



DEPARTMENT OF EARTH SCIENCES

# THE EFFECTS OF REINDEER GRAZING ON MYCELIAL BIOMASS IN THE OROARCTIC



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## Abstract

Climate warming is driving increased vegetation growth and shifts in species composition in tundra regions, which may also lead to changes in mycorrhizal communities. An increase in deciduous shrubs has been observed, however, several studies suggest that herbivores can limit this expansion. This study investigates how reindeer grazing affects mycelial biomass using an enclosure experiment at two Oroarctic sites: Kilpisjärvi (Finland) and Ritsem (Sweden). Ingrowth mesh bags filled with sand have been incubated into the soil at the sites with various incubation times, spanning from one growing season length to two years plus growing season length. The dominant vegetation at these sites includes deciduous shrubs, evergreen ericaceous shrubs, and graminoids. And the main mycorrhizal types, analysed from soil samples, are ectomycorrhiza (ECM), arbuscular mycorrhiza AM and a small proportion of ericoid mycorrhiza (ERM).

Mycelial biomass was estimated using two methods: ergosterol extraction (a fungal-specific biomarker) and loss on ignition (LOI). Results show higher mycelial biomass in enclosure plots compared to grazed (ambient) plots at both Kilpisjärvi and Ritsem, with a statistically significant treatment effect at Ritsem. These findings suggest that reindeer grazing can suppress mycorrhizal growth.

Changes in mycorrhizal composition may also affect soil carbon dynamics. While the expansion of deciduous shrubs could increase above ground carbon storage due to enhanced plant productivity, their associated ectomycorrhizal (ECM) fungi are suggested to have a faster turnover rate than the ericoid mycorrhizal (ERM) fungi that dominate evergreen shrub systems. A shift from evergreen to deciduous vegetation may lead to increased soil carbon respiration and decreasing soil C storage.

*Keywords:* Mycelia, Herbivores, Reindeer, Oroarctic, Mycorrhizal fungi

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# 1 Introduction

The Arctic is currently experiencing rapid warming, at a rate two to four times faster than the global average (Hugelius et al., 2024; Rantanen et al., 2022). This phenomenon, known as Arctic amplification, is driven by a range of positive feedback mechanisms. One major concern in this context is the vast amount of carbon (C) stored in Arctic permafrost soils (Schuur et al., 2015; Parker et al., 2021), that has been accumulating since the last glaciation (Clemmensen et al., 2015). Currently, Arctic tundra ecosystem functions as a carbon sink. However, with continued warming, it risks becoming net carbon source, potentially accelerating global climate change (Hugelius et al., 2024; Ciais et al., 2013).

One consequence of Arctic warming is the change of vegetation, including increased plant productivity and shifts in species composition (Clemmensen et al., 2021). In particular, the expansion of deciduous shrubs, which may simultaneously increase its symbiont, ectomycorrhizal (ECM) fungi (Clemmensen et al., 2021; Vowles & Björk, 2019). Reindeer/Caribou (*Rangifer tarandus*) primarily feed on lichens during winter and on deciduous shrubs, graminoids, and herbs during summer (Ylänne et al., 2021). Selective grazing by reindeer can thus limit the climate-induced growth of deciduous shrubs (Bråthen et al., 2017; Sundqvist et al., 2019b). Moreover, ecosystems of different dominant mycorrhizal fungi are found to have various soil C storage (Averill et al., 2014; Ylänne et al., 2021). As reindeer may be able to alter mycorrhizal biomass and composition through grazing, they may also have an impact on the soil C storage in tundra regions. This project specifically investigates whether reindeer affect mycelial biomass in the Oroarctic.

## 1.1 The role of fungi

Fungi are non-photosynthetic organisms that are involved in the carbon and nutrient cycling (United Nations Decade on Ecosystem Restoration, n.d.). Saprotrophic fungi gain both nutrients and carbon through decomposition of organic material like litter and dead plant material. Mycorrhizal fungi, e.g. arbuscular (AM), Ectomycorrhizal (ECM) and Ericoid mycorrhizal (ERM) fungi, form symbiotic bonds with living plants and receive carbon directly from plant roots in exchange for nutrients (e.g. nitrogen, phosphorus) and contributes to soil C stocks (United Nations Decade on Ecosystem Restoration, n.d)

Studies suggest that different types of mycorrhizal fungal necromass have different decomposition rates due to its chemical composition, which affects how much C accumulates in the soil (Vowles & Björk, 2019; Ylänne et al., 2021). ECM are a widespread mycorrhizal type that primarily associate with deciduous shrubs in the Arctic, such as *Betula nana* (Vowles et al., 2018). ECM fungi produce extramatrical mycelia (EMM) (Vowles et al., 2018), the fungal hyphae found outside of the root system. A majority of Arctic evergreen shrubs are associated with ERM fungi and it has been suggested that deciduous shrub tundra have lower C stocks than adjacent ericaceous heaths (Parker et al., 2015; Vowles & Björk, 2019). ERM fungi have

melanized hyphae which slows down the decomposition of the fungal tissue (compared to the EMM) which allows for more build-up of organic matter in the soil (Clemmensen et al., 2015; Vowles & Björk, 2019). Additionally, the litter of evergreen species decomposes more slowly than of deciduous species, contributing to a slower nutrient cycle. In nutrient-poor environments, evergreen species may have an advantage over faster-growing deciduous species (Vowles & Björk, 2019).

