

Snow Algae Blooming Are Benefitable for Microinvertebrates Assemblages (Tardigrada and Rotifera) on the Seasonal Snow Patches in Japan

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Abstract

Although studies on snow algae and macroinvertebrates have been frequently conducted on snow patches, no attention have been paid to ubiquitous microinvertebrates which in other cold habitats reach high biomass and play various trophic roles. The aim of this study was to search microinvertebrates in seasonal snow patches in Mt. Gassan, in northern Japan, and identify factors determining their distribution associated with snow algal blooming of various coloration (red, green, and yellow). Microscopic observation revealed presence of two major groups of microinvertebrates Tardigrada and Rotifera. Tardigrades and rotifers were the most abundant and frequent in green snow formed by blooming of *Chloromonas* sp., but few in red or yellow snow. Body length of tardigrades increased through the melting season and animals laid eggs on colored snow. These results suggest tardigrades successfully grew and reproduced on snow patches. Taking into account the presence of tardigrades and rotifers mostly in green snow (only few found in red and yellow) with high densities, we may assume green snow patches constitute important and unique low-temperature ecosystems for microinvertebrates in a temperate mountainous forest. Area of snow algae blooming worldwide are unrecognized novel habitat for tardigrades and rotifers.

Introduction

Seasonal snow are one of the most common and at the same ephemeral cold environments in the world¹. Owing to accumulation during winter and melting during the spring, seasonal snow influence adjacent ecosystems such as terrestrial, freshwater and marine ecosystems as a source of water or nutrients². In some cases snow significantly affects whole ecosystems i.e. reduction of seasonal snow might lead soil freezing and damage for forest ecosystems³, or melting snow play important role in supporting nutrient input to glacial ecosystem and biological production there⁴.

Although snow patches are important element supporting the functioning of other ecosystems, seasonal snow provide also specific environments for microorganisms. Despite the harsh conditions of snow surface like a low temperature and high UV irradiation, there are many taxa inhabiting unique snow ecosystems including primary producers (snow algae and cyanobacteria), microbial heterotrophs like fungi⁵, and consumers represented mostly by insects⁶. Activity of microorganisms in seasonal snow could influence their melting by reduction of snow albedo, change air composition and effects on climate by releasing e.g. hydrocarbons, halocarbons, aldehydes, mercury and ⁷. Therefore, each step in understanding snow ecosystems, regrettably one of the fast disappearing, are highly needed to reveal their productivity and biodiversity.

During the melt season, snow algae blooming change the color of snow surface into red, green, yellow or orange⁸. One of the common groups of snow algae are *Chloromonas* and *Sanguina* species, reported in glacial and snow habitat worldwide including Japan; *Sanguina* sp. is common on open snowfields while *Chloromonas* sp. often appears of forest floor⁹⁻¹². Snow algae blooming has been shown to influence

melt rates of snow due to reduction of albedo on the snow surface, at the same providing liquid water for further biological processes^{13,14}, and act as mediator in nutrient and carbon cycles^{7,15}. Potential consumers of algae on snow patches have been also reported^{5,6,16}, but without detailed ecological data explaining links between their densities, biomass, sources and seasonality. Although invertebrates are common apex consumers in snow ecosystems, knowledge on their diversity is related mostly to macroinvertebrates in high mountains^{17,18} with limited data on microinvertebrates particularly tardigrades and rotifers which are one of the most common faunal groups in polar and high mountain ecosystems¹⁹. Data on tardigrades and rotifers densities, biomass, and factors determining their distribution on snow are still unknown.

Tardigrada and Rotifera are small cosmopolitan microscopic (mostly < 1 mm) invertebrates inhabiting virtually most of terrestrial and aquatic ecosystems, from tropical rain forests to glaciers in polar regions^{19,20}. Until now, ca.1400 and 2000 species of tardigrades and rotifers respectively have been described worldwide^{21,22}. Their feeding mode and behaviors (herbivorous, omnivorous or carnivorous; grazers, filter-feeders, predators) and reproduction style (sexual, asexual and parthenogenetic) differ depending on the species²³. Owing to their ability of cryptobiosis which enables them to survive in extreme conditions such as high UV radiation doses, low and high temperature or salinity^{24,25}, they can inhabit various environments worldwide including the coldest ones like cryoconite holes on glaciers²⁶. Although there are many studies devoted to ecology and diversity of tardigrades and rotifers in high mountain or polar bryophytes and lichen habitats^{27,28}, their ecology in cryosphere is still underscore^{29,30}. Tardigrades and rotifers in snow habitats, although important, are particularly overlooked or just anecdotally recorded⁵.

Owing to influence of monsoon, a large amount of snow accumulates during the winter in mountainous areas in Japan. This snow remains until late spring, sometimes occur through the whole year and play a role of a low temperature habitats for temperate psychrophiles (e.g. stonefly³¹). In Northern Japanese snow patches, there were no reports on these microinvertebrates. Such limited data on microinvertebrates on snow may hinder our understanding of trophic networks and functioning of low temperature habitats. In this study, we aimed to search microinvertebrates in the seasonal snow patches and identify the factors determining their distribution on snow in forest in Northern Japanese mountainous area (Fig. 1, 2). Microinvertebrates were collected in late-melt season over two years (2018-2019), and their population density, their body size, biomass, diet and habitat preferences are presented and discussed.

Results

Microinvertebrates on snow surface

We found two groups of microinvertebrates on snow, Tardigrada and Rotifera (Fig. 3 and see supplementary Fig. S1). Springtails (Collembola) and stoneflies have been seen on the snow during sampling but they were absent in the collected samples. We did not detect other invertebrates (e.g.

nematodes, planaria) but in samples we observed abundant ciliates. Tardigrades were represented by two morpho-species belongs to genus *Hypsibius* with smooth or reticular cuticle (see supplementary Fig. S2). Both taxa have typical buccal tube for herbivorous species. Rotifers have not been identified to species level; all specimens represented order Bdelloidea (Fig. 3b). Tardigrades found on the snow represented different size classes (Fig. 4), including juveniles. Exuvia of tardigrade with eggs have been also found, each contained 4-5 eggs (see supplementary Fig. S3a). We also found different sizes of rotifers (not measured due to their shrinking) along with exuvia contained eggs (see supplementary Fig. S3b).

In moss samples, tardigrades representing genus *Hypsibius* and *Macrobiotus* along with heterotardigrades have been found. However, based only on morphology we could not fairly say whether *Hypsibius* sp. in mosses and snow is the same. However, specimens of *Macrobiotus* and heterotardigrades have been not found in any kind of snow during two seasons.

Body length of tardigrades living in green snow were varied during sampling campaigns (Fig. 4). Their body length was 129-386 μm (mean SD: 253 6 μm) in April 2018, 226-452 μm (mean SD: 302 3 μm) in May 2018 and there were significant differences between April and May (Welch's t-test, $t(113) = 7.54$, $P < 0.001$). In May 2019, their body length was 178-457 μm (mean SD: 298 13 μm) and similar to the one in May 2018 and their biomass was .

Snow algae and Chlorophyll a concentrations

Microscopic observations revealed that each colored snow were dominated morphologically different snow algae species (Fig. 5). In green snow, oval shaped cells with green chloroplasts were dominated (Fig. 5a). The length of this algae was 13.0-20.1 μm (mean SD: 17.2 0.1 μm) and width was 7.2-12.7 μm (mean SD: 9.2 0.1 μm). In red snow, oval shaped cells with ribbed cell wall, green/orange chloroplasts and orange secondary carotenoids were dominated (Fig. 5b). The length of this algae was 36.8-63.4 μm (mean SD: 48.2 0.4 μm) and width was 19.9-33.3 μm (mean SD: 28.2 1.5 μm). In yellow snow, triangular cells with yellow chloroplasts were dominated (cell size were not measured, Fig. 5c).

Chlorophyll *a* concentration largely varied across the snow surface. In particular there were significant difference in the concentration between colored snow and white snow (Fig. 6, 7 and Table 1). In samples in April and May 2018, chlorophyll *a* concentration were and in green snow (April and May respectively), in red snow (May), in yellow snow (May) and in white snow (May). Whereas there were no differences in the chlorophyll *a* concentration between green snow and other colored snow (Mann-Whitney test: green and red snow, $U(4, 3) = 6$, $P > 0.05$; green and yellow snow, $U(4, 3) = 5$, $P > 0.05$), there were significant differences between white snow and other colored snow (Mann-Whitney test: green and white snow, $U(4, 4) = 0$, $P < 0.05$; red and white snow, $U(3, 4) = 0$, $P < 0.05$; yellow snow and white snow, $U(3, 4) = 0$, $P < 0.05$). In samples from May 2019, chlorophyll *a* concentration were in green snow and in white snow, and there was significant difference between green snow and white snow (Mann-Whitney test, $U(9, 21) = 0$, $P < 0.001$).

