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ORIGINAL ARTICLE

Warm, rainy winter onset increases the risk of hard, icy snow layers and the occurrence of mycotoxins in reindeer winter pastures

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Abstract

When thick wet snow covers unfrozen ground at the beginning of winter, herders fear the development of a hard, icy bottom snow layer and the appearance of noxious moulds (microfungi) in semi-domesticated reindeer pastures. Such winter onsets were experienced in 2019 and 2021 in the reindeer herding area of Finland, after which significant reindeer losses, along with collapses in calf production and slaughter animals, were encountered. We studied the development of weather and snow conditions in the late autumn and early winter of 2021–2022 and measured snow conditions in March 2022 in 11 reindeer cooperatives. We also collected samples from reindeer winter forage plants for mycotoxin analysis. We found that the weather and snow conditions during the late autumn and early winter of 2021 caused the formation of a hard, icy bottom snow layer and the development of mycotoxins in pastures. Alternariol (AOH) and alternariol monomethyl ether (AME), produced by *Alternaria* spp., were found in all 33 samples (104–2562, 61–808 µg/kg DM) and zearalenone (ZEN) by *Fusarium* spp. in 16 samples (14–206 µg/kg). Certain significant correlations in the concentrations of mycotoxins with snow conditions and ground surface temperatures were found. We assume that besides difficult grazing conditions in the winters of 2019–2020 and 2021–2022, the presence of mycotoxins in pastures has contributed to reindeer losses and reduced the body condition, health, and reproduction of reindeer. As onsets of winters become warmer and rainier, the risk of similar pasture conditions in reindeer herding may increase.

Keywords Reindeer · Pastures · Snow conditions · Mycotoxins · Body condition · Herd productivity

Introduction

In recent decades, the temperatures and precipitation in the Arctic region have increased considerably (Serreze and Barry 2011; Yu and Zhong 2021; Rantanen et al. 2022). Arctic seas store warmth and remain unfrozen much longer than previously, leading to increased evaporation from these water systems and therefore higher air humidity in northern areas, especially in the autumn and early winter (Liu et al. 2012; Bailey et al. 2021; Sato et al. 2022). This results in rainier and warmer autumns and early winters in the Arctic, including the northern parts of Fennoscandia

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(Bintanja 2018). Furthermore, it has been observed that despite the warming trend in autumns and winters, the relatively early arrival of snow in late autumns has occurred in large areas of northernmost Eurasia in recent decades (Dauginis and Brown 2021).

The early arrival of snow was experienced in the late autumns of both 2019 and 2021 in the semi-domesticated reindeer (*Rangifer tarandus tarandus*) herding area of Finland (Kumpula et al. 2020 and 2022), where c. 200,000 semi-domesticated reindeer graze on natural pastures covering a land area of 114,000 km^2. In both years, a thick and wet snow layer already covered an unfrozen and still warm ground in mid-October. After this, alternating frost periods and warm weather with rain-on-snow events transformed the wet snow into a hard, icy bottom layer over the pasture vegetation (Kumpula et al. 2020 and 2022). This made it very difficult for reindeer to forage through the snow, particularly as more snow accumulated later in the winter over this bottom layer.

Ground lichens (*Cladonia* spp.), dwarf shrubs (*Empetrum* sp., *Vaccinium* spp., *Calluna* sp.), sedges (*Carex* spp.), and hays (*Dechampsia* and *Festuca* spp.) form the most important forage plants for reindeer. If plenty of *Cladonia* lichens are available on pastures, they are mainly used by reindeer. However, in the early winter and when the availability of ground lichens is low, dwarf shrubs, sedges, and grass hays comprise the main part of the reindeer diet (Kojola et al. 1995; Kumpula et al. 2015). To graze on these food plants, reindeer must dig craters through the snow, and snow conditions (depth, density, and hardness of snow) therefore considerably affect food availability. In turn, this significantly affects reindeer's body condition and productivity (Kumpula 2001; Kumpula and Colpaert 2003; Helle and Kojola 2008).

In addition to causing problems with food availability for reindeer due to hard, icy snow, rainy autumns and early and wet snow can also create suitable growth conditions for various moulds (microfungi) on the pasture vegetation (Kumpula et al. 2000; Rasmus et al. 2018). This may especially be the case when the ground is still unfrozen and warm, and the temperature is above freezing beneath the snow, with a lot of moisture. In the plant samples collected in such pasture conditions in the winter of 1996–1997 from reindeer winter pastures in northernmost Finland, the microfungi *Mortierella* and *Penicillium* spp., *Trichoderma viride*, *Mucor hiemalis*, and *Leptothyrium* sp. especially were observed to appear plentifully (Kumpula et al. 2000). Some of these microfungi are also known to produce mycotoxins, although the previous study did not investigate the occurrence of mycotoxins in reindeer pasture plants.

During previous kinds of winters, reindeer herders have traditionally feared the formation of noxious moulds (microfungi) on pasture vegetation (e.g. Helle 1980). Although such snow and grazing conditions have occurred relatively rarely in the past, reindeer herders are very familiar with them. They describe these conditions by saying that the "bottom of winter pastures" has developed badly for reindeer foraging (Helle 1980; Kumpula et al. 2000; Turunen et al. 2016; Rasmus et al. 2018). Herders believe that in addition to difficult snow conditions, moulds that develop in these pasture conditions can create significant health risks for reindeer, even leading to their deaths. They have also observed that reindeer tend to avoid grazing in areas where moulds are appearing. The last observation was also supported by the study of Kumpula et al. (2000).

During 2019–2020, over 20,000 adult reindeer were reported to be lost (Finnish Food Authority 2021). In addition, in the subsequent slaughter season, the average calf percentage decreased from 61 to 45%, and the number of slaughtered reindeer decreased from 83,700 to 56,900 animals (Statistics of Reindeer Herders' Association 2021). Furthermore, the total size of the next winter breeding stock decreased by 12,700 reindeer. Similarly, snow and grazing conditions were very difficult for reindeer in the northern parts of the reindeer herding area at the beginning of the winter of 2021–2022 (Kumpula et al. 2022). In the late winter of 2022, bone marrow samples from several hundred dead reindeer were collected by the Finnish Food Authority, but according to the reindeer herders, these data constituted only small parts of real reindeer losses.

As the winters of 2019–2020 and 2021–2022 showed, it is crucial to obtain more knowledge and a deeper understanding of the factors which promote and cause the formation of such conditions in reindeer pastures. The study's objectives were to investigate the development of snow and grazing conditions for reindeer and the potential occurrence of mycotoxins in different kinds of reindeer pastures in the herding area of Finland in the winter of 2021–2022. We especially studied which factors promoted the development of difficult snow and grazing conditions in the late autumn and early winter and promoted the appearance of mycotoxins in pasture plants. We also discuss how such grazing conditions with an appearance of mycotoxins can affect reindeer's body condition and health.

Material and methods

Temperature, precipitation, and snow grid data

Grid data (1 × 1 km) provided by the Finnish Meteorological Institute (FMIClimGrid, https://en.ilmatieteenlaitos.fi/gridded-observations-on-aws-s3) were used to study weather and snow conditions in the autumn and early winter of 2021–2022. First, we compared the average temperature and precipitation in October 2021 to the average corresponding values in the Octobers of 2009–2018 in the reindeer herding area. We then examined the development of daily temperatures, precipitation, and snow depth during October–November 2021 in different parts of the herding area. We also compared the snow depth development in October–November 2021 with the average snow depth developments in the October and Novembers of 2009–2018. We chose this period because the development of snow conditions in the onsets of these winters represented a relatively typical development, and very difficult winters for reindeer were not experienced during this period.

Field site measurements and forage plant samples

Snow and grazing conditions were studied on 33 field sites in the natural winter pastures in 11 reindeer herding cooperatives in northernmost Finland (Fig. 1) between 7 and 22 March 2022. Of these, 22 sites were in dry pine forests, five in dry mountain birch forests, and six in high-elevation open mountain heaths. The studied field sites were selected in an area covering several hectares by homogenous pasture type. With local reindeer herders, we ensured that the field sites were located in pastures and areas where reindeer typically graze during the winter. On each field site, five snow pits of around one square metre were dug in straight lines with 50-m intervals to measure the snow parameters. The measurements included the number (n), height (cm), and vertical hardness (from up to down, g/cm^2) of each clearly identified snow layer, depth (cm), density (kg/m^3), and weight (kg/m^2) of the total snow cover, and finally, the ground surface temperature (°C) beneath the snow. In the last measurement, a long thin thermometer sensor was pushed through the snow on the surface of the ground. The measurements were performed using the methods described by Kumpula and Colpaert (2007). The presence of ice crust or granular ice layers over the pasture vegetation at the bottom part of the snow was also observed.

Available reindeer forage plants were collected from each snow pit. Later, all five samples from each site were combined into a single sample and stored in a freezer to await mycotoxin analyses. The pooled plant samples consisted of ground lichens (mainly *Cladonia* spp.) and dwarf shrubs (mainly *Empetrum nigrum*, *Vaccinium vitis idea*, *Vaccinium myrtillus*, and *Calluna vulgaris*), along with a few grasses and sedges (*Deschampsia* and *Carex* spp.). We pooled the forage plants from the same field site and did not separate different plants in the samples, as this was the only way to obtain the minimum required plant amount per sample for mycotoxin analyses. Additionally, natural reindeer winter forage consists of a mixed composition of ground lichens, dwarf shrubs, sedges, and grass hays (Kumpula et al. 2015).

It was impossible in March to document the actual snow and pasture conditions that existed during the autumn and at the beginning of winter, when the mycotoxins probably developed in the pastures. During the winter, snow accumulation and air temperatures also caused snow metamorphoses that changed snow depth and structure, as well as the

