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Individual internship report

4th year

Linking phenology and carbon exchange under snow cover changes and reindeer grazing in northern boreal ecosystems

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Presentation

During my internship I worked with Johannes Cunow on linking phenology and carbon exchange under snow cover changes and reindeer grazing in northern boreal ecosystems. Our work was divided into two phases.
First, we spent seven weeks doing fieldwork in Oulanka National Park in Finland. We stayed at the research station 25 km south of the Arctic Circle, 13 km west of the Russian border (66°22' N, 29°19' E, 166.5 m a.s.l.). It has 95 beds and can accommodate researchers of all backgrounds. It is close to a forest and a wetland, the two study sites where we have been working. The research carried out by the researchers focuses mainly on 'meteorology, water chemistry of streams and lakes, phenology, ice cover, snow cover, active layer depth, air pollution, as well as different plant and animal species, etc' (*Oulanka Research Station - INTERACT, 2021*).

We then returned to Umea in Sweden to analyse and interpret the data we had collected. The University of Umea was founded in 1965 following a parliamentary decision to establish a new university in the north of Sweden. At the outset, it welcomed between 2,000 and 3,000 students, but soon expanded to a student body of 37,942 in 2023 (*Umeå University, 2023*). They are spread across the 4 faculties (Faculty of Arts and Humanities, Faculty of Medicine, Faculty of Social Sciences and Faculty of Science and Technology), the Umeå Academy of Fine Arts, the Umeå Institute of Design and the 4 schools (Umeå School of Architecture, Umeå School of Business, Economics and Statistics, Umeå School of Education, Umeå School of Sports Sciences). Umeå University offers more than 150 degree programmes and 1,800 courses, including 40 programmes and 450 courses taught in English. The faculties are divided into 39 departments and units, which bring together almost 2,000 researchers. There are 19 research centres, where researchers from different disciplines work in teams on complex topics. An average of 1,100 scientific articles are published each year in international journals, a figure similar to that of the University of Tours (Dambrine, n.d.).
The university has an international reputation, with 810 exchange agreements and 513 partner universities in 58 countries. It is also known for the Nobel Prize won by Emmanuelle Charpentier's team in 2020 for the discovery of the CRISPR-Cas9 gene-editing tool.
The university is governed by the University Board, chaired by the Vice-Chancellor. It consists of 'three representatives for the teachers and three for the students and eight members who are appointed by the Government and who represent the society and industry' (*Umeå University, 2023*). In 2019, it redefined the university's values, namely responsibility for the future, collaborative development of knowledge, and competitiveness and pride.

I worked in the Department of Ecology and Environmental Sciences (EMG). It has about 300 students, 40 research and about 30 PhD students working in biogeochemistry, evolutionary biology, freshwater ecology, marine ecology, mathematical ecology, paleolimnology, restoration ecology and terrestrial ecology (*Umeå University, 2023)*.

I was attached to the Terrestrial Ecology Research Group, which mainly studies ecological processes in boreal forests and mountain regions. This involves trying to understand how climate, topography, nitrogen deposition and different land use regimes (such as land management or forestry) affect northern ecosystems. Examples of such projects include the transport of pollutants across ecosystem boundaries and their ecological effects, the decline of herbivorous insects as revealed by environmental DNA and plant chemical composition, and the synthesis of decomposition and respiration responses across space and time (Umeå University, 2023).

I. Introduction

In the context of global warming, it is essential to study and understand its effects on vegetation and carbon fluxes in northern ecosystems, which are particularly affected. Wetlands, estimated to contain 20-25% of the world's soil organic carbon (Davidson, 2014), have been halved (Gorham, 1999 *in* Mitra et al., 2005). Boreal forests represent about a third of all forests (FAO, 2006) and almost a third of terrestrial carbon stocks (IPCC, 2007; Pan et al., 2011 *in* Bradshaw and Warkentin, 2015). It is therefore necessary to protect them to limit carbon release, but also for their biodiversity.

Extreme winter warming events are becoming more frequent (Bokhorst et al., 2011), leading to earlier spring and plant growth (Pau et al. 2011; Wolkovich et al. 2012; Xu et al. 2013 *in* Blume-Werry et al., 2017). The entire soil biogeochemistry is then altered, leading to 'disturbances in soil microbial activity (Clein and Schimel 1995; Bolter et al. 2005), increased leaching of carbon, nitrogen (N) and phosphorus (Fitzhugh et al. 2001; Joseph and Henry 2008; Haei et al. 2010), and increased losses of soil trace gases (Matzner and Borken 2008; Öquist and Laudon 2008)' (Kreyling et al., 2011) because all the elements of interest to microbes are more readily available and are not closely linked to organic matter that is difficult to decompose.. As a result, ecosystem respiration (ER) is favoured by plants to the detriment of gross primary production (GPP), which means that less carbon is absorbed from the soil (Bokhorst et al., 2011).
This phenomenon of early warming is often associated with periods of intense refreezing. Plants that are no longer protected by snow cover can then suffer significant damage (Bokhorst et al., 2011).
It is important to distinguish between air temperature and soil temperature. Reducing snow cover exposes plants to the temperature of the 'warm' air earlier, so they can develop earlier above the cooler soil. Additional snow cover does the opposite: plants are isolated from 'warm' air for longer but grow in 'warm' soil. What's more, growing in soil increases the RE.

In addition to warming temperatures, lichens, which are 'a symbiotic association between a heterotrophic mycobiont (a fungus) and one or more autotrophic photobionts (green algae and/or cyanobacteria)' (Asplund & Wardle, 2016), are threatened by reindeer grazing, which has led to the almost complete disappearance of lichen cover in some areas (Väre et al. 1995,1996). There are many reasons for this disturbance, including plant consumption, defecation, urination (e.g. Dove & Mayes, 1991; Holechek, Vavra, & Pieper, 1982; Stewart, 1967 *in* Heggenes et al. 2017) and trampling.
These impacts on the lichen caused by reindeer modify 'microclimate (Kershaw & Field 1975), soil respiration (Väre et al. 1996 , Stark et al. 2000), soil nutrients (Olofsson et al. 2001) and fine root biomass (Väre et al. 1996) and may indirectly alter the natural regeneration of woody plants'. (Asplund & Wardle, 2016). Wildlife is also affected, with small animals using the lichen as shelter and larger animals using it as a food source.

Although forestry is the activity that has the greatest impact on lichen cover, reindeer husbandry management is also important. There are different management methods. Norway and Sweden practice rotational grazing, which means that the reindeer do not stay in the same place to graze depending on the season (they generally go to the mountains in summer) (Stark et al., 2002). In Finland, on the other hand, reindeer graze in the same area throughout the year. This increases the pressure on the lichen and limits its ability to regenerate.

This is why we study linking phenology and carbon exchange under snow cover changes and reindeer grazing in nothern boreal ecosystems. Phenology can be defined as 'the study of the timing of recurrent biological events, the causes of their timing in terms of biotic and abiotic forces, and the interrelationship between phases of the same or different species' according to the International Biological Programme Working Group (Lieth, 1974). Here we have concentrated mainly on the phenology of plants in the two ecosystems. Reindeer grazing and warmer temperatures are two major components of the northern ecosystem that influence plant phenology. Grazing and snow are subject

to change, so we want to know whether they interact, reinforce each other, or possibly cancel each other out.

We hypothesise that early snowmelt will induce early phenology, whereas delayed snowmelt will induce later phenology and lower carbon uptake. We also assume that reindeer grazing has a negative impact on the lichen by reducing its biomass, which would result in lower photosynthesis if they were not replaced by mosses or shrubs (lichens are not very productive and grow slowly).

II. Material and methods

1) Presentation of the study sites

Our work focused on 2 ecosystems: a wetland and a boreal forest. The boreal forest is composed of oligotrophic pines on alluvial sediments resting on limestone bedrock. The soil lichen is often called 'reindeer lichen' and consists of *Cladionia mitis, Cladonia rangifera, Cladonia uncialis* and *Cladonia stellaris.* The fen, formerly a lake, is rich in sedges, with a neutral pH and an average thickness of 2m, also resting on a substrate. Each site consists of 36 plots, all marked out with wooden planks (to avoid walking on and therefore damaging the area studied, see figure 1), half of which are fenced off from reindeer grazing by a fence. This makes it possible to compare the results and estimate the potential impact of their activity. The reindeer enclosure was built in 1994 in the pine forest and in 2019 in the fen.

Figure 1 : Photo of a snow removal plot (centre) and an additional snow plot (left) of fen the 14th of May (credit : J. Helson, 2024)

The plots were grouped into blocks of three. For each block, there are three types of snow treatment: a plot where snow has been removed, another where snow has been added and a control plot (see figure 2).

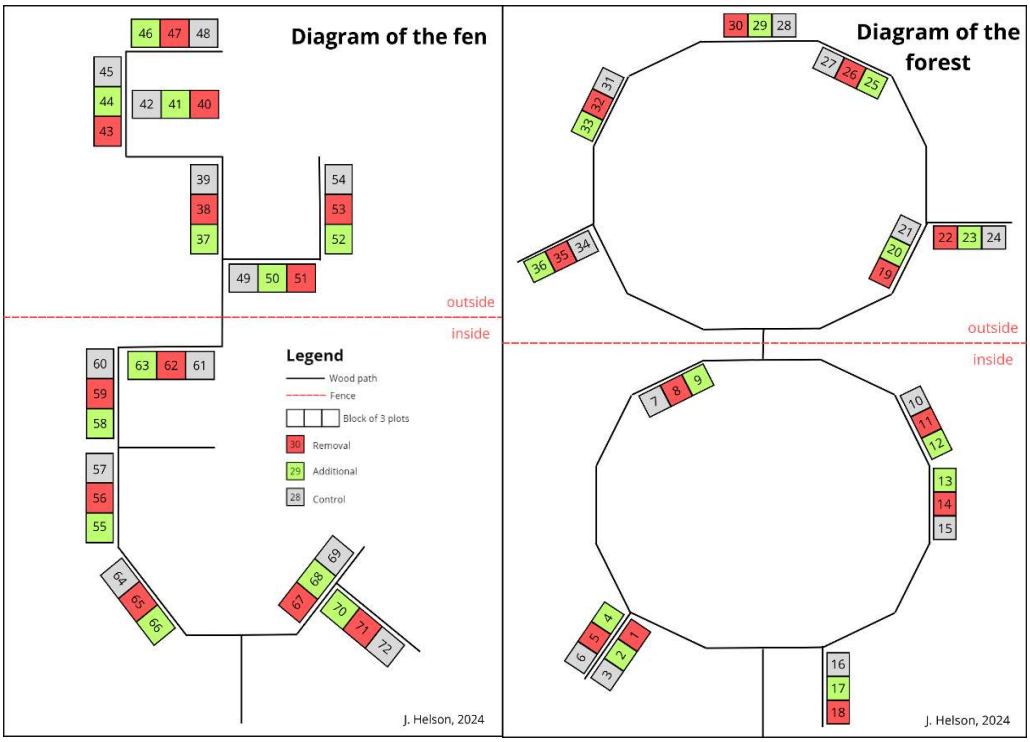


Figure 2 : Diagram of fen and forest plots (J. Helson, 2024)

Our work was divided into three main tasks: scanning the roots with a tube buried in the ground (see figure 3), measuring CO2 fluxes and studying the phenology of each plot. I worked on the phenology about every five days and measured CO2 fluxes the rest of the time. However, Johannes showed me how to scan the roots. I didn't do that part because it was a bit too physical. In order to organise our work as well as possible, we wrote down the tasks we did each day. This allowed us to look ahead to the next few days (see appendix 2). We also checked the weather forecast every day to adapt our work. I attended some meetings with Johannes' supervisors to discuss the progress of the fieldwork.