In contrast, on a global scale, soils in ecosystems dominated by ECM and ERM fungi store more soil carbon than those dominated by AM fungi, as much as 70% more C per unit nitrogen (Averill et al., 2014). ECM and ERM fungi produce nitrogen-degrading enzymes, giving them greater access to organic nitrogen sources. This reduces the nitrogen available to free-living soil microbes, which in turn inhibits the decomposition of soil organic matter, leading to increased carbon storage. This may become a positive plant-soil feedback as the host plant of ECM and ERM get access to N which promotes plant growth (Clemmensen et al., 2021). In return, plants may allocate more carbon to their fungal symbionts through their roots.

### 1.1.2 Ergosterol

Ergosterol is the predominant sterol of the fungi's cell and mitochondrial membrane. The sterol is important for fungal growth, stress adaptations and proliferations (Bhattacharya, 2021). It is also unique for fungi, meaning that plants do not contain this component which makes it a useful biomarker for fungal biomass. Kandeler (2015) indicates that the ergosterol content in arable, grasslands and forests, is between 0.75-12.9  $\mu\text{g g}^{-1}$ . However, the fungal biomass is lower in tundra soils than other biomes, such as boreal forests.

A method to measure the fungal biomass content of an ecosystem is to incubate fine mesh bags (mesh size 50 $\mu\text{m}$ ) into the soil. The in-growth mesh bags (filled with sand) are placed between the organic and mineral horizons where the production of mycorrhizal fungi is the greatest (Wallander et al., 2013). Since the bags are filled with sand saprotrophic fungi are discriminated since they are dependent on organic C for growth (Hagenbo et al., 2017). The size of the mesh comprising the bags allows fungal hyphae to grow but prevents root intrusion (Hagenbo et al., 2018), which makes it possible to analyse the mycelia production of an ecosystem.

### 1.1.3 Shrubification

There is an ongoing shrubification of the Arctic with increased shrub growth, advancing shrubline and shift in plants species (Clemmensen et al., 2015; Vowles & Björk, 2019). Epstein et al. (2012) found in their study that the aboveground phytomass in the Arctic had increased by 20% from 1982 to 2010. Plants species with ECM association, such as *Betula nana*, are becoming more widespread (Clemmensen et al., 2021). *Betula* species may contribute to an increase aboveground C storage as they increase their biomass (Ylänne et al., 2021). However, the total C storage of *Betula* have been found to be smaller than of evergreen shrub heaths, possibly due to their mycorrhizal associations (Parker et al., 2015; Vowles & Björk, 2019; Ylänne et al., 2021).

Furthermore, the increase of woody deciduous shrubs, such as *Betula* and *Salix* can affect the snow cover, soil temperature and nutrient availability (Vowles et al., 2017). The denser canopy decreases the albedo opposite to shrub-free tundra, and the branches allows for more snow accumulation. This acts as insulation which can increase the soil temperature in the winter further leading to higher nitrogen mineralization rates in both summer and winter (Vowles & Björk, 2019). These factors, as well as the increased litter input and the faster decomposing litter (than of the typical competing evergreen species) (Vowles & Björk, 2019) may contribute to a positive feedback loop on the primary production of the deciduous species (Vowles et al., 2017).

In contrast Cahoon et al. (2016) found that areas dominated by *B. nana* are stronger C sink than of areas dominated by graminoids. This because of the *B. nana* have a greater leaf area promoted by greater gross ecosystem photosynthesis. Additionally, some regions of the Arctic are experiencing longer growing seasons which may provide a negative feedback as it lengthens the period of C sequestration (Barichivich et al., 2013; Belshe et al., 2013).

## 1.2 Herbivores

The Arctic is a region of low species richness of large herbivores (Olofsson & Post, 2018). In most of the Arctic it ranges between 0-2 species, the reindeer and muskoxen (*Ovibos moschatus*), though the moose (*Alces alces*) can be found in the Fennoscandian tundra and parts of the Russian tundra as well. Furthermore, it is suggested that due to the recent climate change moose have extended northward in Alaska and colonized tundra regions (Tape et al., 2016). However, the reindeer is the most wide-spread herbivore in the Arctic, and in many areas the only large herbivore (Olofsson & Post, 2018). Additionally, in many areas the reindeer is semi-domesticated, especially in Eurasian Arctic.

Studies suggest that reindeer grazing can limit the growth of deciduous shrubs and hold back the tree line advancement (Bråthen et al., 2017; Sundqvist et al., 2019b; Ylänne et al., 2021). Herbivore grazing can also prevent grasslands from transitioning into shrubland by suppressing the growth of tall shrubs (Bråthen et al., 2017). Furthermore, herbivores may be able to alter fungal biomass and fungal composition (Vowles et al., 2018; Vowles & Björk, 2019; Ylänne et al., 2021). As ERM fungi are found to associate with evergreen shrubs and ECM fungi associate with deciduous shrubs, reindeers can through selective grazing limit deciduous shrubs and its symbiont and potentially promote ERM. Herbivores may thus have a positive effect on the soil C storage as ERM dominated ecosystems are suggested to have a larger soil C storage than of ECM dominated ecosystems (Ylänne et al., 2021).

A study from Finnmark found that reindeer grazing during the summer resulted in a 100 meter lower shrubline than of the area of reindeer exclusion during the summer (Bråthen et al., 2017). They also found a threshold of approximately 5 reindeers per km<sup>2</sup> prevented an increase of shrubs. However the reindeer density in Ritsem (which is studied in this project) is lower than

this threshold (Vowles et al., 2017). Furthermore, it is suggested that herbivores' ability to suppress shrub growth is dependent on the climatic conditions (Vuorinen et al., 2022).

Yläne et al. (2021) mentions the complexity and uncertainty of implementing herbivores as management of the vegetation and C storage as ecosystems of different characteristics, e.g. dominant plants will have different effects. Reindeer could increase soil nutrient turnover and productivity in ecosystems of fast-growing vegetation due to fecal material. In contrast, slow growing ecosystems exposed to reindeer could reduce the litter quality due to defence mechanisms which would then lead to less soil nutrient and productivity. In an experiment by Nauta et al. (2015) they removed all the aboveground biomass of *B. nana* (which was the dominant deciduous plant). This led to an increase of thawing depth and in four years the active layer increased from 5 cm on average to 12 cm which further led to a small-scale permafrost collapse and net release of methane. This study by Nauta et al. (2015) did not focus on the impact of herbivores, but it highlights the vulnerability of the tundra ecosystem.

Nevertheless, because herbivores could limit the climate-induced expansion of shrubs; managing reindeer populations, and potentially reintroducing large herbivores, could be effective strategies for controlling climate-driven increases in vegetation (Olofsson & Post, 2018; Yläne et al., 2021; Bråthen et al., 2017a). Though, the interaction between grazing by large herbivores, mycorrhizal fungi, vegetation changes, and their combined impact on soil carbon storage is not currently well-studied. Gaining a deeper understanding of how different mycorrhizal types influence soil carbon in the Arctic, and how herbivores affect these dynamics, is essential.

### 1.3 Aim and research questions

The aim of this thesis is to investigate how mycelia production in Ritsem (Sweden) and Kilpisjärvi (Finland) is affected by grazing from large herbivores, using an enclosure fence experiment that has excluded reindeer and compare it with ambient plots where grazing occurs. I will quantify the total mycelial biomass using the biomarker Ergosterol. Specifically, I will:

- Investigate if there is a difference in mycelial biomass between enclosed and open areas – do large herbivores have an impact on mycelial production?
- Investigate if there is a difference in the strength of the exclusion effect between Kilpisjärvi (KLP) and Ritsem (RIG), given that they have been fenced since 2020 and 1995, respectively.
- Examine if there are differences between years.

## 2. Method

### 2.1 Study area

Samples used in this study are from Kilpisjärvi (KLP), Finland and Ritsem (RIG), Sweden, latitude 69° 02' 35" N, 20° 48' 22" E and 67° 06' 53" N, 12° 16' 30" E respectively. The sites are located in the Oroarctic tundra, which is characterised by ericoid-graminoid tundra, and at lower altitudes, dwarf birch tundra. However, due to the mountainous landscape, there is no absolute altitudinal boundary between these tundra types. The terrain of the Oroarctic tundra also contributes to heterogeneous climatic conditions (Virtanen et al., 2016).

The RIG plot (Ritsem meadow) has been fenced since 1995 and KLP has been fenced since 2020. The dominant plants of KLP site are *Betula nana*, *Empetrum nigrum*, *Vaccinium myrtillus* and *V. vitis-idaea* (Brachmann et al., 2025). The dominant plants of RIG site are *Deschampsia cespitosa*, *D. flexuosa*, *Carex aquatilis*, *Betula nana* and *Empetrum nigrum* (Sundqvist et al., 2019a; Vowles et al., 2017). The dominant large herbivore of both sites is *Rangifer tarandus* (reindeer).

Analyses (DNA extraction) on soil samples from the sites have previously been done. Both KLP and RIG are dominated by the ECM fungi, followed by AM fungi and a small portion of ERM fungi (Brachmann et al., 2025). Further, both the total number of species and the number of ECM species were more than twice as high in the exclosures compared to the ambient plots at KLP. In contrast, at RIG, the total number of species was twice as high in the ambient plots compared to the exclosures (Brachmann et al., 2025).

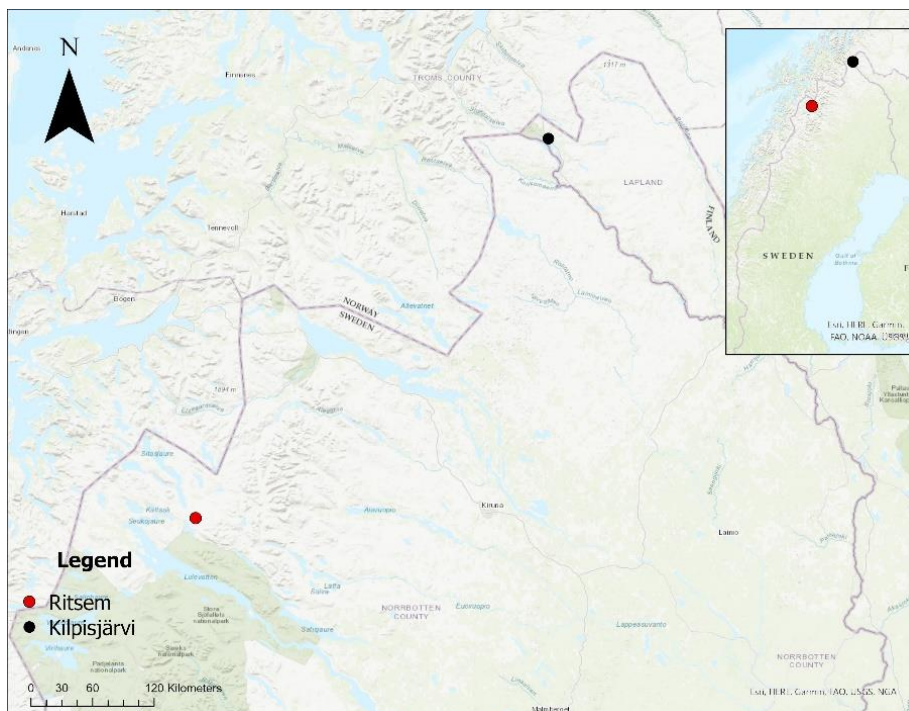


Figure 1. Geographical locations of Kilpisjärvi (black) and Ritsem (red).

One site consists of 48 samples that includes three ambient plots (referred to as A) and three fenced plots (referred to as T as in treatment or enclosure). The three fenced plots in KLP have an area of 1994, 1994 and 562  $m^2$ , and the three fenced plots in RIG are 625  $m^2$  each. There are five different time spans of the incubation time of the sandbags in the soil (Table.1), spanning between June 2020 to September 2022.

*Table 1. Letter notation of the incubation time of the samples, when they were installed and retrieved.*

	Incubation time (Harvest)
A	Growing season length (June-September), with replacement. Either 2020, 2021, 2022
B	1-year, with replacement. Either from June 2020-June 2021, or June 2021 - June 2022
C1	1-year + 1 growing season. June 2020-September 2021
C2	2-year. June 2020-June 2022.
D	2-year + 1 growing season. June 2020 - September 2022

I conducted laboratory work on samples from KLP, and on eight samples from Ritsem shrub heath (RIR). The RIR samples have previously been processed in 2022 but resulted in multiple null values. The additional eight samples processed during this project were insufficient to generate a reliable dataset. Therefore, data from the RIG site, processed in 2022 using the same protocol, were used in the analysis instead. Each harvest group consisted of six samples—three from the ambient plot and three from the fenced plot. However, some ergosterol extractions were below the detection limit, so not all groups retained the full set of three samples per treatment. Specifically, the KLP dataset included six measurements below the detection limit, and the RIG dataset had one.

The results from KLP and RIG were processed in Microsoft Excel to generate charts. Statistical analyses, including ANOVA and Tukey’s honest significant difference (HSD) tests, were performed in Python using the pandas and statsmodels libraries.

## 2.2 Loss on ignition

Approximately 10 grams of each sample are weighed using an analytical balance with a resolution of 0.0001 grams. The samples are placed in crucibles and heated in a muffle oven at 550°C for 10 hours. After the ignition each sample is being weight three times to ensure accuracy. The loss of mass (of organic material) can then be calculated. Because of the mesh grid of the sandbags only mycelia should be able to grow in the samples and the mass loss can thus be assumed to be the weight of mycelia. The carbon mass of mycelia is assumed to be 45% (Ekblad et al., 2016), the weight loss is thus multiplied by 0,45 for the total carbon of the samples.

### 2.3 Ergosterol extraction

The method of ergosterol extraction is done by firstly adding 10% potassium hydroxide (KOH) dissolved in methanol to the samples (10 gram each). The samples were in a sonicator for 15 minutes for cell disruption, followed by 60 minutes in water bath (70°C). To separate the ergosterol from the sample 1 ml of dH<sub>2</sub>O was added to the KLP samples 1-21, and for the other samples molecular water was used. This to ensure the samples were not contaminated with other substances. 2 ml cyclohexane was also added before 5 minutes of centrifugation at 800 x g (RCF). The cyclohexane phase was then transferred into new tubes. 2 ml cyclohexane was again added to the original tube followed by centrifugation, and again transferring the cyclohexane phase to the new tubes to ensure all the ergosterol is extracted from the samples. The cyclohexane was evaporated under nitrogen (N<sub>2</sub>) on a heating block at 40°C. The samples were then dissolved in 400 µl methanol and put in the sonicator for 15 min and water bath of 40°C for 15 min. The samples were filtered into autosampler vials with 0,45 µm Teflon syringe. The samples were then stored in -20°C freezer until they were sent to Lund university for HPLC analysis.

The results were converted from  $\mu\text{g ergosterol per extracted sample}$  to  $\text{g C m}^{-2}$ . To obtain the *fungus biomass* ( $\mu\text{g C}$ ),  $\mu\text{g ergosterol per extracted sample}$  was divided by 0,005 and multiplied by 0,45 as fungus biomass contains 0,5% ergosterol and 45% C. This followed by '*fungus biomass* ( $\mu\text{g C}$ )' \*  $\pi$  to obtain the areal value and lastly convert from  $\mu\text{g C cm}^{-2}$  to  $\text{g C m}^{-2}$ .

### 3. Results

For both KLP and RIG almost all the exclosures (except KLP-A2021 and KLP-C1) shows a higher result of mycelial biomass (Fig. 2). However, the statistical analysis of KLP (Table 2) shows a high p-value (0.43) for treatment, indicating it is not statistically different. At RIG, the treatment effect was significant ( $p = 0,008$ ), with exclosures showing higher mycelial biomass.

Similar trends between the sites of KLP and RIG can be seen. Such as the B-samples (incubation time of one year from June to June) have lower results for the year of 2021-2022 than the previous year for both ambient and exclosures. The yearly differences between the growing seasons (Fig. 2a) of KLP show variational mycelial biomass in both ambient and exclosures. The yearly differences (A-samples) in RIG are quite subtle, but like KLP the exclosures have higher amount of mycelial biomass. The highest amount of mycelial biomass is found in the longest harvest time, the D-samples (June 2020 – September 2022). And the exclosures of the D-samples, have the highest mycelial biomass of both KLP and RIG. Overall, RIG have a higher mycelial biomass.

The standard error of the mean is generally greater for KLP than RIG. Many of the samples of KLP have quite large standard errors. KLP A2022 (T) and KLP C1 (A & T) have standard errors which are greater than the mean value indicating a big variance between the sample values. Note that KLP A2021 does not have an error bar since there was only one sample.

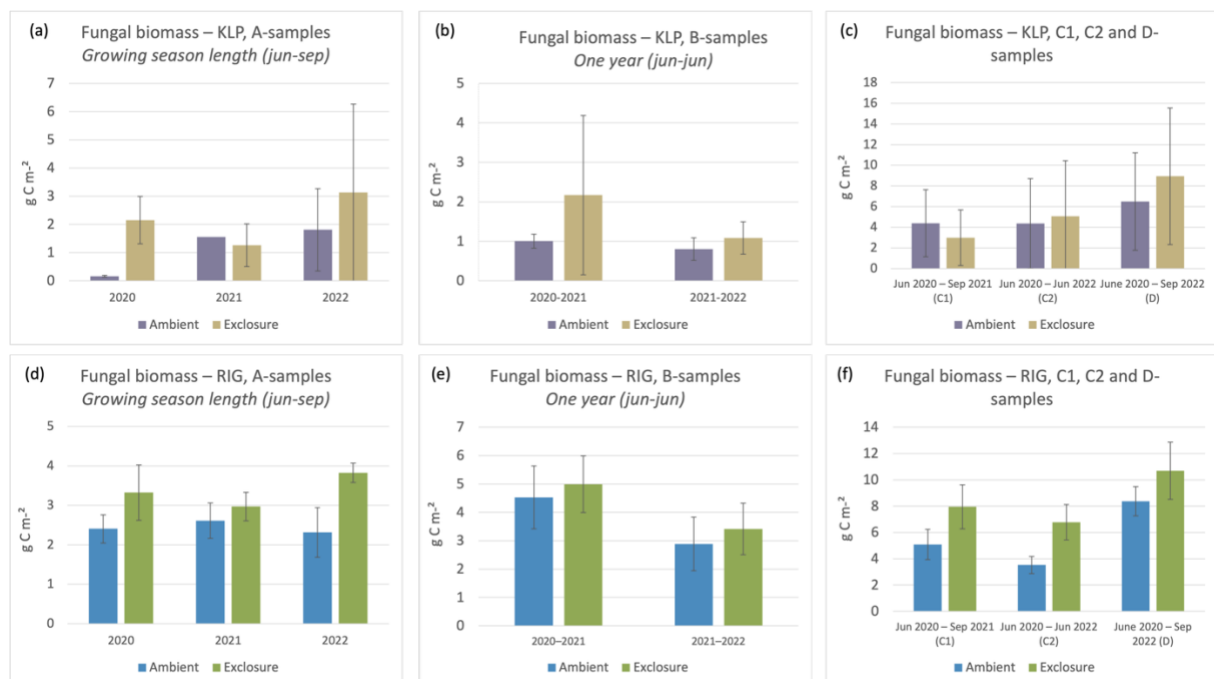


Figure 2. Fungal biomass ( $g C m^{-2}$ ) of KLP (a, b and c) and RIG (d, e and f). Each bar, e.g. 'Ambient 2020' is an average result of three samples. Purple/ blue represents the ambient plots of each harvest time, and beige/green represents the exclosure plots for KLP/RIG. Error bars of the standard error of the mean.

The p-value (0.015) for ‘Harvest’ at KLP indicates a significant effect of harvest time, suggesting that fungal biomass increases with longer incubation periods. For example, fungal biomass in the A-samples (incubated for one growing season) is below  $3 \text{ g C m}^{-2}$  (except A\_2022, T, which slightly exceeds  $3 \text{ g C m}^{-2}$ ). In contrast, the D-samples, which had the longest incubation period (two years plus one growing season), show fungal biomass between 6 and  $9 \text{ g C m}^{-2}$ . Similarly, at RIG, the p-value for ‘Harvest’ was less than 0.001, indicating a significant effect of harvest time. The A-samples in RIG range from 2 to  $4 \text{ g C m}^{-2}$ , while the D-samples range from 8 to  $11 \text{ g C m}^{-2}$  (Fig. 2). These increases were supported by Tukey’s HSD post-hoc tests (Fig. 3), which showed that group D had significantly higher biomass than harvest groups of shorter incubation periods at both sites. Specifically, for KLP, harvest group D differed significantly from A\_2020, A\_2021, B\_20–21 and B\_21–22. At RIG, group D showed significantly higher biomass than all A-samples as well as B\_20–21, B\_21–22 and C2. In addition, C1 samples also exhibited significantly higher biomass than A-samples.

The interaction between treatment and harvest was not significant for either KLP ( $p = 0,22$ ) or RIG ( $p = 0,56$ ).

Table 2. Statistical analysis (ANOVA) of KLP (a) and RIG (b) data of ergosterol extractions.

<b>(a) ANOVA - Kilpisjärvi</b>				
Source	Sum of Squares	df	F	p-value
Treatment	6.342	1	0.646	<b>0.429</b>
Harvest	216.681	7	3.152	<b>0.015</b>
Interaction (Treatment:Harvest)	14.784	7	0.215	<b>0.979</b>
Residual	255.354	26		

<b>(b) ANOVA - Ritsem</b>				
Source	Sum of Squares	df	F	p-value
Treatment	26.043	1	8.025	<b>0.008</b>
Harvest	232.588	7	10.239	<b>&lt;0.001</b>
Interaction (Treatment:Harvest)	13.35	7	0.588	<b>0.761</b>
Residual	100.599	31		

Table 3. Tukey’s HSD post-hoc test for pairwise differences in mean Biomass across Harvest groups. Only significant differences ( $p < 0.05$ ) are shown.

<b>(a) Tukey HSD - KLP</b>					
Harvest Group 1	Harvest Group 2	Mean Difference	p-value (adjusted)	Confidence Interval (lower)	Confidence Interval (upper)
A_2020	D	6,361	0,016	0,789	11,933
A_2021	D	6,381	0,028	0,441	12,320
B_20-21	D	6,248	0,019	0,676	11,819

B_21-22	D	6,773	0,005	1,461	12,086
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<b>(b) Tukey HSD - RIG</b>					
<b>Harvest Group 1</b>	<b>Harvest Group 2</b>	<b>Mean Difference</b>	<b>p-value (adjusted)</b>	<b>Confidence Interval (lower)</b>	<b>Confidence Interval (upper)</b>
A_2020	C1	3.94	0.028	0.268	7.612
A_2020	D	6.663	<0.000	3.162	10.165
A_2021	C1	4.013	0.024	0.341	7.685
A_2021	D	6.736	<0.000	3.235	10.237
A_2022	C1	3.736	0.044	0.064	7.408
A_2022	D	6.459	<0.000	2.958	9.961
B_20-21	D	4.768	0.002	1.267	8.27
B_21-22	D	6.378	<0.000	2.876	9.879
C2	D	4.373	0.006	0.872	7.875

The ergosterol relationship with LOI was poor giving a low R-squared value for both sites, approximately 0,083 for KLP and 0,078 for RIG (Fig. 3). There is no clear trend for either of the sites suggesting a stronger or weaker correlation between the methods for either the ambient or enclosure.

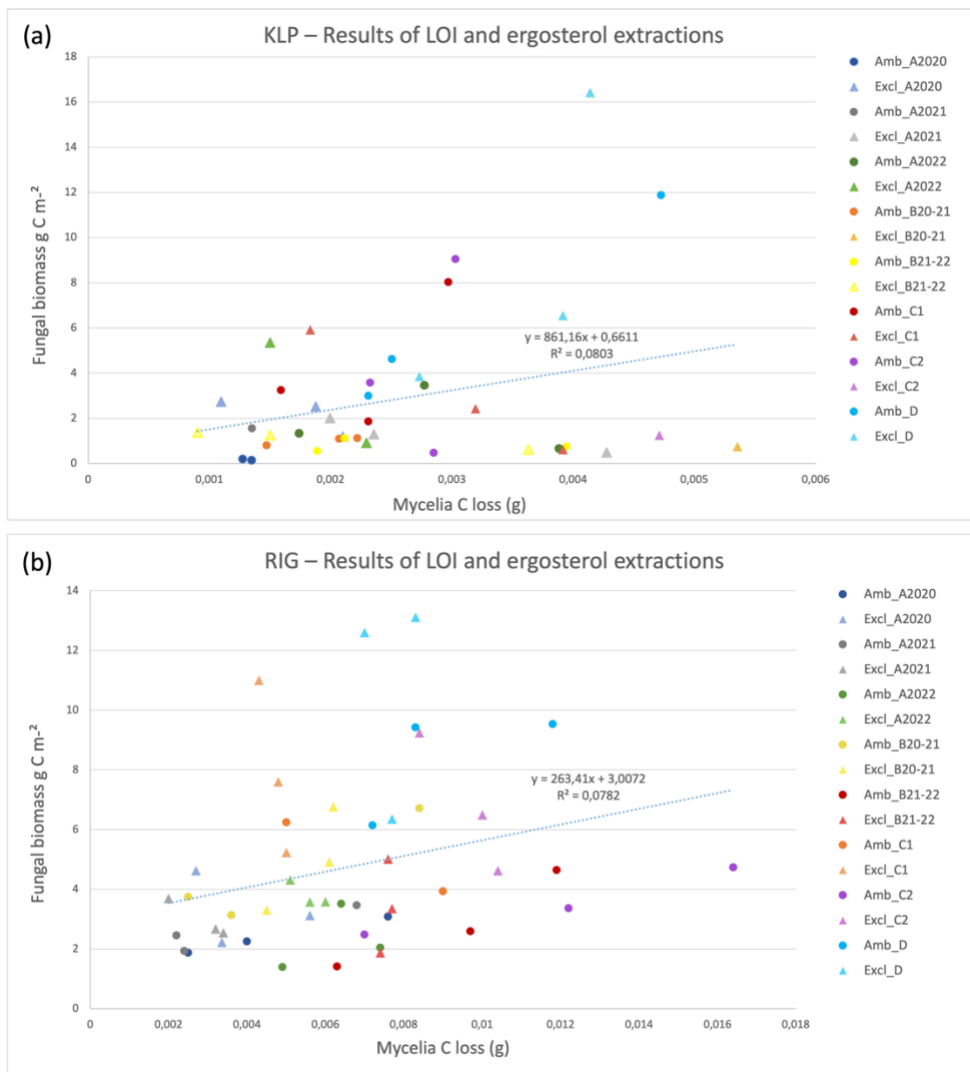


Figure 3. Scatter plot diagram of KLP(a) and RIG (b) - ergosterol and LOI data. Circles represent ambient plots and triangle represent enclosure plots. X-axes represent the LOI data as Mycelia C loss (g), and y-axis represent the ergosterol extraction data as  $g\ C\ m^{-2}$ . The blue dotted line is a linear trend line of both data sets (ergosterol extraction and LOI)

## 4. Discussion

Herbivore exclusion had a positive effect on mycelial biomass at RIG, with higher biomass in enclosure plots compared to ambient plots. While a similar pattern was observed at KLP, where most results showed higher mycelial biomass in the enclosures, the treatment effect was not statistically significant. It is important to note that the enclosure plots at KLP have been fenced since 2020, the same year the incubations began (for A2020, B2020–2021, C1, C2, and D). In contrast, the enclosure plots at RIG have been fenced since 1995. The shorter time length of herbivore exclusion in KLP may be the reason for the non-significant treatment effect. Since the interaction between treatment and harvest time is not significant for either KLP or RIG, this may be because a longer measurement period is needed to detect such interaction. Additionally, the enclosure plots differ in size: KLP plots are 1994, 1994 and 562  $m^2$ , while RIG plots are 625  $m^2$  each. The larger areas of KLP could lead to a greater variety in the vegetation and mycorrhizal communities which may explain the larger standard errors of mean observed at KLP.

In a study by (Vowles et al., 2018), with similar incubation time as the A-samples, they found higher EMM biomass in enclosures of birch forest sites. In contrast to both the birch forest sites and the results from RIG, the same study reported lower mycelial biomass in enclosures at a shrub heath site in Långfjället. At that site, EMM biomass was suggested to be positively affected by grazing. The authors suggested that grazing may stimulate plant carbon allocation to roots as a defence mechanism, which in turn can enhance fungal growth (Babst et al., 2005; Vowles et al., 2018) which may explain the higher EMM biomass observed in the ambient plots of the shrub heath site. The contrasting results between Vowles et al. (2018) shrub heath site and my findings of RIG could reflect local ecological differences, such as vegetation composition and possibly reindeer density. Although, it's also important to note that the methods used to quantify mycelial biomass differed between the studies.

