April 25. 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). This 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. The hourly means of air temperature and solar radiation were obtained from the records. To describe temporal changes in the microbial community structure and chemical solutes within the snowpack, snow samples were collected during the snowmelt season of 2021. Snow samples were collected 11 times (on March 7 and 27; April 10, 24, and 28; and May 4, 9, 12, 16, 18, and 20). Sampling was conducted at approximately 5:00 a.m. (Japan standard time) to eliminate the possibility of the sunlight-led disappearance of microbes from the snow surface

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(Hoham, 1975b; Kawecka, 1986; Ono and Takeuchi, 2025). 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). Snow depth, measured as the actual physical depth of the snowpack, was 320 cm on March 6 and 280 cm by March 27 (Figure 3a). Snowfall occurred around April 9, resulting in fresh snow cover of approximately 8 cm in depth (the snow depth was 223 cm at this time). Between April 24 and May 4, the snow depth gradually decreased from 152 to 132 cm. The snow depth was 6 cm on May 20. Rainfall occurred on April 25, 29, May 2, 10, 17, and 19. Snow samples were collected from five layers across snow depths (Figure 2): an area of 10 × 10 cm^2 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). These samples were collected at three different locations at each sampling time, except on March 7, when they were collected at only one of the three locations. After the appearance of green algal blooms, samples were collected from one blooming snow surface (mainly green snow) and from two snow surfaces adjacent to the bloom. In total, 465 snow samples were collected using a small spatula and preserved in Whirl-Pak bags (B01065WA; Nasco, USA). To avoid potential interference with microbial distribution due to sampling, each sample was collected at a distance of at least 3 m from the others.

Figure 1: Temporal changes at the study site during the study period.

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 an ice auger. Snow core collections were conducted at 5:00 a.m. on April 25 (core length: 180 cm), May 4 (133 cm), 7 (113 cm), 10 (94 cm), 13 (80 cm), 16 (56 cm), and 18 (30 cm). The results of the snow core analyses collected on May 7th have been published (Ono and Takeuchi, 2025). The cores were cut horizontally every 10 cm using a snow saw and preserved in Whirl-Pak bags.

To evaluate the nutrient supply from rainwater in the snowpack, rainwater was collected twice at the study site. Samples were collected from both open and tree-covered areas on May 3 when beech leaves were partially open, and on May 17 when

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beech leaves were fully open. Samples were collected directly into a 50-mL polypropylene bottle attached to a stainless-steel rod.

All the samples were frozen and transported to a laboratory at Chiba University, Japan. The samples were stored in a freezer (-20°C) until further processing. The samples were slowly melted in a refrigerator (5°C) prior to analysis. After melting, the snow samples were separated as follows: 5 mL for chlorophyll *a*, 5 mL for chemical analysis after passing through a sieve to remove plant litter, 10 mL for the dry weight of total insoluble particles, and the remainder for microinvertebrates. Snow core samples, including snow algae and microinvertebrates, were used for microbial analysis. Samples for chemical analysis were separated into 6-mL plastic tubes after the meltwater was passed through a 0.45-µm ion-free disposable filter (13AI Chromatodisk; GL-science, Japan) to remove microbes and particulate dust.

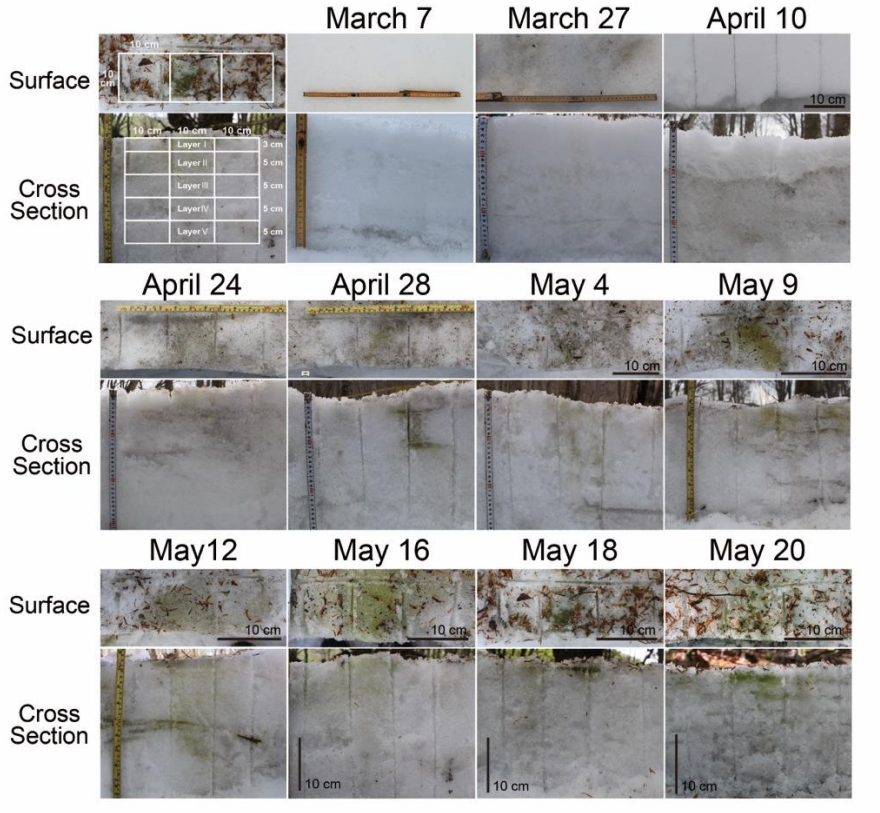

Figure 2: Temporal changes in snowpack conditions during the study period.

2.2 Community structure analysis of microbes

To identify the temporal changes in the abundance of snow algae and fungi, measurements of chlorophyll *a* concentration and microscopic observations were conducted. Chlorophyll *a* is a common indicator of algal biomass (Holm-Hansen et al., 1965), and another method was described by Ono et al. (2021). To calculate the cell concentration of snow algae and fungi, 5–200 µL of each subsample was used. The samples were filtered through a filter holder equipped with a 0.45-µm PTFE membrane

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filter (JHWP01300; Merck Millipore, Germany) and a pump (Linicon LV-125; Nitto Kohki, Japan). The filter was then covered with a cover glass and MilliQ water on a glass slide. Cell counts were performed 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}). For snow core samples, the vertical distributions are described as the proportions of abundance in each layer relative to all layers of the snow core. 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, although this may be possible in the form of vegetative cells (Matsuzaki et al., 2015, 2020).