Relationship between microinvertebrates and snow algae

Tardigrades and rotifers were concentrated mainly in green snow and their population density were different between different snow colors. In April and May 2018, population density of tardigrades were (mean SD), in green snow (April and May respectively), in red snow (May), in yellow snow (May) and 0 in white snow (May) (Fig. 6 and Table 1). In May 2019, population density of tardigrades and rotifers were and in green snow, and 0 in white snow respectively (Fig. 7 and Table 1). Population densities of tardigrades, rotifers and concentration of chlorophyll *a* were significantly correlated in green snow (Pearson's correlation coefficient: tardigrades and rotifers, $r = 0.91$, $P < 0.01$; tardigrades and chlorophyll *a*, $r = 0.87$, $P < 0.01$; rotifers and chlorophyll *a*, $r = 0.84$, $P < 0.01$). Regarding to tardigrades, whole algae cells were observed in their intestine as well as had a green intestine color (Fig. 3a and supplementary Fig. S1).

Discussion

In this study, we revealed that tardigrades and rotifers were concentrated on seasonal snow patches in beech forests in Japan, particularly in green snow. Our observation suggests that snow algae blooming is truly benefitable for tardigrades and rotifers where they have stable and abundant food source. According morphology, we found two tardigrade taxa represented by genus *Hypsibius* and bdelloid rotifers on snow (Fig. 3). The high densities of microinvertebrates, their presence through more than one seasons and various size classes (Fig. 4, 6, 7) suggests that tardigrades and rotifers living on the seasonal snow patches and adapt to snow surface environment. Although snow algae blooming serves a good habitat for tardigrades, not all taxa found in mosses have been found on snow. Under laboratory conditions, tardigrades represented by tardigrade genus *Macrobiotus* which was found in mosses from trees at the study site are well known to feed on algae³², but was absent in snow. We expect that some physiological or dispersal constraints might limit their appearance on seasonal snow patches. Most probably it is a temperature regime, which seems shape tardigrade communities on glaciers²⁶. Regrettably, we cannot say clearly where *Hypsibius* species on snow came from, whether mosses, lichens growing on ground or in tree canopies.

Most probably, microinvertebrates on snow origin from passive dispersal by wind. Hypothetically, if they would be passively transported to snow from surrounding mosses, more taxa should be found during sampling campaigns than only two *Hypsibius*. This observation suggests that even some tardigrades might appear on snow, they are not adapted enough to grow and reproduce in these ecosystems. In fact, previous work conducted in Akashibo snow patches in Japan, reported tardigrades and other microinvertebrates (e.g. Nematoda, Oligochaeta) in snow layer under the snow patches, which probably migrated from soil, however these microinvertebrates did not seem to be active in snow such as growing and reproduction³³. Although the harsh conditions (low temperature and periodic freezing) occurred on seasonal snow patches in Mt. Gassan, due to presence of snow algae, these ephemeral ecosystems supports growth and reproduction of microinvertebrates. Tardigrades and rotifers found in green snow seems to not only be cold lovers, but taking into account high biomass and densities they must benefit from being on snow. From the above, a simple, hypothetical estimation of the microinvertebrates life cycle is as follow (I): dispersal to snow surface by wind from moss growing on tree trunks or canopies

(April), (II): grow and reproduce by eating green snow algae (May), (III): passive transport with melting snow to mosses and spend their life in there (no snowy seasons).

There were no differences of concentration of chlorophyll *a* between colored snow in 2018 (Fig. 6), however, it seems that green snow form the most favorable conditions for growth and reproduction of tardigrades and rotifers in the study area. In green snow, exuvia of tardigrades and rotifers were found (supplementary Fig. S3), body length of tardigrades in May 2018 were bigger than in April 2018 (Fig. 4) which suggest successful growth. At the same time concentration of chlorophyll *a* in the samples also increased in time, which means that along with available food sources tardigrades increased their size and numbers. Although seasonal variability may result in growth of tardigrades body size, such effect has not been widely accepted.

Microinvertebrates were found with the highest population density in green snow, none or low in red, yellow and white snow (Fig. 6, 7 and Table 1). Tardigrades in green snow are typical algae feeders^{32,34} and indeed their intestine were full of algae cells (Fig. 3a and supplementary Fig. S1). At this moment it is hardly to say whether tardigrades and rotifers utilize the same food source on snow, or they use different food; tardigrades eat algae and rotifers might eat some of the associated suspension bacteria or yeasts. Although, the same two dominant algae genera on snow were found in Canada⁵ (*Chloromonas* spp. or *Sanguina* spp.). Authors presented tardigrades, rotifers, mites and springtails in red snow, and showed that both tardigrades and rotifers have the same red intestine content. Regrettably, authors did not presented densities of animals, hence we cannot compare data. In Mt. Gassan, it is known that snow algae which compose green and red snow are mix of several species of genus *Chloromonas*, yellow snow are *Ochromonas* species^{10,11,35,36}. Even though green and red snow may consist of same genus *Chloromonas*, still they are different species, the difference in their color is probably due to the difference in life cycle of snow algae. In green snow, algal cells were mostly motile vegetative cells, which have chloroplasts without any secondary pigment, and were actively swimming using flagella. They can asexually reproduce in the melting snow surface and form visible green snow. In contrast, algal cells were mostly cysts with ribbed and thick cell walls, they contained orange or red colored secondary carotenoids, which are probably astaxanthin. Such secondary pigments are usually produced when snow algae are stimulated by light, for minimizing the amount of light available^{36,37}. Tardigrades belong to *Hypsibius* also found in snow are considered to eat algae by sucking³⁴, so this thick cell walls make it difficult for tardigrades to suck algae. Moreover, tardigrades in laboratory cultures feed on *Chlorella vulgaris*, *Chlorella* sp.,^{38,39} and green algae on snow have similar size (2-10 µm for *C. vulgaris*⁴⁰. It was mentioned that size of algae is not important for their dietary preferences³⁴, by far we cannot say whether size of snow algae affect their dietary preferences. For yellow snow algae, their cell walls and size do not seem to interfere with the tardigrade's dietary so we can assume that tardigrades get something beneficial from green snow algae pigments not from yellow snow algae. However, these relations required exact testing in the future.

Due to simplicity of snow ecosystems, low organic matter content, few primary producers and few consumers, snow ecosystems may facilitate recognition and understanding of ecological processes in the cryosphere. In cryoconite holes which are cylindrical water filled reservoirs contained cyanobacteria, dust, organic matter and mineral particles on glacial ice^{41,42}, although some study showed correlation between tardigrades and rotifers^{30,43,44}, the significant relation between tardigrades or rotifers and any organisms as a typical source of food in cryoconite have not been found^{29,43}. There are two potential scenarios which might explain this unrecognized pattern in cryoconite holes. In comparison with areas of snow algae blooming, cryoconite holes host very diverse biota and rich organic matter, thus diet of invertebrates might be complex which may constrain understanding of food preferences⁴⁵. Secondly, dynamic nature of cryoconite holes in Arctic and alpine regions like rapid ablation, inter-hole water-sediment mixing and stochastic weather events disturb or sometimes destroy habitats on surface of ice like cryoconite holes^{30,46}, hence in dynamic habitats findings of any ecological relations are problematic.

In the seasonal snow patches, meltwater is considered the only way causing removal of biotic components including microinvertebrates. In this case, snow surface was mostly flat and during field works we could not see meltwater flowing on the surface. Therefore animals might migrate along with the movement of snow algae, and maintain stable until they are supported by snow (as a liquid water) and algae (as a food). Nevertheless, population density of microinvertebrates in the seasonal snow patches was lower than that of in the cryoconite holes with note that snow patches and cryoconite holes have different conditions of water (solid or liquid) so the densities cannot be simply compared; in Greenland, average was approx. (looks as same as snow but reached up approx. for tardigrades, approx. for rotifers²⁹, in European Alps, approx. (reached up approx.) for tardigrades²⁶, in Svalbard, reached up approx. for tardigrades, approx. for rotifers⁴⁷, in Antarctica, reached up approx. for tardigrades, approx. for rotifers⁴³. In other cold environments, tardigrades were found in mosses in Antarctica with higher population density and biomass (reached up to approx.) than that of in the seasonal snow patches⁴⁸. Rotifers were found in weathered snow/ice in accumulation zone with lower population density (reached up to) than that of in the seasonal snow patches⁴⁹.

