European Journal of Soil Biology 60 (2014) 77–80

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Short communication

Snow fungi as a food source for micro-arthropods

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ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form
21 October 2013
Accepted 7 November 2013
Available online 19 November 2013
Handling editor: Hermann Verhoef

Keywords:
Acari
Collembola
Food web
Isotope
Winter

ABSTRACT

Snow fungi are often visibly abundant on tundra and forest understory vegetation immediately after snow melt in Nordic regions. Fungal hyphae are a common food source for many terrestrial arthropods and snow fungi could therefore be a potentially important component of an as of yet unexplored winter food web. We compared the abundance of soil arthropods (Acari and Collembola) from paired patches with and without dense infections of snow fungi in the forest understory of a northern Swedish boreal forest after snow melt. Although we did not find increased abundance of these animals when snow fungi were present, Collembola and Acari were sustained on a diet of snow fungi for six months. The isotope signature of the snow fungi clearly differed from humus and other fungal types from literature values obtained from similar boreal forests, suggesting that these fungi may occupy a novel N niche during winter in northern boreal forests. Our study shows for the first time that snow fungi are a potential food source for micro-arthropods during winter and spring. Potentially, snow fungi may represent the basis of an unexplored sub-nivean winter food web but further work is required to assess their importance for community development and winter litter decomposition.

1. Main text

Snow fungi are often observed in tundra and forest understory shrub vegetation in northern biomes immediately following spring snow melt after a period of prolonged snow cover [1–3], and often disappear within days of the snow melting. The impact of these fungi on vegetation can be very negative, resulting in crop losses [4], damage to shrubs [5] and retarded tree seedling growth [6,7]. Further, reindeer illness and even mortality can result after consumption of plants covered by snow fungi [8,9]. Our understanding of the role that snow fungi play in ecosystem processes is limited [1] but the visible abundance of considerable hyphae that can sometimes be observed suggests that they may be able to acquire large quantities of nutrients from their environment during winter [10,11]. At the same time, this hyphae abundance could provide a significant food resource for terrestrial arthropods (Collembola and Acari) that predominantly feed on fungi [12–14]. Earlier work has reported a three-fold higher abundance of Collembola in litter infected by snow fungi compared with litter that was not infected [15]. Therefore, snow fungi could potentially play a direct role in litter decomposition by breaking down organic matter, as well as an indirect role by supplying an abundant food source for faunal groups that play a key role in decomposition [16,17]. The role of snow fungi in the decomposer subsystem or as a food source for soil fauna remains unexplored, despite the fact that many ecosystem processes continue during winter [18,19].

To explore the role of snow fungi during early snow melt for micro-arthropods we studied patches that were and were not infected by snow fungi (*Typhula* spp.) in a pine forest 500 m north of the Swedish University of Agricultural Sciences in Umeå (63°49′ N, 20°15′ E), over an area of 30 m × 30 m. In this area we analysed the natural stable isotopes signatures ($\delta^{13}C$ and $\delta^{15}N$) of snow fungi growing on each of four abundantly occurring types of plant material in the area (*Pinus sylvestris* cones, *Calluna vulgaris* foliage, *Vaccinium vitis-idaea* foliage, and the moss layer consisting of *Pleurozium schreberi* and associated pine needles). For each type of plant material we collected fungi for each of 5 replicate samples immediately after snow melt during April 2012, with each replicate sample representing one plant or occupying an area of 20 cm × 20 cm of moss, and with replicate samples at least 3 m apart. For each sample, all visible fungal hyphae growing over the plant material and up to 1 cm away from it was collected, freeze dried and analysed for $\delta^{13}C$ and $\delta^{15}N$ (Elemental analyser linked to an Isotope Ratio Mass Spectrometer). Following the basic assumption 'you are what you eat' [20], we predicted that the stable isotope signature of the snow fungi should reflect that of its source, i.e., slightly enriched (1–2 units) compared to the different plant

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http://dx.doi.org/10.1016/j.ejsobi.2013.11.006

and litter sources over which it is growing [21]. One way ANOVA was used to test for isotope signature differences among the 4 substrate types.