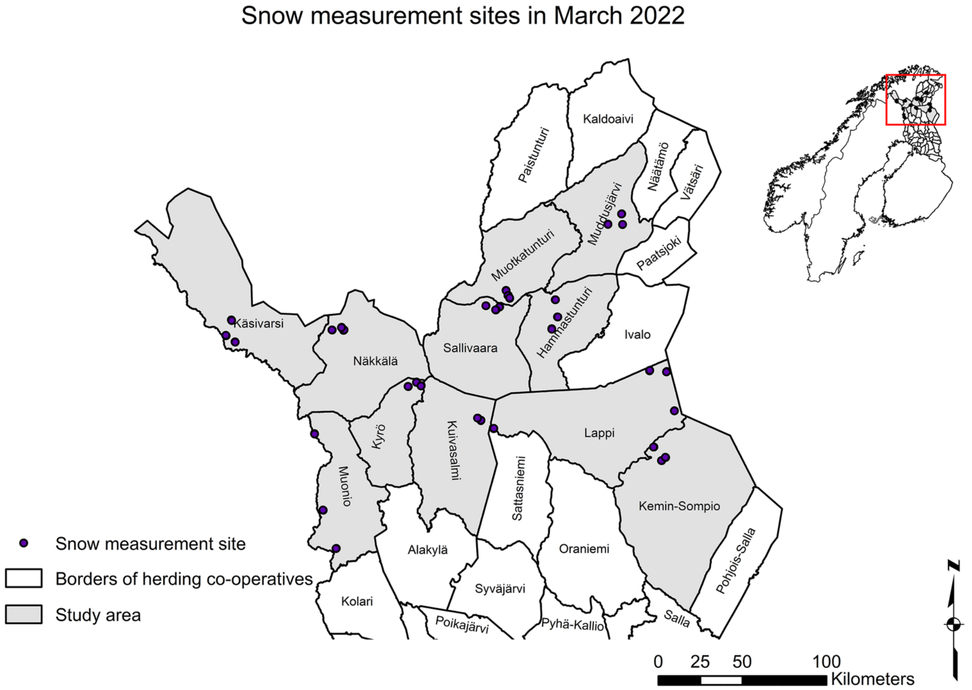

Fig. 1 Field sites ($n = 33$) in the winter pastures of 11 reindeer herding cooperatives (grey) in northernmost Finland. The measurements of snow conditions and reindeer food plant collection were made between 7 and 22 March 2022. Finland's entire reindeer herding area presented in a small map

ground surface temperature. Although our measurements cannot fully capture the actual situation at the beginning of the winter of 2021–2022, they are still a good indicator of the development of the snow and pasture conditions at the onset of winter.

Mycotoxin analyses

Reindeer forage samples were analysed at the laboratory of the Natural Resources Institute Finland (Luke). Currently, it is possible to analyse 33 different mycotoxin compounds in the feed material of ruminants in Luke's laboratory by using the method based on ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS/MS) (Rämö et al. 2020; Manni et al. 2022). These mycotoxins are produced by microfungi belonging to the genera *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* (Table 1).

All 33 fresh frozen pooled samples were weighed and then freeze-dried and reweighed, after which the percentage (%) of dry matter was calculated in each sample. All samples were then homogenised for the mycotoxin analyses. Then the plant samples were analysed according to Manni et al. (2022), with some modifications: mycotoxins were separated by a Waters Acquity UPLC HSS T3 column (1.8 µm, 2.1 × 100 mm) in a gradient run (18 min), in which solvent A was 0.1% formic acid in the water, and solvent B a mixture of acetonitrile and methanol (1:1) (Han et al. 2010). The gradient run was started with 100% of solvent A to detect the most polar mycotoxins (e.g. moniliformin). The ratio of solvent B was increased during the run until 100% of it was reached to separate the most nonpolar compounds (enniatins). Both solvent and matrix-matched calibrations were prepared for quantification of mycotoxin concentrations in freeze-dried samples (µg/kg dry matter). A grass silage sample analysed earlier by Manni et al. (2022) and observed free from the 33 mycotoxins given in Table 1 was used for matrix calibration.

Most of the analysed mycotoxins (23 compounds) were quantified in a filtered raw extract with a multi-point matrix calibration in grass silage extract. If zearalenone (ZEN) was detected in a sample, a second extraction was performed to confirm the result. All the results were confirmed by recovery tests with the same concentrations as in grass silage calibration (six concentration levels) in the reindeer food sample, in which the concentrations of detected mycotoxins were lowest. The recovery test results showed that the grass silage matrix significantly suppressed the MS signal of alternariol (AOH) compared with the solvent calibration, and finally, a multi-point solvent calibration was needed to quantify it.

Raw extracts of the samples were cleaned by dispersive solid phase extraction (dSPE) enriched and dissolved in a water-acetonitrile solution (1:1; v:v; 0.1% formic acid) for the quantification of patulin, moniliformin, and eight trichothecenes (see Table 1) with similarly prepared silage matrix calibration according to Manni et al. (2022) with some modifications: Mycotoxins were separated by a Waters Acquity UPLC HSS T3 column and run with the first UHPLC gradient (Han et al. 2010). These samples were also run with a second gradient run, in which solvent A was 5 mM ammonium acetate in water with 0.1% of acetic acid, and solvent B was methanol, to confirm the patulin and deoxynivalenol results (Braun et al. 2018). Moniliformin's MS signal was too weak to be detected in these samples.

Data handling and statistical methods

The data handling and analyses followed the SAS Enterprise Guide 7.1. For the depth (cm), density (kg/m^3), weight (kg/m^2), and hardness (g/cm^2) of the total snow cover, as well as the hardness (g/cm^2) of the bottom snow layer and ground surface temperature (°C) beneath the snow the Mean, Md, Min, Max, and SD values were calculated in different winter pasture types. The differences in the measured parameters between pasture types were analysed by one-way ANOVA with Tukey's pairwise comparisons. The mean, Md, Min, and Max concentrations (µg/kg dry matter) of found mycotoxins were then calculated in various winter pasture types. The statistical differences in these concentrations between pasture types were then analysed by the Kruskall-Wallis test with Tukey's pairwise comparisons. Finally, Spearman rank correlations were calculated between the different measured snow parameters and the concentrations of observed mycotoxins across all field sites.