The need for holistic studies on snow and biosphere including microbial activity which change the chemical composition of snow is well acknowledged⁷. Many taxa inhabit the seasonal snow^{5,6,16}, however in many cases it is hard to infer which faunal elements are true snow ecosystems element and which randomly feed on snow algae. Herein we showed that tardigrades and rotifers are abundant where snow algae blooming occurred with preferences towards green snow (*Chloromonas* sp.) over the two sampling seasons in Japanese mountain forests. Intestine contents which contained algae cells, various instars and size classes of animals suggests that microinvertebrates are virtual faunal element in snow blooming while snow patches serve as a new arena for studies on biodiversity and ecology of these ubiquitous microinvertebrates. Further studies on the consumption rate by snow algae consumers may bring a new data on how invertebrates suppress algae blooming and how these algae respond on abundant invertebrates. These studies also have possibility to contribute to understanding material cycle such as carbon and nitrogen in snow which related to microbial activity on the snow surface^{50,51}. While it

was also mentioned carbon and nitrogen cycle in snow cover environments and emphasized impact of presence of seasonal snow cover for such cycle in soil ecosystem under the snow⁵², microbial activity in and on snow were not considered. Understanding the relationship between snow and biosphere including microinvertebrates is important in anticipation future changes and biogeocycles in snow ecosystems.

Material And Methods

Study site

The field study was conducted at Yumihiradaira park (38°30' N, 140°00' E: altitude 770 m) in Mt. Gassan, Yamagata prefecture in Japan (Fig. 1). The peak of Mt. Gassan is an altitude of 1984 m above sea level (a.s.l.) and the main mountainous ridge extends from the north to the south along the west coast of the northern part of the Honshu Islands of Japan. This mountain range directly receives the strong winds during winter, which is originally from Siberia and blow over the Sea of Japan. This strong wind contain abundant moisture supplied from the Tsushima Warm Current in the Sea of Japan, and induce heavy snowfall in the mountains⁵³. Vegetation at the study site is dominated by montane broad-leaved deciduous trees (including beech trees shown in Fig. 1) up to the elevation of 1500 m a.s.l. Above the elevation, subalpine bamboo, ash and maple dominate up to the top of the mountains⁵³.

The study site is covered with snow from mid-November until early June. The snow depth usually reaches approximately 4 m in February as maximum depth. According to the meteorological station near the study site (750 m a.s.l.), which is operated by Snow and Ice Research Center, National Institute of Disaster Research in Japan, the daily mean air temperature ranged from approx. -8°C to 4°C in January and February (reached approx. 17°C by sampling collection).

Sampling collection

For analysis of microinvertebrates and snow algae, surface snow samples were collected on April 23rd, May 14th 2018 and May 18-20th 2019 (Fig. 1). Snow depth were approx. 320 cm, 163 cm, 90 cm respectively. Total of 52 samples have been collected. The dimension of the collected snow surface was 10×10×2 cm (length×width×depth; Fig. 2). During the study periods, there were various colors of snow surfaces, and each colored snow appeared patchy in the size of 10 cm to 30 cm in diameter. We classified the snow surface in 4 different colorations, which are visibly identified as white, green, red, and yellow snows as described before³⁵. The different color of the snow is most likely to be shaped by abundance and species composition of snow algae. The 4 different colors of snow were collected in the season of 2018, white and green snow were collected in the season of 2019. In order to compare microinvertebrate taxa with those in the closest snow free habitats, moss samples attached to beech trees at the study site were collected in 2018 (April = 4 samples, May = 4 samples). All snow samples were collected by using sterile stainless-steel scoop, and all moss samples were collected by using small shovel. Immediately after sampling, material were kept frozen and preserved in 50 ml polypropylene bottle (AS ONE CORPORATION, Osaka, Japan) or Whirl-Pak® bags (Nasco, Fort Atkinson, WI, USA). All

samples were transported to the laboratory at Chiba University, Japan and stored in a freezer (-20°C) until further processing.

Microscopic observation of microinvertebrates

Snow and moss samples were melted gradually at refrigerator (5°C) in order to avoid potential thermal shock of psychrophilic invertebrates. After melting, liquid water were moved directly to a petri dish and examined using a MZ125 stereo microscope (Leica Microsystems, Wetzlar, Germany) with FLEXACAM C1 digital camera (Leica Microsystems, Wetzlar, Germany). Moss samples were put a petri dish with adding MiliQ water and left 2-3 days for waiting on animals' recovery. After 2-3 days, moss were removed after shaken using tweezers. Invertebrates have been counted and put into 6 ml glass tube (AS ONE CORPORATION, Osaka, Japan) with 70% ethanol for preservation. Some individuals were used for taking picture using a BX51 phase contrast microscope (OLYMPUS, Tokyo, Japan) with DP21 digital camera (OLYMPUS, Tokyo, Japan). Population density of tardigrades and rotifers in snow were calculated based on counted number of tardigrades or rotifers per volume of melted water.

Tardigrades were mounted on microscope slides in a small drop of Hoyer's medium then examined under the following light microscopes BX51 and Olympus BX53 with phase-contrast (PCM) and another with differential interference contrast (DIC). Pictures were taken using a DP21 digital camera, cellSens Entry 1.12 software (Olympus, Tokyo, Japan) or Quick PHOTO CAMERA 3.0 software (Promicra, Czech Republic). For species identification the keys and species descriptions were used^{54,55} to tardigrades. Rotifers presumably have been represented by more than one species, but have not been identified. For calculation of biomass of tardigrades, we measured their body length and width using pictures taken under Olympus BX53 and measured in Image J and Quick PHOTO Camera 3.0 software. We excluded from our analysis those animals not suitably orientated for morphometry (shrunken or bent). Body length was measured without length of legs and width of the body between legs and was measured. Biomass (dry weight, W) of each specimens were calculated based on formula⁵⁶: if body length (L) and width (D) were 4:1; , 5:1; . We used these formulae that takes the closer ratio.

Microscopy of snow algae and measurements of the chlorophyll a concentration

Melted snow was used for microscopic observation of snow algae and measurements of the chlorophyll a concentration which expressed concentration of algae⁵⁷. Firstly, put 20-50 µm melted water on slide glass and observed snow algae by taking picture using a BX51 phase contrast microscope with DP21 digital camera. Length and width of snow algae were measured in Image J. For measurements of the chlorophyll a concentration, Welschmeyer method which quantify with fluorescence was used; melted water (approx. 90-150 mL per sample) were filtered through a glass micro filter (Whatman® glass microfiber filters gf/f 25 mm, Cytiva, Tokyo, Japan), and put them into 8 ml sterilized tube (60.542.024, SARSTED, Nümbrecht, Germany) with 6 ml of N,N-dimethylformamide (DMF) to extract chlorophyll. After storing tubes in refrigerator for 2-3 days to wait extracting pigment, fluorescence intensity were measured by using a *Trilogy Laboratory Fluorometer* (TURNER, CA, USA). Chlorophyll a concentration were obtained

from fluorescence intensity based on molecular extinction coefficient⁵⁸ ($88.74 \text{ L}^{-1} \text{ g}^{-1} \text{ cm}^{-1}$), and a calibration curve created by measuring fluorescence intensity and absorbance (664 nm) of *chlorophyll a* from *spinach* (C5753-1MG, Sigma-Aldrich Japan, Tokyo, Japan) with a fluorometer and a spectrophotometer (UV-mini 1240, Shimadzu, Kyoto, Japan).

Statistical analysis

To test differences between invertebrate biomass and densities, and chlorophyll *a* in white and colored snow and seasonal changes of tardigrade body size, two statistical tests were used. The nonparametric Mann-Whitney U test for testing differences of population densities of tardigrades and rotifers or concentration of chlorophyll *a* between white and colored snow, and the Welch's t-test for testing differences in seasonally changes of body length of tardigrades. All statistical analysis were calculated using R software⁵⁹.

Declarations

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Author contributions

Collecting material for research: M.O and N.T; manuscript concept: M.O, N.T and K.Z; microscopy: M.O, N.T and K.Z; chlorophyll: M.O; statistical analysis: M.O. All authors edited and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. Number of snow samples, population density of microinvertebrates and concentration of chlorophyll *a*. Inv.: population density of microinvertebrates (tardigrades or rotifers, indL⁻¹), Chl-a: concentration of chlorophyll *a* (µgL⁻¹), N/A: could not see colored snow, - : rotifers were present but did not count

Year	Month		Snow patches			
			White	Green	Red	Yellow
2018	April	n	4	4	N/A	N/A
		Inv.	0, -	7.3×10 ³ , -	N/A	N/A
		Chl-a	0	2.9×10 ²	N/A	N/A
	May	n	4	4	3	3
		Inv.	0, -	7.1×10 ³ , -	7, -	7, -
		Chl-a	28	1.3×10 ³	1.0×10 ³	1.0×10 ³
2019	May	n	21	9	N/A	N/A
		Inv.	1.2×10 ² , 0	1.0×10 ³ , 5.5×10 ²	N/A	N/A
		Chl-a	1.4×10 ²	4.4×10 ²	N/A	N/A

Figures



Figure 1

Study site (a) in April, 2018 when beech leaf did not open, (b) in May, 2018 (same in May, 2019) when beech leaf opened and there were bud scale on snow.



Figure 1

Study site (a) in April, 2018 when beech leaf did not open, (b) in May, 2018 (same in May, 2019) when beech leaf opened and there were bud scale on snow.

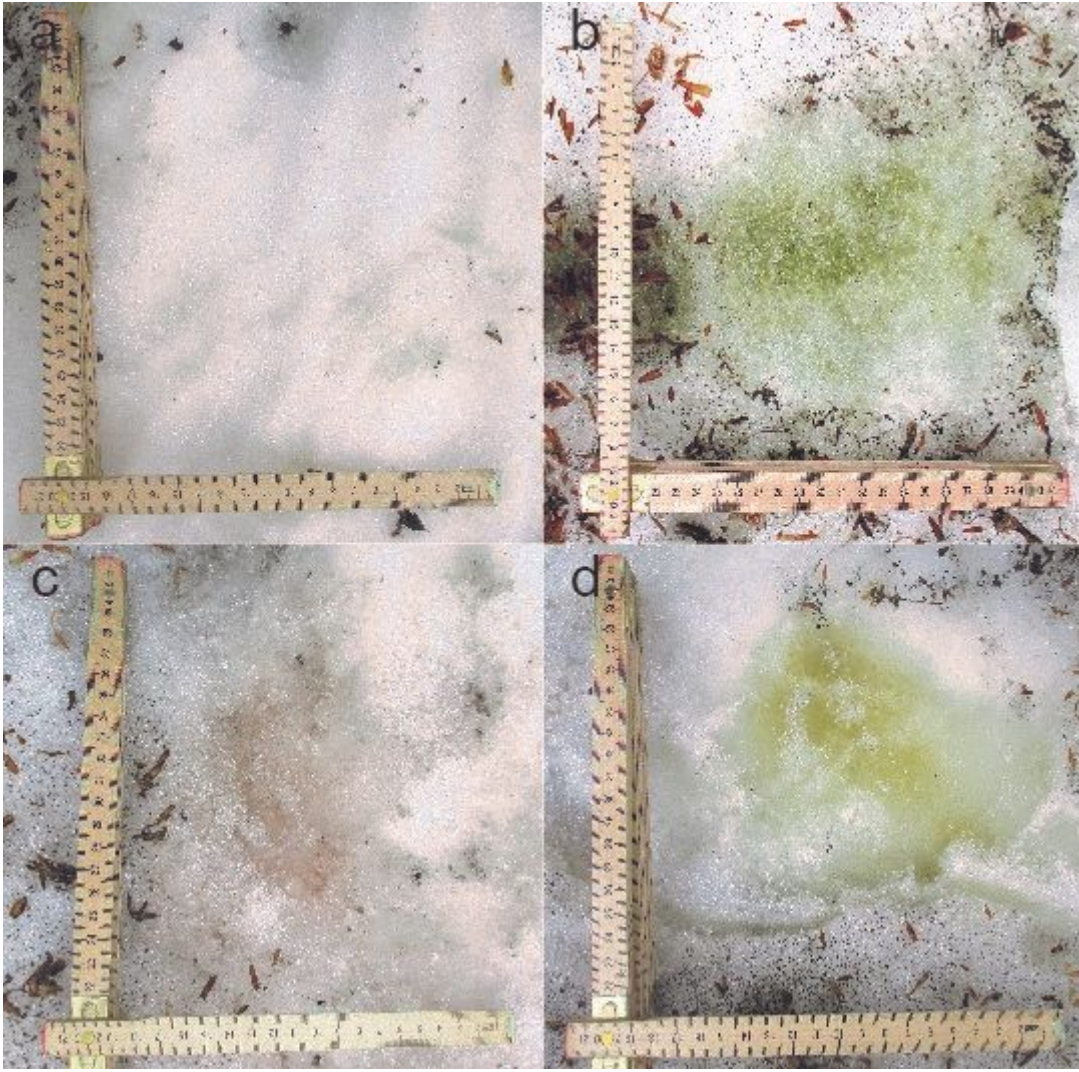


Figure 2

Snow surface collected in 2018. (a) white snow, (b) green snow, (c) red snow, (d) yellow snow.

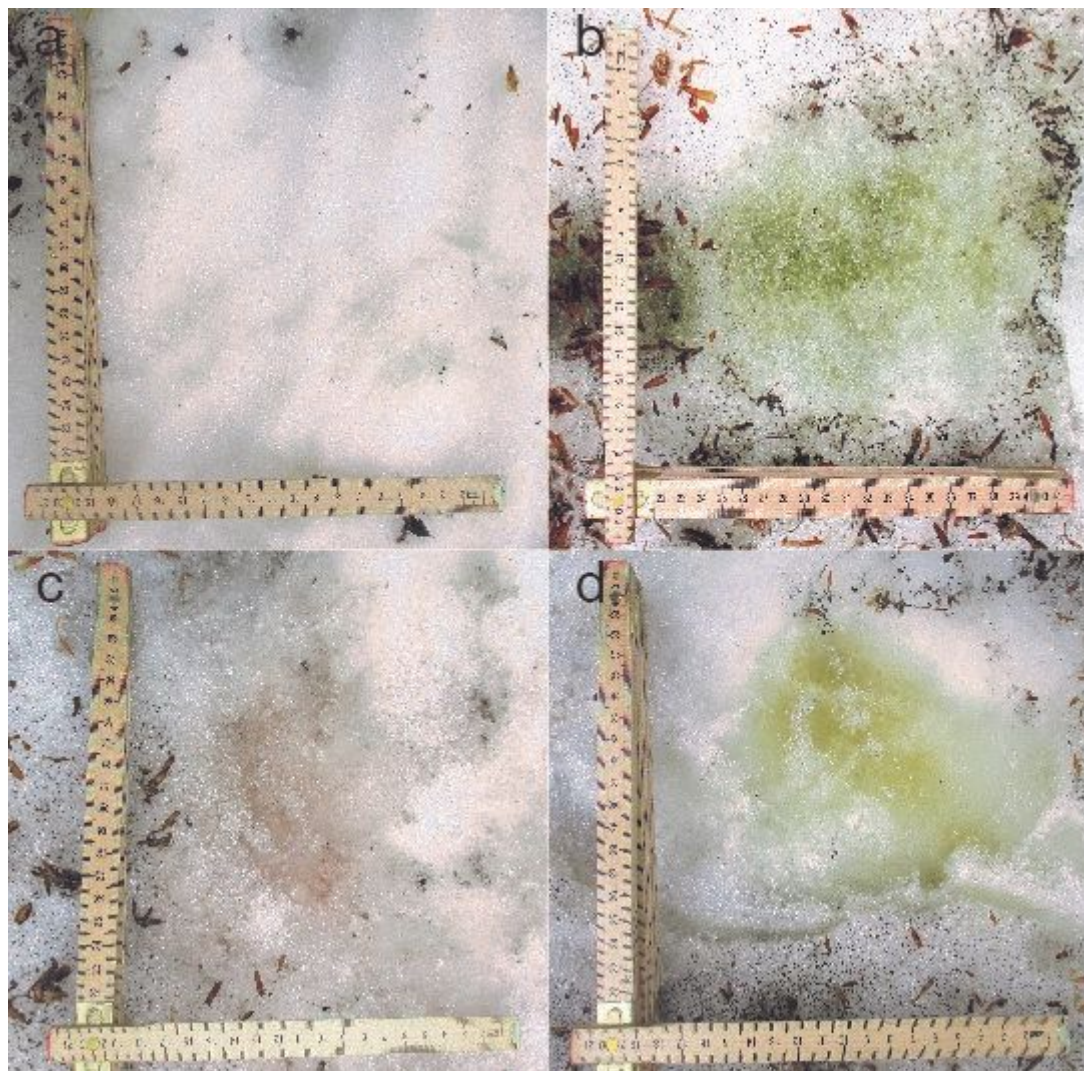


Figure 2

Snow surface collected in 2018. (a) white snow, (b) green snow, (c) red snow, (d) yellow snow.