To quantify the abundance of Collembola and Acari in snow fungi infected areas we sampled the litter + underlying soil for each of two separate sites (200 m apart) in the same forest as that from which the snow fungi were collected during April 2012. For each site we selected 9 replicate pairs of samples; one member of each pair had visible presence of snow fungi while the other member did not. To ensure the same litter components were present, the two samples for each pair were taken as close together as possible and were always between 10 and 20 cm apart; pairs of samples were always at least 2 m apart. Each litter sample consisted of the top 3 cm of the litter + soil, within a 10 cm diameter core. Each litter sample was placed in a Tullgren heat extractor [22] for one week to extract and quantify the soil arthropod community. The temperature gradient of the tullgren was from 35 °C at the top to 20 °C at the bottom. Identification of Collembola followed Fjellberg [23,24]. Acari were determined following Krantz et al. [25] into three major groups: Prostigmata (also containing any Astigmata), Oribatida and Mesostigmata. For each of the two sites, ANOVA was used to test for the effects of snow fungi presence versus absence on the abundance of species and groups of arthropods, with the 9 pairs of samples serving as replicate blocks. We used a Principal Component Analyses (PCA) on the Collembola species data to detect differences in community assembly in the presence or absences of snow fungi.

Five additional paired (fungal infected and uninfected) litter samples were collected from one of the two sites and extracted over water so that arthropods could be used for stable isotope analyses; individuals of these arthropods, were, after identification, immediately freeze-dried for isotope analyses [26]. We performed $\delta^{15}N$ and $\delta^{13}C$ analysis of each of two taxa, i.e., *Lepidocyrtus lignorum* and Phthiracaridae, and because of the limited amount of material available had to pool samples so that for both infected and uninfected litter we had $N = 2$ and 3 independent replicates for *L. lignorum* and Phthiracaridae respectively. The stable isotope signatures of arthropods should reveal if they feed on snow fungi, i.e., if they are enriched in $\delta^{15}N$ and $\delta^{13}C$ compared to the fungi [27,28]. We used a two way ANOVA to test for the effects of micro-arthropod taxa and the presence versus absence of snow fungi on the stable isotope signatures. The inclusion of $\delta^{13}C$ has proven to be a useful measure in separating potential food sources for arthropods in addition to $\delta^{15}N$ [29,30].

To observe if the micro-arthropods would feed from and survive on a diet of snow fungi, we presented Collembola and Acari with snow fungal hyphae (a bundle of hyphae of 2 cm × 2 cm × 0.5 cm) on top of moist filter paper in three 4 cm diameter petri-dishes, each containing a different arthropod species − 1: *Folsomia quadrioculata* (one individual); 2: *Entomobrya nivalis* and *Tomocerus* sp. (one and two individuals respectively); and 3: a mixture of Phthiracaridae and Brachypylina (five individuals of each). A new bundle of fresh hyphae was added to each petri dish when all hyphae were consumed by micro-arthropods. Petri-dishes were kept in the dark in a refrigerator at 6 °C. Feeding activity of arthropods was monitored twice a month by microscope from the start of May until the end of October, and the presence of fecal pellets and disappearance of snow fungi was recorded.

The abundance of micro-arthropods was not affected by the presence of snow fungi in the field (P always > 0.05 according to ANOVA; Table 1) suggesting that micro-arthropods did not aggregate towards snow fungi. Further, there was no evidence for a specific snow fungi Collembola community (Fig. 1). The stable isotope ratios indicate that two dominant micro-arthropods, *Lepidocyrtus lignorum* (Collembola) and Phthiracaridae (Oribatida), did

Table 1
Soil arthropods from litter with and without presence of snow fungi in a boreal forest understory after snow melt in early spring. There were no significant differences in abundance between snow fungi infected and non-infected areas for any of the individual species or groups according to ANOVA (P always > 0.05). Values are mean abundance ($n = 9$) per litter sample (individuals per 78.54 cm^2) with SE between brackets.