Ylänne et al. (2021) studied reindeers' ability to alter soil fungal communities in year-round grazing areas compared to winter-only grazing areas. They found that year-round grazing had a higher fungal abundance, and higher abundance of evergreen ericaceous dwarf shrubs which were linked to ERM fungi. The increased fungal biomass in long-term enclosures at RIG supports the interpretation that reindeer can suppress fungal growth through their grazing.

### 4.1 Yearly differences

While no significant treatment effect was observed at KLP, there was a significant harvest effect and the Tukey's HSD test confirms that D-samples have significantly higher biomass at both KLP and RIG. This is expected, as longer incubation times allow more time for mycelial growth, leading to increased fungal biomass. This trend was observed at both KLP and RIG.

The mycelial biomass of the growing-season samples (A-samples) varied between years at both sites, with slightly greater variation at KLP. Additionally, fungal biomass was generally higher at RIG than at KLP, both for the A-samples and for samples with longer incubation times. These differences may be explained by contrasting vegetation patterns between the two sites. The samples incubated from June 2021 to June 2022 (B-samples) had lower fungal biomass than those from the previous year. This trend is also somewhat reflected in the summer-season results (Fig. 2a, b, d, e). The smaller amount of biomass during this period may be related to temporary climatic conditions affecting fungal growth.

## 4.2 Method

During the ergosterol extraction process, an unexpected white substance was observed at the bottom of several test tubes, which did not dissolve in methanol. In response, the protocol was slightly modified by adding 15 minutes of sonication. Despite this adjustment, the white substance remained. The substance is suspected to be an oily residue; however, if it did contain ergosterol, the measured values may underestimate the actual ergosterol content. For future studies, it would be ensuring to identify this substance and, if necessary, further refine the extraction protocol.

The LOI method is simpler and less time-consuming than of the ergosterol extraction method. While the in-growth mesh bags only allow for mycelial growth and exclude plant roots, it is worth noting that water-soluble organic carbon may still be present in the samples (Almeida et al., 2022). As a result, LOI measurements may be somewhat biased. Ergosterol extraction could be a more accurate method for estimating mycelial biomass, as ergosterol is unique to fungi (Kandeler, 2015). The methodological differences and what they measure may explain the low correlation observed between the mycelial biomass measurements.

Hagenbo et al. (2018) found that the fungal communities captured in ingrowth mesh bags vary with forest age and that the method is less biased in young forests. The authors emphasize that data from ingrowth mesh bags in older forests should be interpreted with caution. However, if this applies older and younger tundra ecosystems is not stated.

## 4.3 Further studies

As KLP have not been fenced for a long time it will be interesting how the enclosure experiment will affect the mycelial growth as time goes on, and if there will be a significant effect of treatment as longer time has passed. As grazing have been suggested to alter mycorrhizal composition it would be useful to analyse the fungal types of the samples from KLP and RIG which could give a more clarification on herbivores impact on specific fungal types. Furthermore, see how fungal types progress over an extended period. Additionally, Yläne et al. (2021) found that saprotrophic fungi were more abundant in winter-only grazing areas and

positively associated with ECM fungi. Since ECM fungal tissue decomposes more easily than ERM fungal tissue, this combination of ECM and saprotrophic fungi may lead to faster decomposition rates and hinder soil carbon accumulation. A suggestion for future studies is to investigate how herbivores impact not only mycorrhizal types but saprotrophic fungi as well to understand how soil C storage may change.

Bråthen et al. (2017b) found in their study that 5 herbivores/km<sup>2</sup> prevented grassland to transform into shrublands. However, treatment effect was still significant at RIG even though RIG have a lower reindeer density (Vowles et al., 2017). This suggest that the same threshold is not accurate for all regions, but is it possible to find an accurate threshold of reindeer (or herbivore) density for various regions? Additionally, global warming is expected to continue (Parker et al., 2015), it is critical to assess how further warming might influence fungal and plant biomass, the herbivore population, and their ability to alter the tundra vegetation.

## 5. Conclusion

This study of enclosure experiment in the Oroarctic show that the mycelial biomass is higher in the fenced plots than the ambient (grazed) plots. Although, the treatment effect was not statistically significant at KLP, possibly due to the shorter duration of grazing exclusion (since 2020), compared to RIG, which has been fenced since 1995. The greater fungal biomass in enclosures suggests that reindeer exclusion have a positive effect on mycelial biomass, whereas grazing by reindeer may suppress it.

Furthermore, this finding is important in the context of soil C storage whereas ERM fungi are associated with higher soil C stocks than those dominated by ECM fungi and reindeer are known to limit deciduous shrubs (which associate with ECM) (Vowles & Björk, 2019; Ylänne et al., 2021). In contrast, ecosystems dominated by ERM- and ECM fungi can have lower soil C storage than AM-dominated systems due to AM fungi inability to access organic nitrogen which allows for faster decomposition of soil organic matter by free living microbes (Averill et al., 2014).

More research is needed to further understand how herbivores affect fungal biomass through altering the vegetation and further how this may influence soil carbon accumulation and respiration. This is particularly important in the context of the ongoing climate warming and continuous shifting tundra vegetation and expansion northward.

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