Figure 3 : Photo of the tube in the soil to scan the roots (credit : J. Helson, 2024)

2) Phenology

1) Fen

In the fen we set up 40 x 80 cm rectangular quadrats to study the phenology of the graminoids *Carex lasiocarpa*, *Carex rostrata, Carex chordorrhiza, Trichophorum sp:* and the shrub *Betula nana*. We chose the location by trying to make it as representative of the plot as possible, but not too close to the collar to measure CO2, the tube to scan the roots and the edges of the plot. These elements can be a source of potential disturbance to plant development. Installing quadrats ensures that data collection is standardised. This makes it possible to compare data from different times, places and treatment conditions.

The installation was carried out gradually as we were dependent on the snow melt. In fact, the height of snow was surprisingly high.
What's more, the development of these different species doesn't start at the same time. We started with *C. lasiocarpa* and *C. rostrata, then B. nana, C. chordorrhiza* and finally *Trichophorum sp:*.

For *C. lasiocarpa, C. rostrata* and *C. chordorrhiza* we tagged with marking sticks five individuals using sticks. We measured the length of the stem of *C. lasiocarpa* by sticking a ruler into the ground, while for *C. rostrata* and *C. chordorrhiza* we measured the smallest part of the green coloured plant (active photosynthesis). *Trichophorum sp*: being a species that grows in groups, it is difficult to identify a particular individual. This is why we used the Me method. This consists of calculating the average length of five shoots (or less if there were not many shoots) on three occasions. As *B. nana* is a shrub, we did not observe length, but rather the developmental stages proposed by Sweet et al. (2014), namely first leaf bud, first leaf visible, first leaf opening, first leaf expanded (see figure 4).

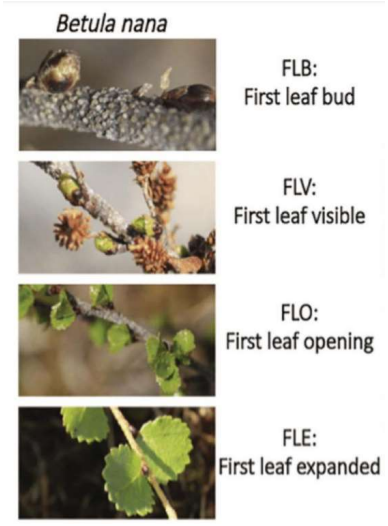


Figure 4 : Development stages of vegetative reproduction of Betula nana (Sweet et al., 2014)

2) Boreal forest

In the forest, we studied the phenology of *Vaccinium myrtillus, Vaccinium vitis-idaea, Empetrum nigrum* and *Pinus sylvestris*.
For the shrubs, we set up subplots in the same way as for the fen. However, we didn't identify any individual. The aim was to note the most advanced stage of vegetative and reproductive development within the plot, an adapted methodology from the ITEX phenology protocol for tundra plant. If there was no vegetative or reproductive stage within the sub-plot, we considered the individuals in the

surrounding area. In addition, phenological stages are often synchronised within a local population. As a result, plants just outside the plot are likely to be at similar stages of development to those inside, ensuring that the data remain consistent and meaningful.

For *Vaccinium vitis-idaea, Empetrum nigrum*, we used the criteria determined by Käärmelahti (2018) (see figure 5, appendix 3-4, 6-7)

Phenophase	**Species**	
Vegetative	***Vaccinium vitis-idaea***	***Empetrum nigrum***
Bud swollen	Bud noticeably swollen, pink	Bud still white, but swollen
Bud green	Green coloration on the bud for the first time	Green coloration on the bud for the first time, starts from the sides
Leaf unfolded	First leaf clearly separated from the bud	First leaf clearly separated from the bud
Leaf expanded	First leaf clearly flat, no need to be mature size	First leaf achieved a mature form
Reproductive		
Flower budding	Inflorecence bud visible	Flower bud visible
First petals	Shape of petals recognizable on the first bud of the inflorecence	Opening on the tip of flower bud, petals visible
Flower open	All flowers of inflorescence open	Anthers approx. 5 mm, flower ready to be pollinated
First fruiting	Fruitset visible	Fruitset visible

Figure 5 : Phenological stages of Vaccinium vitis-idaea and Empetrum nigrum (Käärmelahti, 2018)

For *V. myrtillus*, we used the following classification:
-vegetative phase : bud swollen, first leaf separated, first leaf expanded, all the leaves expanded
-reproductive phase : flower visible, petals visible, no petal, dark pink

At the end of our work period, we counted the number of individuals per subplot and estimated the cover of *V. myrtillus, Vaccinium vitis-idaea, Empetrum nigrum*, lichen and litter.
We also estimated the damage caused by the frost period. To do this, we counted the number of 'healthy' and damaged leaves (50% or more of the surface blackened). To determine which plants to study, we threw a stick at random and it pointed to a plant. We did this with four plants to the right of the plot, four plants to the left and two by the roadside (ten plants per plot). We carried out this task twice: once at the beginning of the thaw (early May) and once before we left (18 June), so that we could compare the number of damaged and undamaged plants. This also allowed us to see if habitat type (lichen-dominated or *V. myrtillus*-dominated) and snow treatment had an effect on plant regeneration. The second time we also counted the number of new leaves.

For *P. sylvestrus* we identified 20 trees between 1.5 and 2 m high, ten on each side of a path. For each tree, we selected with ribbons three buds undamaged buds at the end of a branch, preferably in different directions. (see figure 6). Every four/five days, we measured their length using digital callipers (see figure 6).

Figure 6 : Photo of shaft links and digital callipers (credit : J. Helson, 2024)

We also studied the phenology from a qualitative point of view by identifying the development stages for bud breaking and elongation and the male flower phenology theorised by Debazac (1966) in Reference protocols for assessment of trait and reference genotypes to be used as standards in international research projects (2012) (see figure 7). We did not observe any female flower phenology because they are generally higher up in the canopy.

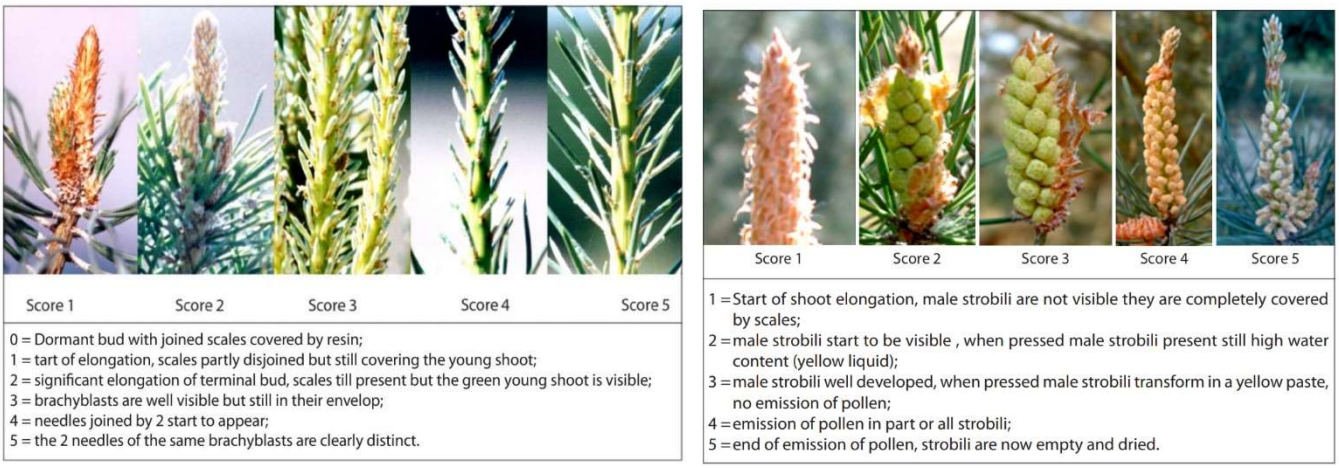


Figure 7 : Scoring system adopted for bud breaking and elongation in Pinus sylvestris (on the left) Scoring system adopted for male flower phenology (on the right) (Debazac, 1966 in Debazac, E. F., 2012)

3) Measurements of CO2 fluxes

Ecosystem respiration is the sum of respiration by living organisms (plants, animals and micro-organisms) in an ecosystem. It represents the total release of carbon by an ecosystem through respiration. Gross primary production (GPP) is the total amount of carbon (in the form of CO2) assimilated by photosynthesis in an ecosystem. Net ecosystem exchange is the net balance of carbon exchange between an ecosystem and the atmosphere. It is therefore the difference between carbon assimilation through photosynthesis (GPP) and carbon loss through total ecosystem respiration (ER): NEE=GPP-ER

To measure net ecosystem exchange (NEE) and ecosystem respiration (ER), we used a transportable transparent chamber (Vaisala model GMP373), which allowed us to sample CO2 fluxes in time and

space. These measurements were accompanied by temperature and humidity measurements (see figure 8). We alternated between plots inside and outside the enclosure to obtain distributed data throughout the day. In this way, all plots inside the enclosure were measured not only in the morning and afternoon, but halfway through both parts of the day.