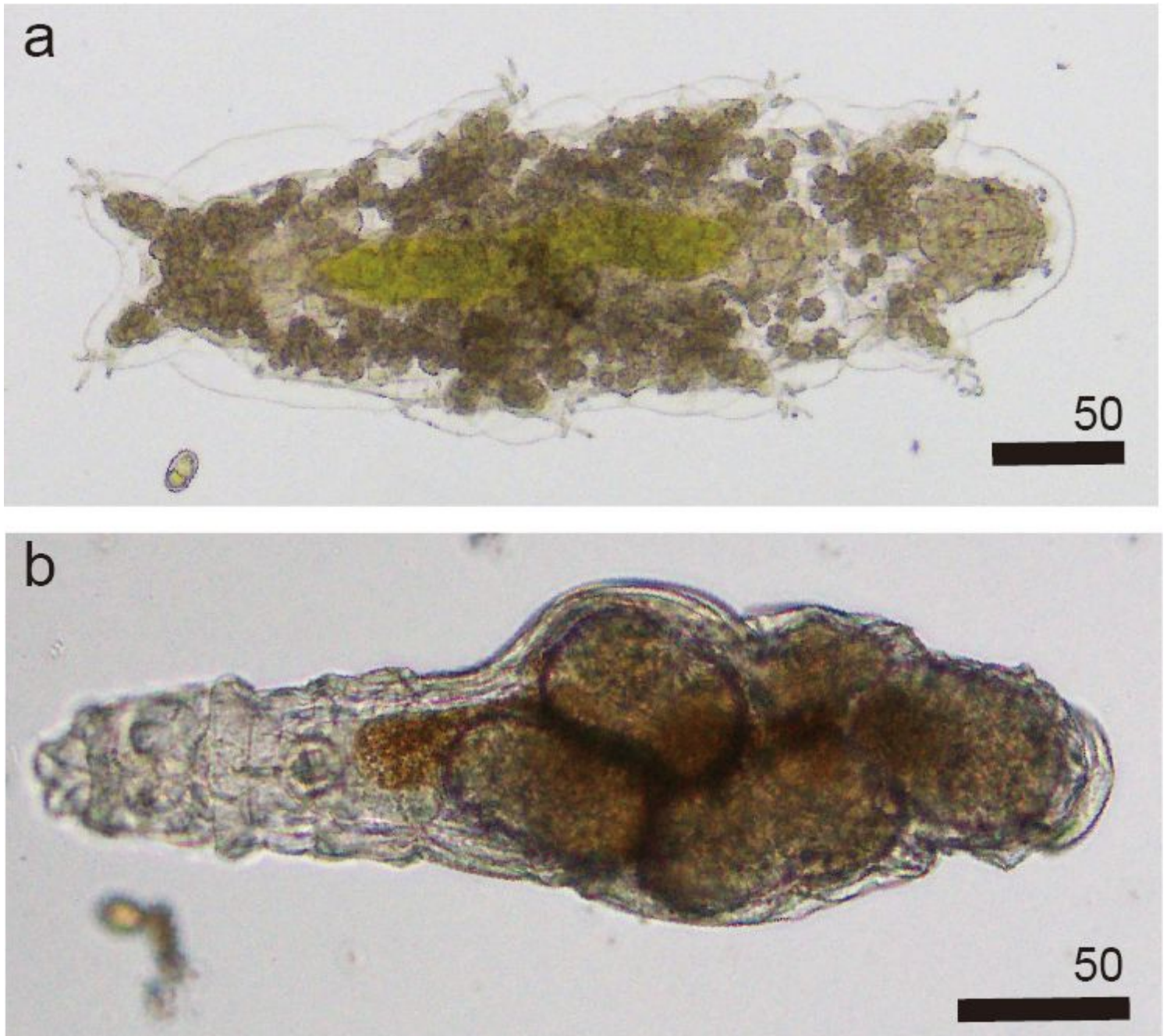


Figure 3

Microinvertebrates in snow. (a) *Hypsibius* sp. with full of algae in their intestine (Light microscope: LM), (b) bdelloid rotifers (LM). All scale bars in micrometers.

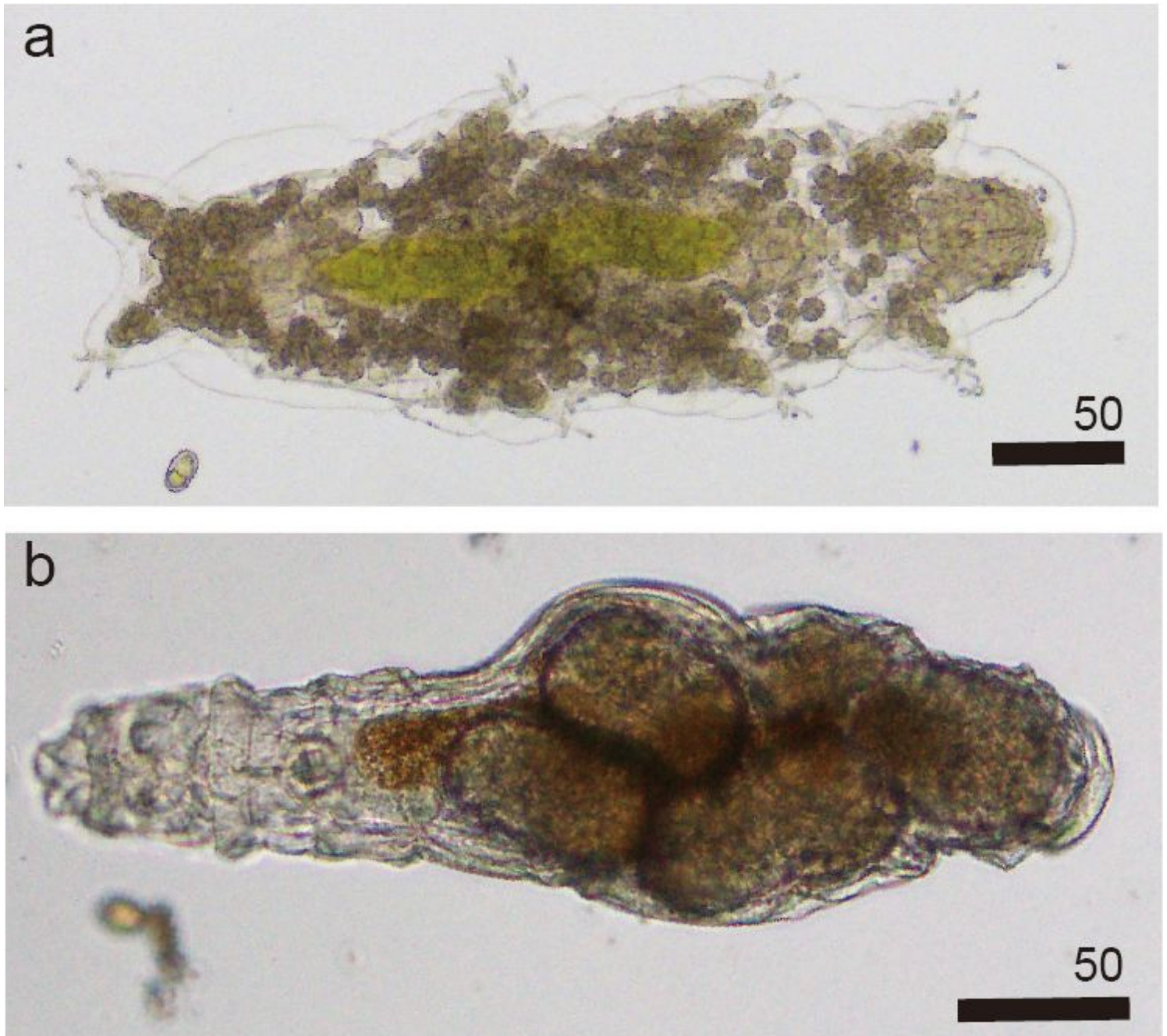


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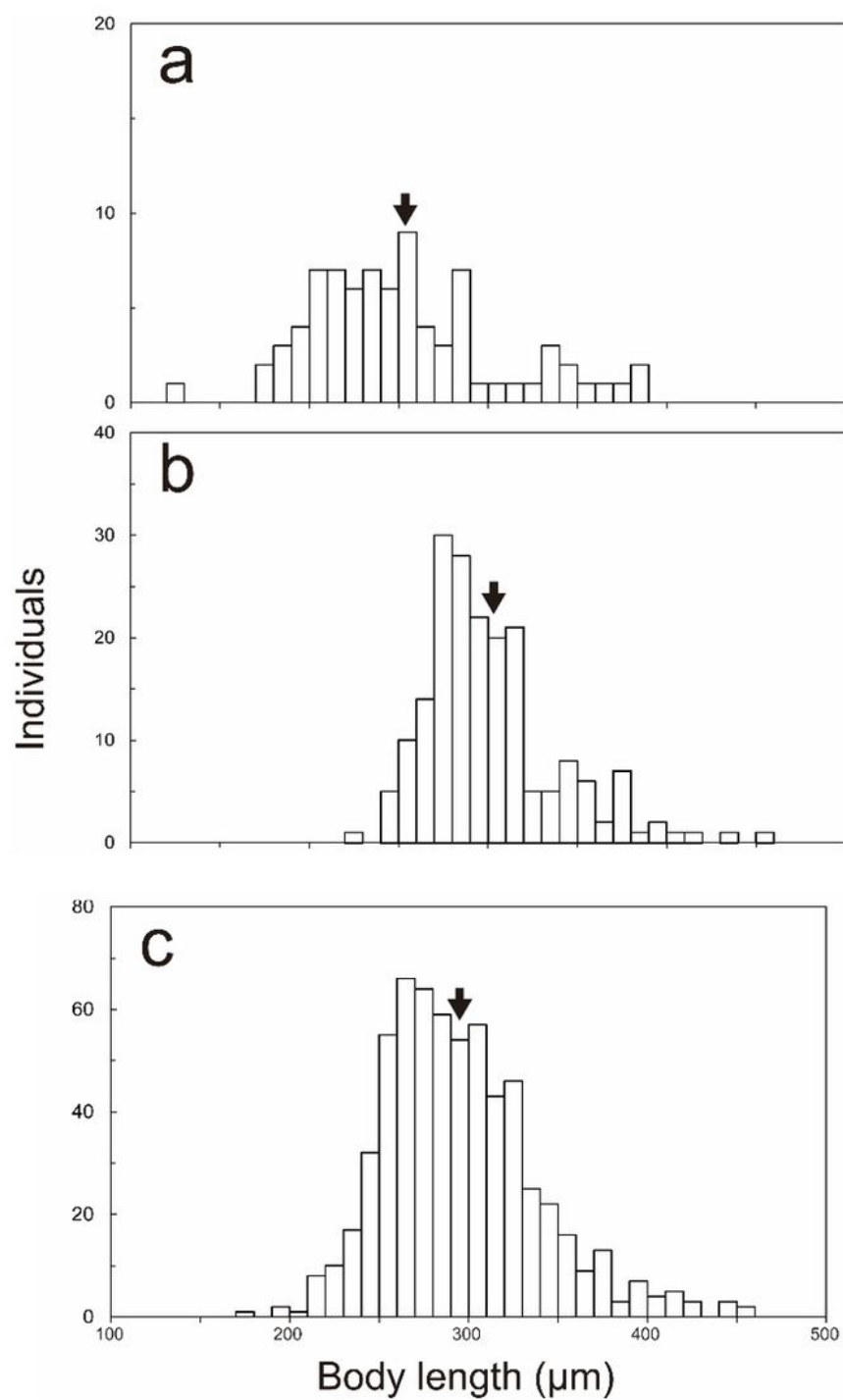


Figure 4

Body size (expressed here as body length) of tardigrades living in green snow (10 μm size increments). (a) collected in April, 2018, (b) collected in May, 2018, (c) collected in May, 2019. Mean values are shown by the arrows.

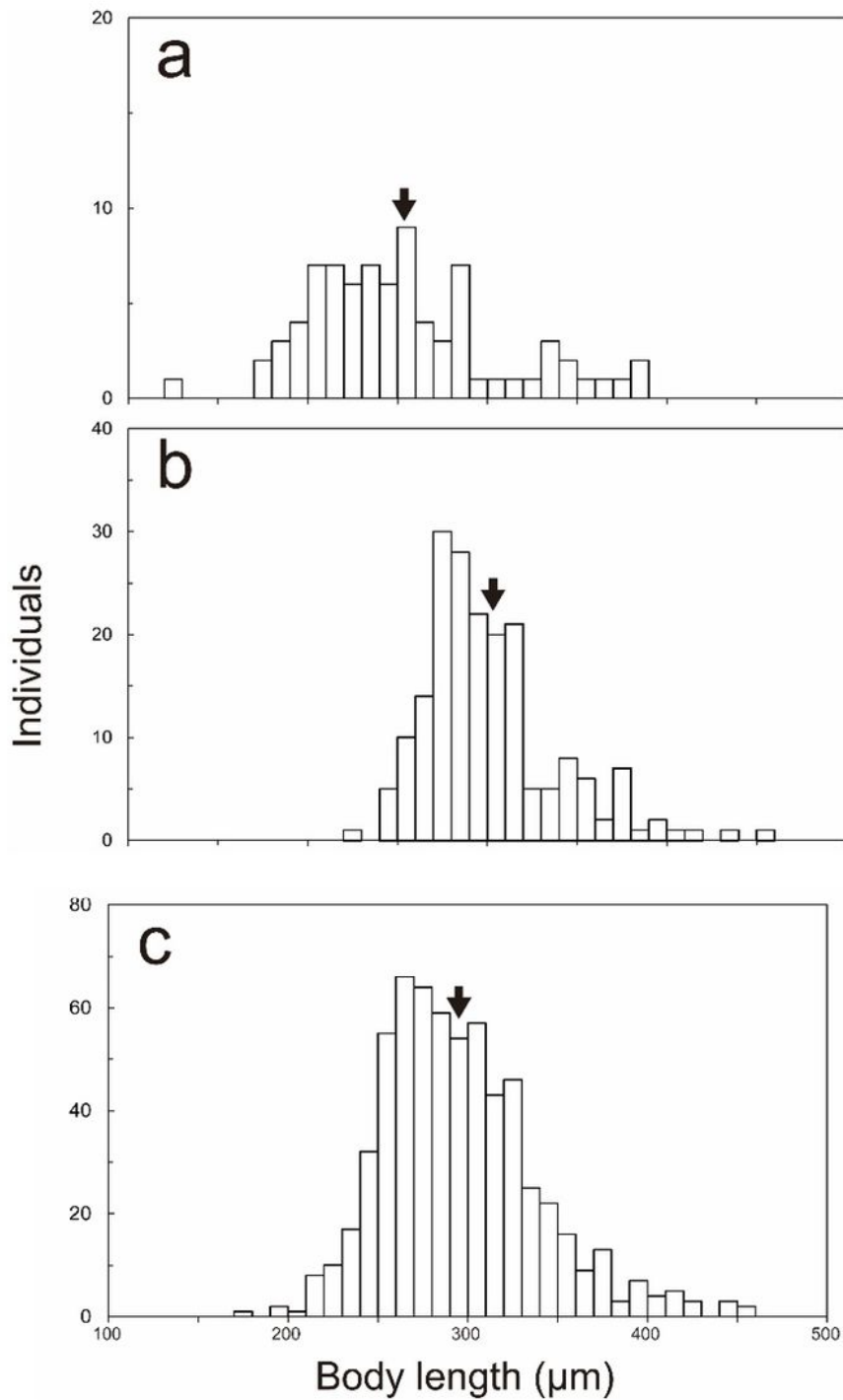


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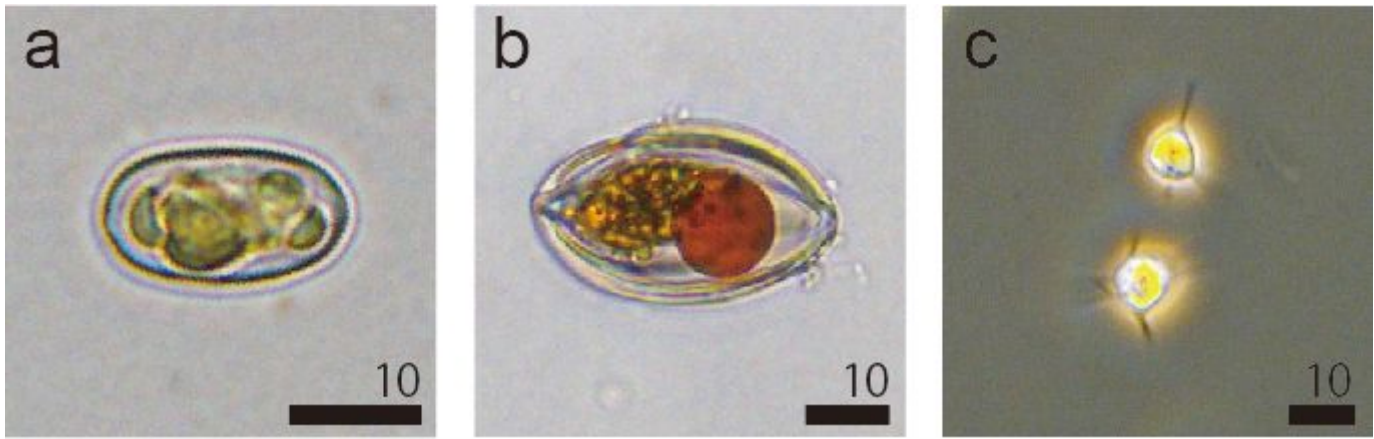


Figure 5

Abundant snow algae species collected in May, 2018. (a) green snow algae (LM; *Chloromonas* sp.), (b) red snow algae (LM; dormant state of *Chloromonas* sp.). (c) yellow snow algae (PCM; *Ochromonas* sp.). All scale bars in micrometers.