To analyze the abundance of microinvertebrates, the melted snow sample remaining after sample processing was transferred to a Petri dish. Tardigrades and rotifers were counted using a stereomicroscope (MZ125; Leica Microsystems, Germany). The population density of the microinvertebrates (individuals L^{-1}) was calculated using the number of observed microinvertebrates and the water equivalent of the snow sample. Some individuals were mounted onto glass slides in a drop of Hoyer's medium (Degma, 2018) and observed under a phase-contrast microscope (BX51; Olympus, Japan) to identify the species, based on the description by Ono et al. (2021, 2022). The body size distribution of tardigrades in white snow on April 24 and alga-blooming snow was measured using the pictures taken by a digital camera (DP21; Olympus, Japan) and Image J 1.52 software. Body length was measured without leg IV length, and body width was measured between legs II and III (Ono et al., 2021).

2.3 Elution of tree-derived litter

Elution experiments were conducted to investigate the leaching of chemical solutes from plant litter into meltwater in the snowpack. Dried plant litter (0.05 g) from green algal-blooming snow samples from May 20 was placed in plastic Petri dishes. MilliQ water (50 mL) was added to each dish, and 5 mL was collected after 10 min, 24 h, 72 h, 120 h, 168 h, 240 h, and 336 h. The water samples were filtered using a 0.45-μm ion-free disposable filter with a 5-mL syringe and then preserved in 6-mL plastic tubes.

2.4 Analysis of chemical solutes and insoluble particles

To elucidate the relationship between snow-ice microbes and environmental conditions, the concentrations of chemical solutes and the abundance of insoluble particulates in the samples were analyzed. The concentrations (μEq kg^{-1}) of major chemical solutes (cation: Na^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}; anion: Cl^-, SO_4^{2-}, NO_2^-, NO_3^-, PO_4^{3-}) were quantified using an ion chromatography system (anion: AQUION, Thermo Fisher Scientific, USA; cation: ICS-1100, Thermo Fisher Scientific, USA). The NO_2^- levels were below the detection limit in all samples in this study. The dry weights of insoluble particulates in the samples were quantified by dividing them into three components (plant litter, organic matter, and inorganic matter). The plant litter in the samples was collected using a sieve and placed in a plastic Petri dish. They were then dried at 60°C for 1–2 days and weighed. Ten milliliters of the remaining sample were transferred to ceramic crucibles. The crucibles were then dried at 60°C for 1–2 days and subsequently subjected to combustion at 500°C for 3 h in an electric furnace (SSTR-13R; ISUZU, Japan); only

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inorganic matter remained in the crucibles. The mass concentrations (g L^{-1}) of organic and inorganic matter in the samples were calculated from their dry weights.

2.5 Statistical analysis

Welch's t-test was used to examine the differences in cell concentration, population density, chlorophyll *a* concentration, chemical solutes, and dry weights of insoluble particles between green and white snow. The test was applied to both the entire snow pit and surface layer (I). Welch's t-tests were used to compare the concentrations of chemical solutes in rainwater between tree-covered and -free areas before and after budburst. Differences in the body lengths of tardigrades between April 24 and May 20 were also assessed using the same test. Pearson's correlation coefficients were obtained for each microbe and environmental condition. Canonical Correspondence Analyses (CCA) were used to reveal the relationships between microbes and the chemical conditions within the snowpack. All statistical analyses were performed using the R version 4.4.2 (R Core Team, 2024).

3 Results

3.1 Meteorological conditions at the study site during snowmelt season

The air temperature was above 0°C during daytime since the beginning of the study period (Figure 3b). The minimum temperature was recorded in the morning (2:00–5:00), which was occasionally below the freezing point. After April 27, the minimum temperatures were never below the freezing point and gradually increased until the end of May. The daily mean solar radiation on the snow surface in a tree-free area ranged from 100.2 to 305.5 W m^{-2}, exhibiting small trends of increase or decrease during the measurement period (Figure 3c). In contrast, that in a tree-covered area varied from 81.0 to 185.3 W m^{-2} (55–64% of that of tree-free area) until May 11 and subsequently decreased to 22.5 W m^{-2} (22% of that of tree-free area) on May 16 (Figure 3d). This subsequent decrease matched the timing of beech tree budburst above the snow surface.

The study period was divided into three phases based on the forest conditions: Period A (March 7–April 27), in which the daily minimum temperature was below the freezing point; Period B (April 28–May 14), in which the daily minimum temperature was above the freezing point and the beech trees in the forest had not yet budded; and Period C (May 15–May 20), in which the daily minimum temperature was above the freezing point and the trees had sprouted.

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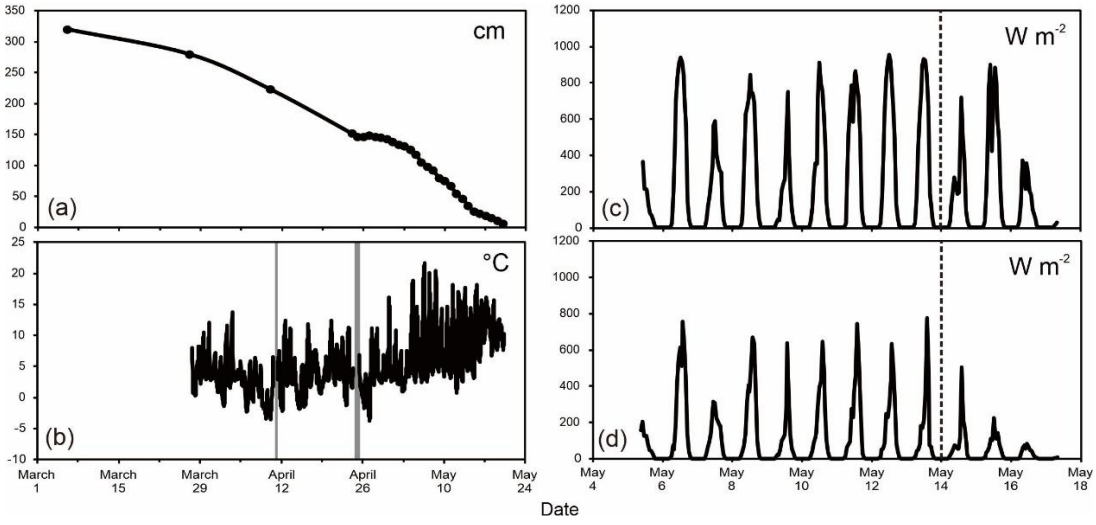

Figure 3: Meteorological conditions recorded at the study site. (a) Snow depth and (b) air temperature in the tree-covered area during the study period (March 1 to May 24). Gray-shaded periods represent intervals of missing data caused by instrument maintenance. Hourly mean intensity of solar radiation on the snow surface in the (c) tree-free and (d) tree-covered areas from May 4 to May 16. The timing of beech tree budburst is indicated by the dashed lines in (c) and (d).