Before inserting the chamber into the collar, the chamber was balanced to ensure relatively stable conditions within the chamber. The recording time was programmed to 5 minutes, during which it is possible to observe NEE and ER. However, we shortened this time when the signals were strong. We looked for linear plots because respiration and photosynthesis are relatively linear processes. When this was not the case, we repeated the measurements. At the same time, we noted the time of day, the photosynthetic photon flux density (PPFD), the plot number and the weather, all of which were useful for analysis.

Figure 8 : Photo of the chamber and the Vaisala GMP373 instrument (credit : J. Helson, 2024)

4) Root scans

A camera is inserted into a transparent tube de 120 cm with an insertion angle at about 45 degrees in the ground (see figure 9). Before inserting it, we need to absorb the moisture with a cloth (see figure 9). This prevents damage to the electronics and eliminates condensation inside the tube, which would otherwise blur the image. This will prevent damage to the equipment and ensure that the pictures are good. The camera is pushed in further and further, with a total of seven steps in the forest and six steps in the fen. The CI-600 operating software version 4.0.4.57 connected to the camera records the images. These are processed by the root detector software which identifies all the roots (see figure 9). This is a convolutional neural network (often referred to as AI) with a U-net architecture. The task of these networks is called 'segmentation'. It identifies the segments that belong to the roots, the soil and other objects such as the shiny silver band at the top of the image.
In this way, it is possible to estimate the density of the root system over space and time (see appendix 8). This data can then be compared with CO2 fluxes (the 2 devices in the soil are relatively close together). This task was repeated for all 36 plots, every six days.

Figure 9 : Photos of the equipment used to scan the roots (left) and to clean the tube (right) (credit : J. Helson, 2024)

III. Results

1)Results of CO2 measurements

1) Meteorological data

To make sense of the CO2 measurements, it is important to put them into context with meteorological data. That's why we downloaded from the website of the Finnish Meterological Institute (FMI) data on air temperature, snow depth and precipitation (see figure 10). These parameters inevitably influence the development of plants, their roots and their photosynthesis/respiration.

The blue areas represent the winter periods, while the orange areas represent the period on site (from 30 April to 20 June). The weather station automatically records the temperature every ten minutes or so. In summer, the daily highs and lows are almost always at the same time of day, whereas in winter they vary much more, resulting in more irregular peaks.

Temperatures in winter 2024 were lower than in 2023, with an average of -15°C and -10°C respectively (see figure 10). There was also a large range between winter values (35°C in 2024) and summer values (25°C in 2024). Similarly, summer temperatures are higher in 2024 than in 2023, averaging 20°C and 15°C respectively.

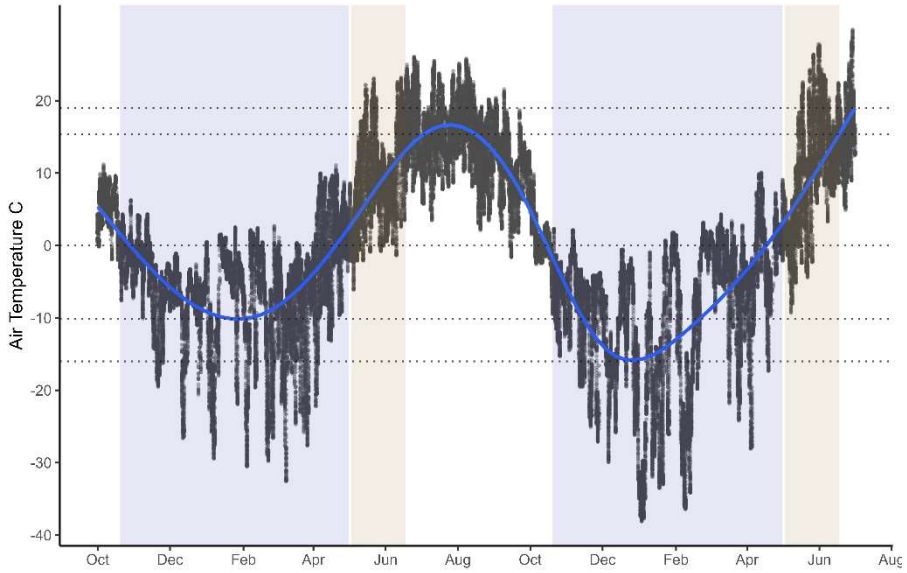
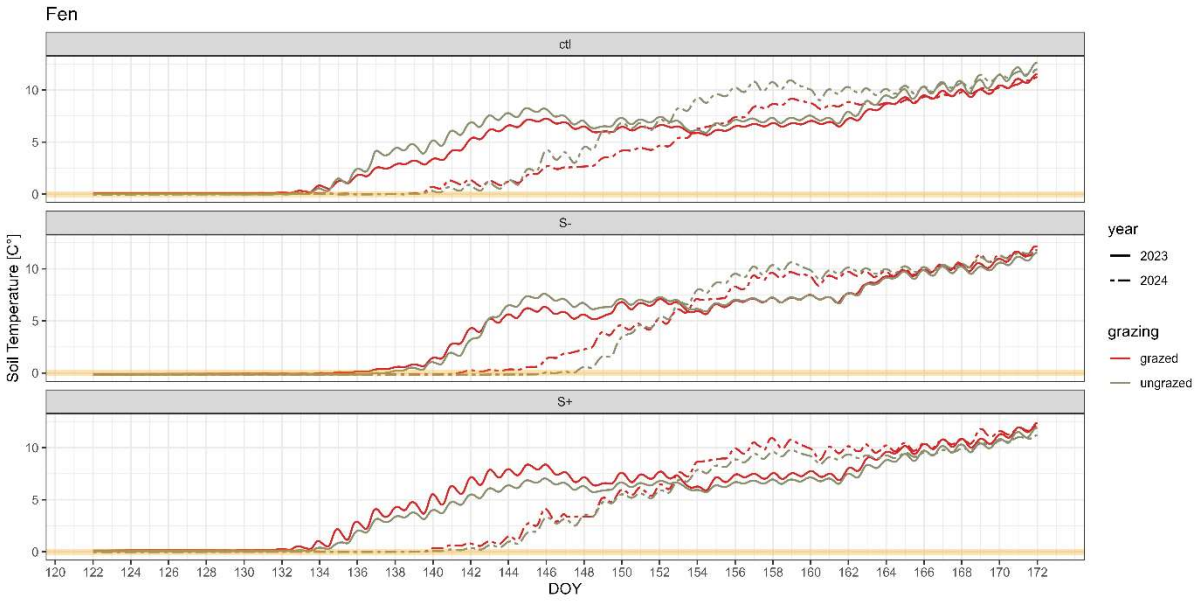


Figure 10 : Air temperature at the Oulanka research station from October 2022 to June 2024 (source : weather station at the Oulanka research station, J. Cunow, 2024)

It was also surprising to see so much snow so late in the season. In fact, on 1 May there was still almost 50 cm of snow. It then melted almost completely within 3 days (from 14 to 16 May). There was also a short period of frost on 8 May. This affected the development of the plants, which were weakened by the cold spell (especially *Vaccinium vitis-idaea*). The snow melted 7 to 10 days later than last year.

Plant phenology depends not only on atmospheric temperature but also on soil temperature.
In the fen, it is interesting to note graphically that the soil temperature curves for the same year behave in a similar way regardless of the snow treatment (see figure 11). In fact, in 2024, the soil temperatures rise sharply until the 158th day of the year (DOY) and then increase very slightly. In 2023, soil temperatures rise sharply until the 146th DOY, fall slightly until the 149th DOY and more or less stabilise until the 162nd DOY. Although the curves behave differently from year to year, it is interesting to note that the curves eventually converge.

The main difference lies in the date when the temperature increase begins. In 2023, it is from the 133rd DOY for the control and snow addition plots and from the 136th DOY for the snow removal plots. In 2024, the start of the temperature increase is later. In fact, it is from the 140th DOY for control and snow addition plots and from the 142nd DOY for snow removal plots.
In addition, the soil temperatures of the grazed plots where snow has been added are higher than those where snow has not been added, regardless of the year. Conversely, the grazed plots have lower temperatures than the ungrazed plots, regardless of the year.



**The yellow line represents the freezing point of water.*

Figure 11 : Changes in soil temperature in the fen according to different snow treatments and grazing in 2023 and 2024 (J. Cunow, 2024)

The soil temperature curves in the forest have similar profiles to those in the fen (see figure 12). However, the start of the temperature increase is different. For the control plots, temperatures start to rise on DOY 134, for the snow removal plots on DOY 130 and for the snow addition plots on DOY 138. In addition, the difference between grazed and ungrazed plots is smaller than in the fen.

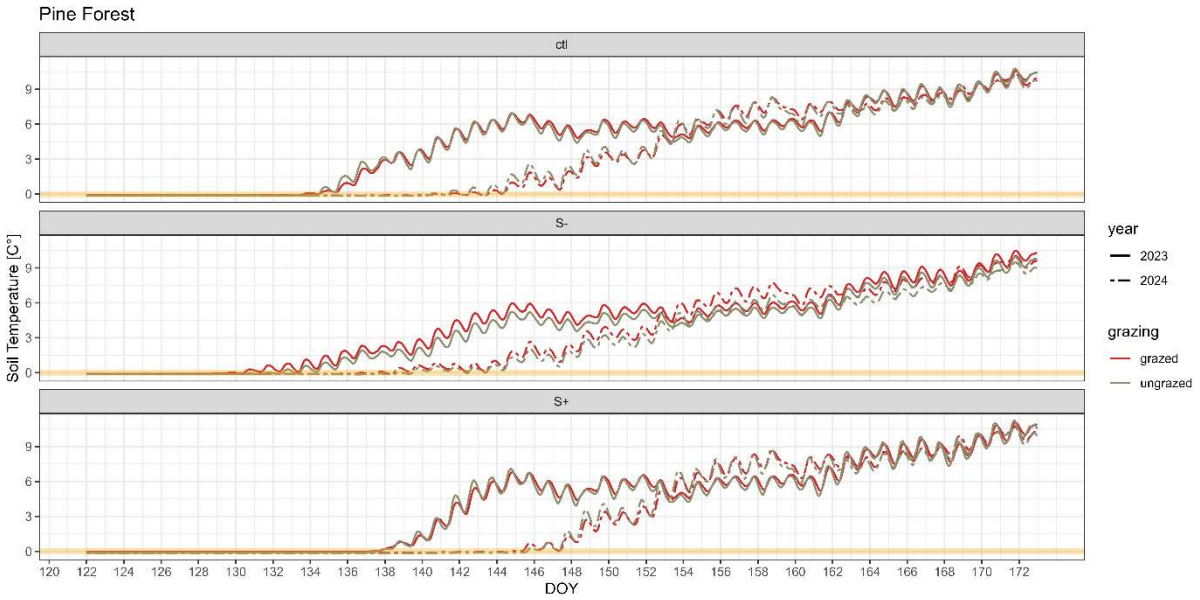


Figure 12 : Changes in soil temperature in the forest according to different snow treatments and grazing in 2023 and 2024 (J. Cunow, 2024)

2) Fen results

Measurements of CO2 in the fen make it possible to estimate the respiratory activity of the plants according to the different snow treatments (removal, additional, control). This makes it possible to assess the impact of snow depth on respiration.
For all three treatments, we can see graphically that ecosystem respiratory activity (RE) increases until 7 June and then starts to decrease (see figure 13). This can be explained by the fact that temperatures have fallen. Since photosynthesis is partly dependent on temperature, it has also decreased.
The plots with additional snow had lower respiratory activity than the plots with snow removed and the control. Respiratory activity was higher on the control plots than on the plots with snow removed.