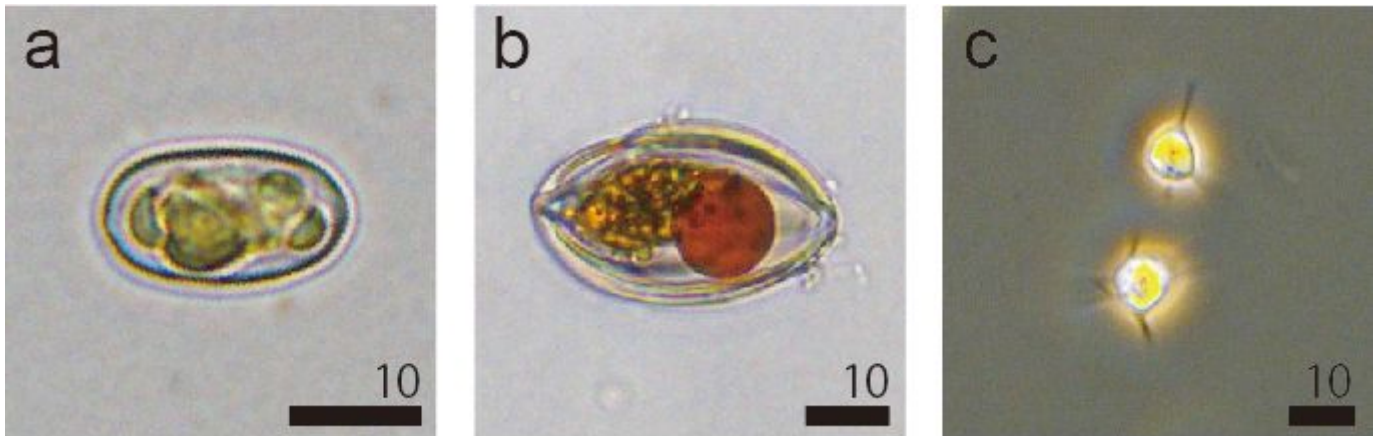


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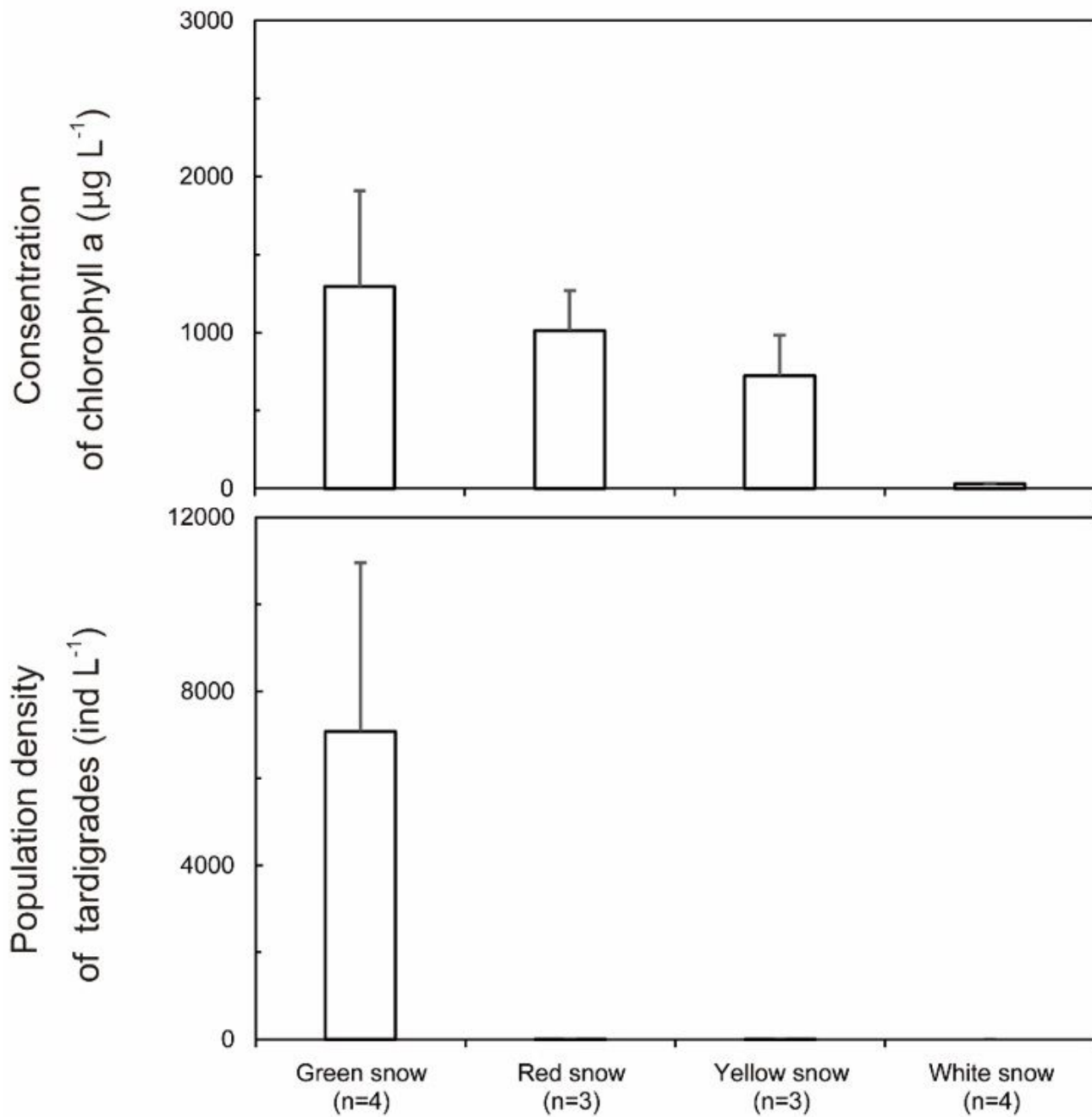


Figure 6

Population density of tardigrades and concentration of chlorophyll a in each color of snow collected in May, 2018. n: number of snow samples, error bar - standard error.

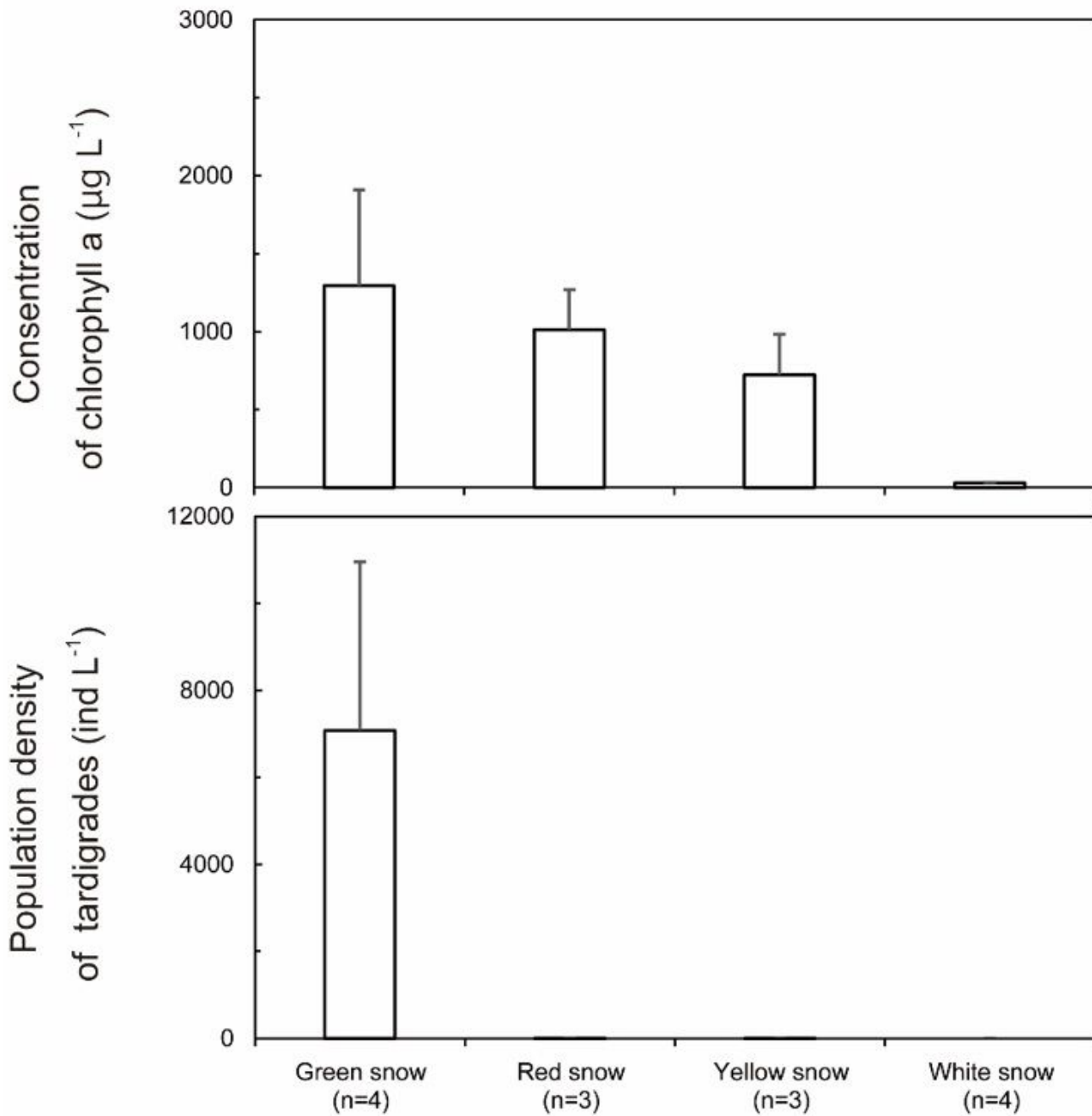


Figure 6

Population density of tardigrades and concentration of chlorophyll a in each color of snow collected in May, 2018. n: number of snow samples, error bar - standard error.