3.2 Snow-ice microbes in the snowpack

Microscopic observations of snow samples revealed various microbes, as reported by 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*).

3.3 Temporal changes in and vertical patterns of abundance of snow-ice microbes

The chlorophyll *a* concentration in the upper layers above 13 cm generally increased throughout the study period (Figure 4). Chlorophyll *a* was detected in the subsurface layers (III and V) for the first time during sampling (March 10, Period A). Subsequently, it increased mainly in Layer I during Period A. In the case of algal-blooming and adjacent snow, chlorophyll *a* increased in Layers I and III at the beginning of Period B. During Period C, the chlorophyll *a* content increased continuously in Layers I and II.

Temporal and vertical changes in snow algae abundance showed different trends for each morphotype. Type A showed a trend similar to that of chlorophyll *a*. Type B was first observed in Layers II and III in the middle of Period A (April 10). It then increased drastically in Layers I–III until the middle of Period B, but only in the algal-blooming snow. It decreased towards the end of Period B, but increased again in Period C. Types C and D were first observed at the end of Period A (April 24) in Layers I–II and I–III, respectively. They continuously increased in Layer I of the alga-blooming snow throughout Periods B and C. In the adjacent snow, Type B was distributed over the entire snowpack on April 28, whereas it was concentrated on the

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snow surface in the alga-blooming snow. Types A, C, and D in the adjacent snow showed similar temporal and vertical distribution patterns to those observed in the alga-blooming snow.

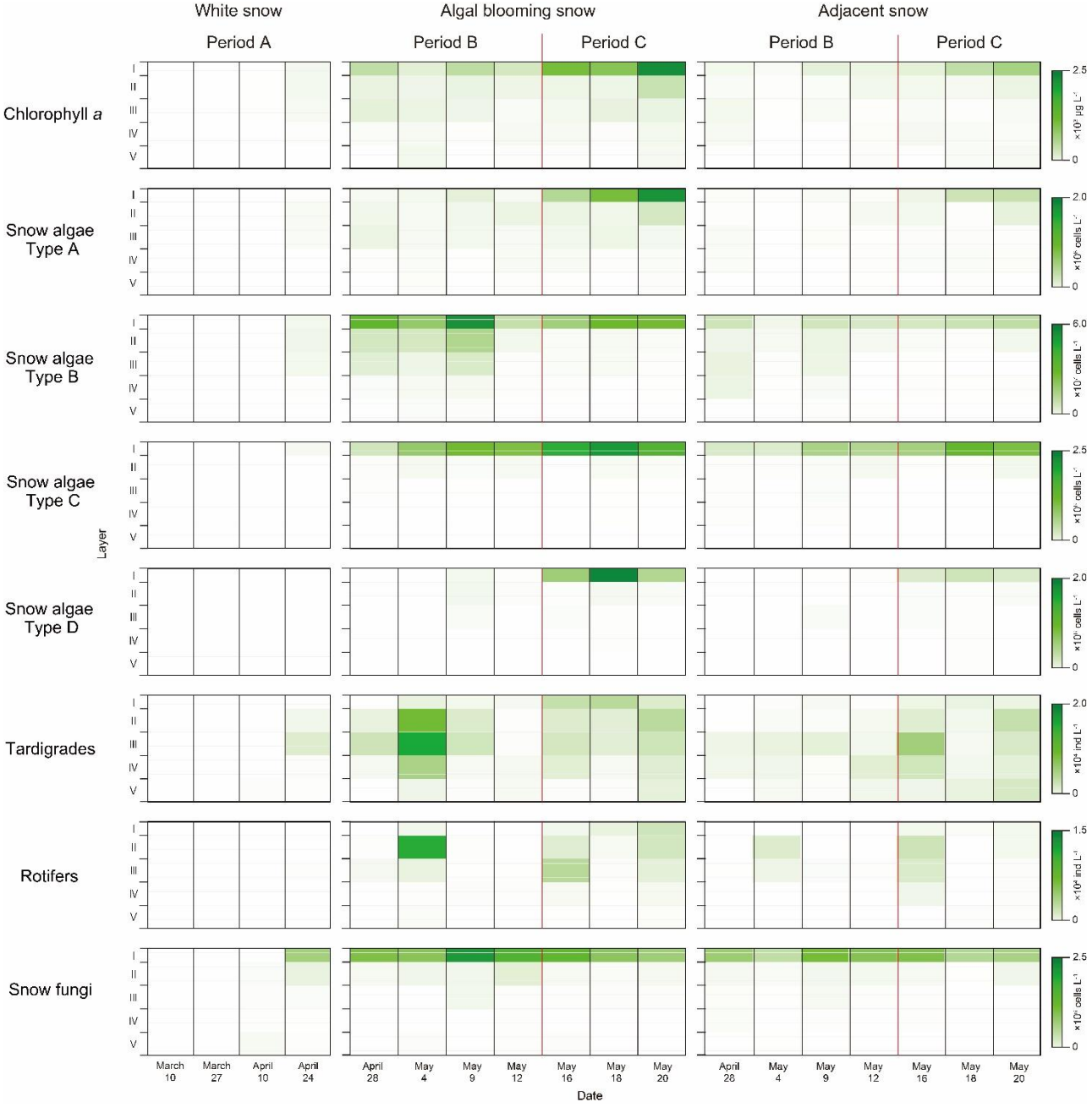

Figure 4: Temporal and vertical changes in microbial abundance in the upper layers of the snowpack. Temporal and vertical changes in algal-blooming and adjacent snow surfaces were observed after the emergence of algal blooming. The red line indicates the time at which the forest trees sprouted.

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The two microinvertebrates showed an appearance pattern distinct from that of the snow algae within the snowpack. They mainly appeared in the subsurface layers (II and III), and their populations varied significantly throughout the study period. Tardigrades and rotifers first appeared in the middle of Period A in Layer V (March 27) and layers III–V (April 24), respectively. Subsequently, their abundance increased in all layers during Period B (May 4) but decreased by the end of it. They increased again in all the layers during Period C. In the adjacent snow, the vertical distributions of tardigrades and rotifers differed from those of the algal-blooming snow, with no increase in Layers I–IV observed in the alga-blooming snow on May 4, whereas the vertical distribution was similar for the remainder of the period.

Snow fungi also showed a distinct pattern of appearance from those of algae or microinvertebrates; they appeared mostly in the surface layers and increased during Period B but decreased in Period C. Few individuals of *Chi. nivalis* appeared in all layers (I–V) in the middle of Period A (March 27). In both the algal-blooming and adjacent snow, their abundance in the surface layers (I and II) increased until the middle of Period B (May 9), and then decreased in Period C.

The abundance of microbes was two to five times higher in the algal-blooming snow than in the adjacent snow. The contrast was more significant in the surface layer (I), and their abundance was 2–6 times higher than that in the adjacent snow. However, this was not observed for Type D algae or tardigrades (Table S1).

3.4 Temporal changes in snow-ice microbes

Analysis of the snow core samples revealed that the snow-ice microbes (snow algae, tardigrades, and rotifers) were concentrated in the layers from the surface to the depth of 30 cm during the study period (Figure S1). Chlorophyll *a* concentrations were significantly higher in the surface layers above the depth of 30 cm than in the lower layers from late April to early May (73–98% of the total content in the snow core). After mid-May, they were significantly higher in the layers above the depth of 10 cm (68–82%). Tardigrades were mostly concentrated in the surface layers above the depth of 20 cm during the study period (71–95%). Rotifers were also concentrated in the surface layers above the depth of 20 cm during these periods (74–100%); however, they were exceptionally found at the 120–130-cm depth on April 25 and 60–70-cm depth on May 13.

3.5 Temporal changes in body size distribution and species composition of tardigrades within the algal-blooming snowpack

The mean body length of the tardigrades increased over time and their size distributions differed between the snow surface and subsurface layers (Figure 5). Tardigrade body lengths in the subsurface layers ranged from 210 to 423 µm on April 24, and significantly increased to 242–444 µm by the final sampling on May 20 ($t = 6.28$, $p < 0.01$). A similar trend was observed on the snow surface; however, only smaller individuals (<200 µm) were observed on the snow surface.

The species composition of tardigrades differed between the snow surface and subsurface layers. *Hypsibius* sp. dominated both snow surface and subsurface layers throughout the season. *H. nivalis* consistently accounted for a higher proportion on the snow surface than that found in subsurface layers. Between Period B and the early stages of Period C, *H. nivalis* comprised 12.3–23.3% of the tardigrade population on the snow surface and 0–7.5% in the subsurface layers.

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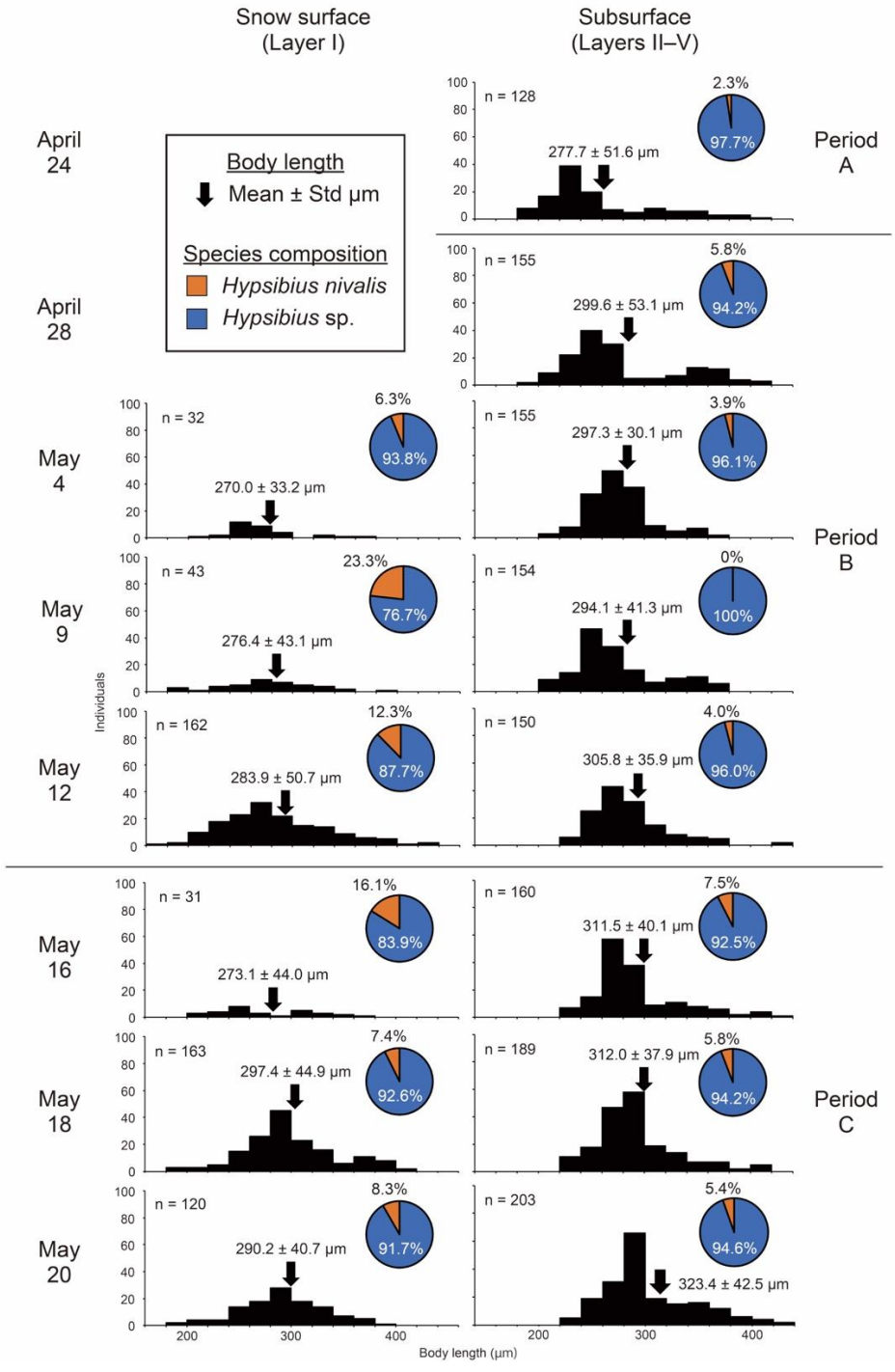

Figure 5: Temporal changes in body size distribution and species composition of tardigrades living in snowpacks. Histogram of body length expressed in 20-µm increments. The species composition of tardigrades at each sampling time is displayed next to the histogram of body length.

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3.6 Temporal and vertical changes in chemical solutes within the snowpack

Most chemical solutes in the snowpack were concentrated in the surface layer; however, each solute exhibited distinct temporal changes during the study period (Figure 6). The concentrations of Na^+, Cl^-, SO_4^{2-}, and NO_3^- were higher in Layers I and II than in the lower layers during Period A, particularly on April 10, when snowfall occurred. Thereafter, no further increase in concentration was observed. The concentrations of NH_4^+, PO_4^{3-}, and K^+ remained low during Periods A and B and increased in surface layers I and II during Period C. This increase was particularly remarkable for algal-blooming snow surfaces. The concentration of K^+ in the surface layer increased exponentially throughout Periods B to C in the algal-blooming snow. The concentrations of Mg^{2+} and Ca^{2+} in Period A were higher than those in the other periods, particularly in Layers I and II on April 10. Although no significant variation was observed during Period B, their concentrations increased again in Period C in both algal-blooming and adjacent snow, particularly in Layer I.

3.7 Temporal changes in the amounts of insoluble materials on the snow surface

Measurements of the dry weights of insoluble materials on snow surfaces revealed that they changed temporally; however, their patterns differed among the types of material (Figure 7). The total insoluble material on the snow surface was below the detection limit throughout Period A. Plant litter increased sharply from the middle of Period B (May 9) to the end of Period C (May 20). The amount of organic matter, excluding plant litter, increased from Period B (May 4) until the end of Period C. The amount of inorganic matter did not show an increasing trend, but fluctuated throughout Periods B and C. These trends were similar for both the algal-blooming and adjacent snow. The amounts of insoluble material collected on April 28 and May 20 showed that plant litter, organic matter, and inorganic matter were approximately twice as much in algal-blooming snow than in the adjacent snow (Table S1).

3.8 Chemical solutes in rainwater

Analysis of chemical solutes in rainwater collected from open and tree-covered areas revealed that nutrient concentrations increased after the budburst of beech trees in both areas (Figures 8 and S2). The NH_4^+ and K^+ concentrations in rainwater collected from the open area increased by 55% and 65%, respectively, after the budburst. PO_4^{3-}, K^+, and Mg^{2+} concentrations in rainwater collected from tree-covered areas increased by 230%, 326%, and 15%, respectively. The concentrations of all chemical solutes in rainwater measured in this study were significantly higher in tree-covered areas than in open areas, with the exception of NO_3^- and Ca^{2+} (Table S2).

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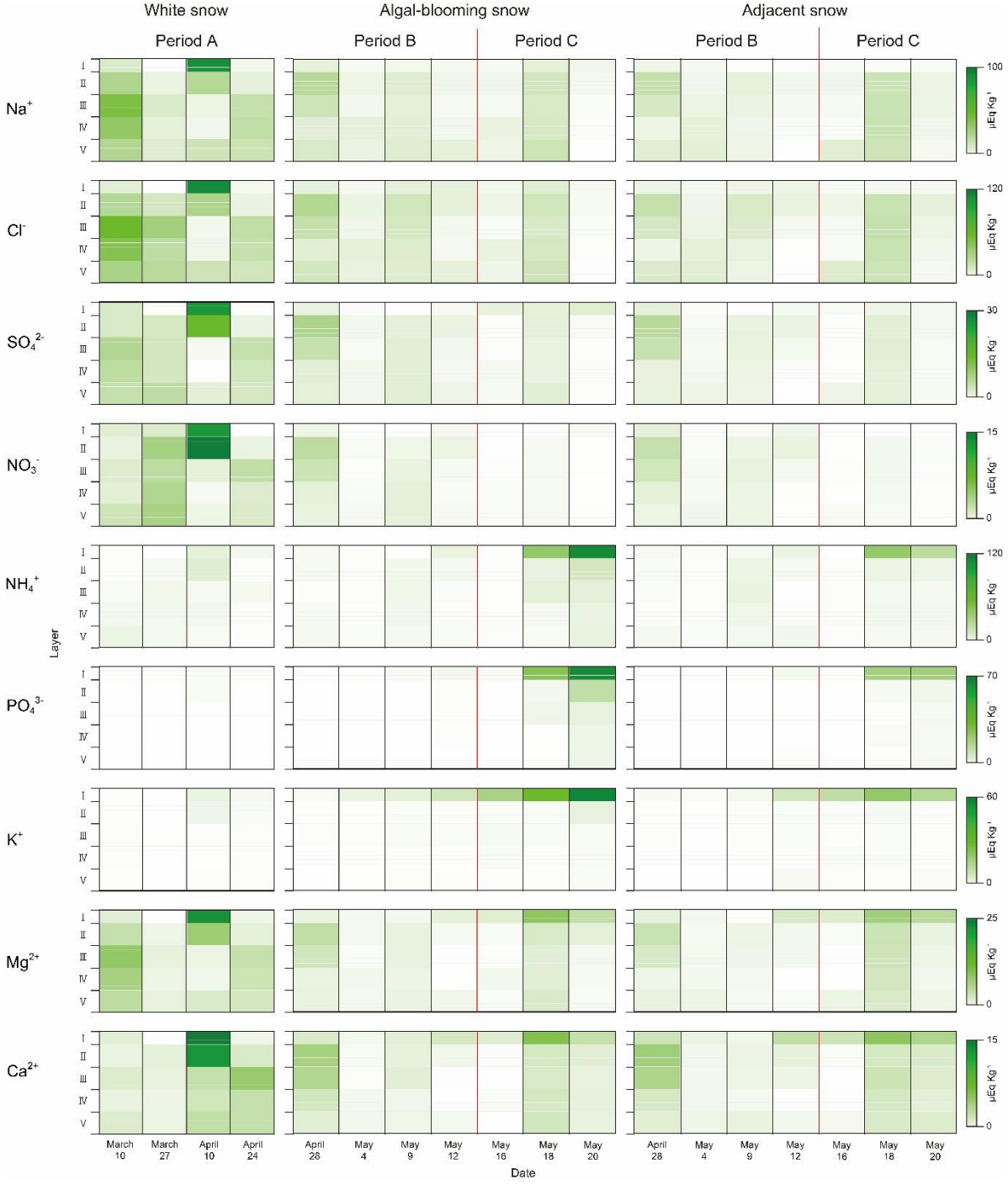

Figure 6: Temporal and vertical changes in the concentration of chemical solutes in the upper layers of snowpack. Temporal and vertical changes in and adjacent snow surfaces were observed after algal bloom. The red line indicates the day when the forest trees sprouted.

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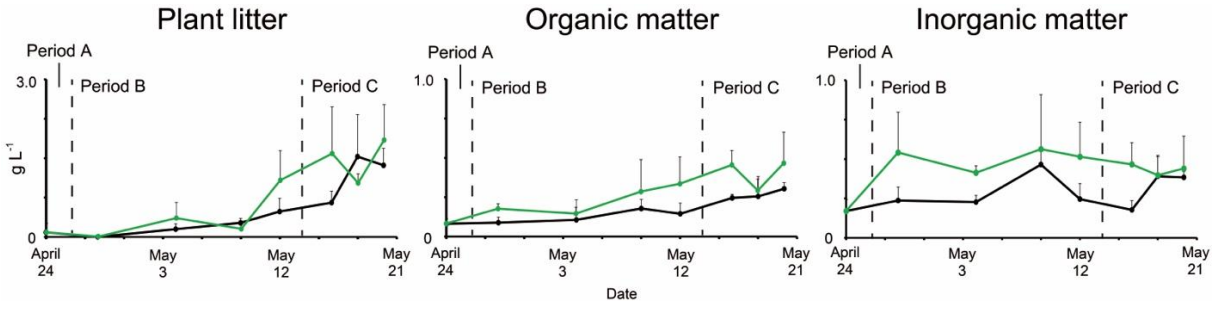

Figure 7: Temporal changes in the dry weight of insoluble particles. The dry weights of insoluble particles on algal-blooming and adjacent snow surfaces are shown as green and black lines, respectively. The dashed lines represent the boundaries of Periods A, B, and C.

3.9 Elution experiment of solutes from tree-derived litter

The elution experiment revealed that NH_4^+, PO_4^{3-}, and K^+ were eluted from the tree-derived litter on the snow surface into the water. The concentrations of Na^+, Cl^-, SO_4^{2-}, and NO_3^- in the water did not change throughout the experiment. In contrast, the concentrations of NH_4^+, PO_4^{3-}, and K^+ increased at the beginning of immersion and remained high at 336 h (Figure 8). Simultaneously, the concentrations of Mg^{2+} and Ca^{2+} gradually increased during 336-h immersion (Figure S3).

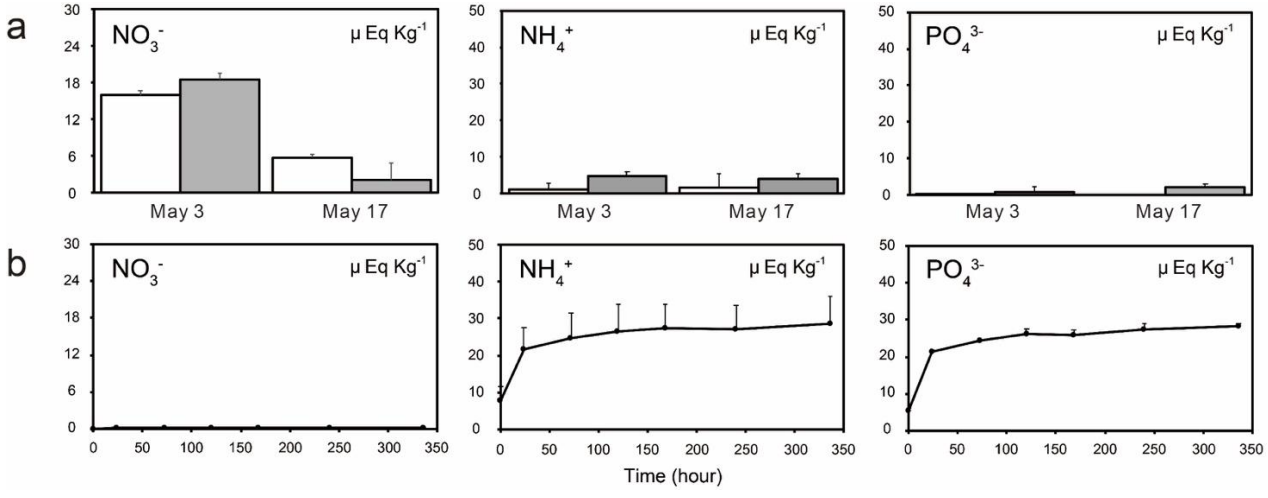

Figure 8: Concentration of nutrients in (a) two rainwater samples collected during Periods B (May 3) and C (May 17) and (b) results of elution experiments. The nutrient concentrations in the rainwater samples collected from the open and tree-covered areas are shown using white and gray bars, respectively.

4 Discussion

Temporal and vertical changes in the microbial community and environmental conditions indicated that microbial activity throughout the snowmelt season was distinct among the three periods (Periods A–C): the initial appearance of microbes (Period

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A), formation of algal blooms (Period B), and further blooming aided by the additional supply of nutrients (Period C). The following sections discuss the factors influencing the microbial activity during each period.

Despite the insulating properties of snow, studies have shown that temperature fluctuations can affect snow temperatures down to depths of 20–30 cm, especially during frequent melt–freeze cycles.

4.1 Settlement of microbes into the snowpack (Period A)

The vertical distribution of microbes during Period A indicated that the biological activity within the snowpack did not enhance immediately after the onset of snowmelt. During Period A, the microbial abundance remained low. On April 24, at the end of Period A, an increase in total microbial abundance was observed in Layers I–III (Figure 4). In addition, the body size distribution of tardigrades showed no juveniles within the snowpack (Figure 5). These findings suggest that microbial growth and reproduction do not occur during the first month after snowmelt onset. One possible reason for the lack of biological activity during this period is the number of freeze–thaw cycles in the surface layer. Although snow has strong insulating properties, temperature variations at the surface can influence the thermal regime of the snowpack up to depths of 20–30 cm, which corresponds to the sampling depth in this study, as observed in other regions and under various snow depth conditions (Zhang, 2005; Chen et al., 2013; Hirashima et al., 2015). During this period, the daily minimum temperature was below the freezing point, indicating that the snowpack had frozen. The snow algae and microinvertebrates observed in this study may have had difficulty remaining active under freezing conditions. 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 in the form of cysts (Procházková et al., 2018; Procházková et al., 2019a). Similarly, tardigrades found in snowpacks do not exhibit specific tolerance to freeze–thaw cycles, unlike some other tardigrades in glaciers that are well adapted to freeze–thaw cycles (Zawierucha et al., 2023). Compared with previous research, our findings indicate that Period A corresponds to a phase in which microbes are active but their growth is limited by the freezing air temperatures.

4.2 Emergence of algal blooms in snow (Period B)

The temporal changes in snow algae and chemical solutes observed during Period B suggest that the nitrate (NO_3^-) present in the snowpack was utilized for the growth and blooming of snow algae. During Period B, Types A and B algae initially increased between April 24 and 28, coinciding with the appearance of green snow patches (Figure 4). Among these, Type B algae exhibited exponential growth. In addition, nitrate concentrations remained high during this period. These results suggest that algal growth occurred through the utilization of available nutrients during this period. Previous research has shown that snow algae are present in environments with high NO_3^- concentrations (Hanzelová et al., 2018). 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 could be of atmospheric origin.

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The increase in microbial abundance during Period B suggests that the activity of other microbes was induced by algal blooms. The drastic increase in the population density of microinvertebrates and fungi coincided with the emergence of algal blooms in the snow (Figure 4). For microinvertebrates, significant positive correlations were observed between the abundance of tardigrades and both Types A and B algae, as well as between rotifers and Type A algae (Figure S5). The body size distribution of the tardigrades revealed no juveniles during this period, indicating absence of reproduction (Figure 5). These results suggest that microinvertebrates migrate toward and assemble where food resources are available. This is consistent with the results of a previous study (Ono et al., 2021). The abundance of snow fungi *Chi. nivalis* showed a significant positive correlation with that of Type B algae (Figure S5), suggesting that germination of the fungi was likely promoted by the presence of snow algae. A significant correlation between the abundance of *Chi. nivalis* and algae have also been reported from other snowpacks (Hoham et al., 1993; Yakimovich et al., 2020). These studies have suggested that algal exudates are nutritionally significant for fungal germination.

Algal growth at the end of this period was likely limited by nutrient depletion and the life cycle stages of the microinvertebrates changed during the same period. In the late Period B, abundance of all the microbes did not increase (Figure 4), and nutrient concentrations during majority of this period were below 1.0 μEq kg^{-1} (Figure 6), suggesting that their abundances had achieved their environmental carrying capacity due to depletion of nutrients. A previous study showed that snow algal growth reaches its maximum at a nitrate concentration that is more than 56 times higher than the concentration observed during this period (Broadwell et al., 2023). Furthermore, a decrease in Type B algae and a consistent increase in Type C algae were observed from late April to late May. Notably, the increase in Type C algae during the snowmelt season coincided with a decrease in NO_3^- , showing a significant negative correlation (Figure 4, S5). These results suggest that Types B and C represent different life stages of the same algal species. Type C seems to be an elliptical cell before becoming dormant cells (Type B) of *Chloromonas hindakii*, *Chloromonas miwae*, and/or *Chloromonas nivalis* (Muramoto et al., 2008; Matsuzaki et al., 2019; Procházková et al., 2019a). Changes in algal life stages have been previously reported at the same study site (Muramoto et al., 2010), and nitrogen depletion has been suggested as a factor in the formation of dormant spores of snow algae (Hoham and Duval, 2001). These results also suggested that the microinvertebrates reproduced and hatched from the eggs at this time. The population density of microinvertebrates decreased in both algal-blooming and adjacent snows (Figure 4), whereas tardigrade juveniles were observed on the snow surface during this period (Figure 5). They likely laid eggs in the subsurface layers, and their egg-containing exuviae (previously reported in Ono et al., 2021) reached the snow surface as the snow melted, and eventual hatching occurred at the surface layer. The eggs of *Hypsibius dujardini*, the same genus inhabiting snowpacks, require 4–4.5 days to hatch (Gabriel et al., 2007). If this period also applies to tardigrades in snowpacks, it can be inferred that the observed increase in individuals results from the hatching of eggs laid in early May.

4.3 Enhancement of microbial growth by nutrients from forest canopy (Period C)

Changes in microbial abundance and snowpack conditions suggested that the budburst of beech trees triggered the regrowth of microbes in the snowpack during the late snowmelt season. The increase in Types A and B algae between May 16 and 20

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coincided with increases in NH_4^+, PO_4^{3-}, and K^+, and a significant positive correlation was noted between their abundance and the concentration of chemical solutes (Figure 4, S5). This suggests that algal growth in snow was promoted by the supply of nutrients to the snowpack. Previous studies have shown that NH_4^+ and PO_4^{3-} in snowpacks are limiting factors for algal growth (Hoham and Duval 2001; Jones 1991; Suzuki and Watanabe 2000; Hanzelová et al. 2018). A laboratory experiment also showed that high P availability promoted blooming of snow algae (*Chloromonas rosae* and *Chloromonas typhlos*; Almela et al., 2024).

Temporal changes in plant litter and nutrients and elution experiments on plant litter indicated that the increase in nutrient concentrations was due to the supply of litter and other insoluble particles deposited on the snow surface. Nutrients (NH_4^+ and PO_4^{3-}) increased on May 18 in both the algal-blooming and adjacent snow (Figure 6). Because high concentrations of NH_4^+ and PO_4^{3-} were detected in the elution experiment, nutrients were likely supplied by plant litter deposited on the snow surface and utilized by snow algae. A recent study reported an increase in nutrients associated with budburst on Mt. Gassan in Yamagata Prefecture (Suzuki and Takeuchi, 2023). The abundance of snow algae and nutrients in snowpacks are known to increase with tree budburst (Hoham, 1976; Hoham et al., 2008). These studies suggest that tree-derived litter deposited on the snow surface may be a source of nutrients that induces the growth of snow algae.

The timing of the decrease in solar radiation in 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. A decrease in solar radiation, the budburst of beech trees, and an increase in snow algae were observed on May 15 and 16 (Figures 1, 3, and 4). The relationship between solar radiation and algal blooms has been previously described. Excessive light intensity can reduce the photosynthetic activity of snow algae and cause cellular damage (Remias et al., 2005; Procházková et al., 2023). Reduced solar radiation may contribute to the uptake of nutrients at the snow surface and an increase in chlorophyll *a* (Suzuki and Takeuchi, 2023; Ono and Takeuchi, 2025). Based on these studies, it is possible that beech tree budburst contributes to enhanced algal growth on nutrient-rich snow surfaces, possibly by prolonging the period of favorable conditions for algal activity.

The vertical distribution of microinvertebrates during this period indicates increased microinvertebrate activity, similar to that of snow algae. Similar to snow algae, microinvertebrate populations increased again on May 16, following the beech tree budburst (Figure 4). However, unlike algae, they were concentrated in the subsurface layers. Their abundance and vertical distribution can be attributed to the availability of food resources and intensity of solar radiation. As observed during Period B, microinvertebrates were concentrated in the layers where algal cells were abundant. The high population density of tardigrades on the snow surface may have been caused by larval hatching. The intensity of solar radiation may still be above the limit that inhibits the activity of microinvertebrates within the snowpack, despite the budburst. Consequently, they are likely to be active within the snowpack rather than on the snow surface. Because snow algal abundance increased in the adjacent snow during this period, it is likely that algal growth in the adjacent snow facilitated a subsequent increase in microinvertebrate populations.

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4.4 Possible effects of changes in climate and forest phenology on microbial communities within snowpacks

The vertical distribution of microbes in the snow pits and cores indicates that microbes were active within the snowpack throughout the snowmelt season. Microbes in the snowpack were distributed from the surface to the depth of 30 cm during the snowmelt season (Figure S1), and their vertical distribution varied according to the biota, with snow algae concentrated near the surface (0–3 cm deep) and microinvertebrates widely distributed within the snowpack (3–23 cm deep). These results are consistent with those of a previous study suggesting the presence of a Microbial Active Snow Surface (MASS) layer on a diurnal scale (Ono and Takeuchi, 2025). The results of the present study demonstrated that the MASS layer was maintained throughout the melting season.

While the intensity of solar radiation and nutrient concentrations on the snow surface have been considered as key factors determining the depth of the MASS layer, this study suggests that multiple factors influence their distribution throughout the melting season. 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. Another important factor is the beech tree budburst, which influences nutrient supply and reduces solar radiation reaching the snow surface.

Future climate change is expected to alter the meteorological conditions, which could affect the biological activity within snowpacks. For example, global warming may lead to a decrease in maximum snow depth and earlier disappearance of snowpacks (Wakazuki et al., 2015; Kawase et al., 2020; Urban et al., 2023). Under such conditions, snowpacks may not persist until Period C. In addition, an increase in the mean temperature could cause an earlier onset of above-freezing conditions, thereby shortening the duration of Period A. However, this might not occur, as previous studies have suggested that the timing of snow disappearance would shift earlier at a faster rate than the onset of snowmelt (Urban et al., 2023). Nevertheless, earlier snowmelt could still increase the likelihood that snowpacks would disappear during Period A or B, potentially disrupting biological processes within the snowpack.

Additionally, phenological shifts due to rising temperatures, such as the earlier budburst of beech trees, may have triggered earlier snow algal blooms. If budburst occurs earlier (Vitasse et al., 2009; Čufar et al., 2012), the transition from Period B to C, which is characterized by increased biological activity driven by nutrient input, may also occur earlier, potentially advancing the timing of peak microbial growth. In addition to phenological changes, shifts in the distribution of tree taxa have also been predicted in Japan (Matsui et al., 2004). These shifts may alter the chemical composition supplied from the elucidate of plant litter, significantly affecting the microbial activity within snowpacks in forested areas. During Period C, enhanced algal growth coincided with the timing of beech tree budburst, likely driven by an increase in nutrient input from fresh litter or throughfall. Therefore, future shifts in tree species composition could substantially alter the nutrient regimes in snowpacks, reshaping microbial dynamics.

Because these conditions vary across regions, future studies should examine biological activity 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

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onset of Period C may occur earlier in response to an earlier budburst; however, its duration is expected to remain relatively unchanged. To better understand microbial dynamics in snowpacks, future research should focus on clarifying how variations in snow depth and phenology interact with microbial activity.

5 Conclusion

Temporal and vertical changes in the abundance of snow-ice microbes within the snowpack in the forested area of Mt. Gassan in Yamagata Prefecture were investigated, and the factors influencing these changes were discussed. This study demonstrates that microbial communities exhibit distinct seasonal dynamics in response to environmental conditions, with clear shifts in microbial activity between the defined periods of the melting season (Periods A–C). During Period A, microbial activity began, but remained limited because of sub-freezing air temperatures. Period B was characterized by an increase in microbial growth with increasing temperature; however, this growth was constrained by the depletion of available nutrients. In Period C, nutrient input after the budburst of trees, coupled with shading effects, increased microbial activity, particularly on the snow surface. These findings suggest that temporal changes in microbial activity in snowpacks are regulated by snow depth, temperature increase, and the phenology of trees above the snow surface, highlighting the complex interplay between physical and biological factors in shaping snow ecosystems. The results of pit and core sampling suggest that microbes in the snowpack are active not only on the snow surface but also within the snow, referred to as the Microbial Active Snow Surface (MASS) layer, throughout the snowmelt season, and that their abundance and vertical distribution change with nutrients and solar radiation. Further studies are required to clarify the effects of different tree species on biological activity and to better quantify the response of snow ecosystems to climate change.

Competing interests

The contact author declares that none of the authors have any competing interests.

Acknowledgement

Sampling and all analyses were supported by JSPS KAKENHI (19H01143, 20K21840, 20H00196, and 21H03612) and the Arctic Challenge for Sustainability II (ArCS II, Program Grant Number JPMXD1420318865) for N. T. Making and publishing of the manuscript was supported by JSPS KAKENHI (22J11017, 22KJ0471, and 24KJ0118) for M. O. We would like to thank Editage (www.editage.jp) for English language editing.

Author contributions

Sampling for research: M. O., K. K., D. S., F. K., K. W., and S. U.; manuscript conception: M. O. and N. T.; microscopy: M. O.; chlorophyll *a*: M. O.; chemical analysis: M. O.; and statistical analysis: M.O. All authors have edited and reviewed the manuscript.

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