Table 1 The studied 33 mycotoxins produced by different microfungi, of which analyses were made for the plant samples in Luke's laboratory with ultra-high performance liquid chromatography–mass spectrometry (UHPLC-MS/MS)

Microfungi species	Analysed mycotoxins (33 compounds) in the reindeer food plant samples
Alternaria spp.	Alternariol, alternariol monomethyl ether, and tenuazonic acid
Aspergillus spp.	Aflatoxins B1, B2, G1, G2, ochratoxin A, sterigmatocystin, and cyclopiazonic acid
Fusarium spp.	Beauvericin, enniatins A, A1, B, B1, fumonisins B1, B2, B3, moniliformin, zearalenone and trichothecenes: deoxynivalenol, 3- and 15 -acetyl deoxynivalenols, diacetoxyscirpenol, fusarenon X, HT-2-toxin, nivalenol, and T-2-toxin
Penicillum spp.	Mycophenolic acid, ochratoxin A, patulin, penicillic acid, roquefortine C, citrinin, and cyclopiazonic acid

Results

Development of weather and snow conditions in early winter 2021–2022

According to the data produced by the Finnish Meteorological Institute, October 2021 was slightly warmer in the southern and middle parts of the reindeer herding area than the average October temperatures during 2009–2018 (Fig. 2). However, the total precipitation in October 2021 was considerably higher throughout the herding area than the average October precipitation in 2009–2018 (Fig. 3). Moreover, the first snow depth in October 2021 was significantly deeper than the average snow depth in the Octobers of 2009–2018, and the permanent snow came early (Fig. 4). Typically, there is less than 10 cm of snow at the end of October in the reindeer herding area, but especially in the north-western part of the herding area, a much deeper wet snow layer covered the ground in mid-October. By the end of October, the average snow depth in this area increased to 40 cm (Fig. 4).

The first snow was very wet and covered unfrozen ground. However, on 20 and 25 October, cold days changed the wet snow into a very hard snow layer over the vegetation in pastures (Fig. 4). The snow layer then also increased and was deepest on 30 October. Due to warm and rainy days again on 2–5 November, the snow again became wet, and it partly melted. Especially in the southern part of the herding area, the snow had nearly completely melted. However, in the northern and especially in the north-western parts of the herding area, a 10–20-cm-thick layer of wet snow remained on the pastures. It was frozen again after 5 November, forming a hard and icy cover over the pastures (Fig. 4).

Snow and pasture conditions in late winter 2021–2022

Based on our field data for March 2022, the measured average snow hardness was 1437–9847 g/cm^2 (Table 2). The bottom layer of snow especially was icy and very hard (5859–12,632 g/cm^2) on nearly all measurement sites, indicating that the very wet snow in the early winter had frozen and formed a hard and icy layer over the ground that persisted throughout the winter (Table 2). All ground surface temperatures were below zero in the March measurements, with the lowest temperatures occurring on mountain heaths, and the highest in pine forests. It is very likely that these ground temperatures fell from degrees above zero to degrees below zero when the winter progressed.

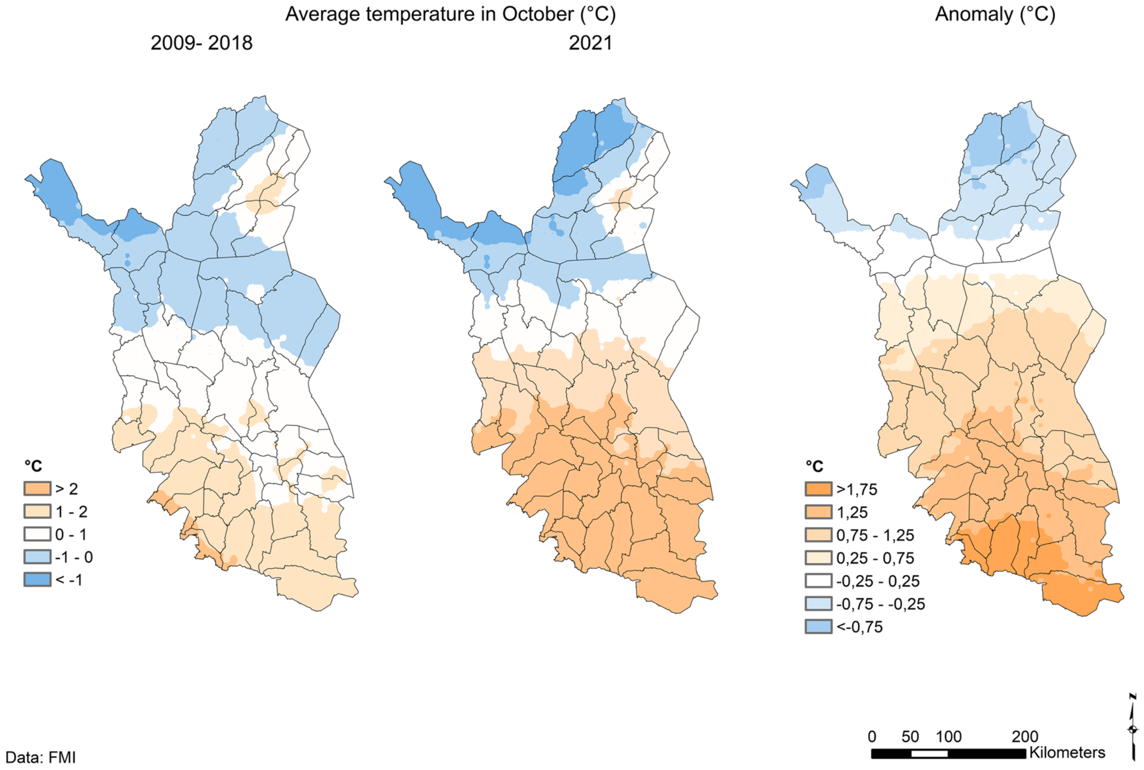

Fig. 2 Average October temperature (°C) during 2009–2018 and in 2021 in the reindeer herding area. Map on the right: Anomalies calculated by comparing the average temperature in October 2021 with the average October temperature in 2009–2018. Data: Finnish Meteorological Institute

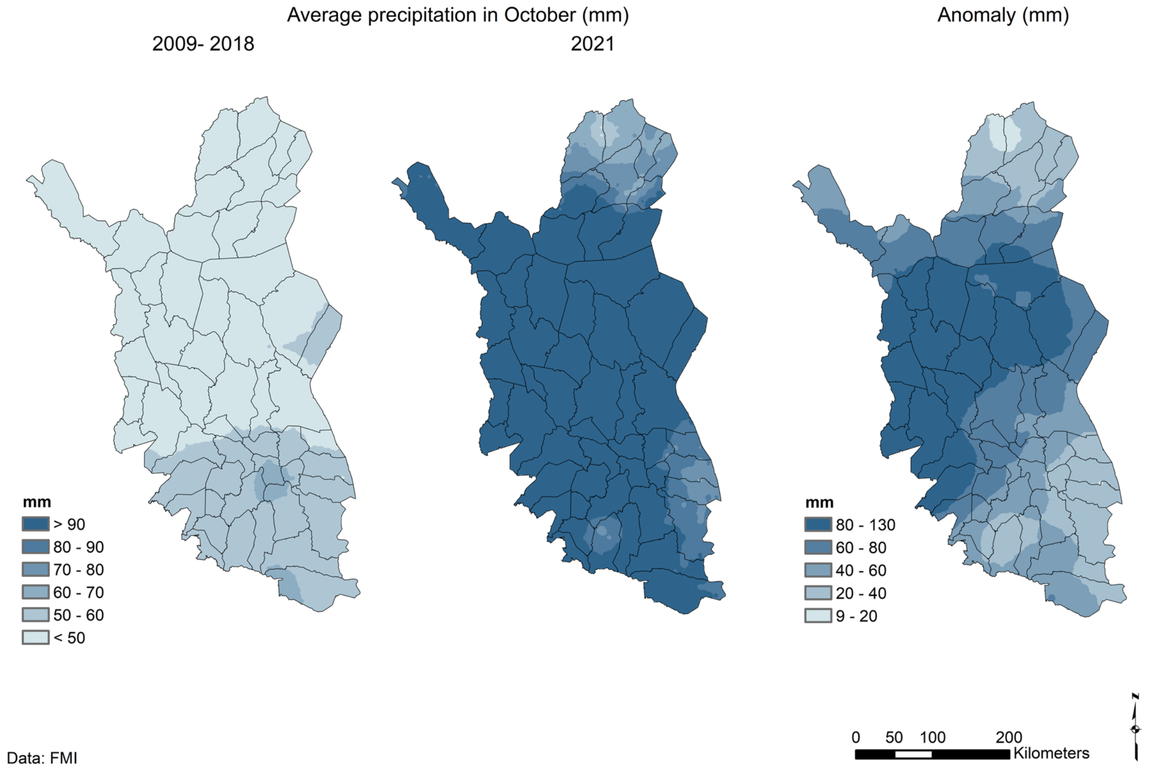

Fig. 3 Average October precipitation (mm) during 2009–2018 and in 2021 in the reindeer herding area. Map on the right: Anomalies calculated by comparing October 2021 precipitation with the average October precipitation in 2009–2018. Data: Finnish Meteorological Institute

On the field sites, the mean depth of the bottom snow layer varied between 11.2 and 19.6 cm, and it was very hard and icy (Table 2). Its mean hardness varied between 5158 and 12,632 g/cm^2. However, there were no statistical differences between the pasture types in the bottom snow depth and hardness. In contrast, the statistical differences between pine forests/mountain birch forests versus open mountain heaths were observed in the total depth ($P = 0.008$), density ($P < 0.001$), hardness ($P < 0.001$), and layer numbers ($P = 0.026$) of the snow, as well as ground surface temperatures ($P < 0.001$).

Concentrations of mycotoxins in the winter forage plant samples

Alternariol (AOH) and alternariol monomethyl ether (AME) were found in all 33 samples. The measured concentrations of AOH in the plant samples varied between 104 and 2562 µg/kg (Table 3). Although its average concentrations did not differ statistically significantly between pasture types ($P = 0.063$), its mean values, 998 µg/kg in mountain birch forests, 671 µg/kg in pine forests, and 280 µg/kg on open mountain heaths, indicated some differences. Respectively, the measured concentrations of AME in all samples varied between 61 and 808 µg/kg, and there were no significant differences in its concentration between pasture types ($P = 0.152$), with the variation of mean values 250–345 µg/kg.

The mycotoxin zearalenone (ZEN) was detected in 16 samples, with values of 14–206 µg/kg (Table 3). When all 33 observations were included in the test, the concentration varied significantly between pasture types ($P < 0.001$). All samples from open mountain heaths contained ZEN, with an average of 73 µg/kg. In the mountain birch forests, the average concentration of ZEN was 27 µg/kg and in pine forests 21 µg/kg respectively (including zero value samples). According to Tukey’s test, the concentration of ZEN differed only between open mountain heaths and pine forests ($P < 0.001$).

Relationships between measured pasture conditions and mycotoxins

The concentration of AOH in the plant samples was positively and significantly correlated with the total snow depth ($P = 0.005$) and weight ($P = 0.027$), as well as with the ground surface temperature under the snow ($P = 0.007$). Additionally, there was a significant negative correlation between the AOH concentration and total snow hardness

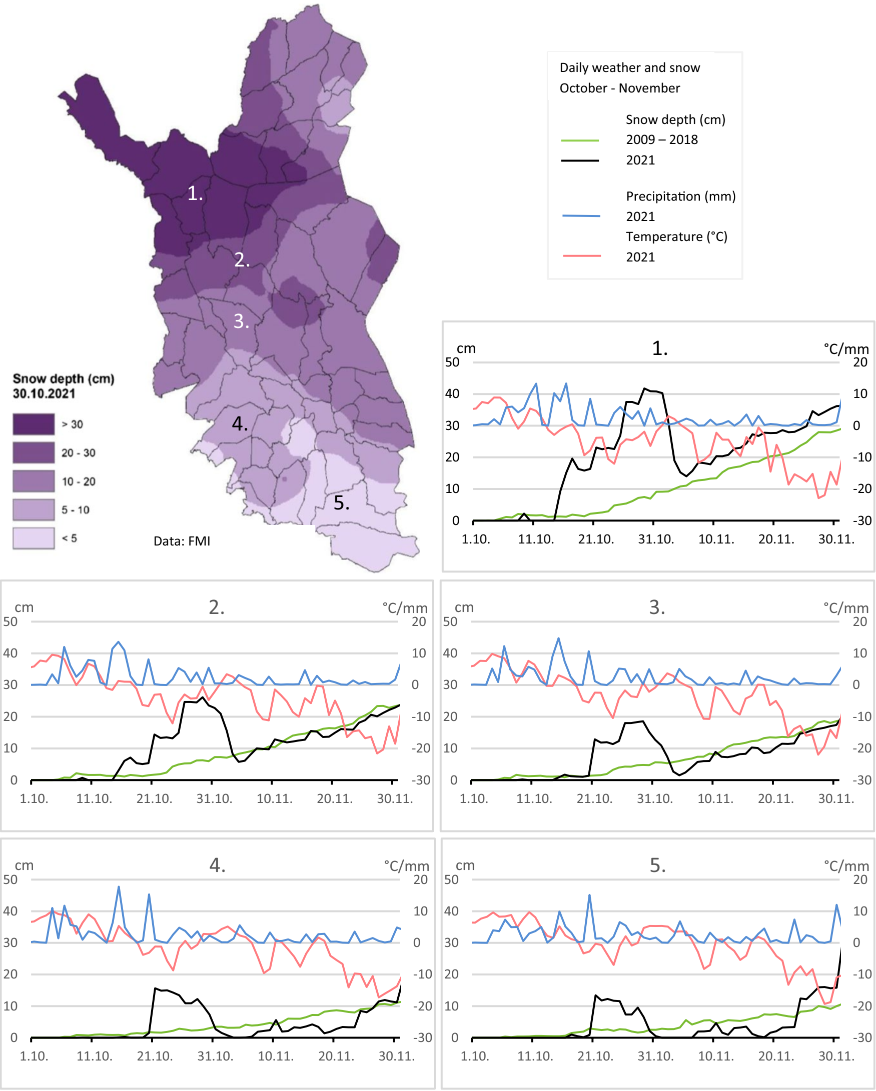

Fig. 4 Average snow depth (cm) in different parts of the reindeer herding area on 30th October 2021 (large map) and the developments of the daily mean temperature (°C), precipitation (mm), and snow depth (cm) during October–November 2021 in different areas (separate graphs). Developments of average daily snow depth in the same period and in the same areas in 2008–2018 are also shown. Data: Finnish Meteorological Institute

($P = 0.013$), but the correlation between the AOH concentration and the bottom snow hardness was a little over the significant level ($P = 0.065$) (Table 4). The AME concentration showed no significant correlations with the measured parameters. The ZEN concentrations were positively and significantly correlated with snow density ($P = 0.001$) and total snow hardness ($P = 0.010$) and negatively with ground surface temperature ($P = 0.009$). The ZEN concentration also had a nearly significant and negative correlation with the number of snow layers ($P = 0.051$).

Discussion

A very hard icy bottom snow layer appeared on reindeer pastures at the beginning of the 2021–2022 winter. In the measurements in March 2022, its hardness (5859–12,632 g/cm^2) was much harder than in earlier studies (50–600 g/cm^2) made at the same time during a typical winter (Kumpula et al. 2015). This made it very difficult for reindeer to dig through the snow to access forage, especially as more snow accumulated later on the pastures. Despite intensified supplementary feeding in pastures, many reindeer herding cooperatives, especially in the northern part of the herding area, reported poor body condition and health and nutrition problems in reindeer. Furthermore, hundreds of reindeer losses were also reported during the winter of 2021–2022 (Kumpula et al. 2022).

We also identified three different mycotoxins (AOH, AME, and ZEN) produced by the *Alternaria* and *Fusarium* species in the pasture plants collected from beneath the snow on these field sites. Only Burkin and Kononenko (2011) have previously studied the occurrence of mycotoxins in reindeer forage plants. In reindeer lichen (*Cladonia* sp.) samples collected from the Murmansk and Karelia areas of Russia between the autumn and late winter of 2009–2010, they

Table 2 Mean, SD, and statistical differences in one-way ANOVA in the measured snow parameters and ground surface temperature under the snow on field sites ($N=33$) in different pasture types in 11 reindeer herding cooperatives in northernmost Finland. Each field site had five measurement points

Measured parameter	Pine forest ($n=22$)		Mountain birch forest ($n=5$)		Open mountain heath ($n=6$)		One-way ANOVA	
	Mean	SD	Mean	SD	Mean	SD	*P*-value	Tukey's test
Snow depth (cm)	72.0	8.9	71.6	26.9	52.7	6.7	*0.008*	*pf > omh; mbf > omh*
Snow density (kg/m^3)	219.7	20.5	234.4	15.3	287.8	34.1	*< 0.001*	*pf < omh; mbf < omh*
Snow weight (kg/m^2)	158.4	26.9	167.0	62.9	151.1	16.8	*0.729*	-
Total snow hardness (g/cm^2)	1437	1215	2728	3610	9847	5476	*< 0.001*	*pf < omh; mbf < omh*
Bottom snow depth (cm)	18.1	11.2	19.2	9.7	19.6	5.0	*0.936*	-
Bottom snow hardness (g/cm^2)	8101	8332	5859	5158	12,632	6992	*0.325*	-
Number of snow layers (n)	4.1	0.5	3.8	0.4	3.4	0.4	*0.026*	*pf > omh*
Ground surface temperature (°C)	−0.6	0.5	−1.0	1.3	−2.5	1.1	*< 0.001*	*pf > omh; mbf > omh*

pf, pine forest; *mbh*, mountain birch forest; *omh*, open mountain heath; < and > refer to the direction of statistical difference between pasture types by < 0.05 significance in Tukey's pairwise comparisons

Table 3 Concentrations of three observed mycotoxins (μg/kg dry matter) in reindeer food plant samples collected from 33 the measurement sites in different winter pasture types. Statistical differences between pasture types have been analysed using Kruskall-Wallis test

Pasture type	Alternariol (μg/kg dry matter)					
	N	Mean	Median	Min	Max	SD
Pine forest	22	671.2	497.9	104.0	1840.5	447.4
Mountain birch forest	5	998.1	513.7	306.1	2562.4	965.8
Open mountain heath	6	280.3	290.8	133.2	436.9	134.9
Kruskall-Wallis test				Chi-Sq	DF	Prob
				5.40	2.0	*0.063*
Pasture type	Alternariol monomethyl ether (μg/kg dry matter)					
	N	Mean	Median	Min	Max	SD
Pine forest	22	250.1	207.7	61.3	807.6	173.5
Mountain birch forest	5	345.3	324.7	136.1	568.5	164.7
Open mountain heath	6	308.4	321.9	168.1	461.7	99.3
Kruskall-Wallis test				Chi-Sq	DF	Prob
				3.79	2.0	*0.152*
Pasture type	Zearalenone (μg/kg dry matter)					
	N (*N**)	Mean	Median	Min (M*)	Max	SD
Pine forest	22 (7)	20.9	0.0	0.0 (0.3)	206.1	48.4
Mountain birch forest	5 (4)	27.3	33.3	0.0 (8.2)	40.4	16.2
Open mountain heath	6 (6)	72.9	74.5	49.5	90.0	13.7
Kruskall-Wallis test				Chi-Sq	DF	Prob
				12.72	2.0	*< 0.001*
Tukey's *t*-test					*pf < omh*	*< 0.05*

*N**, numbers of samples where zearalenone was found; *M**, minimum found zearalenone concentration; *pf*, pine forest; *omh*, open mountain heath; < and > refer the direction of statistical difference between pasture types by < 0.05 significance in Tukey's pairwise comparisons

found several different mycotoxins belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*. Later, Kononenko et al. (2012) also reported that lichen species in the genera *Cladonia and C. islandica* contained various types of mycotoxins. In our study, the average AOH concentration in different pasture types varied between 280 and 998 μg/kg, which are higher concentrations than Burkin and Kononenko (2011) found (243 μg/kg).

As such, AOH, AME, and ZEN appear to be relatively common in various livestock pastures around the world. In the grass plant samples collected from livestock pastures in Argentina, 60 fungal metabolites toxic to ruminants were detected, including AOH, AME, and ZEN (Nichea et al. 2015). The median concentrations of AOH in their samples varied between 17 and 65 μg/kg, for AME, between 11 and 13 μg/kg, and for ZEN, between 3 and 7 μg/kg respectively.

Table 4 Spearman rank correlation coefficients (*R*) with their probabilities (Prob) between the measured parameters and different mycotoxin concentrations on the field sites

Measured snow parameter	Spearman rank correlation	AOH (µg/kg)	AME (µg/kg)	ZEN (µg/kg)
Snow depth (cm)	*R*	0.47	0.11	−0.17
	Prob	*0.005*	*0.544*	*0.359*
Snow density (kg/m^3)	*R*	−0.16	0.23	0.55
	Prob	*0.372*	*0.203*	*0.001*
Snow weight (kg/m^2)	*R*	0.39	0.21	0.18
	Prob	*0.027*	*0.244*	*0.316*
Total snow hardness (g/cm^2)	*R*	−0.43	0.15	0.44
	Prob	*0.013*	*0.417*	*0.010*
Bottom snow depth (cm)	*R*	−0.07	−0.23	0.01
	Prob	*0.700*	*0.195*	*0.952*
Bottom snow hardness (g/cm^2)	*R*	−0.32	0.18	0.26
	Prob	*0.065*	*0.328*	*0.141*
Number of snow layers (*n*)	*R*	0.18	−0.07	−0.34
	Prob	*0.327*	*0.701*	*0.051*
Ground surface temperature (°C)	*R*	0.46	−0.16	−0.45
	Prob	*0.007*	*0.361*	*0.009*

These median concentrations were notably lower than those discovered in our samples, which ranged between 291 and 498 µg/kg for AOH, 208 and 325 µg/kg for AME, and 0 and 75 µg/kg for ZEN (Table 3). In contrast, research conducted in New Zealand by Di Menna et al. (1987) observed the occurrence of ZEN in 17% of the sheep pasture plant samples, with high concentrations ranging from 400–4000 µg/kg. In addition, in pastures in the Czech Republic, Skládanka et al. (2011) found ZEN in grass plants at levels of 4–173 µg/kg.

Our work showed that different mycotoxins can also be formed in the natural pastures of reindeer in similar weather and snow conditions as in the late autumn and early winter of 2021–2022. Despite favouring warm and very humid conditions, the minimum growth temperatures of the *Alternaria* species range from 2.5 to 6.5 °C, and even lower temperatures, from 0 to −5 °C, have been reported in cooler regions (Juan et al. 2016). Furthermore, it was confirmed that *Alternaria* species are capable of growing and synthesising AOH and AME at both cool and room temperatures. For ZEN production, high humidity and temperatures ranging from 15 to 28 °C have been shown to promote ZEN synthesis (Velluti et al. 2000 and 2001; Llorens et al. 2004). It has been suggested that a shift in temperature from warmer to cooler conditions can enhance the production of ZEN (Blaney and Dodman 2002; Martins and Martins 2002).

In our observations, AOH and AME were found both in pine and mountain birch forests, and ZEN mostly on open mountain heaths. Correlation analyses also showed that concentrations of AOH in pasture plants increased when the ground surface temperature in March, and the depth and weight of the total snow layer increased, while an increase in the hardness of the snow decreased the concentration of AOH. In contrast, the denser and harder the total snow was, and the lower the ground surface temperature was, the higher the concentrations of ZEN observed in the samples. The concentration of ZEN was also negatively correlated with the number of layers in the total snow cover.

The observed differences in mycotoxin concentrations between pasture types may be explained by the pasture type-connected differences in snow and weather conditions. Based on our results and the previously presented literature (Juan et al. 2016), we assume that during the early winter, the development of AOH is promoted by the unfrozen and still warm soil, which is suddenly covered by a deep and wet snow cover, thereby also keeping the temperature under the snow layer above freezing and maintaining its high humidity. In contrast, ZEN could already be generated mostly by the wet and warm late autumn before the arrival of snow. A rapid transition from warmer temperatures to cooler conditions, followed by the freezing of the wet snow, may also enhance ZEN production in pastures characterised by shallower snow cover areas, such as open mountain heaths (see also Blaney and Dodman 2002; Martins and Martins 2002).

According to Escriva et al. (2017), *Alternaria* toxins have been shown to have harmful effects on animals, including cytotoxicity (toxicity to cells), fetotoxicity (toxicity to the foetus via the placenta), and teratogenicity (causing malformations or defects in an embryo or foetus). They have been associated with various pathologies, ranging from haematological disorders to oesophageal cancer. Bansal et al. (2019) observed that different exposures to AOH on mice's skin caused oedema, irritation, and inflammation. According to Schoevers et al. (2020), AOH disturbs the maturation of the ovum, as well as embryo implantation and development. den Hollander et al. (2022) found that the cytotoxic effects of AOH and AME were at the same level, but they have significantly stronger effects when combined.

ZEN is a compound that has also been observed to disturb the reproduction of animals (Hou et al. 2015). It has also been linked with early puberty (Massart and Saggase 2010), and it may lead to fertility problems and foetal developmental issues in ruminants raised in pastures (Aranega and Oliveira 2022). Furthermore, Balázs et al. (2021) conducted a study of the combined cytotoxic effects of mycotoxins, observing in their in vitro study that reduced ZEN metabolites, AOH, and the phytoestrogen genistein (GEN) could aggravate ZEN-induced toxicity.

No studies of how different levels and combinations of different mycotoxins affect reindeer health and reproduction are available, but we assume that besides difficult snow and grazing conditions, they may reduce reindeer body condition, health, and reproduction in winters like those of 2019–2020 and 2021–2022. In a large questionnaire sent to reindeer herders during the winter of 2019–2020 (Kumpula et al. 2020), herders reported a rapid weakening of reindeer body condition and health at the beginning of the winter, including diarrhoea, ulcers, abscessed inflammations and necrosis in the mouth, inflammations in the limbs and different parts of the body, weak utilisation and refusal of food, and apathetic behaviour. Furthermore, several thousand reindeer deaths were reported during the winter of 2019–2020 (Kumpula et al. 2020), and in the following slaughter season, calf percentages declined considerably. These effects on reindeer very closely resembled those observations which reindeer herders also made during the winter of 2021–2022 especially in the 19 northern cooperatives, where snow and pasture conditions were evaluated to be the most difficult (Kumpula et al. 2022).

Conclusions

Temperatures and precipitation are expected to rise especially in November–March in northern Finland (Räisänen 2016; Ruosteenoja and Jylhä 2021). Furthermore, in this area, the water equivalents (water content) of the mean annual snowfall and the average annual maximum 1-day snowfall are predicted to increase during the winter. As the total precipitation has been observed to be positively correlated with temperature in colder areas (Räisänen 2021), this probably means more wet snow and rain-on-snow events in cold areas during milder winters, although snow days will tend to decrease.

The realisation of this development means that warmer and rainier onsets of winters with unstable weather, rain, and snow events are becoming more common in northern tundra, mountain, and forest areas. In this scenario, the risks of encountering winter onsets when both hard and icy snow layers and mycotoxins develop on reindeer pastures increase. This will also erode the productivity and profitability of the semi-domesticated reindeer herding system in the north. However, in the changing climate, much additional research is required to understand the developments of snow conditions and the appearance of mycotoxins in reindeer winter pastures more broadly.

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Author contribution Jouko Kumpula: main author and responsible writer of the manuscript, responsible researcher for the whole study, data collection, and handling. Sari Rämö: responsible researcher for the mycotoxin analyses and main writer of the mycotoxin part of the manuscript. Leena Holkeri: assisting in the mycotoxin analyses. Antti-Juhani Pekkarinen: participating researcher in the field data collection and manuscript writing. Jukka Siitari: assisting in the collection and handling of data, making figures. Heikki Tuomenvirta: enabled the use of FMI's weather data, participating researcher in the writing of the manuscript. Ilari Lehtonen: assisting researcher in the weather data handling and participating in the writing of the manuscript. Sirpa Rasmus: assisting research in the writing process of the manuscript, especially in its climatic part.

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