The graph below shows the respiratory activity of a block of three plots (in µmol/m^2/s) as a function of time (in days). Each point represents data from one plot. The non-linear curves were fitted using a locally estimated scatterplot smoother (loess). The advantage of loess is that the coefficient changes over time. The first minute of the recording was discarded because this is the 'adaptation' period during which the plants begin to respire. This is because the energy captured by the light is still present in the dark and plants still use it to continue photosynthesis for some time, even in the absence of light. This is a metabolic switch.

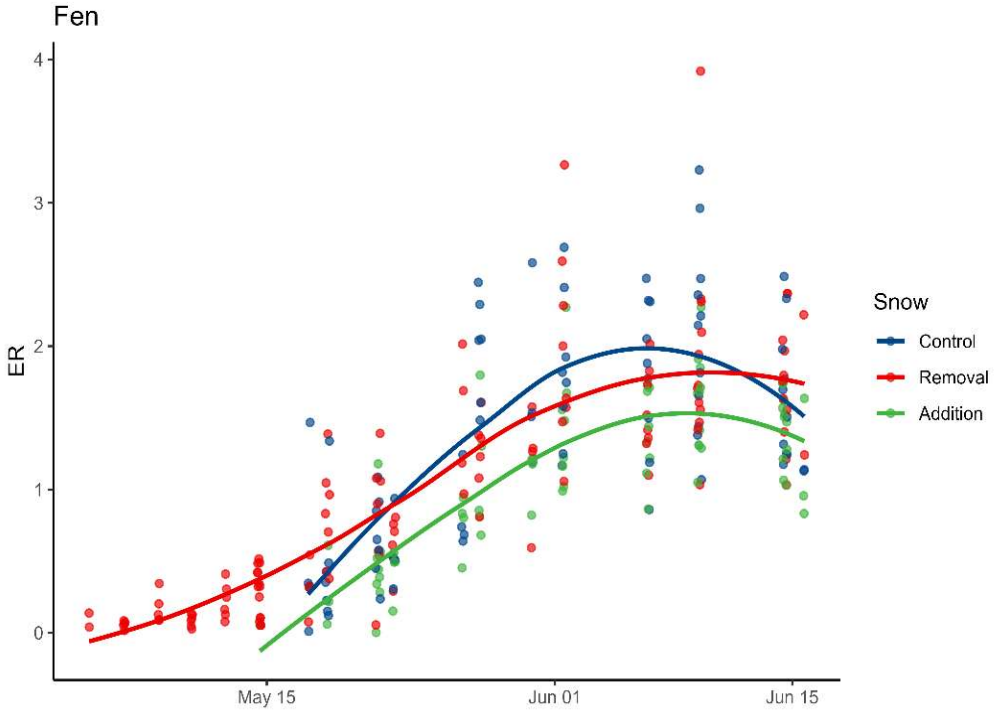
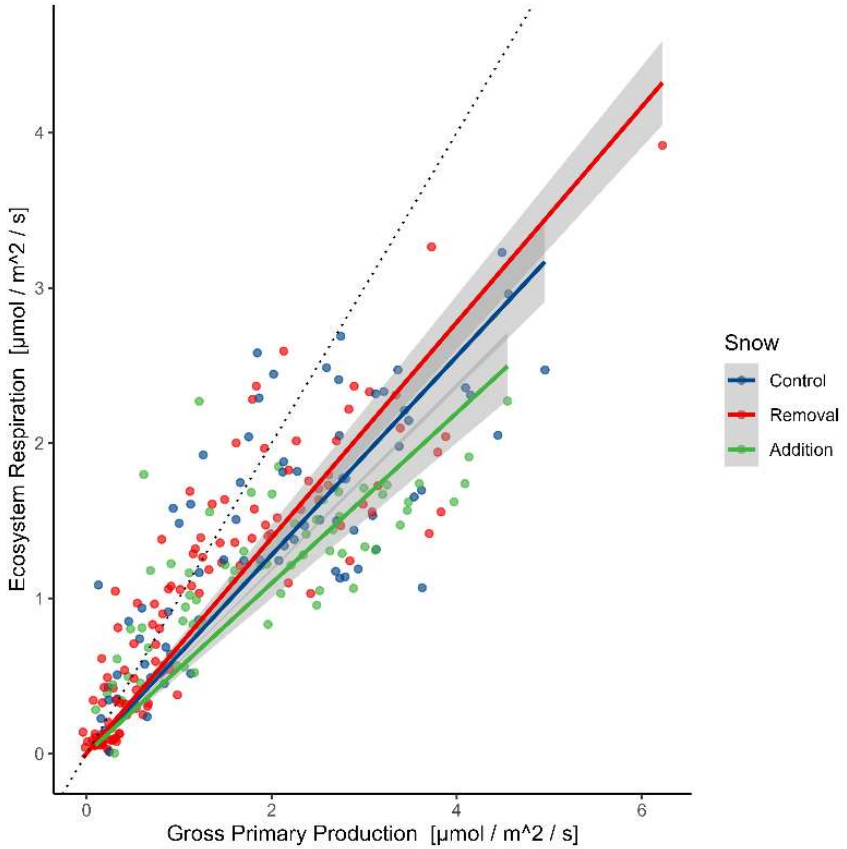


Figure 13 : Respiration of the fen ecosystem (ER) as a function of time (J. Cunow, 2024)

If we compare gross primary production (GPP) with ecosystem respiration, we can see that the ecosystem performs more photosynthesis than respiration (see figure 14). In fact, for 2 µmol/m^2/s of GPP, there is only around 1 µmol/m^2/s of ER for plots with more snow. The photosynthetic activity of the snow additional plots is higher than that of the control plots, which is itself higher than that of the snow removal plots.



**The dotted line shows the 1:1 ratio between ER and GPP (the equivalent of NEE).*

Figure 14 : Respiration in the fen ecosystem as a function of gross primary production (J. Cunow, 2024)

3) Forest results

The principle is the same as for the fen, except that the type of habitat must be taken into account. Plots with less sunlight (due to higher tree density) are dominated by *Vaccinium myrtillus.* On the other hand, more open areas are dominated by lichens. Respiration varies according to habitat. In fact, the respiration of the *Vaccinium*-dominated plots is higher than that of the lichen-dominated plot (see figure 15). This can be explained by the presence of trees and mosses (less present in plots dominated by lichens), which respire and therefore release CO2 in addition to the plants present in the plot. The curves are fairly linear and relatively similar regardless of the snow treatment. However, the curve for the *Vaccinium* dominated plot with additional snow is slightly higher than the others.

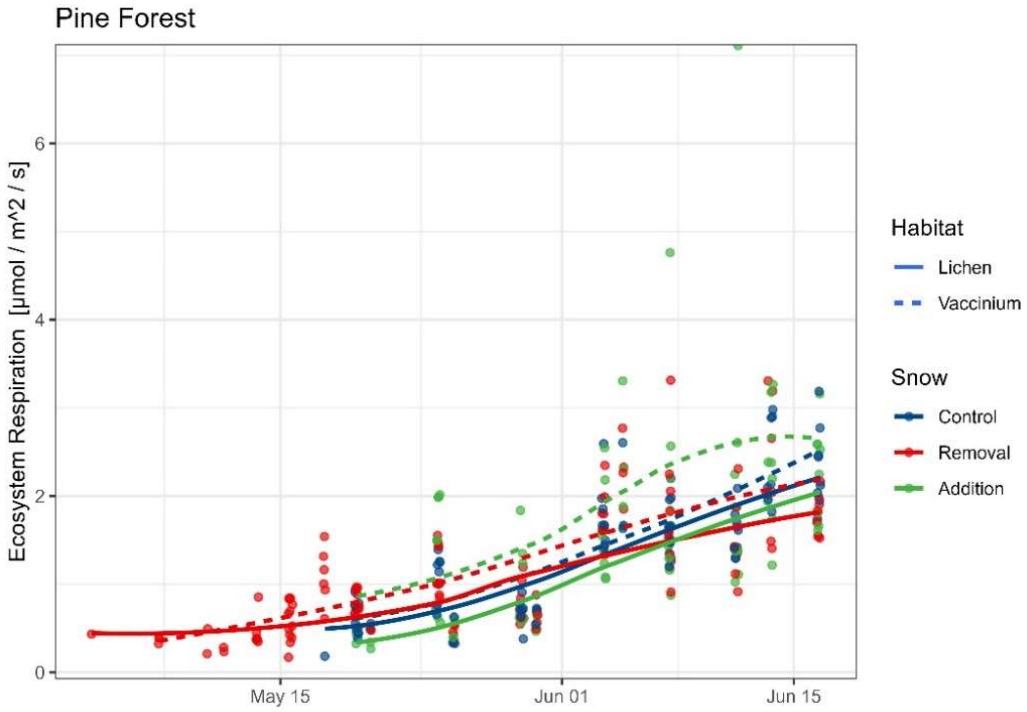


Figure 15 : Respiration of the pine forest ecosystem (ER) as a function of time (J. Cunow, 2024)

We also compared ecosystem respiration between the fenced plots and those exposed to reindeer grazing. The ER seems to be similar between the habitats for the same snow treatment. Slopes seem to have similar coefficients for plots with snow removed and control plots. The ER appears to be greater for plots with additional snow. The ER is lower for lichen plots exposed to reindeer grazing than for those not exposed, regardless of the snow treatment (see figure 16). On the other hand, the grazed plots dominated by *Vaccinium* showed higher respiration than the non-grazed plots.

This figure contains several graphs showing ecosystem respiration as a function of time according to different criteria: snow treatment (removal, additional, control), habitat (Vaccinium, lichen) and grazing (grazed or fenced). The straight lines were fitted using linear regression.

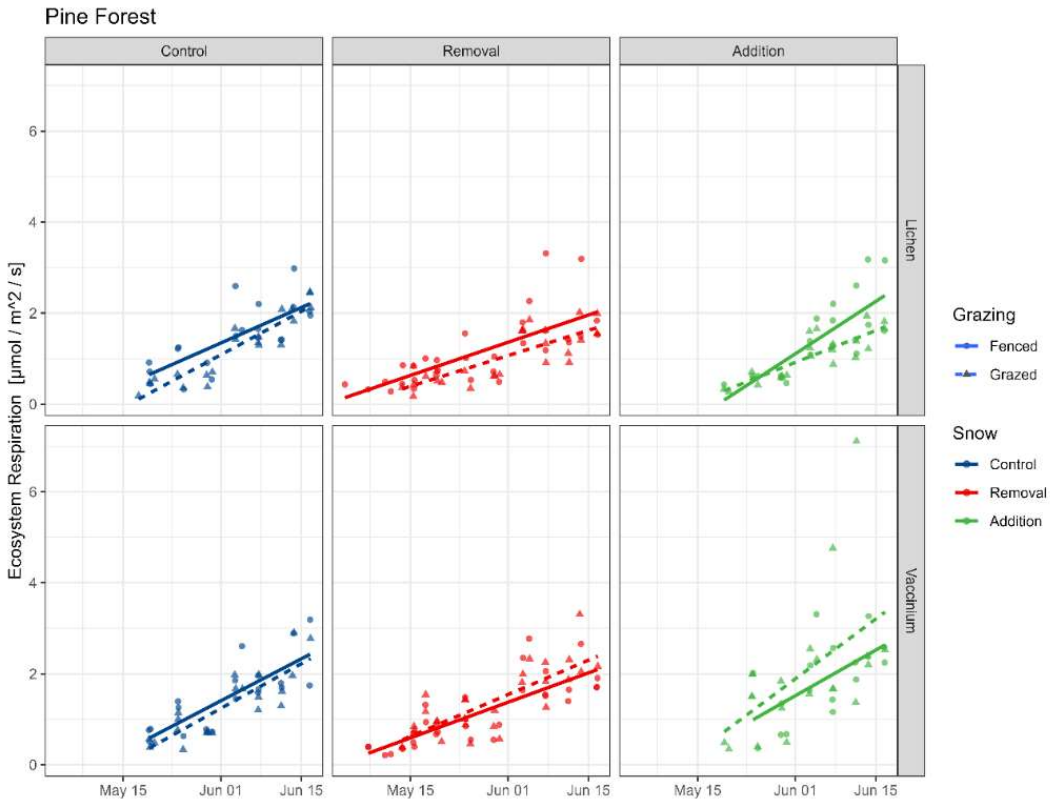


Figure 16 : Respiration of the pine forest ecosystem (ER) as a function of time according to habitat and grazing (J. Cunow, 2024)

Figure 17 shows that there is almost as much respiration as photosynthesis for the control plots and the plots where the snow was removed. Plots with more snow had slightly more respiration than photosynthesis. It is important to note, however, that the GPP of the trees is not included, which alters the balance of the ecosystem.

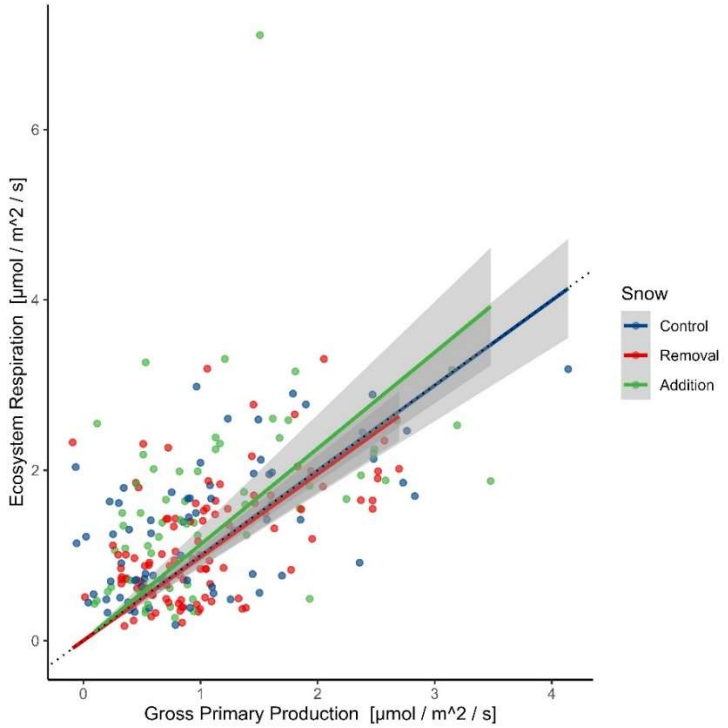


Figure 17 : Respiration in the fen ecosystem as a function of gross primary production (J. Cunow, 2024)

2) Results of phenology

1)Fen

In order to process the elongation data for *Carex lasiocarpa* and *Carex chordorrhiza*, we discarded the values with a difference of plus or minus 10%. Measurement errors may be due to the ruler not being inserted to the same depth (depending on the moisture content of the peat). It may also be due to the plant not being completely parallel to the ruler when measured (curved), which means that the measurement is smaller than the actual size.

We then performed the Wilcoxon test for dependent values to determine whether there were significant differences between the 5 values from the same plot, i.e. to know if we have selected shoots of a similar size. These are paired values because the 5 selected plants are located in the same plot, which has specific morphological, ecological and hydraulic proprieties. This test was carried out for each plot and for all the dates on which elongation was measured. The result was that there was no significant difference between each *C. lasiocarpa* plant in the same plot.

We then calculated the mean of these 5 values for each plot. As we checked graphically that the distribution was normal, we used a linear regression model and to test whether there was a significant difference between the means of the 3 different treatment groups for each date. For *C. lasiocarpa*, plant elongation was significantly greater in removal snow plots than in control plots, except on 5 and 17 June (see figure 18). There was no significant difference between the plants in the additional snow plots and those in the control plots. The relatively low multiple R-squared (between 13.36% and 19.01%) does not fully explain the variations in the observed variable. The minimum value is 127.7 mm for the control and 149.1 mm for the suppressed snow (see figure 18). The maximum values for the controls were 302.3 mm and 350.2 mm for the removal snow plots, representing growth rates of 136.73% and 134.88% respectively. Although the average elongation of the snow removal plots is greater than that of the controls and additional snow plots, they follow the same trend.
It is important to note that these results show the behaviour of overwintered *C. lasiocarpa* shoots that have already reached their initial height. Newly formed shoots may behave differently.

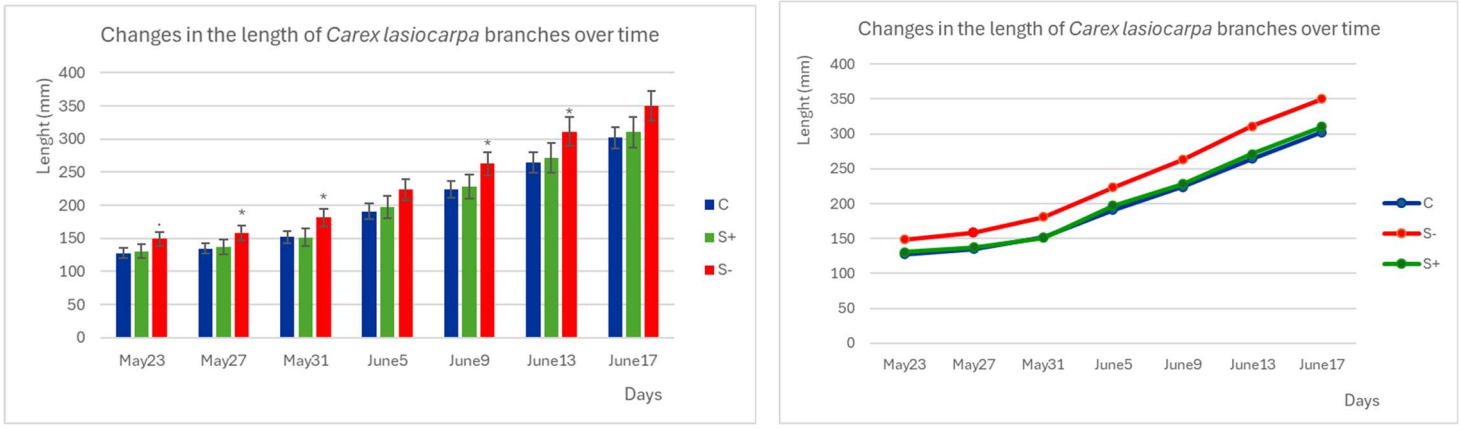


Figure 18 : Changes in the lenght of Carex lasiocarpa (J. Helson, 2024)

For *Carex chordorrhiza*, the Wilcoxon test showed no significant difference between the 5 plants in each plot. The linear regression also showed no significant difference between the different treatments, whatever the date (see table 1). However, these results should be treated with caution as the multiple R-squared is relatively low (between 4.37% and 12.97%).

Table 1 : Summary of linear regression of Carex chordorrhiza (J. Helson, 2024)

Date	Treatment	Estimate standard	Error	Pr(>|t|)	
May23	C	10.167	1.249	1.9e-07	***
	S-	2.278	1.612	0.175	
	S+	0.600	1.766	0.738	
May27	C	15.733	1.336	6.79e-10	***
	S-	1.806	1.724	0.309	
	S+	2.061	1.889	0.290	
May31	C	23.058	1.517	1.03e-11	***
	S-	3.175	1.959	0.122	
	S+	2.325	2.145	0.293	
June5	C	36.625	3.693	1.02e-08	***
	S-	3.731	4.768	0.444	
	S+	4.242	5.223	0.427	
June9	C	45.417	5.173	6.36e-08	***
	S-	7.833	6.678	0.256	
	S+	9.750	7.316	0.199	
June13	C	52.083	6.521	2.51e-07	***
	S-	7.806	8.418	0.366	
	S+	8.817	9.221	0.352	
June17	C	59.392	7.224	1.66e-07	***
	S-	8.442	9.327	0.377	
	S+	9.708	10.217	0.355	

2)Forest

The study of the phenology of *Vaccinium myrtillus, Vaccinium vitis-idaea and Empetrum nigrum* shrubs is qualitative. The statistical variables are therefore qualitative, independent and ordinal. Each developmental stage was assigned a number (from 1 to 8). This allowed us to carry out a Kruskal-Wallis rank sum test for the reproductive and vegetative phases of each shrub, in order to determine whether there was a significant difference in the stage of development according to the three snow treatments (additional, removal, control). For each date, we carried out six tests. The test indicates whether there is a significant difference between the groups but does not specify which specific groups show a significant difference. The results showed that there was no significant difference between the treatments for any of the shrubs (see table 2). We therefore did not need to carry out a post-hoc test (such as Dunn with Bonferroni, Dunn with Holm or Dunn with Benjamini-Hochberg) to identify the specific groups between which there were significant differences.

Table 2 : Kruskal-Wallis rank sum test for Vaccinium myrtillus, Vaccinium vitis-idaea and Empetrum nigrum (J. Helson, 2024)

		May22		May28		June1		June4		June8		June12		June16	
Shrub	Stage	X^2	p-value	X^2	p-value	X^2	p-value	X^2	p-value	X^2	p-value	X^2	p-value	X^2	p-value
V. myrtillus	vegetative	1.5306	0.4652	1.2083	0.5465	1.2347	0.5394	2.445	0.2945	No diff		No diff		No diff	
	reproductive	1.5306	0.4652	0.98063	0.6124	0.72905	0.6945	1.7273	0.4216	1.2585	0.533	No diff		0.21333	0.8988
E. nigrum	vegetative	1.1004	0.5768	2.09	0.3517	No diff		8.512	0.01418	1.9	0.3867	No diff		No diff	
	reproductive	2.3545	0.20529	4.3393	0.1142	1.3633	0.41502	0.58641	0.7459	No diff		No diff		No diff	
V. vitis idaea	vegetative	2.1875	0.335	0.22727	0.8926	0.25455	0.8805	0.18984	0.9094	0.8642	0.6491	1.4583	0.4823	2.8438	0.2413
	reproductive	3.0033	0.2228	2.9533	0.2284	0.60041	0.7407	1.2884	0.5251	2.1875	0.335	No diff		1.0294	0.5977

* no diff: all sub-plots are at the same stage of development

IV. Discussion

1) Results of CO2 measurements

1)Fen

The control plots have a higher ER than the other plots. This can be explained by more optimal conditions for microbial respiration and decomposition of organic matter, in particular due to more stable temperatures.
The fact that the GPP per ER in plots with additional snow is higher than that in control plots or plots with removal snow can be explained by the fact that snow is a thermal insulator, protecting plants from frost. This stimulates microbial activity, which improves the formation of carbon-rich humus and the decomposition of organic matter. In addition, additional snow reduces water loss through evapotranspiration during cold spells. As a result, plants experience less water stress, making them less stressed and more able to photosynthesise.
As the absorption potential is higher, there is a potential relocation of carbon in the soil (which can then be recycled as CO2), the emissions (ER) are not higher. One possible explanation is that the soil is still much colder and needs more time to warm up, inhibiting the growth of microbes.

Conversely, snow removal plots produce less GPP per RE. This means that the direct benefits to plants in terms of growth (capital gains) from snow removal are lower than the indirect benefits (energy savings).
Furthermore, if refreezing events occur after the snow has melted, plants can be damaged or even die, leading to a significant reduction in GPP (Bokhorst et al. 2009 *in* Rixen et al., 2022). This decrease also leads to a decrease in ER and therefore carbon sequestration for the following season (Treharne et al. 2019; Treharne et al. 2020 *in* Rixen et al., 2022).
Gross primary production therefore depends on the microclimate, making it difficult to produce generic models.

The fen is mainly dominated by graminoids (*Carex*), but there are also shrubs such as *B. nana*. Sweet et al (2014b) have shown that warmer temperatures favour the development of taller and taller shrubs. This is because 'low soil temperatures limit the translocation of nutrients and carbohydrates from underground reserves to tussock crowns and rhizomes (e.g. Chapin, Johnson and McKendrick 1980)' for low vegetation (Sloan et al., 2015). These regions, dominated by tall shrubs, are not affected in the same way by early snowmelt. In fact, these landscapes dominated by shrubs retain more snow, which ultimately lengthens the winter season. Roots are therefore better protected from the cold and can maintain relatively high winter microbial activity and nutrient mineralisation (Schimel et al., 2004; Wahren et al., 2005; DeMarco et al., 2011 *in* Sweet et al., 2014), giving them a developmental advantage over graminoids.
The phenology of plants in shrub ecosystems is then delayed. However, this delay is compensated by rapid leaf development. In the end, the photosynthetic period will not be significantly shorter than that of the smallest shrubs, with no effect on soil carbon uptake (Sweet et al., 2014). Ultimately, it is the microclimate that determines carbon release or uptake and plant phenology (Sannimari Käärmelahti, 2018).

2) Forest

The lichen plots exposed to reindeer grazing showed lower respiration activity than those that were not. We can hypothesise that the plant biomass is lower due to reindeer grazing on the lichen. In addition, the ER of the additional snow plots is higher. This can be explained by the fact that snow provides

plants with a physical barrier against some herbivores (Rixen et al., 2022). The duration of exposure to reindeer was shorter and therefore less grazing and trampling of the plant cover occurred. Trampling leads to a reduction in vegetation cover, biomass and diversity (e.g, Holtmeier, 2015; Koster, Berninger, Koster, & Pumpanen, 2015; Olofsson, 2006b; Suominen & Olofsson, 2000 *in* Heggenes et al., 2017b) and makes it particularly sensitive to wind and water erosion (Belnap & Gillette, 1998; Pegau, 1970; Wielgolasky & Kjelvik, 1973 *in* Heggenes et al., 2017b), which may lead to its disappearance in certain areas. Heggenes et al. (2017b) showed that the moisture content and thickness of the lichen played a major role in its ability to recover from this disturbance. The lichen that remained under the snow for longer may have been able to absorb more water and therefore be wetter, making it more resilient to trampling. It is difficult to predict the evolution of lichen cover in relation to temperature, as the weather tends to warm (which can dry it out), but also to wet it (Heggenes et al. 2017).

The disappearance of lichens also has an impact on pine seedlings. When lichen density is lower, nutrient fluxes increase and allelopathy disappears, which benefits the growth of pine seedlings. However, lichen also acts as an insulator for the roots of pine seedlings. It insulates them from high summer temperatures and very cold winter temperatures (Väre et al., 1996) and limits soil desiccation (Kershaw and Field, 1975). In addition, *Cladina spp*. provide an important regeneration niche for Scots pine (Steijlen et al., 1995). If the lichen density is lower, the pine roots are less insulated from extreme temperatures, which can weaken them. The positive effects of lichens on pine seedlings outweigh the negative ones.

In contrast, the ER of blueberry-dominated plots with additional snow and exposed to reindeer grazing is higher than that of fenced plots. This could be explained by the fact that they increase their growth rate to compensate for biomass losses (McNaughton, 1983). This leads to an increase in photosynthetic activity and consequently respiration.

2)Phenology results

1)Fen

The elongation of *Carex lasiocarpa* was significantly higher in the snow removal plots than in the control plots. This can be explained by the fact that they had earlier access to light and higher air temperatures, which increases photosynthetic activity. However, in addition to above-ground growth, there is also below-ground growth. If root phenology is not adapted to early snowmelt, this can lead to a mismatch between available resources and plant uptake (Blume-Werry et al., 2017). This could delay and limit leaf growth. As *Carex* root growth is relatively short-lived, it would be advantageous to start it early in the season to avoid running out of nutrients, the main factor limiting its development (Sloan et al., 2015). This may give them a competitive advantage over species that grow later and consequently have fewer nutrients.
Furthermore, we know that the addition of snow provides good starting conditions for microbes because the soil is already 'warm'. However, the phenology of the plants in these plots is similar to that of the control plots. We can therefore rule out soil phenology as an explanation. Another explanation could be that the more favourable winter conditions led the microbes to deplete labile carbon sources (i.e. sugars and organic matter easily decomposed and require little energy input). As a result, plants lack replenishment at the start of their growth. It would therefore be very interesting to measure winter CO2 fluxes as part of a future project.

For many plant species living in high latitudes, the growth of plants above and below ground is asynchronous (Abramoff & Finzi 2015 in Blume-Werry et al., 2017). As the air temperature warms up faster

than in the soil, growth of the above-ground part starts earlier in the summer season than that of the roots (Steinaker & Wilson, 2008 in Abramoff & Finzi, 2014). This can lead to excessive asynchrony, which can be detrimental to the aerial part of the plant. Plants that are able to adjust their growth both above and below ground may then have a competitive advantage. In any case, changes in plant phenology lead to numerous changes in reproductive function, population dynamics and physical condition (Cleland et al. 2012; Diez et al. 2012; CaraDonna, Iler and Inouye 2014 in Blume-Werry et al., 2017) and ecosystem processes such as carbon, water and nutrient cycling (Richardson et al. 2013 in Blume-Werry et al., 2017).
The fact that *C. chordorrhiza* elongation showed no significant difference between treatments could be explained by later growth in the season. The snow had already melted in the removal snow, additional snow and control plots. Plants in the snow removal plots were therefore unable to benefit from more light or higher air temperatures. As *C. chordorrhiza* grows more from aerial shoots than *C. lasiocarpa*, soil temperature is perhaps less important for this growth strategy.

2)Forest

No significant difference was found between the different snow treatments for the phenology of *Vaccinium myrtillus, Vaccinium vitis-idaea and Empetrum nigrum*. This could be explained by the fact that the snowmelt delay between the additional snow plots and the control/snow plots was not long enough to observe a significant difference in shrub phenology. The thaw period may also have influenced plant development. Plants that were more advanced in their development may have been weakened, whereas plants in plots with extra snow were protected from this cold period. The delay may therefore have been made up in the end.
Nevertheless, Sannimari Käärmelahti (2018) has shown that *V. vitis-idaea* postpones its phenophases on early snowmelt to protect itself from potential frost periods and to ensure the presence of pollinators by not flowering too early.

In contrast, *E. nigrum* seems to start its spring phenology as soon as the snow melts, even if it is early. Sometimes it may even begin to develop before the snow melts. Stemkovski et al (2020) have shown that pollinators emerge relatively early after snowmelt but are still less sensitive than plants. This leads to asynchrony, which can reduce pollination rates. Furthermore, this early emergence can lead to a second pollinator reproduction episode (Altermatt, 2010). If this occurs too early in the season, individuals will not be adapted to the autumn season, which could pose problems for the long-term survival of the species (Van Dyck et al., 2015). This new generation can pollinate pollen-limited plants, allowing them to reproduce more. However, the advantages are outweighed by the disadvantages of asynchronous phenology between pollinators and plants (Stemkovski et al., 2020).
In short, *V. vitis-idaea* is 'more sensitive to changes in snow conditions and *E. nigrum* to have more plasticity in utilizing the lenghtened snow free period'. (Sannimari Käärmelahti, 2018)

V. myrtillus bud burst later in the season when winter temperatures were warm (Bokhorst et al. 2008 in Rixen et al., 2022). Conversely, Preece et al. 2012 showed that experimental winter ice encasement (ROS simulation) caused earlier spring budburst. The phenology of *V. myrtillus* therefore depends directly on meteorological conditions.
Furthermore, root cover was found to be lower in snow removal plots than in control and additional snow (Kreyling et al., 2011). This can lead to problems with nutrient transport, which can limit plant growth and even reduce photosynthesis.

V. Conclusion

There are a number of interrelated components to this research question. It is therefore not possible to give a generic answer. Respiratory and photosynthetic activity as well as plant phenology are strongly influenced by microclimate.
For example, frost events can lead to a decrease in GPP as a result of plant damage (Bokhorst et al. 2009 in Rixen et al., 2022) and thus carbon sequestration. Vegetation also plays a role. When landscapes are dominated by tall shrubs, snow is retained, which delays the phenology of the shrubs. However, this photosynthetic period, although shorter, will not absorb less CO2.

Furthermore, the plant species studied do not respond in the same way to the different snow treatments. This can be explained by different adaptation strategies, greater or lesser sensitivity to temperature and microclimate. For example, the elongation of *C. lasiocarpa* is greater in snow removed plots than in snow added plots and controls. Conversely, there was no significant difference in the elongation of *C. chordorrhiza*.
No significant differences between the 3 snow treatments were found for forest shrubs. However, we assume that our results are not necessarily relevant on a seasonal scale, especially since Sannimari Käärmelahti (2018) was able to show that *V. vitis-idaea* postpones its phenophases with early snowmelt and that *E. nigrum* starts its spring phenology as soon as the snow melts. Bokhorst et al (2008) also showed that *Vaccinium myrtillus* starts budding later, waiting for more favourable temperatures. Comparing our current data with those from previous or subsequent years would allow us to draw more precise conclusions.

We have not been able to show directly that reindeer grazing has a negative effect on lichens. However, this does not mean that the management of reindeer husbandry in Finland should not be questioned. It is a complex issue with many parameters. For example, reindeer husbandry has been an integral part of the Sami culture for hundreds of years. So it's difficult to change their customs completely. In Sweden, they decide who can own reindeer and who cannot. This leads to conflicts over land use with the Swedish and Finnish governments, who sometimes want to use the land for forestry, which is the most harmful activity for lichens.

Figure 19 : Photos of reindeer grazing or walking (credit : J. Helson, 2024)

Reflecting on the experience

During my internship I learnt more about research work, which was completely foreign to me. I realised that it is all about regularly questioning what we are doing and adapting our work. What potential impact could our manipulations have on the ecosystem? How can we quantify them? How can we try to limit them if necessary? I was also surprised to learn that it was necessary to consult scientific articles to find out how to study plant phenology. I wouldn't have thought of that myself, although it's obvious now.

The work on site was quite physical in the sense that we had to do the same movements over and over again. It wasn't easy at first to live on the research station, which was isolated and not very busy at the start of the season. However, it was very pleasant to work outside, especially with the nice weather we had!
As far as the work at the university was concerned, I was completely free in terms of my working hours. The challenge was to work independently and not to hesitate to ask Johannes to help me with my work. Perhaps I should have asked Johannes for more help, but I think that's due to a certain shyness...

I had problems processing the data. It's easier to use Rstudio for advanced statistics. However, I had never used this software before and found it difficult to get to grips with... As I don't master this software, I wasted time doing certain tasks ‘more manually’ using Excel. That's why I decided to use ChatGPT for the R codes. I sent it my requests, specifying as I went along what I was expecting. I then adapted the code because it's not always very reliable or optimised.
I realised that research is about trial and error, trying things out, asking questions, starting again... It's a tedious process and one that I found a bit difficult to get to grips with in the sense that I like to be efficient. But it allowed me to really think for myself and look for answers in the scientific literature.

I don't project myself into the world of research because I find it too abstract. Perhaps this is due to the topic of the thesis, which is still quite specific and which I would associate more with basic research. Nevertheless, Johannes was passionate and exciting. He taught me to have a sharper eye and to pay more attention to the world around me. He made me more curious and questioning. When I go for a walk, I find myself stopping to observe a bud, a flower, etc.

Beyond the professional aspect, this experience has enriched me personally. First of all, I was able to improve my level of English, speaking with Johannes as well as the owner of my room and the Swedes I met. I was able to experience fieldwork in ecology in a magnificent place, an opportunity that won't come along again soon. During my days off, I was able to go for a few walks and take in some superb scenery...! Once I got back to Sweden, I was able to discover their typical dishes, their way of life and their 'traditional' dances...

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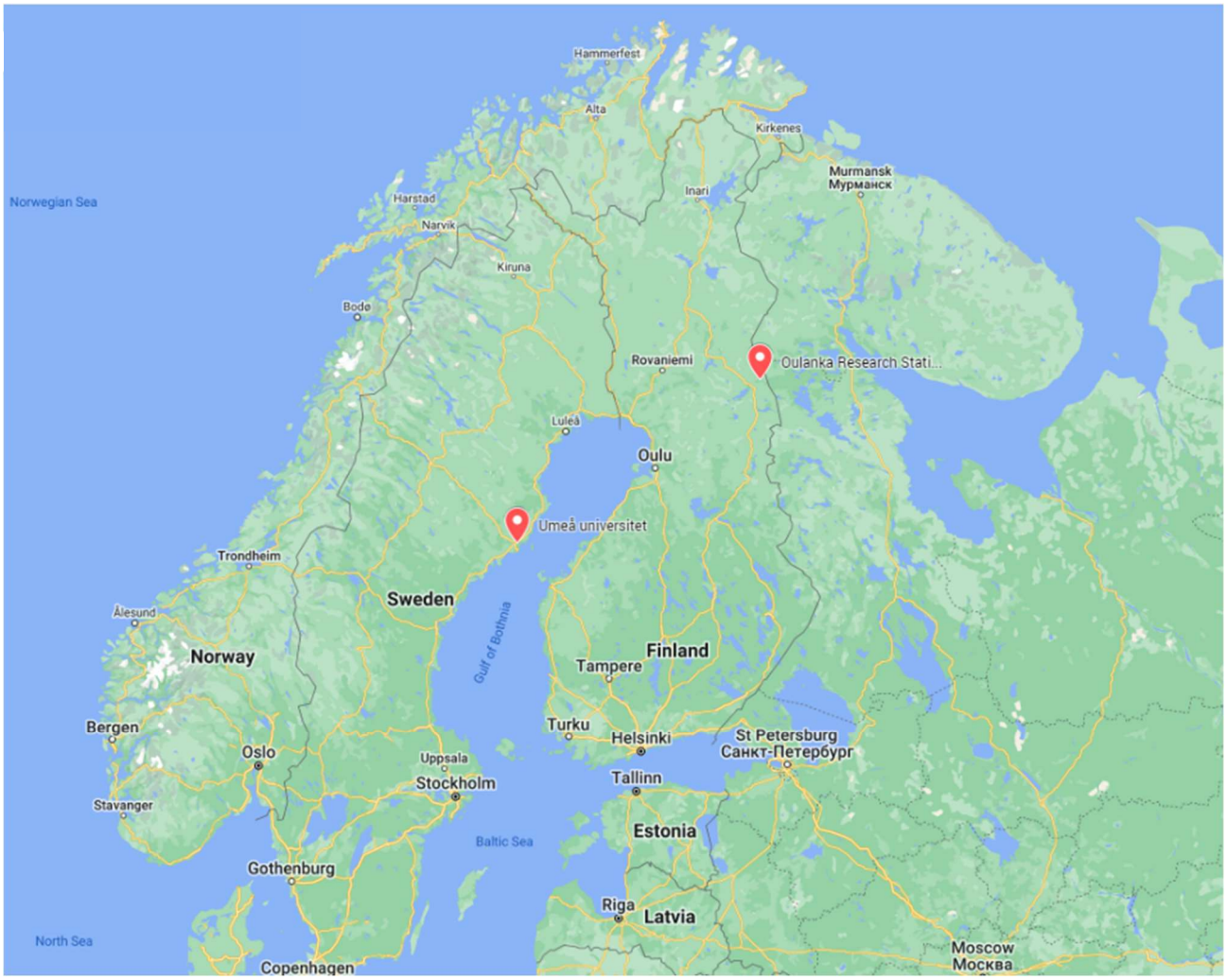
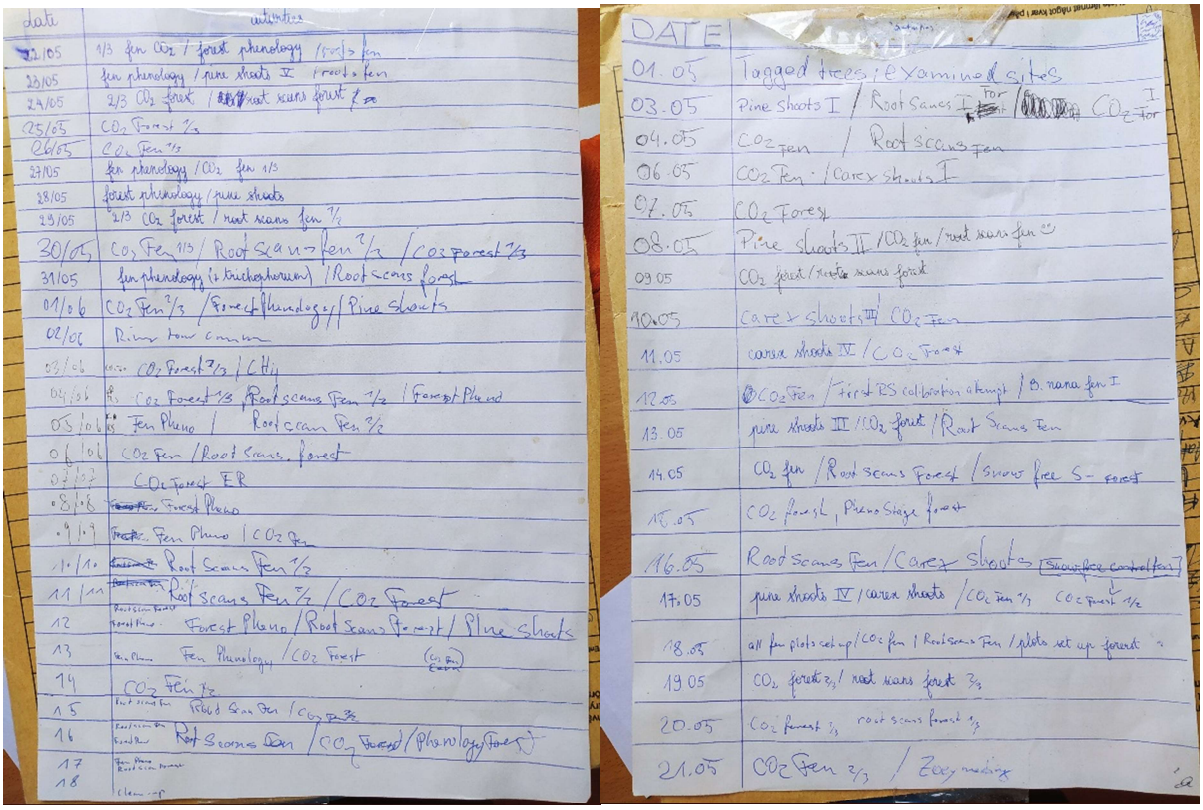
Appendices

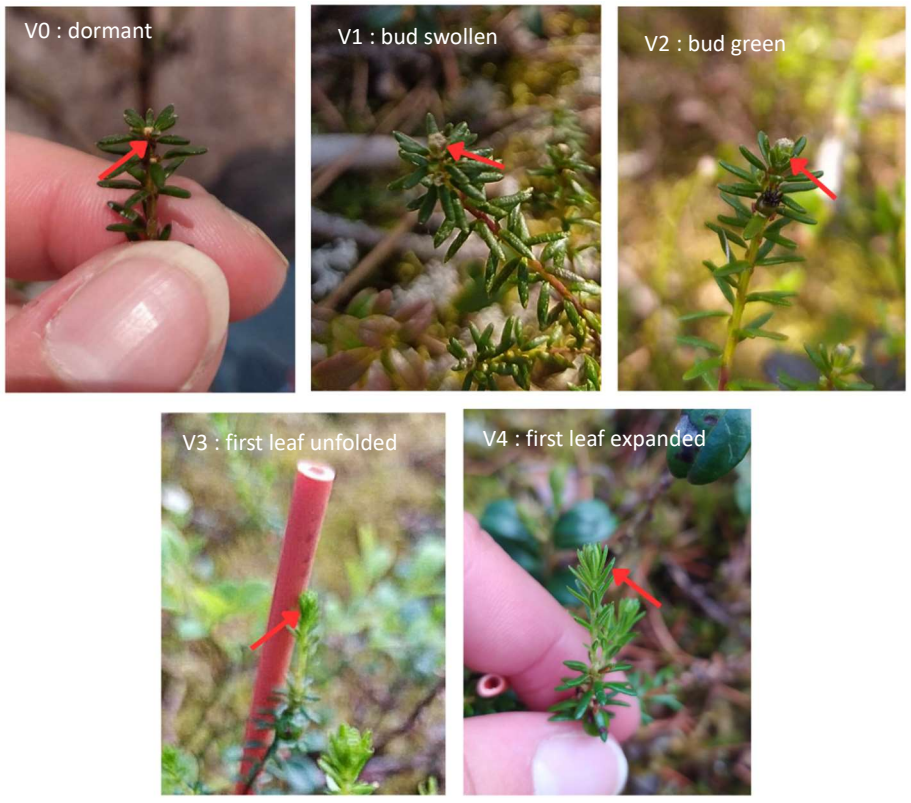
Appendix 1 : Location of Umea university and the Oulanka research station (Googlemaps, 2024)



Appendix 2 : On-site work schedule (credit : J. Helson, 2024)



Appendix 3 : Phenological stages of the vegetative phase of Empetrum nigrum (credit : J. Helson, 2024)

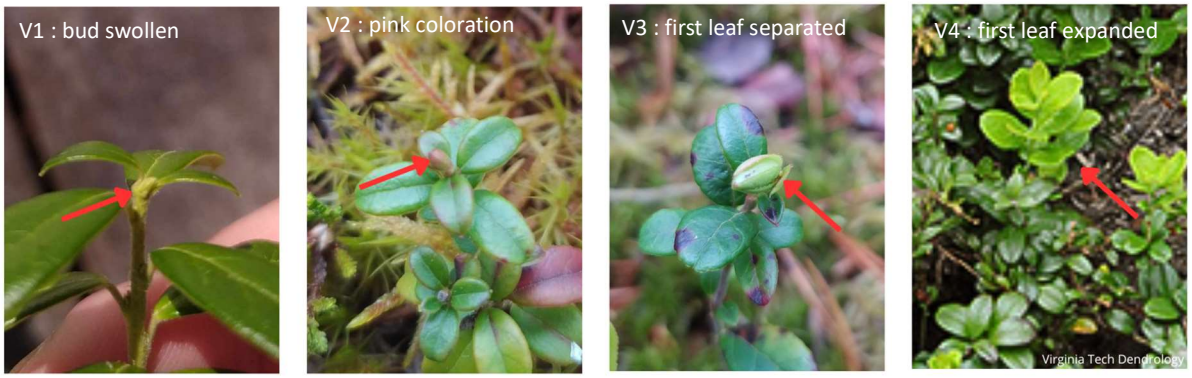


Appendix 4 : Phenological stages of the reproductive phase of Empetrum nigrum (credit : J. Helson, 2024)

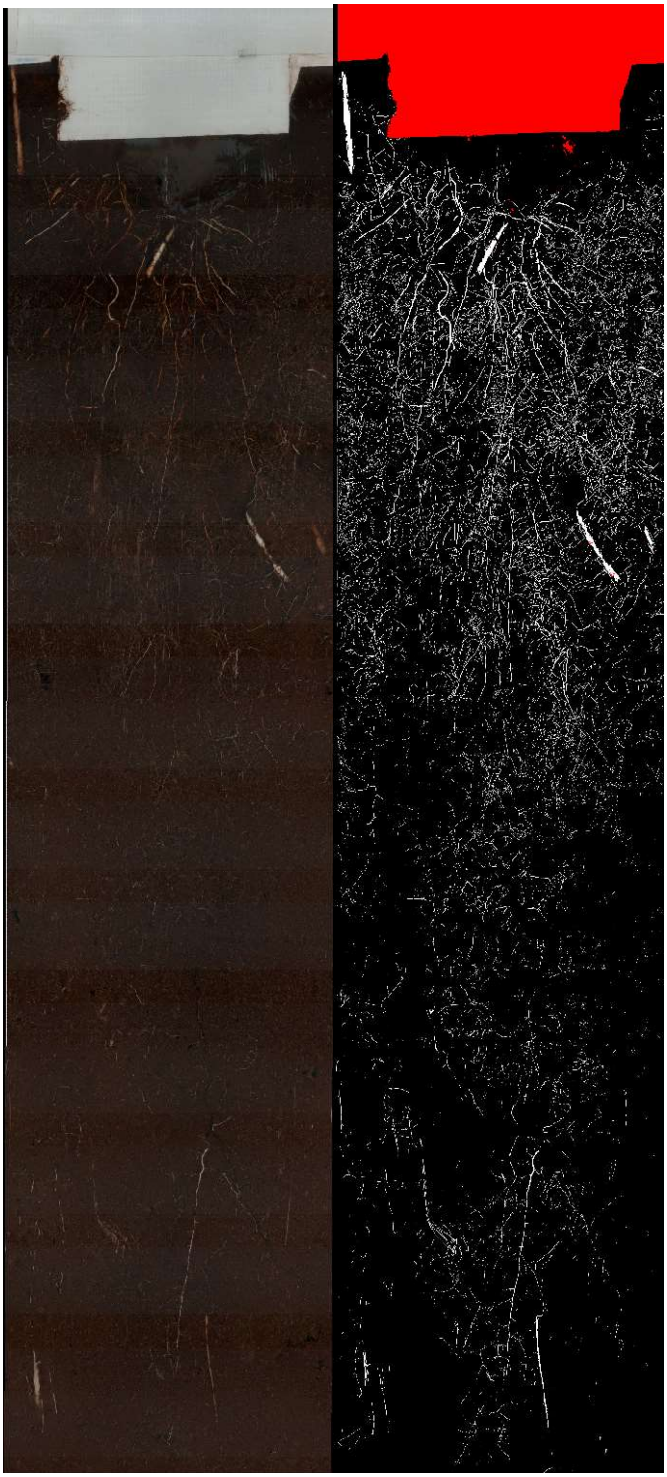
Appendix 5 : Phenological stages of the reproductive phase of Vaccinium Myrtillus (credit : J. Helson, 2024)

Appendix 6 : Phenological stages of the reproductive phase of Vaccinium vitis-idaea (credit : J. Helson, 2024)

Appendix 7 : Phenological stages of the vegetative phase of Vaccinium vitis-idaea (credit : J. Helson, 2024)



Appendix 8 : Root scans without AI analysis (left) and with analysis (right) (J. Cunow, 2023)



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2023-2024

Title: linking phenology and carbon exchange under snow cover changes and reindeer grazing in northern boreal ecosystems

Summary : My internship consisted of two parts. First we went to Oulanka National Park for 7 weeks to collect data (CO2 flux, phenology of certain plants and root scans). We had 2 study sites (a boreal forest and a wetland) with 36 plots each. Measurements were then taken for each plot. I then analysed some phenological data using R and wrote my internship report. Johannes guided me in my work and recommended a number of scientific articles to enrich my thinking.

Key words : research, field work, statistical analysis, phenology

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