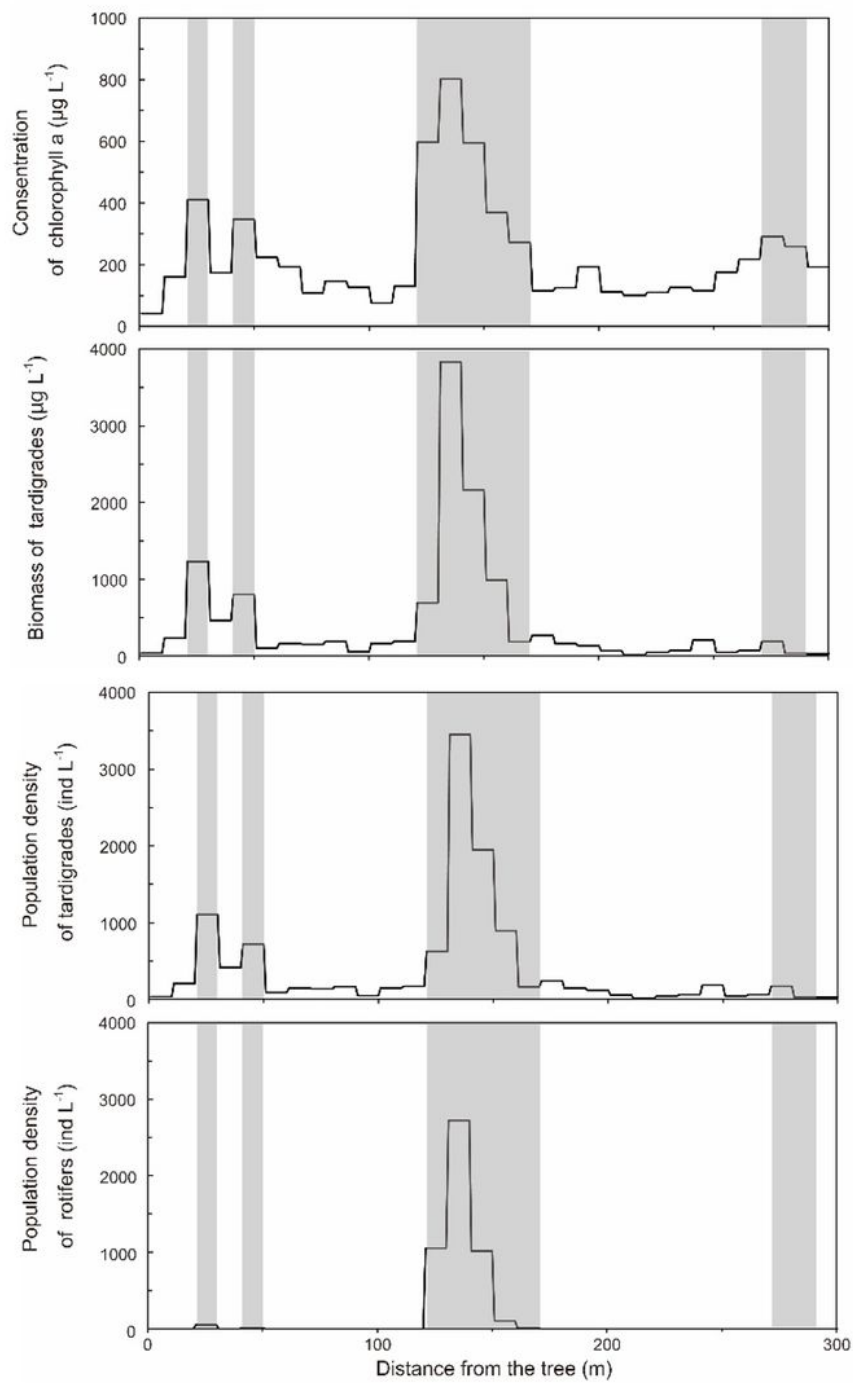


Figure 7

Concentration of chlorophyll a, biomass of tardigrades and population density of tardigrades and rotifers collected in May, 2019. Green snow is shown as gray zone.

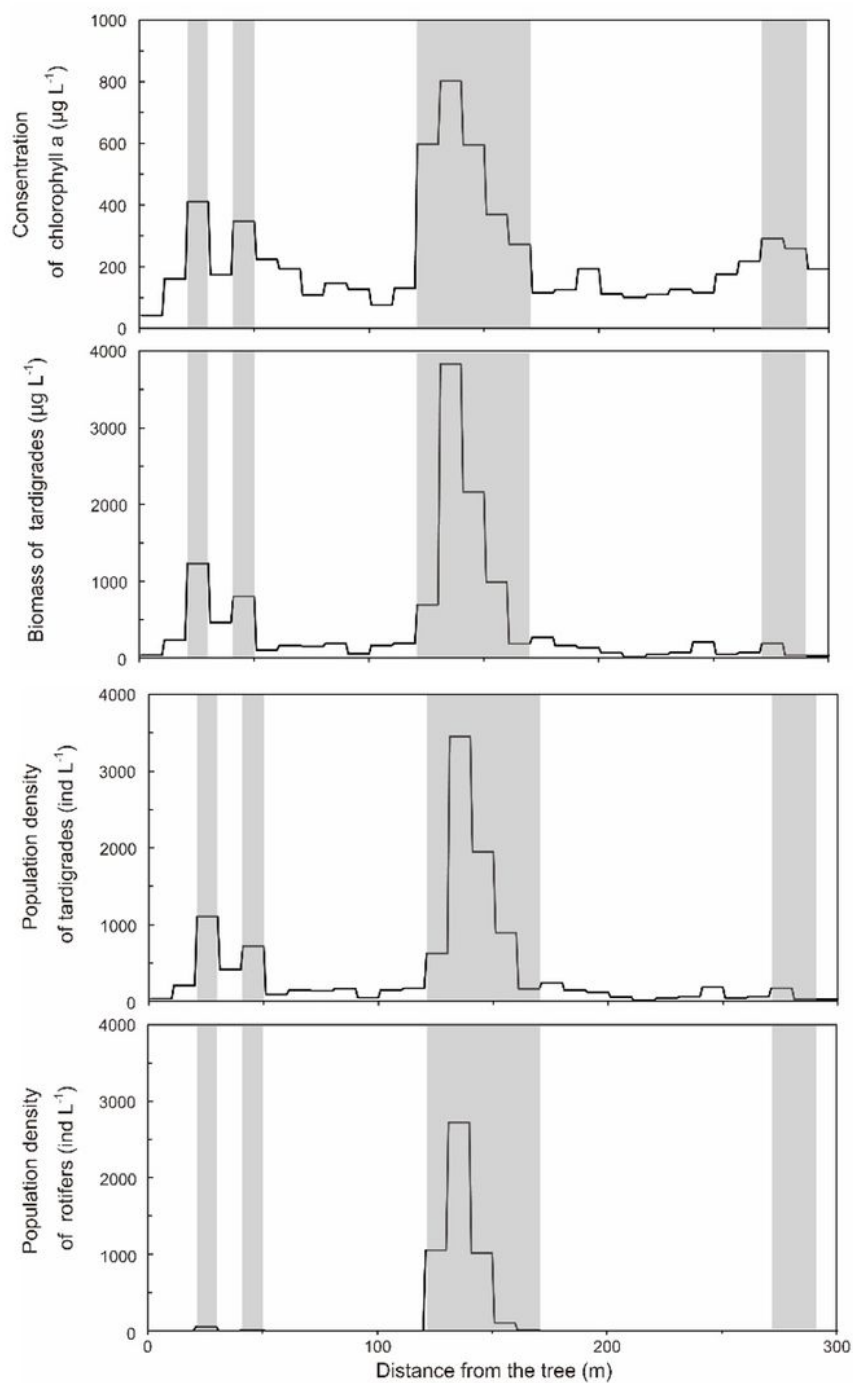


Figure 7

Concentration of chlorophyll a, biomass of tardigrades and population density of tardigrades and rotifers collected in May, 2019. Green snow is shown as gray zone.

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