	Site 1		Site 2	
	Snow fungi presence		Snow fungi presence	
	Yes	No	Yes	No
Collembola				
Eudaphic (total)	29.0 (10.8)	30.8 (8.9)	39.3 (8.4)	67.6 (27.7)
Isotomiella minor	5.7 (3.0)	12.3 (5.2)	27.3 (7.8)	58.0 (26.5)
Megalothorax minimus	0.3 (0.3)	0	0	0.1 (0.1)
Onychiurinae	18.1 (9.6)	12.9 (3.0)	7.6 (2.6)	9.2 (1.8)
Paranura sexpunctata	0	0	0.3 (0.2)	0.2 (0.2)
Tullbergiinae	4.9 (2.2)	5.6 (3.6)	4.0 (3.8)	0
Xenyllodes armatus	0	0	0.1 (0.1)	0
Hemidaphic (total)	8.7 (3.5)	7.2 (2.3)	66.7 (16.6)	60.8 (27.6)
Anurophorus sp.	3.9 (1.5)	5.3 (2.1)	3.9 (2.5)	4.1 (2.9)
Folsomia quadrioculata	0	0	32.2 (11.4)	39.2 (25.9)
Friesea sp.	1.1 (1.1)	0.6 (0.3)	25.6 (7.8)	15.4 (5.3)
Neanura muscorum	0.1 (0.1)	0.1 (0.1)	1.6 (0.8)	1.2 (0.5)
Parisotoma notabilis	3.6 (1.5)	1.2 (0.8)	3.4 (1.4)	0.8 (0.4)
Epidaphic (total)	7.2 (1.0)	11.6 (3.3)	43.1 (22.0)	28.9 (15.3)
Dicyrtoma fusca	0	0	0.1 (0.1)	0
Isotoma viridis	0.8 (0.7)	0.9 (0.7)	1.2 (0.4)	1.2 (0.7)
Isotomurus sp.	0.4 (0.3)	0.3 (0.3)	0	0
Lepidocyrtus lignorum	4.7 (0.8)	9.7 (3.4)	33.0 (19.8)	18.9 (11.6)
Orchesella bifasciata	0.2 (0.1)	0.3 (0.2)	0.1 (0.1)	2.1 (1.0)
Sminthurus viridis	1.1 (0.9)	0.3 (0.2)	7.9 (3.7)	5.0 (2.5)
Tomocerus sp.	0	0	0.8 (0.4)	1.7 (0.7)
Total	44.9 (10.9)	49.6 (12.0)	149.1 (39.8)	157.2 (45.0)
Acari				
Prostigmata	101.2 (35.8)	180.3 (95.7)	197.2 (40.4)	166.7 (50.0)
Oribatida	137.1 (76.2)	105.3 (31.7)	676.0 (228.2)	488.0 (91.5)
Mesostigmata	7.1 (3.6)	13.4 (5.1)	10.9 (3.6)	14.0 (4.2)
Total	245.4 (96.1)	299.1 (125.6)	884.1 (259.8)	668.7 (118.7)

show somewhat lower values for $\delta^{15}N$ (ANOVA: $F_{1,6} = 6.4$, $P = 0.045$) when collected from litter infected by snow fungi, and these values were closer to the more negative $\delta^{15}N$ values of the snow fungi (Fig. 2). However, $\delta^{13}C$ values were not affected in the arthropods by the presence of snow fungi (ANOVA: $F_{1,6} = 0.3$, $P = 0.648$). Laboratory observations showed numerous (i.e., hundreds of) black fecal pellets visible among the snow fungal hyphae in the Petri dishes with Oribatid mites and Collembola, strongly suggesting that they had been feeding on the snow fungi. Indeed, snow fungal hyphae were completely consumed within 4 weeks by *Entomobrya nivalis* and *Tomocerus* sp., and any new fungal hyphae presented during the feeding trial were also rapidly consumed. *Tomocerus* sp. remained alive on this diet for at least 6 months. *Folsomia quadrioculata* had grown in size and undergone at least two molting stages in the feeding trial. The feeding observations indicate that snow fungi are a potential source of food for micro-arthropods during winter under the snow layer, and spring until shortly after snowmelt. The $\delta^{15}N$ signature provides some support for this as well but the $\delta^{13}C$ signatures indicate that snow fungi are definitely not the only food source, as the difference between snow fungi and the micro-arthropods is too high to encompass one trophic step [31]. This is in accordance with the recognized generalist feeding preference of these animals [29,32,33].

Marginally non-significant differences in stable isotope composition were found between the snow fungi collected from the four sampled types of plant material, i.e., pine cones, *Calluna vulgaris*, *Vaccinium vitis-idaea* and the moss layer (ANOVA ^{15}N: