

Nuuk Ecological Research Operations

# BioBasis Manual

Conceptual design and sampling procedures of the biological monitoring programme within NuukBasic



3<sup>rd</sup> edition, 2019



**BioBasis Manual – Conceptual design and sampling procedures of the biological monitoring programme within NuukBasic.**

3<sup>rd</sup> edition, February 2019.

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Front cover illustration: *Silene acaulis* flowering in the Kobbefjord valley. Photo by: Katrine Raundrup

## Preface

BioBasis Nuuk monitors key species and key processes across plant and animal populations and their interactions within the terrestrial and limnic ecosystems in Kobbefjord, Nuuk, thereby documenting the intra- and inter-annual variation, resilience and long-term trends. Emphasis is on biodiversity, abundance and composition, phenology, reproduction and predation as important components in the structure and functioning of the low arctic ecosystems.

Additional biotic (e.g. NDVI) and abiotic parameters (e.g. snow-melt patterns, soil moisture and water chemistry) related to biota are monitored, complementing other subprograms.

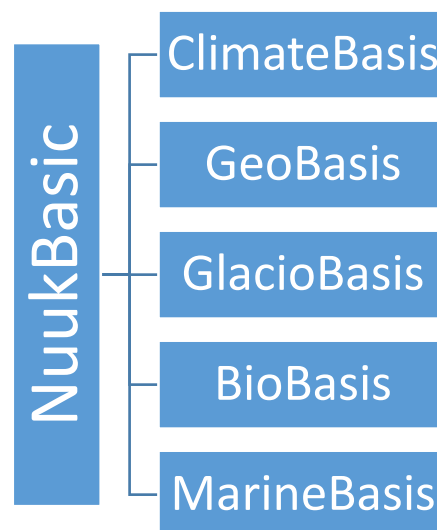
BioBasis aims at maintaining the integrity of the long core time series available from Kobbefjord, Nuuk, whilst developing the monitoring to embrace new methodologies and questions. Field protocols for monitoring elements that are no longer part of the BioBasis monitoring programme can be found in previous versions of the manual.

The BioBasis subprogram is part of a larger set of subprograms (ClimateBasis, GeoBasis, GlacioBasis and MarineBasis), which combined, is referred to as NuukBasic. NuukBasic together with corresponding programmes in ZackenbergBasic (since 1995) and DiskoBasic (since 2017) form the integrated monitoring and long-term research program on ecosystem dynamics and climate change effects and feed-backs in Greenland, Greenland Ecosystem Monitoring (GEM; [www.g-e-m-dk](http://www.g-e-m-dk)).



**NERO** 

**Nuuk Ecological Research Operations**



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## 1 Introduction

The NuukBasic programme was initiated in 2007 by the National Environmental Research Institute, Aarhus University, in cooperation with the Greenland Institute of Natural Resources. NuukBasic is funded by the Danish Energy Agency and the Danish Environmental Protection Agency as part of the environmental support programme DANCEA – Danish Cooperation for Environment in the Arctic. The present manual describes methods and sampling procedures of the subprogram BioBasis. The manual is updated regularly. The latest version can always be found at [www.nuuk-basic.dk](http://www.nuuk-basic.dk).

NuukBasic is a climate change effects monitoring programme with the monitoring sites located close to Nuuk in west Greenland. The programme studies the effects of climate variability and change on marine and terrestrial ecosystems. In terms of scientific concept, NuukBasic is equivalent to the investigations carried out in ZackenbergBasic, at Zackenberg Research Station in Northeast Greenland ([www.zackenberg.dk](http://www.zackenberg.dk)).

The terrestrial monitoring area is located at the inner parts of Kobbefjord approximately 20 km east of Nuuk as seen in figure 1 and 2. The local climate is low arctic with a mean annual temperature of  $-0.1^{\circ}\text{C}$  (2008-2017). The warmest month is July (average  $10.6^{\circ}\text{C}$ ) while the average temperature in March is  $-8.2^{\circ}\text{C}$  (coldest month). The annual mean precipitation in Kobbefjord is around 800 mm and maximum snow depths vary strongly between 0.3 and 1.3 m at the climate station. The drainage basin is located in an alpine landscape with mountains rising up to 1400 meter above sea level and with glacier coverage of approximately  $2\text{km}^2$ . Geologically, the area is relatively homogenous with precambrium gneisses as basement throughout the drainage basin. For a presentation of the Kobbefjord geology please refer to the note on “Kobbefjord geology” by Thebaud and Lebrun which can be found in the cabin in Kobbefjord.

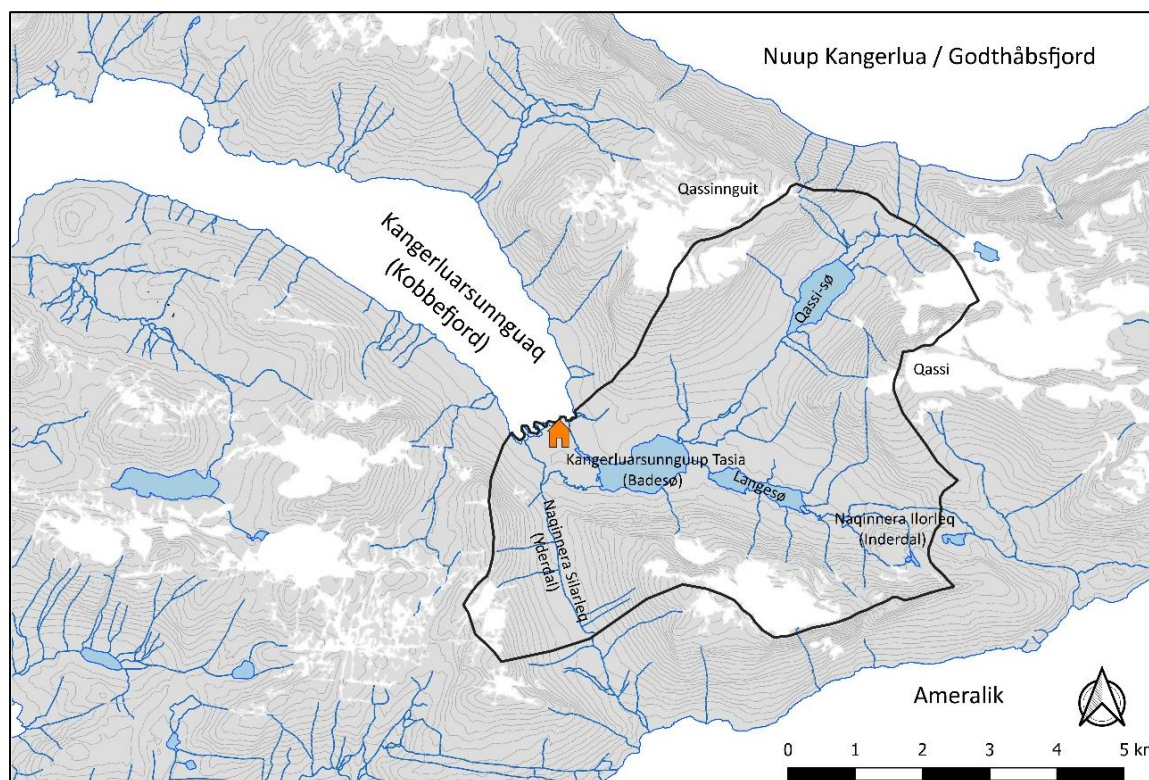


Figure 1. The study area. The orange symbol marks the location of the cabin. Nuuk is located further east (outside the figure to the left).

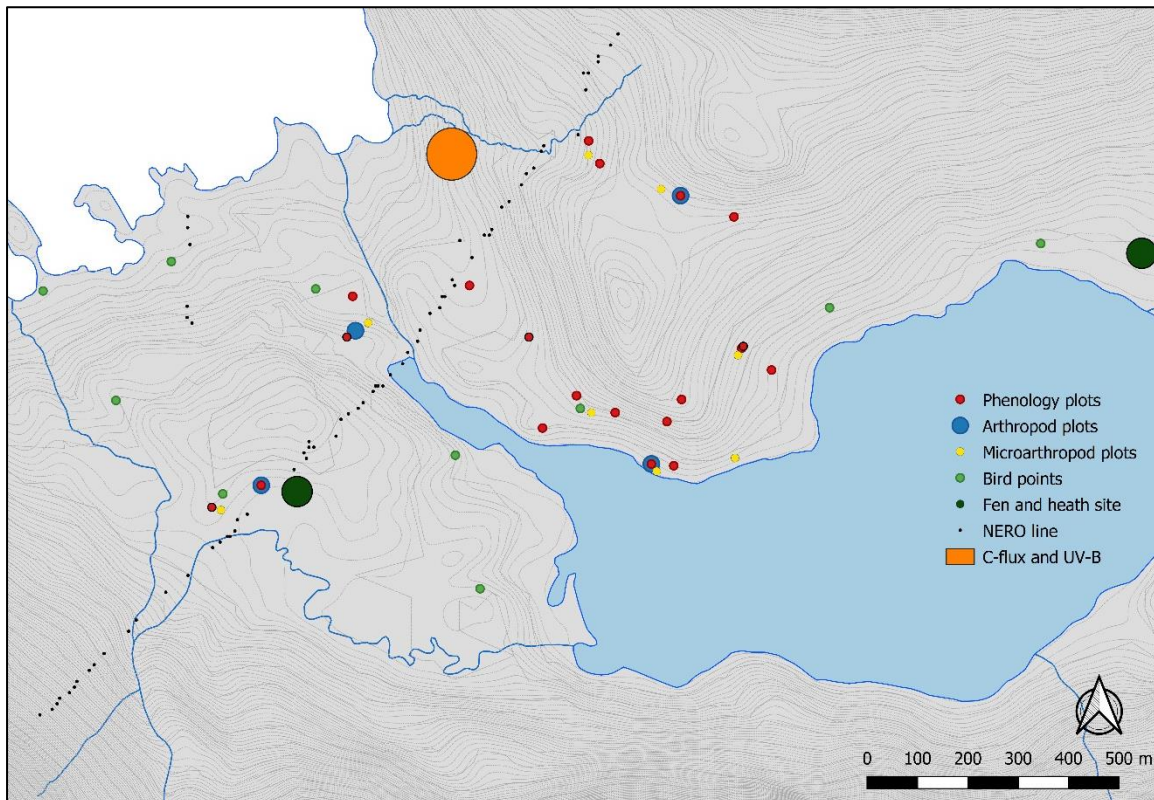
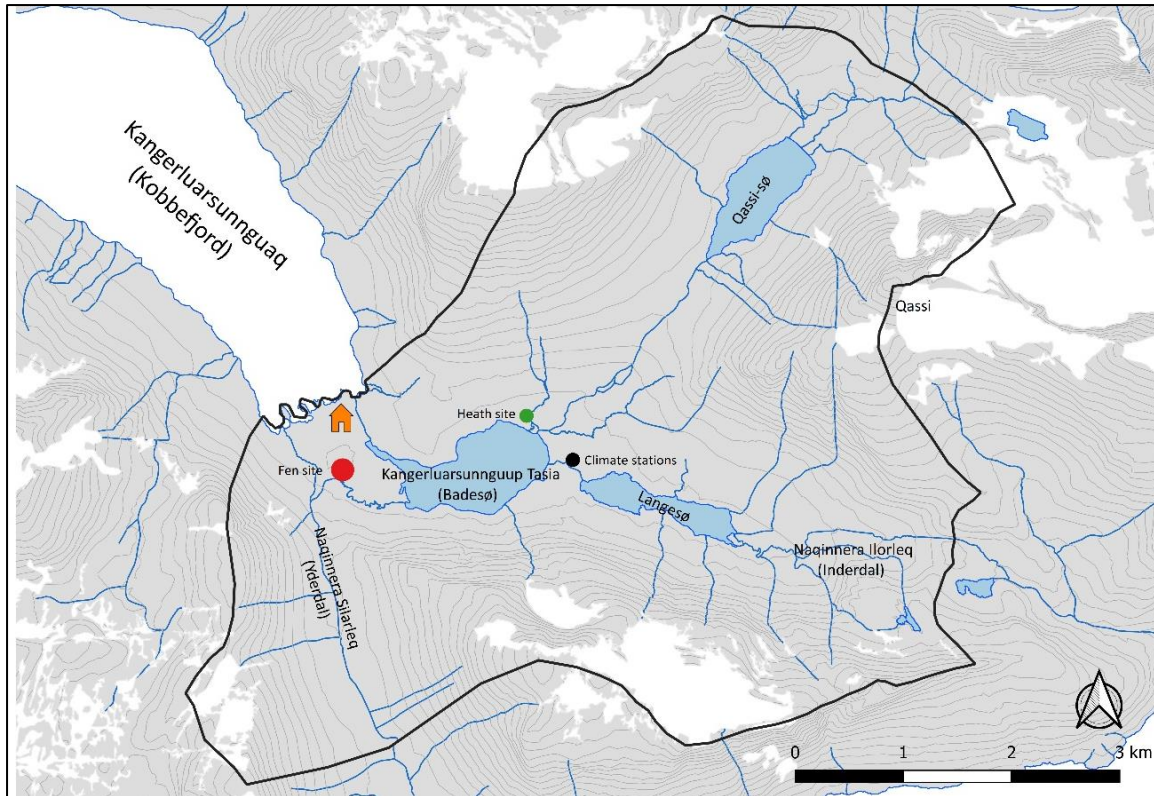


Figure 2. Upper figure: Close up of the BioBasis study area. The climate station is marked by black dot, the heath site by green dot and the fen site by red dot. The orange symbol marks the location of the cabin. Lower figure: Close up of area with most plots. The two dark green dots refer to the GeoBasis stations: Fen site and Heath site. Please note that some of the plots are very close together and points on the map may be overlapping.

## 2 Overview of monitoring elements

Table 1 gives an overview of the monitoring elements.

Table 1. Overview of monitoring elements.

Monitoring element	Species, treatment, sample	Number of plots, samples	Sampling frequency	Sampling object	Sampling period
<b>NERO line</b>	Plant communities and species		Every 5 <sup>th</sup> year		
<b>Phenology</b>	<i>Salix glauca</i>	4	Weekly	Buds, flowers, senescent	May-October
	<i>Loiseleuria procumbens</i>	4			
	<i>Silene acaulis</i>	4			
<b>Total count of flowering shoots</b>	<i>Salix glauca</i>	4	Once per season at peak flowering	Flowers (Sal, Loi, Sil), individual plants (Eri)	Depending on phenology
	<i>Loiseleuria procumbens</i>	4			
	<i>Silene acaulis</i>	4			
	<i>Eriophorum angustifolium</i>	4			
<b>NDVI</b>	Along NERO line		Monthly		May-October
	In phenology plots incl. <i>E. angustifolium</i> and <i>Empetrum nigrum</i>	20	Weekly	Plot	May-October
<b>CO<sub>2</sub>-flux</b>	Control, C	6	Weekly	Plot	May-October
	Increased temperature, T	6			
	Shading, S	6			
	Long growing season, LG	6			
	Short growing season, SG	6			
	Vegetation analysis, pin-point analysis	30			
<b>UV-B</b>	Control, C	5	3 times per season	Plot	Weekly in weeks 29-31
	Mylar film, B	5			
	Teflon film, F	5			
<b>Arthropod</b>	All taxonomic groups	4, 32 samples	Weekly	Specimens	May-October
<b>Microarthropod</b>	All taxonomic groups	8	3 times per season	Specimens	June-September
<b>Birds</b>	Passerines	13	Weekly	Specimens	May-October
	Passerine nests	Ad hoc	As often as possible	Nests	Breeding season
<b>Mammals</b>	Arctic fox, caribou	Ad hoc	When observed	Specimens	All year
<b>Lakes</b>	Water chemistry, zoo- and phytoplankton, Chl <i>a</i> , DOC	2 lakes	5 times per season		Ice-free period
	Flora	2 lakes	Once per season		
	Fauna (Arctic char, sticklebacks)	1 lakes	Every 5 <sup>th</sup> year		
	Isotopes	2 lakes	Every 5 <sup>th</sup> year		

### 2.1 Plants

#### 2.1.1 The NERO line

A permanent vegetation transect (the NERO line) was established in July 2007, in order to monitor future changes in the distribution and composition of vascular plant species in the plant communities (Bay et al. 2008). Surveys of the transect take place with 5 year intervals and have been done in 2007, 2012 and 2017. In 2010, mosses and lichens were included in the monitoring programme.

The concept relies on the assumption that changes in the distribution of plant communities can be observed by tracking changes of boundary lines between vegetation zones. Thus, each boundary between vegetation zones has been marked by a peg. The species composition of the vegetation zones is documented by Raunkjær analyses. Immigration of new species is assumed to be documented by the surveys with five-year intervals. The same concept is used for the ZERO line in Zackenberg in high arctic North East Greenland (Fredskild & Mogensen 1996).



### 2.1.2 Reproductive phenology

It is expected that plant phenology will give an early and distinct response to climate change. This has already been convincingly shown in Zackenberg (Post et al. 2009). In Nuuk we monitor three species: *Salix glauca*, *Loiseleuria procumbens*, and *Silene acaulis*. These species were chosen because they are widely distributed in the area, they cover a spectrum of different growth forms (deciduous dwarf shrub, evergreen dwarf shrub, and cushion forming herb), and they are functionally comparable to species monitored in Zackenberg.

For each species, four monitoring plots were established in 2007/2008. The specific sites of the plots were chosen in order to cover the ecological amplitude of the species with respect to duration of snow cover, difference in soil moisture and altitude. The size of each plot varies depending on the abundance of individual flowering shoots of the species in question.

### 2.1.3 Total flowering

Total flowering is a proxy of maximum annual productivity. Total flowering in the reproductive phenology plots is monitored in *Salix glauca*, *Loiseleuria procumbens*, *Eriophorum angustifolium*, and *Silene acaulis*. The number of flowers is counted at peak flowering as the total number of buds, flowers (catkins in *Salix* and flowering stems in *Eriophorum*) and senescent flowers (catkins in *Salix* and flowering stems in *Eriophorum*).

### 2.1.4 Normalized Difference Vegetation Index (NDVI)

NDVI is a measure of vegetation greenness and used as an index of plant production and vigorousness. The progression in vegetation greenness is monitored along the NERO line, in the plant phenology plots and in the CO<sub>2</sub> flux plots by measuring NDVI with a scanner. The scanner measures the spectral reflectance of the plant canopy.

### 2.1.5 CO<sub>2</sub> flux plots

The CO<sub>2</sub> flux is important for understanding the balance between emission and uptake of CO<sub>2</sub> in the vegetation. The monitoring documents the present state, but also provides data from manipulations simulating increased temperature and increased cloud cover. This will provide an indication of whether the low arctic vegetation type dwarf shrub heath functions as a sink or a source of CO<sub>2</sub> now as well as in the future, where increased temperatures and cloud cover is expected.

### 2.1.6 UV-B exclusion

UV-B radiation will increase as a result of the expected depletion of the ozone layer in the atmosphere (Jansen et al. 1998). UV-B radiation has damaging effects on higher plants as e.g. normal growth and development may be affected. We monitor the effect of increased UV-B radiation on plant stress indirectly by measuring chlorophyll fluorescence in three series of plots: Controls, plots with a filter excluding UV-B, and filter controls with a film without exclusion of UV-B. Measurements of chlorophyll fluorescence are carried out on individual *Betula nana* and *Vaccinium uliginosum* leaves in a mesic dwarf shrub heath dominated by *Empetrum nigrum* and with *Betula nana* and *Vaccinium uliginosum* as subdominant species.

## 2.2 Arthropods

Arthropods are sampled by means of yellow pitfall traps. Window traps are used for flying insects. The traps are emptied weekly throughout the snow free season. Samples are stored at the Greenland Institute of Natural Resources (GINR) and shipped to, sorted and identified at the

Department of Bioscience, Aarhus University, Silkeborg. Samples are stored at Naturhistorisk Museum, Aarhus.

### 2.3 Microarthropods

Soil cores are collected with a soil auger (soil corer) from which the organisms are extracted in a heat extractor by gradually heating. Microarthropods are identified at Department of Bioscience, Aarhus University, Silkeborg.

### 2.4 Birds

The avifauna is monitored with special emphasis on passerine birds representing the highest trophic level monitored in Kobbefjord. Breeding phenology (first egg dates, hatching, fledging) is monitored throughout the season on an ad hoc basis.

Weekly counts of birds are carried out at census points during the snow free season. Other bird observations are recorded ad-hoc during the entire field season.

### 2.5 Mammals

Only few terrestrial mammals occur in the study area and only very sporadically: Arctic fox (*Vulpes lagopus*), arctic hare (*Lepus arcticus*), and caribou (*Rangifer tarandus*). All observations of mammals are recorded ad-hoc. If arctic fox dens are discovered, reproduction will be followed.

### 2.6 Lakes

The two sampling lakes are located in the Kobbefjord catchment area in the bottom of Kobbefjord (Badesø / Kangerluarsunnguup Tasia: 64,13°N, 51,36°W and Qassi Sø: 64,15°N, 51,31°W).

Monitoring include ice cover, water chemistry, DOC (dissolved organic carbon), chlorophyll  $\alpha$ , physical conditions, species composition of plankton, vegetation, bottom organisms and fish. Physical-chemical parameters, DOC, chlorophyll  $\alpha$ , phytoplankton and zooplankton are monitored monthly during the ice-free period.

## 3. Detailed manual

Appendix 1 gives an example of the activities during a monitoring season in Kobbefjord. In the following paragraphs procedures are described in detail for all monitoring elements.

### 3.1 Plants

#### 3.1.1 The NERO line

The NERO line was established in 2007. It is described in detail in Bay et al. (2008). Surveys of the vegetation transect are done with 5 year intervals. Surveys have been done in 2007 (establishment), 2012 and 2017 and is planned to be done in 2022. The location of the line is shown in figure 3. See also Bay et al. (2008). The analyses of the transect are only done by trained and experienced botanists.

The NERO line monitoring also includes pin-point analyses as a reference of species composition in the different vegetation types. The sites with pin-point plots are shown in figure 3. Pin-point analyses are performed in seven distinguishable and dominating vegetation types in the Kobbefjord area with five replicas in each vegetation type:

1. Dwarf shrub heath

2. Snow patch
3. Herb slope
4. Fen
5. Copse
6. *Deschampsia* – *Juncus* community
7. Salt marsh

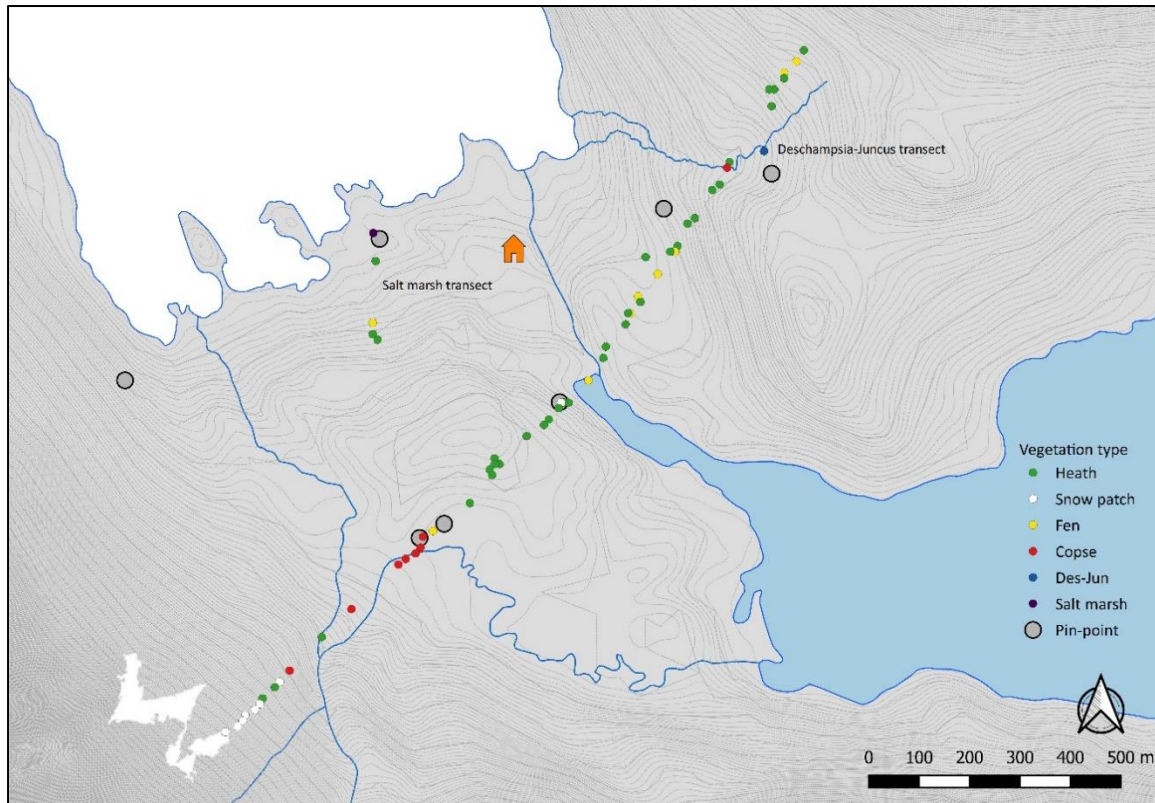


Figure 3. The NERO line. The dots show the positions of the pegs and the colour indicate the vegetation zone northeast of the peg. Numbering of the pegs starts in the south-western corner. The break in the black line to the north-east marks a steep slope that was not analysed by Raunkjær analyses. The short line represents a salt marsh at the coastal zone. The black square indicates the position of the characteristic, but rare, plant community dominated by *Deschampsia flexuosa* and *Juncus trifidus*, which is found on south facing, dry slopes. Grey circles mark the sites of pin-point analyses.

### Input of data into database

Data from the Raunkjær circle analyses are entered into a data file named “NERO\_line\_Raunkjær\_data” with the columns: Peg no., Plot no., Year, Month, Day, Observer, Vegetation type, Species name and Raunkjær value. Uncertain species identifications are marked cfr. (confer). Fertile specimens are indicated by (F) added next to the Raunkjær value.

Data from the pin-point analyses are entered into a data file named “NERO\_line\_pin-point\_data” with the columns: Date (YYYY/MM/DD), Vegetation type, Plot (1 – 5), NS (1 – 10), WE (1 – 10), Layer (1 – 3), Species, Status (Live/Dead), Height (cm), Remarks. See NERO line manual (Bay et al. 2008) for further explanation.

Digital pictures, original data sheets, and data files are kept at the Greenland Institute of Natural Resources.

### 3.1.2 Reproductive phenology

Monitoring of reproductive phenology consists of weekly counts of buds, flowers, and senescent flowers to monitor the proportion of the phenological stages of the species: Northern willow (*Salix glauca*), Trailing azalea (*Loiseleuria procumbens*), and Moss campion (*Silene acaulis*).

#### Species to be monitored

Three species: *Salix glauca*, *Loiseleuria procumbens*, and *Silene acaulis*. These species have been chosen due to the fact that they

- Are commonly found in the area.
- Cover a spectrum of different growth forms (deciduous dwarf shrub, evergreen dwarf shrub, and cushion forming herb/dwarf shrub).
- Are comparable to species monitored by BioBasis in Zackenberg.

There are four plots for each species. The size of each plot varies (see table 2) depending on the abundance of individual flowering shoots of the species in question.

#### Frequency of sampling

Censuses of *Salix glauca*, *Loiseleuria procumbens*, and *Silene acaulis* are made at weekly intervals in the snow free season (normally May – October). Total counts of flowers are done once a year at peak flowering. Total counts includes counting *Eriophorum angustifolium* flowering stems in four plots.

#### Equipment

- Map with position of study plots (or alternatively GPS)
- Camera
- Data form Appendix 2/ Notebook.

#### Location and marking of study plots

The location of the 12 plots are shown in Figure 4. The plots are marked with angle pegs in each corner, with one peg (the A peg) carrying a nametag with the plot-ID. They are divided into four subplots (quarters A, B, C, and D) separated by pegs at the centre where the diagonals cross and at the midpoint of each side – see Figure 5. The lettering starts at the corner with the plot-ID and continues clockwise around the centre. Coordinates, dimensions etc. can be found in table 2.

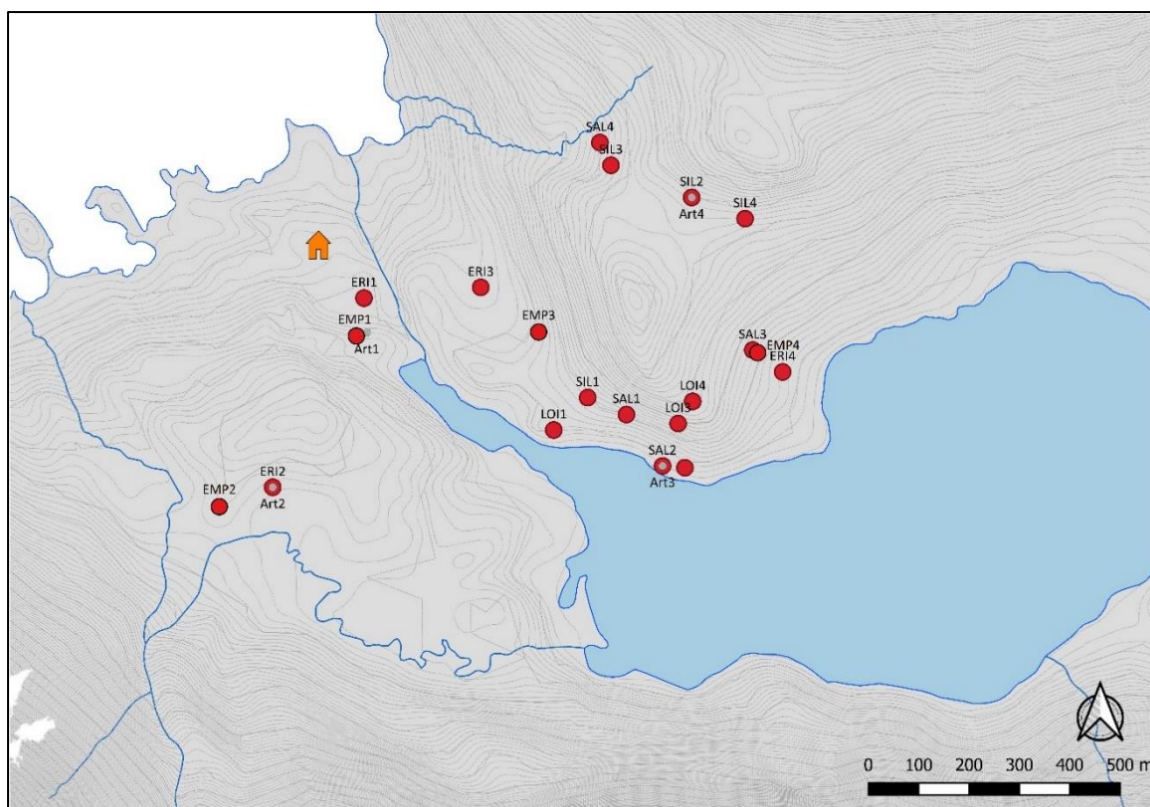


Figure 4. The locations of the plant reproductive phenology plots and plots for annual total counts of flowering shoots for *Salix glauca* (SAL1-SAL4), *Silene acaulis* (SIL1-SIL4), and *Loiseleuria procumbens* (LOI1- LOI4). Note that for *Eriophorum angustifolium* (ERI1-ERI4) only total counts of shoots are monitored. The locations of the *Empetrum nigrum* plots (EMP1-EMP4) for NDVI measurements are included along with the location of the four arthropod plots (marked with grey dots). Coordinates can be found in table 2.

Table 2. Positions and sizes of plant reproductive phenology and *Empetrum nigrum* plots. Positions are given in decimal degrees.

Species	Plot	Latitude	Longitude	Plot dimensions (m)
<b><i>Eriophorum angustifolium</i></b>	ERI1	64.1346	-51.3837	4*10
	ERI2	64.1312	-51.3873	10*10
	ERI3	64.1348	-51.3789	12*17
	ERI4	64.1333	-51.3666	5*9
<b><i>Salix glauca</i></b>	SAL1	64.1325	-51.3729	7*11
	SAL2	64.1316	-51.3714	8*8
	SAL3	64.1337	-51.3678	6*9
	SAL4	64.1374	-51.3741	4*5
<b><i>Silene acaulis</i></b>	SIL4	64.1361	-51.3681	5*12
	SIL1	64.1328	-51.3745	5,5*7
	SIL2	64.1364	-51.3703	11*11
	SIL3	6.4137	-51.3736	7*11
<b><i>Loiseleuria procumbens</i></b>	LOI1	64.1323	-51.3759	1.8*3.85
	LOI2	64.1316	-51.3705	1.7*2.95
	LOI3	64.1324	-51.3708	1.6*2.6
	LOI4	64.1328	-51.3702	1.6*3.0
<b><i>Empetrum nigrum</i></b>	EMP1	61.1339	-51.3839	
	EMP2	61.1308	-51.3894	
	EMP3	61.1336	-51.1336	
	EMP4	61.1337	-51.3675	

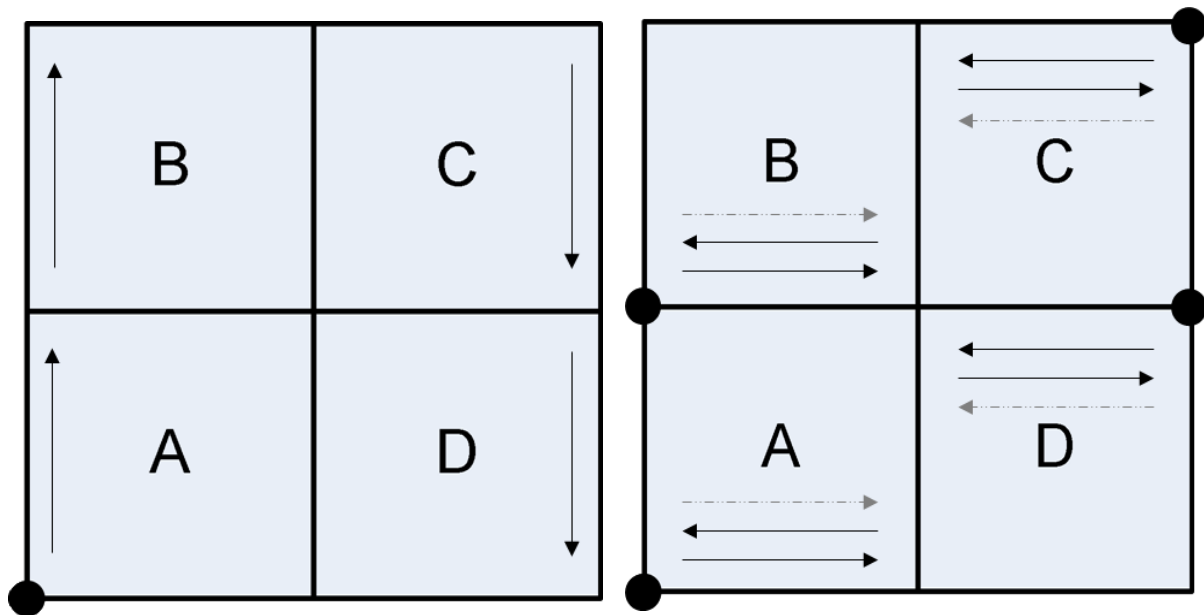


Figure 5. Lettering of subplots in plant phenology plots (left). The dot indicates the corner with the plot ID. Arrows indicate the clock-wise direction to walk when measuring NDVI. Overview of counting direction applied in phenology subplots A-D (right). Black dots represent starting points for counting in each subplot and arrows the counting direction. Begin at the peg with the plot-ID and count from left to right, as shown by the arrow. Continue counting by moving back from right to left. This procedure (left -> right, right -> left) is continued until a total of 50 (*Silene*) or 100 (*Salix*, *Loiseleuria*) is reached, or to the end of the subplot, whichever comes first. Then continue to the next subplot, starting at the next peg, indicated by the next black dot in the figure.

### Sampling method

The following observations and censuses are entered into the relevant data forms for all plots:

- Time
- Cloud cover
- Plot number
- Snow cover
- Number of buds
- Number of flowers (catkins in *Salix*) – note that for *Salix* both male and female plants can be found in the plots (SAL1, SAL2 and SAL3: both male and female plants; SAL4: only male plants)
- Number of senescent female catkins as indicated by presence of hairs (*Salix*)
- Number of senescent flowers (*Loiseleuria* and *Silene*)
- Total number of flowers (*Salix*, *Silene* and *Loiseleuria*) and flowering stems (*Eriophorum*)
- Remarks (include information on e.g. larvae or fungi infected leaves)

Before counting, take a photo of the plot in which as much as possible of the plot is included. As a minimum the metal rod with the ID-tag must be included in the photo. Download photos and rename in the following format SpeciesPlot\_Date: SSSP\_YYMMDD (e.g. ERI1\_180628, SAL4\_180926).

Data forms are found in Appendix 2. The data from the weekly counts are entered into data files with columns relevant for each species. The basic data are: Year, Month, Day, Photo no., Observer, Species (SAL, LOI, SIL), Plot (1-4), Subplot (A-D), Snow (percent in subplot), Cloud cover (x/8), Buds (actual numbers counted, not percent), Flowers, Senescent (flowers), Total (sum of buds, flowers and senescent flowers), and Remarks. Please note, that there are species specific columns in the database files.

During snow melt in May/June, percentage snow cover in each sub subplot is estimated at each sampling trip. If any plant part is visible above the snow layer, the cover is given as 99%. If any ground/vegetation cover is free, no more than 98% can be stated.

When visiting *Silene* plots, samples of a total of 50 flower buds, flowers or senescent flowers (or capsules) are recorded within each subplot. In the *Salix* and *Loiseleuria* plots a total of 100 buds, flowers and senescent flowers (catkins in *Salix*) are recorded. This is done by counting the different phenological stages until a total of 50 (*Silene*) or 100 (*Salix*, *Loiseleuria*) is reached. Start counting at the peg carrying the plot-ID, and count from left to right, as shown in figure 5b. The procedure of counting left to right, right to left) is continued until a total of 50 (*Silene*) or 100 (*Salix*, *Loiseleuria*) is reached, or to the end of the subplot, whichever comes first. Continue to the next subplot, starting at the next peg, indicated by the next black dot in figure 5b.

In general, flower buds are defined as flowers not yet open, flowers are open giving insects access to the reproductive organs, and senescent flowers as flowers that have lost all petals or with all petals almost or fully faded or brown. In some of the final stages, flower stems from the preceding year may interfere with the counts. However, such old stems are always dry and stiff; stems of this year are soft and fleshy.

For each species, the following sampling procedures apply in particular.

### **Salix**

The sampling unit is catkins, not individual flowers. Most flowers from one catkin emerge the same day, and they also wilt at the same time. Hence, catkins are recorded as buds (Figure 6), when no stigmas or anthers are visible, and as male (Figure 7) and female (Figure 8) flowers as soon as anthers (m) or stigmas (f) are visible (they are often both red in the early stages, but the colour may vary).



Figure 6. *Salix glauca* buds. It is not possible to discriminate between male and female flowers at this stage.



Figure 7. *Salix glauca* male flowers.



Figure 8. *Salix glauca* female flowers.



Both senescent flowers and fruits continue to be recorded as 'flowers' until they have exposed seed hairs (Figure 9). From the time of exposure of the first hairs on top of the splitting capsules, they are recorded as 'senescent'. Notice that fruits may be affected by larvae so that they expose seed hairs from the bottom of the capsules (excreta from the larvae are often visible among the seed hairs). These capsules must not be recorded as having seed hairs exposed, but should be recorded separately. In Kobbefjord, this has not yet been observed. Senescent male flowers are not counted.



Figure 9. Senescent *Salix glauca* female flowers with hairs.

Fruits infected by fungi (yellow and twisted) should be recorded separately (yet still included in the number for 'flowers', i.e. the infected fruits appear twice in the data forms). This has not been observed in Kobbefjord yet. Also, infections by insects should be recorded.

## Silene

*Silene acaulis* grows in cushions (Figure 10) and one or a few specimens may dominate the subplots.

Flower buds are reddish or light purple (Figure 11). Senescent flowers (Figure 12) have wilted petals and empty pollen anthers or appear as empty “cups” (Figure 12).



Figure 10. *Silene acaulis* cushion.



Figure 11. *Silene acaulis* flowers in the foreground. Buds in different developmental stages are marked with the arrows.



Figure 12. *Silene acaulis* senescent flowers, some with wilted petals (within circle). Buds or flowers eaten by insects in the foreground.

### **Loiseleuria**

*Loiseleuria procumbens* is a matted shrub with pairs of tiny, oblong, closely set leaves and abundant clusters of small flowers (see Figure 13). In Greenland, plants grow no taller than 10 cm. The plant is creeping, multi-branched, and mat forming, with 2-5 pink, bell-shaped flowers in terminal clusters and evergreen leaves with rolled edges.



Figure 13. *Loiseleuria procumbens* buds (fully closed), flowers (open) and senescent flowers from last year (upper right corner).

### Input of data into database

Data from the weekly censuses are entered into a data file named “Phenology\_Plots” with the columns: Year, Month, Day, Photo no., Observer, Species (SAL, LOI, SIL), Plot (1-4), Subplot (A-D), Snow (percent in subplot), Cloud cover (x/8), Buds (actual numbers counted, not percent), Flowers, Senescent (flowers), Total (sum of buds, flowers and senescent flowers), Larvae, Fungi and Remarks. Please note that there are species specific columns in the data file.

#### 3.1.3 Total flowering

##### Species to be monitored

Northern willow (*Salix glauca*), Trailing azalea (*Loiseleuria procumbens*), Moss campion (*Silene acaulis*), and Cotton grass (*Eriophorum angustifolium*). See previous section for descriptions of *Salix glauca*, *Loiseleuria procumbens*, and *Silene acaulis*. *E. angustifolium* is described below.

##### Eriophorum

The sampling unit is flowering stems, not individual flowers or spikes. There are two or more spikes on each stem. Figure 14-15 shows different stages of *Eriophorum* flower development.



Figure 14. *Eriophorum angustifolium* with immature spikes (yellow anthers visible).



Figure 15. *Eriophorum angustifolium* with mature spikes (conspicuous white perianth bristles).

### Frequency of sampling

Once per season. Total counts of *S. glauca*, *L. procumbens*, and *S. acaulis* are made at peak flowering. The optimal time for total counts of *E. angustifolium* is when most or all spikes have reached senescence.

### Equipment

- Map with position of study plots (or alternatively a GPS)
- Pieces of cord totalling 100 m
- Flower sticks
- Tally counters
- Data form Appendix 2/ Notebook.

### Location and marking of sampling plots

The plots are divided into four subplots (A, B, C, and D) separated by steel pegs at the centre where the diagonals cross and at the midpoint of each side. The lettering starts at the corner with the plot-ID tag and continues clock-wise around the centre. Coordinates, dimensions etc. are found in table 2. The plots are identical with the plant reproductive phenology plots shown in figure 4.

### Sampling method

Tighten a cord around each subplot. In large plots, subsections are established by placing two additional cords with about 0.5 or 1 m intervals from one end of each subplot, whereupon the summed number of flower buds, flowers, and senescent flowers, respectively, are counted between each cord. Move one cord at a time and repeat the process until the entire plot is covered. In small plots, sticks may be used instead of cords. In the *Salix* plots, male and female catkins are counted separately. Catkins that have been grazed, but can still be identified as either male or female, are included.

### Input of data into database

Data from the yearly registrations are entered into a data file named "Phenology\_Plots" with the columns: Year, Month, Day, Photo no., Observer, Species (SAL, LOI, SIL, ERI), Plot (1-4), Subplot (A-D), Snow (percentage in subplot), Cloud cover (x/8), Buds (actual numbers counted, not percent), Flowers, Senescent (flowers), Total (sum of buds, flowers and senescent flowers), and Remarks. Please note, that there are species specific columns in the data file.

#### 3.1.4 Normalised Difference Vegetation Index (NDVI) in plots and along the NERO line

The NDVI-instrument measures the spectral reflectance of the plant canopy. The progression in the vegetation greenness is followed in the plant phenology plots and from 2008-2016 along the vegetation transect (the NERO line). Due to instrument failure (old instrument: handheld Crop Circle TM ACS-210) the line has not been measured since. The new instrument (RapidSCAN CS-45) can not be used for measurements along the NERO line. For information regarding measurements along the NERO line please refer to the previous manual (Aastrup et al. 2015).

### Species or taxonomic groups to be monitored

All plants in the reproductive plant phenology plots.

### Frequency of sampling

Plant phenology plots: Weekly in connection with the plant phenology censuses.

### Equipment

- Map of plant phenology plots or GPS
- RapidSCAN CS-45
- Camera
- Notebook

### Location and marking of sampling plots

Plant phenology plots: See Table 2 and Figure 4.

### Sampling method

NDVI is measured using the RapidSCAN CS-45 (See Figure 16). It integrates a data logger, graphic display, GPS, crop sensor and a power sources in one small instrument. Please refer to the

RapidSCAN CS-45 manual for in depth information on the instrument. Data is collected and stored on a flash file for late retrieval to a PC using the RapidTALK software.



Figure 16. Equipment for measuring NDVI, the handheld RapidSCAN CS-45. Photo from Holland Scientific homepage.

The procedure is:

1. Turn the RapidSCAN CS-45 on by moving the clicker forwards
2. Wait for GPS signal (may need to turn the instrument on and off a few times)
3. Press "OK" for "Log" mode
4. Pull clicker backwards for logging each of the subplots (A-D)
5. Press "OK" after logging subplot D
6. Turn "OFF" between plots

Scans are conducted by moving the sensor steadily forward (ca. 1 meter per second) approximately 75 cm above the vegetation. Refer to the RapidSCAN CS-45 manual for more information.

The sampling order specified in table 3 must be applied. The RapidSCAN CS-45 does not name the files so it is extremely important that the sampling order is followed! If for some reason the order is changed, remember to make a clear note in your notebook. Also, always measure all plots in the order A-D (see Figure 5a). At each visit, note under Remarks the presence of snow (snow in subplot) and if the vegetation is moist.

Table 3. Walking order of phenology plots including Empetrum plots for measuring NDVI.

Plot abbreviation	Plot name
ERI1	Eriophorum 1
EMP1	Empetrum 1
ERI2	Eriophorum 2
EMP2	Empetrum 2
ERI3	Eriophorum 3
EMP3	Empetrum 3
LOI1	Loiseleuria 1
SIL1	Silene 1
SAL1	Salix 1
SAL2	Salix 2
LOI2	Loiseleuria 2
LOI3	Loiseleuria 3
LOI4	Loiseleuria 4
ERI4	Eriophorum 4
EMP4	Empetrum 4
SAL3	Salix 3
SIL4	Silene 4
SIL2	Silene 2
SIL3	Silene 3
SAL4	Salix 4

All measurements are conducted only on the AB and the CD sides of the plots (Figure 5a). Place yourself at the plot number plate, just outside the plot. Hold the sensor perpendicular to your walking direction approx. 75 cm above the ground. Switch on the NDVI logger (follow the procedure described above), and walk slowly (approximately 1 m per second) along the sides indicated by arrows on figure 5a. Use the clicker switch to log the NDVI along each of the four sides in the plot. Only one NDVI value is generated per subplot corresponding to the average NDVI. Hence, four scans (equal to four NDVI measurements) are made in each of the vegetation plots.

Ideally all plots should be measured on the same day. If the vegetation is wet, the measurements must be postponed to the following day.

#### Input of data into database

Data is downloaded from the RapidSCAN CS-45 to the computer using the appropriate cable. Each file holds the following variables: Plot (where 1-4 corresponds to the subplot sides A-D), NDRE (Normalized Difference Red Edge Index), NDVI, RE (Reflectance Red Edge), NIR (Reflectance Near Infrared), R (Reflectance Red), Latitude, Longitude, Latitude, Elevation, HDOP (Horizontal Dilution of Precision), Fixtype (always GPS), Date, Time, N (Number of samples per scan), MaxNDRE (Maximum NDRE), MaxNDVI (Maximum NDVI), MinNDRE (Minimum NDRE), MinNDVI (Minimum NDVI), StdNDRE (Standard deviation NDRE), StdNDVI (Standard deviation NDVI), CVNDRE (Coefficient of variation NDRE), CVNDVI (Coefficient of variation NDVI).

Data are entered into a data file named "Plot\_NDVI" with the following columns: Year, Month, Day, DOY, Date, Observer, Species, Plot, Section, NDRE, NDVI, RE, NIR, R, Latitude, Longitude, Elevation, HDOP, Fixtype, N, MaxNDRE, MaxNDVI, MinNDRE, MinNDVI, StdNDRE, StdNDVI, CVNDRE, CVNDVI.

Pictures are downloaded and renamed according to the procedure described in 3.1.2 Reproductive phenology.

#### 3.1.5 Normalised Difference Vegetation Index (NDVI) in CO<sub>2</sub> flux plots

The progression in the vegetation greenness is followed in the CO<sub>2</sub> flux plots. Spectral reflectance of the plant canopy as well as the incoming radiance is measured using two sensors.



### **Species or taxonomic groups to be monitored**

All plants in the CO<sub>2</sub> flux plots.

### **Frequency of sampling**

Weekly in connection with the CO<sub>2</sub> flux measurements.

### **Equipment**

- Map of CO<sub>2</sub> flux plot positions
- GPS with positions of CO<sub>2</sub> flux plot
- A handheld SpectroSense 2+ system including two light sensors (marked 38291 and 38294 respectively) which calculates the greening index (NDVI). See figure 17
- Data form Appendix 3 or 4 / Notebook.

### **Location and marking of sampling plots**

CO<sub>2</sub> flux plots: See figure 20. The position of the midpoint of the plots is 64.1361°N/51.3831°W.

### **Sampling method**

NDVI in the CO<sub>2</sub> flux plots is measured with the SpectroSense 2+ system using two light sensors. Data is not stored, and must be written in the “Remarks” section on either data form 3A or 3B.

The procedure is:

1. Fasten the 4 metal legs/rods loosely to the Plexiglas plate using the accompanying screws and bolts. The legs should be fixed but able to turn slightly to fit into the metal frame in the plots – one leg in each corner. This ensures identical measurement height every time.
2. Unscrew the bolt on the light sensors and attach them to the Plexiglas plate as follows: Attach the sensor marked 38294, without a diffuser, facing downwards in the middle of the “square” made out of the 4 bolts from the legs. Attach the sensor marked 38291, with a diffuser, facing upwards outside the “square”.
3. Connect the light sensor marked 38291 (with a diffuser) in the “Current C1/C3” (plug no. 2 from the left) on the device. See figure 17.
4. Connect the light sensor marked 38294 (without a diffuser) in the “Current C2/C4” (plug no. 3 from the left) on the device. See figure 17.
5. Place the 4 legs in the corners of the metal frame in the plot (double check that the sensors are placed correctly). See figure 18.
6. Turn on SpectroSense 2+ with the ON button.
7. Tap the MENU button and scroll down to MENU “6-NDVI” using the arrow keys on the right hand side. Press ENTER.
8. Initially the NDVI value fluctuates but should reach a fairly stable value after a few seconds. Write the value (with 3 decimals) in the data form 3A or 3B in the remarks column.
9. Continue to the next plot. Do not dismantle the setup between plots. The SpectroSense 2+ may turn off automatically at some point. In that case start at #6.
10. When all plots have been measured dismantle the legs from the plexiglas plate and unscrew the light sensors but leave the sensor cables in the device.

The easiest way of sampling the plots is by following the same route as when measuring CO<sub>2</sub> flux. It may be favourable to do the NDVI measuring when the flux measurements are completed.

### **Input of data into database**

Data is stored in a data file named “ITEX\_NDVI” with the following columns: Year, Month, Day, Day of Year (DOY), Plot, Treatment, Snow, NDVI and Remarks.

Insert light sensor  
marked 38294  
here

Insert light sensor  
marked 38291  
here

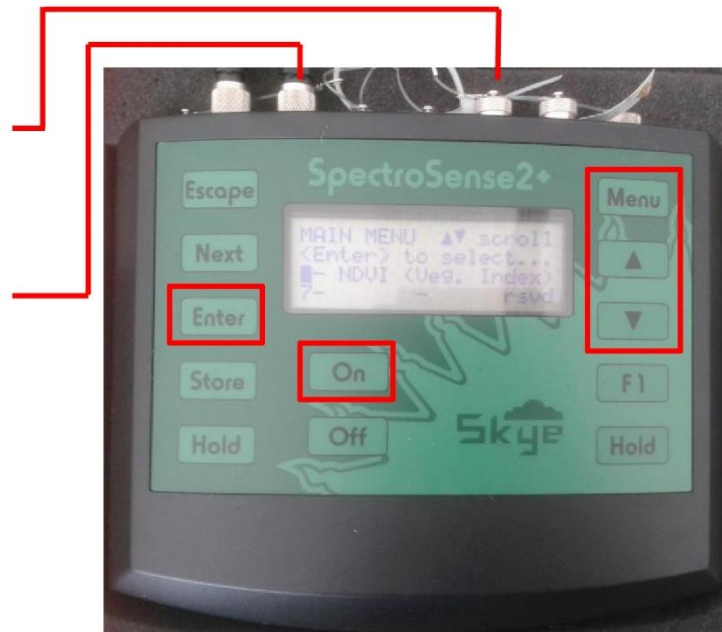


Figure 17. The SpectroSense 2+ device for measuring NDVI in the CO<sub>2</sub> flux plots. Attach the light sensors as indicated in the figure.

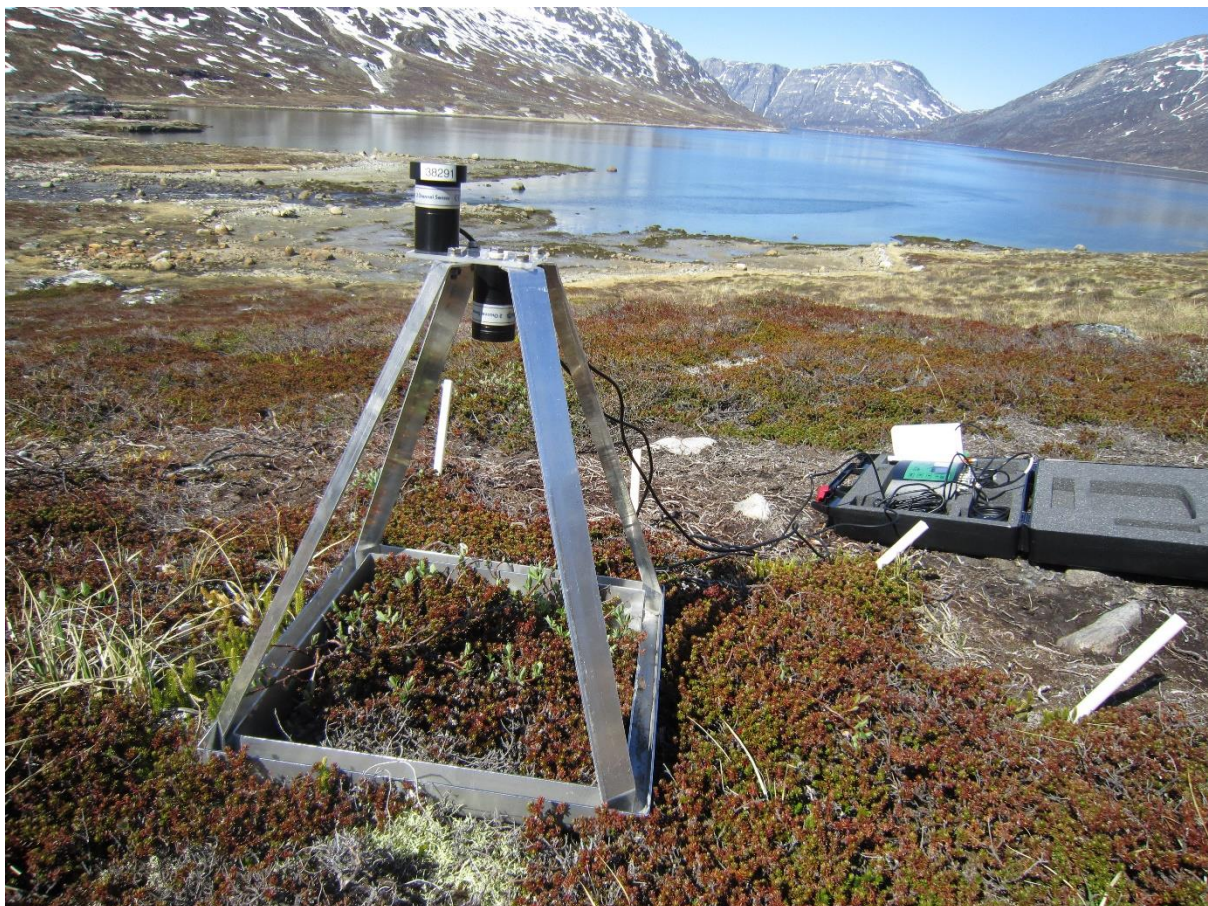


Figure 18. The light sensors attached to a plexiglas plate with 4 legs positioned in each of the corners of the flux plot metal frame. Notice the light sensor with the diffuser marked 38291 is facing upwards while the sensor without a diffuser (marked 38294) is facing downwards.

### 3.1.6 CO<sub>2</sub> flux plots

The ratio between the release of CO<sub>2</sub> from respiration and decomposition of organic matter, and the uptake via photosynthesis is measured. The ratio is called Net Ecosystem Exchange (NEE).

#### Species or taxonomic groups to be monitored

The vegetation in the CO<sub>2</sub> flux plots which is dominated by *Empetrum* heath with *Salix* as subdominant species. The reproductive phenology of *Salix* is followed in all plots. Soil moisture is measured in all plots. Soil temperature is recorded by wireless GeoPrecision mini data loggers.

#### Frequency of sampling

CO<sub>2</sub> flux is measured weekly. All plots should be measured between 9 AM and 3 PM, and on the same day.

#### Equipment

- ITEX-chambers (OTCs) incl. bolts (only for set-up)
- Hessian tents (2.2m x 2.2m – only for set-up)
- Electrical tubes for the hessian tents (only for set-up)
- External hexagonal frame for the hessian tents (already located in the six S-plots)
- Wireless GeoPrecision mini data loggers (already located in each plot)
- Wireless GeoPrecision USB-dongle for data retrieval (bring once in autumn)
- EGM4 – see Figure 21
- TRP-2 probe for measuring CO<sub>2</sub>, PAR (photosynthetic active radiation) and temperature
- Handheld computer
- Plexiglass measuring chamber (PMC) – measuring 33x33x34 cm (LxWxH).
- Theta-probe for soil moisture measurements
- Black plastic bag adjusted for the PMC
- Sticky tack
- External 12V battery
- Ruler
- Camera
- Data forms Appendix 3 and 4 / Notebook.

#### Location and marking of sampling plots

30 plots are located in a mesic dwarf shrub heath dominated by *Empetrum nigrum* and with *Salix glauca* as subdominant species. The heath is facing west.

Figure 19 gives an overview of the site and Figure 20 shows the relative positions of the plots.

The setup consists of 5 treatments each with 6 replicates: Control (C), increased temperature (T, ITEX chambers/OTCs), Shading (S, hessian tents), long growing season (LG, removal of snow during spring) and short growing season (SG, addition of snow during spring).

Temperature is increased at T plots by ITEX chambers (OTCs), see Figure 22. This way temperature is expected to increase 1-2 °C during the growing season (for further information, see Molau & Mølgaard, 1996). The shading treatment (S) is carried out by erecting dome shaped hessian cloth tents over the plot causing approximately 60% reduction of incoming light (Havström et al., 1993).

Short and long growing season plots were intended to be differentiated by respectively adding to and removing snow from SG and LG plots during snow melt in spring causing plots to be exposed earlier (LG) or later (SG) than in control plots. These two treatments have however not been

implemented, but the layout of plots on a slope naturally cause differentiation of snowmelt. The plots thus work as additional control plots.

In each of the 30 plots, a metal frame of 35x35 cm has been inserted permanently into the soil. The frame is used for weekly measurements of CO<sub>2</sub> fluxes in the system by the closed chamber technique. The metal frames were placed in spots where *E. nigrum* and *S. glauca* dominated the vegetation. The metal frames are not to be removed by the end of the season.



Figure 19. Overview of CO<sub>2</sub> flux plot site with Hessien tents for shadowing and ITEX chambers (OTCs) to increase temperature.

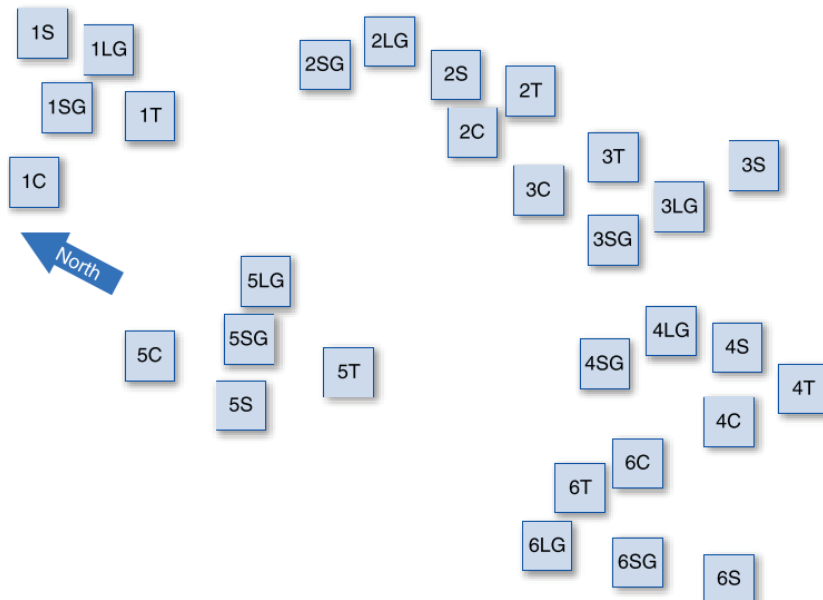


Figure 20. Detailed map showing the position of the CO<sub>2</sub> flux plots. The position of the midpoint is 64.1361°N/51.3831°W. For explanation of the abbreviations, please see text.

### Sampling method

The CO<sub>2</sub> flux treatments are established as soon as possible in the season (make note of the date). By the beginning of the season, check that the metal frames are level and adjust if needed. Do not adjust the metal frames later or in connection with the CO<sub>2</sub> flux measurements!

The six S-plots are equipped with hessian tents by erecting three electrical tubes in an external hexagonal frame over each plot. Each end of the tubes is placed in the external frame on opposite sides creating a dome. The tubes should be long enough to stand one meter over the vegetation at the highest point of the dome. Cover the plot with a piece of hessian and secure it with tent pegs and rocks.

The six plexiglas sides of each OTC are bolted together on site. A wireless GeoPrecision mini data logger is already placed approximately 2 cm horizontally into the soil. The data loggers are programmed to log the temperature every 30 minutes.

Before the start of the season, replace the old Soda lime in the EGM with fresh Soda lime. This is done in the institute lab. See the EGM manual for further details.

Before beginning, the CO<sub>2</sub> flux measurements in a plot take a digital ortho-photo covering the entire area inside the metal frame, and take three soil moisture measurements outside the metal frame but inside the plot using the ThetaProbe. See the ThetaProbe manual for further details.

Measurements of the chamber height is only done three times during the season (beginning, mid and end of season) in order to avoid unnecessary tear on the vegetation. Chamber height is the height (cm) from the upper edge of the metal frame to the ground. Measurements are taken in the middle of each of the four sides.

### Measurement of CO<sub>2</sub> flux

1. Make sure the EGM4 is set to take measurements manually (Figure 21). This is done by turning on the EGM4 and pressing the 2SET button followed by the 5RECD button. The recording mode should read 1REC:M. If not press 1REC to get the desired mode. Furthermore, check that the handheld computer (Handheld Algiz 8X) is set to take automatic measurements every 10 seconds. This is done by starting up the PP Systems Transfer Software, and pressing the "File" bar. Go to "Preferences" and make sure the "Record/Block Interval" is set at 10.
2. In the field insert the TRP-2 probe into the plexiglas measuring chamber (PMC). The probe has a cable and a black tube. Connect the cable and the tube from the probe to the EGM4 (in: black tube). Then connect the clear tube from the PMC to the EGM4 (out: clear tube). Seal the entrance to the chamber with sticky tack (Figure 21 and 22). Do not turn on the EGM4 before the probe is connected.
3. Connect the computer to the EGM4 using the proper cable.
4. Turn on the EGM4 and press 1REC for record by pushing button 1. The EGM4 has to heat up to approximately 56°C (it may take a while depending on the surrounding temperature). Following the "heating-up" the EGM4 calibrates to the CO<sub>2</sub> level in the air (the level varies between approximately 380 and 400 ppm). This happens automatically at start-up and then at intervals of about 20 minutes, at which point the EGM4 will write "Counting Zero" (See Note below).
5. Start the PP Systems Transfer Software programme on the computer. Press the "Logging" bar and choose the "Auto logging" bar. Select your designated folder and name your file. Press "Save" and then "Pause" immediately afterwards.

6. When the EGM4 has run the calibration (called “Counting Zero”) place the PMC in the metal frame in the first plot to be measured. Make sure the PMC handle does not cast a shadow on the TRP-2 probe with the PAR (photosynthetic active radiation) measuring device. While measuring gas flux the EGM4 automatically measures the PAR and the temperature in the PMC.
7. The PP Systems Transfer Software is set to automatically take a measurement every 10 seconds. Take 13 measurements under light conditions. Start the first measurement by pressing “Resume” on the computer. After the 13th measurement press “Pause” on the computer. Lift the PMC off the frame. Aerate the PMC making sure the CO<sub>2</sub> level returns to that prior to measuring. Monitor the CO<sub>2</sub> concentration on the EGM4 or the computer. Place the PMC on the frame again and cover it completely with the black plastic bag. Press “Resume” on the computer. After yet another 13 measurements press “STOP” on the computer. Lift the PMC and aerate it while walking to the next plot.
8. When all plots have been measured turn off the EGM4 and computer.

NOTE: The EGM4 may run a calibration while measurements are being taken, which interrupts the measuring, that has to be started over. To avoid interruption by calibration, make sure to “Count Zero” (Press N/Z) after every second plot measurement.

#### *Measurement of soil moisture*

Take three measurements in each plot by pressing the sensor into the soil, read out the soil moisture, and enter data into the form Appendix 3.

#### *Reproductive phenology of Salix*

Reproductive phenology of *Salix glauca* is followed in all plots according to the procedure described in section 3.1. Please remember to distinguish between flowers inside and outside the metal frame permanently inserted in the soil. Data are entered into the form Appendix 4.

#### *Species composition of plots*

Species composition of the vegetation inside the metal frame is monitored every 5 years, coinciding with the survey of the NERO line. This is done by pin-point analyses and supplementary registration of all remaining species (those not hit by pin-point intercept) within the metal frame.

#### **Laboratory work**

None.

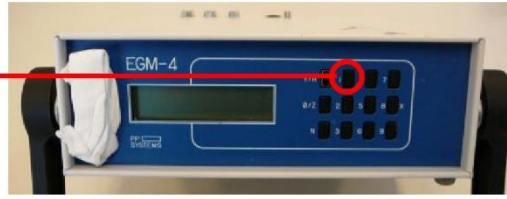
#### **Input of data into the database**

Data is saved directly on the handheld computer. The raw files are saved in a separate folder (named by the date of measurement) and renamed to include the type of treatment (e.g. 1c, 1t and 1s.dat). Data is stored in a data file named “C-flux” and reorganized and supplemented with the following columns: Year, Month, Day, DOY, Hour, Min, Plot, Treatment, Light, Photo\_no, Recno, Cloud cover, Observer, Soil moisture, Chamber height, and Remarks. All files are merged into one, covering the whole season.

Download digital pictures and rename them to include plot name and date (e.g. ITEX\_1C\_090602). Save under “Photos” in the folder named “C-flux”.

Data from the wireless GeoPrecision mini data logger is downloaded once a year at the end of the season. Save the individual files using the plot name (e.g. 5C), and save in a separate folder named “C-flux\_temperature”.

After turning the EGM4 on, press the 1REC button



Handheld computer or tablet is connected here

TRP-2 probe is connected here (2 ports)

Clear plastic tube is connected here



Figure 21. Upper figure shows the EGM4 which is used for measuring CO<sub>2</sub> concentrations. The lower figure shows the top of the instrument.

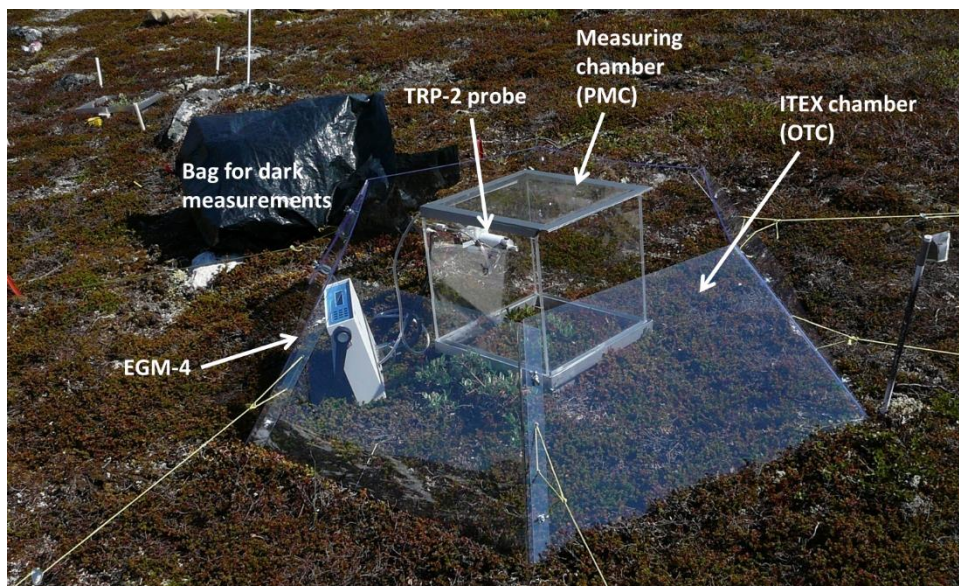


Figure 22. Measurement of CO<sub>2</sub> flux in an ITEX-hexagon open-top chamber with a measuring chamber (PMC) fitted with a TRP-2 probe connected to the EGM4. The black plastic bag is used for dark (respiration) measurements.

### 3.1.7 UV-B exclusion

The impact of ambient UV-B radiation on the vegetation is studied in a mesic dwarf shrub heath by placing filters approximately 10 cm above the vegetation. At the time of peak plant growth the chlorophyll *a* fluorescence is measured as an indicator of plant health. The impact of UV-B exclusion has not been measured since 2017. In 2018, the experiment was set up, but no measurements were done.

#### Species or taxonomic groups to be monitored

A mesic dwarf shrub heath (facing WSW) dominated by crowberry (*Empetrum nigrum*) and with dwarf birch (*Betula nana*) and bog bilberry (*Vaccinium uliginosum*) as subdominant species. The effect of UV-B radiation is measured on *Betula nana* and *Vaccinium uliginosum*.

#### Frequency of sampling

Measurements of chlorophyll fluorescence of leaves of *Betula nana* and *Vaccinium uliginosum* is carried out three times with one week intervals at the peak of plant growth (week 29 to 31).

#### Equipment

- HandyPea fluorimeter (PEA: Photosynthesis Efficiency Analyzer) – See figure 23 and further description below.
- 80 leaf clips
- Data form Appendix 5 / Notebook
- Camera
- 5 Frames with UV-B filter (Mylar film, 0.25 mm - with exclusion of UV-B)
- 5 Frames of filter control (Teflon film - without exclusion of UV-B).



Figure 23. Handy PEA with clips.



The Handy PEA chlorophyll fluorimeter consists of a control unit. The chlorophyll fluorescence signal received by the sensor head during recording is digitized within the Handy PEA control unit. Up to 1000 recordings may be saved in the memory of Handy PEA chlorophyll fluorimeter. Saved data can be viewed onscreen but has to be transferred to a computer for storage and further analysis.

The sensor unit consists of an array of 3 ultra-bright red LED's optically filtered to a peak wavelength of 650 nm, which is readily absorbed by the chloroplasts of the leaf. The LED's are focused via lenses onto the leaf surface to provide even illumination over the area of leaf exposed by the leaf clip (4 mm diameter).

### Location and marking of sampling plots

The UV-B plots are located west of the CO<sub>2</sub> plots. Figure 24 and 25 gives overviews of the plots.

There are three series of plots with five replicates:

1. Control - no treatment: C1-C5
2. UV-B filter (Mylar film, with exclusion of UV-B): B1-B5
3. Filter control (Teflon film, without exclusion of UV-B): F1-F5.

Each treatment plot measures 60 cm x 60 cm; the plots are marked with aluminium tubes at each corner and covered with a frame with the appropriate filter placed approximately 10 cm above the vegetation. During summer, the vegetation may grow as tall as the filter, which can then be lifted.



Figure 24. Overview of UV-B plots.

### Sampling method

Before establishing the UV-B plots, filters on frames are checked carefully, and changed if necessary. There are two filter types, Teflon (filter control: the thinnest and most flexible film) and Mylar (excludes UV-B: thicker and less flexible). Frame positions are given by small sticks within each plot.

UV-B plots are checked regularly during the entire field season, and repaired if necessary. Specifically, filters in the UV-B plots must be checked after heavy rain or wind.

By the end of the field season, all equipment at UV-B plots are taken down and brought back to the institute.

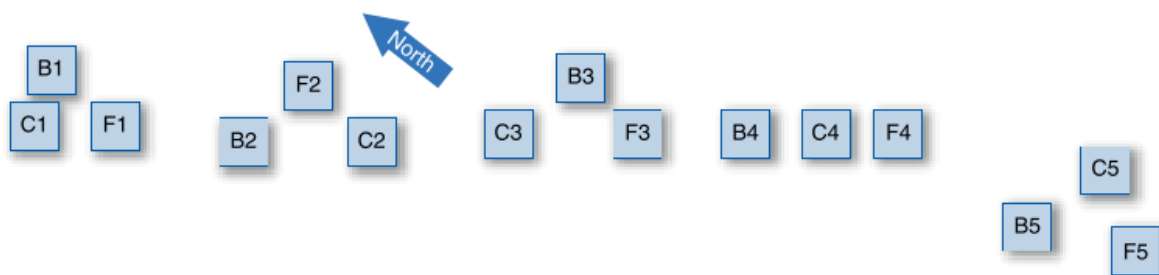


Figure 25. Schematic presentation of the location of UV-B plots.

### Sampling procedure

1. Select five green, “healthy-looking” leaves or shoot-tips of *Betula nana* and *Vaccinium uliginosum* in each plot.
2. Mount leaf clips on all leaves – preferably without removing the leaf from the branch. Mount on one species at a time. Make sure that the leaf is visible through the hole in the clip and push the shutter to cover the hole so the leaf material is in complete darkness.
3. Keep the shutters closed for at least 30 minutes. The closure time may be longer.
4. Switch on the Handy PEA, open “Main menu” and turn the arrow on the screen to “Measure”.
5. Fit the sensor head to the clip; uncover the hole by pushing the shutter back. Start measuring by pushing “OK” on the Handy PEA or push the black button on the sensor head. During the measurement a number of parameters appear on the screen. Note that the “Fv/Fb” should be about 0.8. If something goes wrong step three must be repeated before carrying out a new measurement.
6. Accept to store the measurement. Note which measurement number corresponds to each plot.
7. Repeat the sampling now measuring on *Vaccinium uliginosum* leaves.
8. Take a photo of each plot at each measurement round.

### Input of data into database

When all measurements have been completed, data must be transferred to a computer by use of the Handy PEA programme. Make sure that all data has been transferred to the computer before clearing the memory in the Handy PEA.

Data are downloaded from the Handy PEA using the PEA Plus software, and the raw files are saved in a separate folder named to include the date of the measurements (e.g. yymmdd.pcs). Data are stored in a data file named “HandyPea” and are supplemented with the following columns: Year, Month, Day, Observer, Species, Treatment, and File no.

## 3.2 Arthropods

Surface living arthropods are captured in yellow pitfall traps. Flying arthropods are captured in window traps.

### Species to be monitored

All taxonomic groups of arthropods.

### Frequency of sampling

The traps are emptied weekly. If bad weather prohibits visits to the fjord or proper handling of the samples, the traps must be emptied on the earliest day of convenience.

### Equipment

For field work:

- Maps of the plots (or alternatively a GPS)
- 32 yellow (Pantone no. 108U) plastic cups, 10 cm in diameter and 8 cm deep, called pit-falls (permanent location). At the beginning of the season check if all cups are placed properly
- Extra yellow pit-falls for emptying the permanent pit-falls
- 4 window traps (permanent locations)
- A garden trowel with sharp edge (most relevant in the beginning of the season)
- 5 L container for water
- 5 L container for wastewater
- Detergent: Odour free detergent (Coop Änglemark Bluecare Dishwashing fluid, concentrated, without perfume, colour and preservation agent)
- Salt (NaCl) without iodine and anti-caking agent
- 20 Metal pegs (to be used in the fen area)
- 1 lady's stocking per emptying bout (light brown/skin colored, 20 denier)
- A pair of flat tweezers
- 36 plastic containers with lids
- 2 L of 70% ethanol
- Small bottle with tip (for rinsing the stocking and pit-fall with alcohol)
- Disposable syringes for removal of surplus water
- Ethanol resistant labels (see Appendix 13)
- Pencil
- Data form Appendix 6 /note book.

### Location and marking of sampling plots

The location of the study plots are shown on Figure 26. Each plot measures 10 x 20 m and is made up of eight 5 x 5 m squares marked with metal pegs in each corner. Each plot is identified with an ID-label, and subplots (with one trap each) are lettered A-H clockwise from the number plate, see figure 27.

### Sampling set up

A set of eight pitfall traps has been established in each plot. Each trap is composed of two plastic pit-fall cups fitting into each other, so that the upper one can be lifted and emptied without disturbing the surrounding soil. The traps have been permanently positioned within squares, each of 5 x 5 m. Previously (prior to 2016) the traps were moved randomly each year. This procedure has been abandoned due to slow rate at which the vegetation and soil surface regenerate. Repositioning of the traps each year caused considerable damage to the plots and would eventually render them non representative of the surrounding areas.

Cups are placed upside-down during the winter. At the start of the season (i.e. on the day when the traps have appeared from the snow), new clean (washed with a little biodegradable, non-perfumed detergent) upper cups are placed inside the 'wintering' ones.

If there is any risk of the cups floating due to water in the lower cup, two metal pegs must be placed along each cup to keep them in position.

The upper cup of the trap is filled 2/3-3/4 with water (approx. 1 litre needed per station) added three drops of detergent and a spoonful of salt as killing agent, preservation and to prevent freezing. Use 2 spoonfuls of salt and 5 drops of detergent for the window traps.

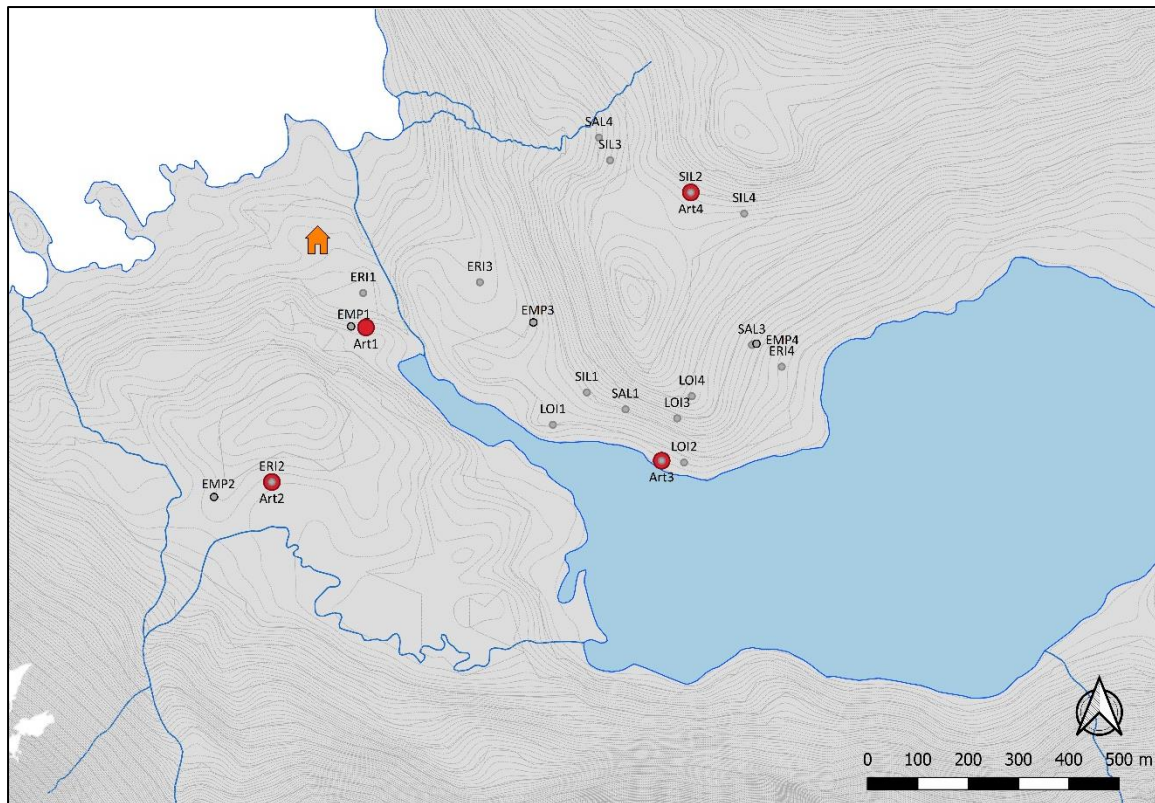


Figure 26. Location of arthropod plots. Art1: *Empetrum nigrum* heath, Art2: Fen, Art3: *Betula nana* / *Salix glauca* heath, Art4: Abrasion. Window traps are located at Art2 and Art3. Phenology plots are marked with grey dots for reference.

### Emptying the traps

Prepare and print labels on water resistant paper before field work. Labels should contain date, plot (Art1-4 or Wart1-4) and subplot (A-H). Remember to write the date on the label, if that was not included in the printing. Samples from each of the pit-fall traps and window traps are kept separate.

1. Open a 100 ml container and insert the correct pre-printed label.
2. Stretch the stocking over the top of a spare cup.
3. Pour the trap liquid through the stocking into the spare cup.
4. Check the pit-fall for remaining arthropods. Should any still remain in the trap flush with ethanol down into the 100 ml container.
5. Reposition the pit-fall in the soil.
6. Empty the catch from the stocking into the 100 ml container by turning the stocking upside down on top of the container.
7. Rinse the inverted stocking with ethanol using a small squeeze bottle.
8. Check for arthropods in the stocking. All remaining invertebrates must be removed carefully from the stocking using tweezers and put into the container.
9. Fill the container approx.  $\frac{3}{4}$  with 70% ethanol. More is needed if there are many insects. In extreme cases, the sample may be divided between 2 containers.

10. Pour the water (now with no arthropods) back into the cup repositioned in the soil.

Window traps are emptied using a plastic spoon with a mesh net. Before emptying a window trap a spare cup is filled (approx. 50%) with water from the trap. Use the spoon with the net to catch all arthropods in the trap. Rinse the spoon in the spare cup now and then. The spare cup is emptied as described above (1. – 10.) when all arthropods have been fished out of the window trap.

Water may need to be added (to compensate for evaporated water), removed (after rain) or replaced (e.g. if the water smells or if foxes have urinated or defecated in the pit-fall). When water is replaced make sure to add a little salt and detergent. Bring all contaminated water back in a wastewater container and pour it into the sink in the “lab-room” in the cabin.

Bring extra pit-fall cups on each round, along with equipment for setting up traps, in case a trap has been destroyed, e.g. by a fox. Any failures such as flooded or floating cups, fox faeces etc. must be recorded. This includes occurrence of fungi and/or algae in the water. In that case, cup and water must be replaced.

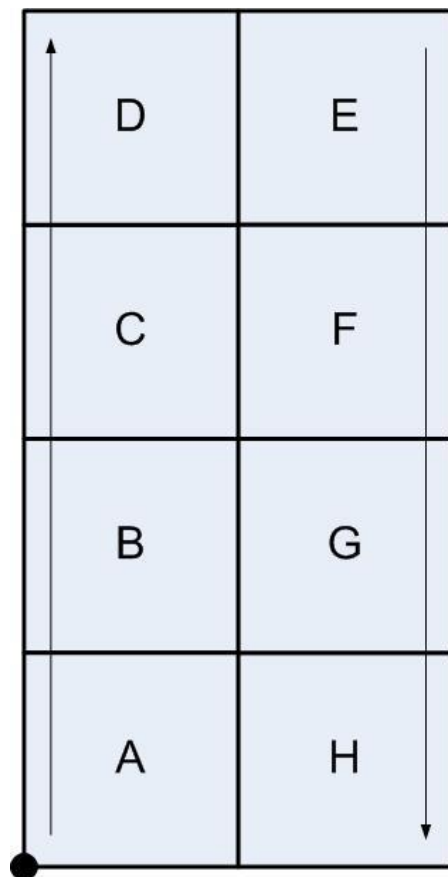


Figure 27. Schematic diagram showing positions of arthropod subplots A-H

Note the full hour of the day, when the traps in each plot are emptied.

During snow melt (and after snow fall in late summer / autumn) the snow cover (%) is estimated for each subplot. If any plant part is visible above the snow layer, the cover is given as 99%. If any ground/vegetation cover is free, no more than 98% can be stated.

Never touch the traps with mosquito repellent or sun cream on your fingers!

**Ending the season**

At the end of the catching season the trap liquid must be collected from all the traps. The waste water is emptied in the sink in the “lab-room” in the cabin (or brought back to the institute if the

drainage system has been closed for the winter). Arthropod samples are kept at GINR before they are shipped to the Department of Bioscience, Aarhus University, Silkeborg, for sorting and identification.

### Laboratory work

None.

### Input of data into database

After the weekly emptying of the pit-fall traps, the following data are entered into a data file named "Arthropods": Year, Month, Day, DOY, Hour, Observer, Trap (Art or WArt), Plot (1-4), subplot (A-H), Snow, Days (days since last emptying), Remarks. Under Remarks, date of opening and closing together with relevant observations about the traps are stated. This includes any disturbance that may influence the efficiency of the traps such as flooding, drying out, icing, dirt, faeces, adding/removing water and vandalism by mammals including humans.

After sorting, the total number of individuals per group is entered into a data file (please refer to the GEM database metadata file for further information).

## 3.3 Microarthropods

### Species to be monitored

All microarthropods.

### Frequency of sampling

Sampling of microarthropods is done in 8 plots (4 habitats with 2 replicates; figure 28). Four samples from each plot are collected three times during the season corresponding to: spring (after snowmelt), summer and autumn (before the snow appears). Extraction is very slow in wet samples. Therefore, samples should only be taken when soil moisture is low i.e. a few days after a rain event.

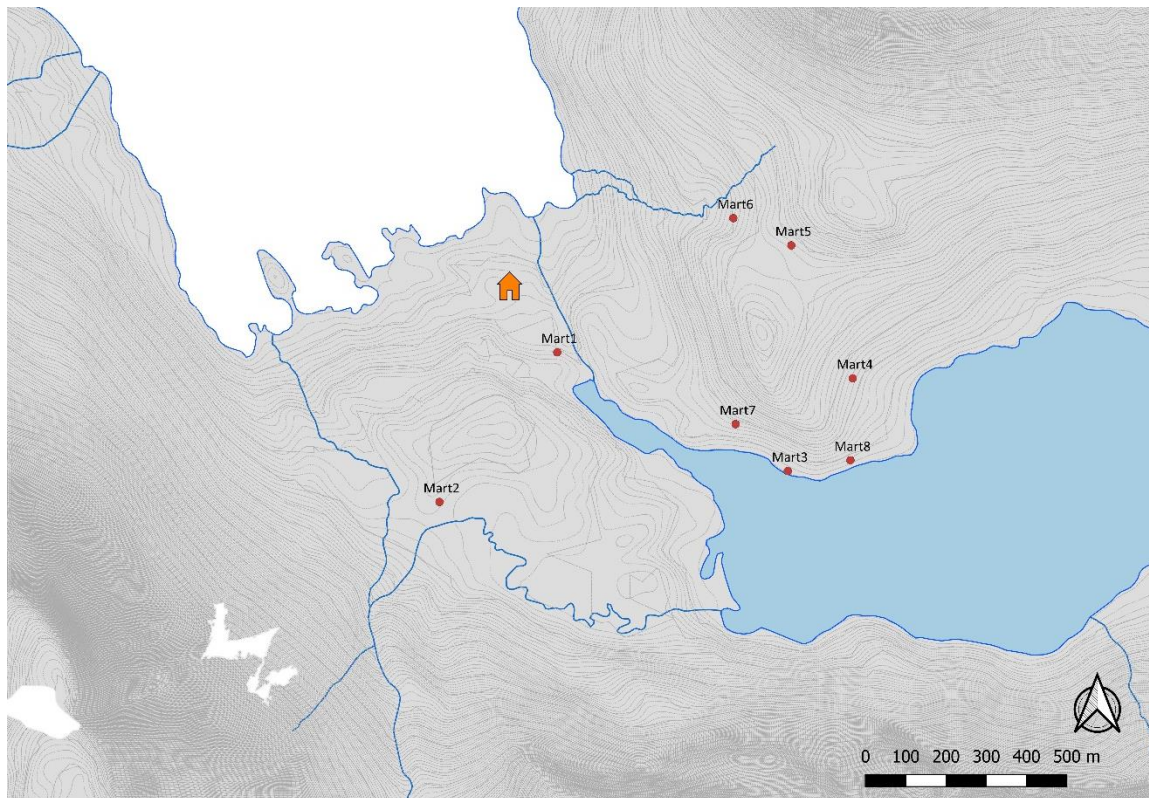


Figure 28. Location of plots for microarthropod sampling.

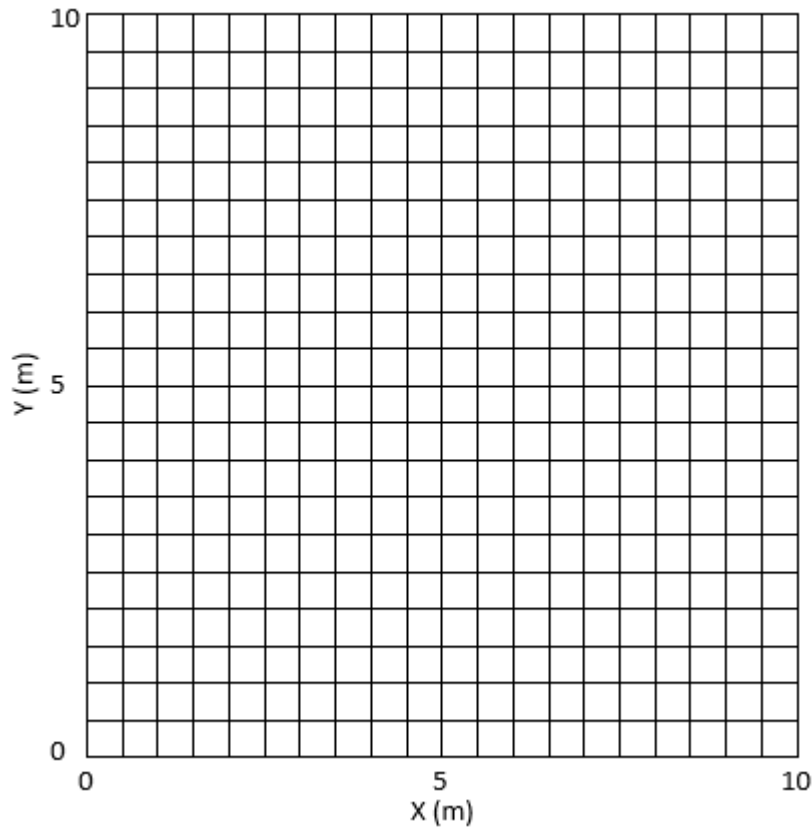


Figure 29. Microarthropods sampling grid in 10x10 m plots with grid size: 0.5 x 0.5 m.

### Equipment

- Map/GPS with positions of plots
- Soil auger
- 34 microcosm tubes made of Plexiglas (height 5.5 cm and diameter 6 cm)
- 64 DBIdut lids (size 89B)
- Knife to cut roots etc.
- Transportation box
- Table with random sampling points (Appendix 15)

The yearly sampling programme consists of collecting microarthropod samples from:

4 habitats \* 2 plots \* 4 subsamples \* 3 sampling occasions = 96 samples.

Each plot is marked with 4 pegs. To ensure enough undisturbed sampling points for several years each plot is divided into a ½-meter square grid with two axes (x, y; figure 29). The peg with the ID-tag (Mart1-8) is the starting corner for the square grid (x, y = 0m, 0m). The table with random sampling points provides 4 sets of random numbers (x, y coordinates) from where the soil samples are to be taken. The same set of random numbers are used for all 8 plots on each sampling occasion.

### Sampling method

1. Two microcosm tubes are inserted into the soil auger, which is then closed and ready for use.
2. The point of sampling is found using the random sampling table.

3. The soil auger is placed vertically at the sampling point so it touches the soil surface.
4. At sites with dense vegetation it may be necessary to use a knife to cut around the soil auger before pushing it down into the soil or peat. Take care not to damage and compress the soil/peat core.
5. Push the soil auger vertically 5.5 cm downwards so that the lowest tube is just filled with soil. The soil surface has to level the upper rim of the lowest tube. The soil auger is open in the top so that you can follow how the soil is pressed into the tube. The function of the upper tube is only to keep the lower tube fixed. While pushing the soil auger down turn it from side to side thereby avoiding compression of the soil in the tube.
6. Tilt the soil auger from side to side to loosen the soil core at the bottom and carefully pull the soil auger including the soil core up.
7. Open the soil auger and carefully remove the tube including the soil core. Place a labelled DBIdut lid at the top immediately to keep organisms on the soil from escaping. Make sure that the lid is properly marked with the sample name (e.g. Mart4C).
8. Turn the tube around and cut surplus soil away with the knife so the soil surface levels the bottom of the lower tube. Place a DBIdut lid in the bottom of the tube.
9. Place the tubes in a box with the top of the sample facing upwards.

Store the samples at low temperature in a shaded place, and avoid excess bumping during transportation. On arrival to the laboratory, the samples are stored in the dark at 5°C until extraction no later than two days after sampling.

### **Laboratory work**

#### *Extraction of microarthropods*

The extractor holds 36 samples, so all samples from one sampling batch are extracted at the same time. When the extractor heats the samples from above the microarthropods will move towards colder soils lower in the samples. They will eventually move through the mesh and drop into the benzoic acid.

#### *Equipment*

- Extractor with temperature sensor and data logger
- Manual for extractor
- Insulation foam
- Cooling circulator with anti-freeze fluid and tubes (for connection to extractor)
- 32 soil samples
- 32 meshes with a mesh size of 1x1 mm
- 32 extraction cups with saturated benzoic acid (14.5 g benzoic acid and approximately 1 ml detergent per 5 L)
- Detergent
- 32 lids for extraction cups (only to be used if for some reason you are not able to pour the samples into the 100 ml containers the same day the extraction is finished)
- 96% ethanol (may be denatured if pure ethanol is not available)
- 100 ml containers with lids
- Ethanol resistant labels (see Appendix 14)

#### *Extraction procedure*

1. One day before extraction: Start the cooling circulator connected to the extractor as the samples should not be extracted at bottom temperatures higher than 5°C. Fill the base of



- the extractor with water (will be cooled by the circulating anti-freeze fluid running in the pipes at the base of the extractor).
2. At the day of extraction: Pour 0.5 cm of a saturated solution of benzoic acid and detergent (14.5 g benzoic acid in 5 L water added roughly 1 ml detergent) in each of the extraction cups. Mark each extraction cup with the sample name.
  3. Put the extraction cups into the extractor
  4. For each sample: Take a microcosmos tube containing a soil sample. Carefully remove the upper lid and place the mesh on the tube with the sample.
  5. Place a suitable cup above the soil sample unit and turn the cup with the sample around.
  6. Remove the DBIdut lid from the bottom (now at the top after turning the microcosmos tube and mesh) and sweep surplus soil down into the cup.
  7. Place the microcosm tube with the mesh on the corresponding extraction cup in the extractor. Make sure the soil surface is facing downwards.
  8. Pour any surplus soil into the soil sample.
  9. Place the insulating material around the samples when all samples are in place in the extractor. The insulation around the tubes must be placed carefully so that no soil particles will drop into the extraction cups. Fill surplus holes in the insulation with the black insulation foam circles.
  10. Connect one temperature sensor into the soil of one of the samples in the extractor for regulation of temperature. Connect three temperature sensors to a data logger to follow the temperature during the extraction in the benzoic acid liquid, just above the mesh and on the surface of the soil sample facing the heater.
  11. Close the extractor.
  12. Turn on the extractor and press the green start button. The extractor will now heat the samples according to this schedule:
    - 30°C for 48 hours
    - 40°C for 48 hours
    - 50°C for 48 hours
    - 60°C for 24 hours, terminated manually by switching off the power supply, but it may be continued until all the samples are dry on the down-facing surface on the mesh. The cooling system should ensure that the temperature of the benzoic acid solution is minimum 4°C and maximum 20°C throughout the extraction.
  13. The extraction is stopped manually by turning the power off.
  14. Check that the samples are dry on the surface facing downwards after termination of the pre-programmed extraction process. If some samples are still wet, continue the extraction at 60°C until the samples are dry. Samples with high organic content can be divided in two, to ensure complete extraction.
  15. Turn off the extractor and cooling circulator.
  16. Throw the soil away.
  17. Empty the benzoic acid holding the microarthopods from the extraction cups into a 100ml container. Clean the sides of the cup with 96% ethanol using a squeeze bottle, spraying the sides down to ensure any remaining microarthopods are flushed into the container.
  18. Make sure that the amount of ethanol added is in the proportion one part benzoic acid to two parts of ethanol (resulting in approximately 70% ethanol). If necessary to obtain this proportion divide the sample into two plastic cups. Put a pre-printed water resistant label into the container with the date and name of the sample.
  19. Brush the nets clean. Wash the tubes.

20. Store the samples with lids closed tightly until identification at Department of Bioscience, Aarhus University, Silkeborg.

### 3.4 Birds

Monitoring of birds consists of two elements: Breeding phenology of small passerines on an ad hoc basis and weekly samplings of bird observations at permanent points.

#### 3.4.1 Breeding phenology of passerines

##### **Species to be monitored**

The passerine bird species Northern wheatear (*Oenanthe oenanthe*), Snow bunting (*Plectrophenax nivalis*), Lapland Bunting (*Carduelis flammea*), and Common Redpoll (*Calcarius lapponicus*) are monitored in the study area indicated in figure 2 and from census points as shown in figure 30.

##### **Frequency of sampling**

During June and July on an ad hoc basis. Nests of breeding passerines are located ad hoc and the located nests are followed as frequently as possible until the chicks have left the nest.

##### **Equipment**

- Binoculars (10x)
- GPS
- Data form Appendix 7 / Notebook
- Sticks for marking of nests

##### **Sampling method**

At all visits at located nests note:

- Date
- Species
- Number of eggs/chicks
- GPS position
- Take close up photo of the nest and chicks.

##### **Input of data into database**

The position of nests is entered into a data file named "Bird\_nests.xls" with the following columns: Species, Date, Observer, GPS-position, Number of eggs, number of chicks, and Remarks.

#### 3.4.2 Point sampling

The primary objective of this study is to monitor the birds in the Kobbefjord valley. It is, however, also a very good opportunity to watch for other kinds of wildlife. The main focus is on the small passerines.

##### **Species to be monitored**

The passerine bird species Northern wheatear (*Oenanthe oenanthe*), Snow bunting (*Plectrophenax nivalis*), Lapland Bunting (*Carduelis flammea*), and Common Redpoll (*Calcarius lapponicus*).

##### **Frequency of sampling**

Weekly during the entire field season.

##### **Equipment**

- Binoculars (10x)
- Clock

- Data form Appendix 7 / Notebook.

### Location and marking of sampling plots

The observation points are located by GPS (Figure 30 and table 4).

Table 4. Positions of bird observation points A-M. Positions are given in decimal degrees.

Point	Latitude	Longitude
Bird A	64.134685	-51.385105
Bird B	64.135155	-51.391187
Bird C	64.134592	-51.396234
Bird D	64.13239	-51.39359
Bird E	64.131052	-51.38916
Bird F	64.129385	-51.37833
Bird G	64.131761	-51.379398
Bird H	64.132669	-51.374116
Bird I	64.134509	-51.363874
Bird J	64.135639	-51.355553
Bird K	64.133636	-51.344558
Bird L	64.132841	-51.336278
Bird M	64.131031	-51.326204

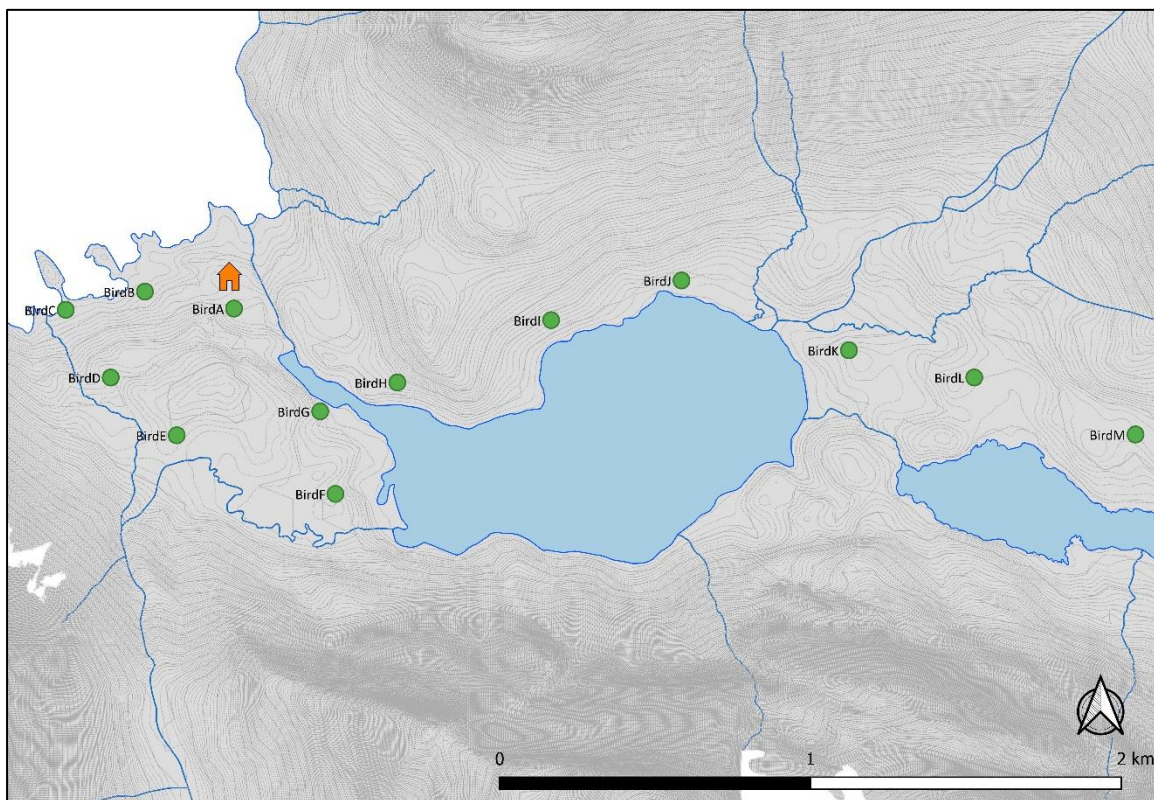


Figure 30. Bird observation points. The field cabin is indicated by the orange symbol close to the observation point BirdA.

### Sampling method

Record species, number, sex and age, special behaviour, etc. Watch for 10 minutes at each point. In the records, indicate clearly whether the observation was done within the first 5 minutes (initial phase) or the second 5 minutes (observation phase). The first period represents birds having been disturbed; the latter period represents birds resident in the area. If a bird is seen within the initial phase AND the observation phase let it only count in the observation phase.

### **Input of data into database**

The number of birds observed at each observation point is entered into an data file named "Birdcountings" with the following columns: Year, Month, Day, DOY, Observer, Time, Point (A-M), Obsperiod (1 or 2), Cloud cover (x/8), Species (NW, SB, LB, RP or other), Age (Juvenile, Adult, Unknown), Gender (M, F, Unknown), Number, and Comments.

## 3.5 Mammals

### **Species to be monitored**

All mammal species.

### **Frequency of sampling**

Ad hoc continuously during entire field season.

### **Equipment**

- Binoculars (10x)
- Note book.

### **Location and marking of study plot**

Entire monitoring area, see Figure 2.

### **Sampling method**

Keep watch for everything with fur. Record geographical position, group size, sex and age, special behaviour etc. All personnel are encouraged to supply observations and submit these on the observation sheet in the cabin.

See observation sheet in Appendix 7 (Random observations).

### **Input of data into database**

Data from Appendix 7 are entered into a data file named "Random Obs." with the following columns: Year, Month, Day, Observer, Location, GPS-position, Species, and Remarks.

## 3.6 Lakes

Physical-chemical and biological (phytoplankton, zooplankton, fish and macrophytes) parameters.

### **Parameters monitored monthly**

- Ice cover
- Water temperature
- Water transparency
- pH
- Conductivity
- Chlorophyll *a*
- Total nitrogen
- Dissolved nitrogen (nitrite/nitrate and ammonium)
- Total phosphorus
- Dissolved phosphorus (phosphate)
- Dissolved organic carbon (DOC)
- Phytoplankton (all taxonomic groups)
- Zooplankton (all taxonomic groups)
- Submerged macrophytes (all taxonomic groups) – only once per year.

### 3.6.1 Water samples

#### Frequency of water sampling

Five times per year, during the ice-free period (normally June – October). First sampling must be done as early in the season as possible – preferably, when there is still some ice on the lake. Sampling is done approximately every 4 weeks. If the ice-free period is shorter than 5 months, the period between samplings is reduced to fit five samplings during the ice-free period.

Ice cover is recorded continuously via automatic cameras operated by GeoBasis.

#### Location and marking of sampling spots

The two sampling lakes are located in the Kobbefjord catchment area in the inner part of Kobbefjord (Badesø / Kangerluarsunnguup Tasia: 64.13°N, 51.36°W and Qassi Sø: 64.15°N, 51.31°W; Figure 31). There is one sampling station at the deepest position in each of the lakes. The station is marked with a buoy anchored at the bottom. At the buoy-line 2 temperature loggers are mounted at 2m and 10m depth. If possible, a sediment trap is attached at the buoy-line at 10m (Qassi Sø) and 15m (Badesø). It has to be collected and stored during winter. Positions of the sampling stations are saved in the GPS.

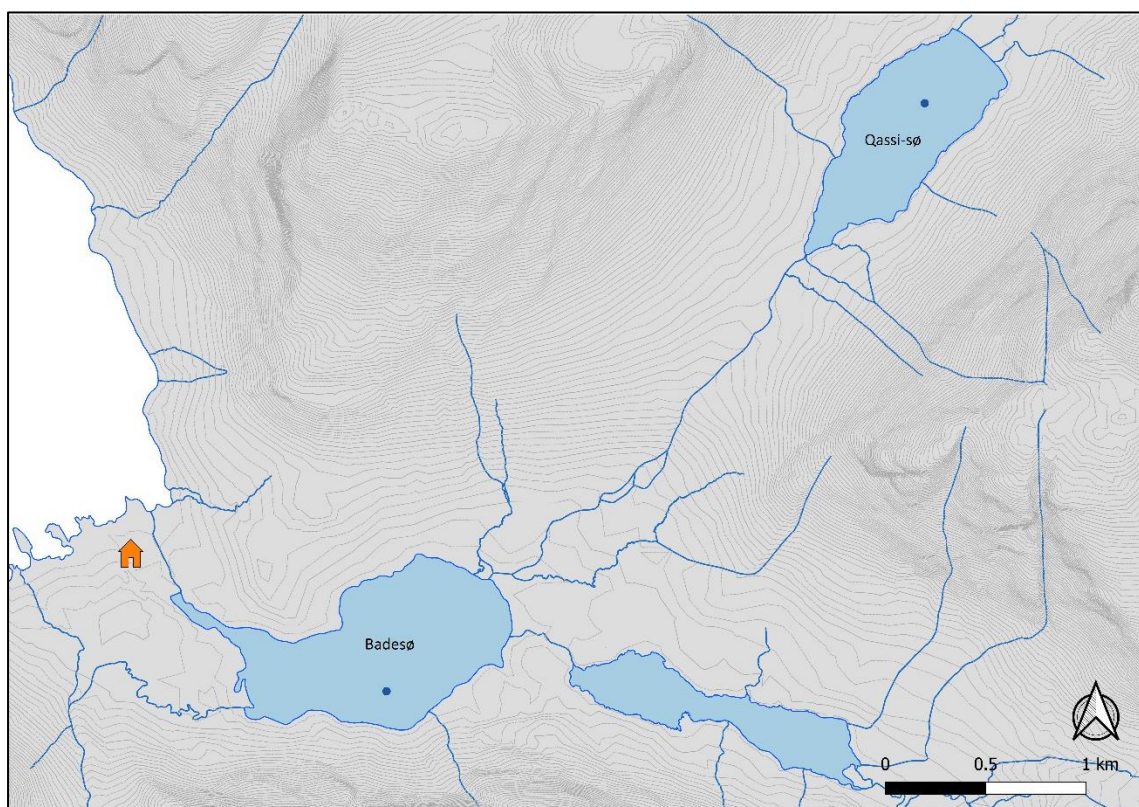


Figure 31. Location of the sampling station in each of the two monitored lakes.

#### Equipment for monthly sampling

- A rubber dinghy with oars and outboard motor
- Pump for the dinghy
- Plastic bottles for sediment (two per lake, only during the first and last visit)
- A water sampler
- 30 litre tub
- Zooplankton filter

- Plastic funnel for 60 ml zooplankton bottles
- Squeeze bottle
- 2 x 60 ml brown glass bottles including 2% Lugol solution for zooplankton (2.5 ml Lugol) and phytoplankton (1 ml Lugol) samples
- Plastic bottles for water chemistry (250 ml), DOC (250 ml) and chlorophyll *a* (2 l)
- Tinfoil
- Secchi disc with rope with decimetre measure
- HOBO Optic USB Base Station and TidbiT v2 Coupler for the HOBO
- StowAway TidbiT Temperature Logger (only in the beginning of the season)
- Depth sounder with thermometer
- Life jacket
- Survival suit.

#### Equipment for annual vegetation sampling

- Plant rake with robe
- Depth sounder with thermometer
- GPS
- Data forms Appendix 10 and 11.

#### Equipment for laboratory work

- Whatmann GF/C filters (47 mm) for Chl *a*
- Tinfoil
- Small plastic tubes for Chl *a* filters
- Equipment for analysing DOC
- Detailed list of chemicals etc. to be used for analysis – see Appendix 12.

#### Sampling methods

At each sampling date, time, cloud cover (x/8), wind speed (calm, light, weak, hard, etc.) and ice cover (% of lake area) is recorded. Use data form Appendix 8A for recording data in the field.

Bring water sampler, tub, Secchi disc, depth sounder, sampling bottles, oars and a filled outboard. Go to the sampling station and tie the dinghy to the buoy.

Before sampling, rinse all sampling gear and bottles with lake water. Measure the transparency with the Secchi disc at an accuracy of 0.1 m on the light side of the boat. Keep the face close to the water surface; lower the disc until it disappears and pull it slowly upwards until it is just visible – this is the Secchi depth.

Take a depth integrated pooled water sample of approximately 25 litres from 0.5 m below the surface to approximately 0.5 m above the bottom. Start from the top and avoid any sediment in the sample. If the lake sediment is disturbed and occur in the sampler a new sample has to be taken. Wait 10 minutes or move the boat away from the first sampling spot. Measure the water temperature both at the surface and at every water sample through the water column.

While in the dinghy, stir the water in the tub well and take:

1. Approx. 100 ml water sample in a 250 ml bottle for water chemistry
2. Approx. 150 ml water sample in an *unused* 250 ml bottle for DOC (dissolved organic carbon)
3. 2 litres of water for chlorophyll *a*

4. One 60 ml sample for phytoplankton enumeration (preserved with 1 ml Lugol solution)
5. 15 litres filtered through 20 µm filter. Release the filter and transfer the collected zooplankton to a 60 ml bottle. Rinse well with water from the pooled sample (preserved with 2.5 ml Lugol solution)

All sample bottles are kept dark (e.g. the samples to be used for chlorophyll *a*, water chemistry and DOC measurements should be wrapped in tinfoil) and cold.

At first and last visit in the season: Empty the sediment traps (see text below) into two plastic bottles. At the same time, data from the two TidbiT v2 temperature loggers is downloaded. Make sure the temperature loggers are logging with 2-hour intervals. One logger is fitted 2 m below the surface, and named “lake name”-1 and the other one 10 m below the surface, named “lake name”-2. The sediment traps are placed in their right positions again. Every 2 years the TidbiT v2’s are replaced.

After each sampling the dinghy is placed at the same spot at Qassi Sjø together with the outboard and other sampling gear. The dinghy is thoroughly secured to the big rock using rope. At Badesø the dinghy is secured after each sampling and taken back to the cabin after the last visit in the season. The outboard motor used at Badesø is taken back to the cabin after each sampling. The outboard at Qassi Sjø is brought back at the end of the season.

### 3.6.2 Submerged vegetation

In August, submerged vegetation is monitored. The method to be used for the macrophyte studies is a transect investigation to obtain a relatively good overall description of submerged macrophyte distribution, density and diversity. The macrophyte diversity is very low and only 2/3 species/taxa are found in the monitored lakes: *Callitriche hamulata* (intermediate water-starwort), moss and in 2018 *Myriophyllum alterniflorum* (alternate water-milfoil) was found in Badesø close to the river outlet from Langesø.

In each lake, approx. 150 observation points are included. If great spatial variability in plant cover occurs, more points may be needed in order to provide an adequate description.

The observation points should follow straight lines from one shore towards the other (parallel to each other and perpendicular to the shore) to a depth of 2x maximum depth of the growing vegetation (min. 5 meters and max. 15 meters). All observation points are marked on a GPS.

At each observation point, water depth, total macrophyte coverage (%), and the species/taxa are recorded (Appendix 11). All observations are recorded in the field as in Appendix 11.

The investigation is undertaken by two people; one conducts all observations (degree of total coverage, species identification, and water depth). The other person operates the dinghy, enters all observations in the data sheet and saves the coordinates of all observation points into a GPS. Observations are made using a plant rake with 1 catch at each observation point and estimating the coverage using a 0-5 scale, where 0 = 0 %; 1 = >0 - 5 %; 2 = >5 - 25 %; 3 = >25 - 50 %; 4 = >50 - 75 % and 5 = >75 - 100 %.

#### *Example of basic analyses of data from the transect investigation*

The following observations were obtained in a transect investigation of a 75 ha lake: 25\*0%, 32\*3%, 40\*15%, 23\*38%, 19\*63% and 11\*88%, i.e. a total of 150 observations.

The relative plant-covered area (RPA) is calculated as follows:

$(25 \cdot 0\% + 32 \cdot 3\% + 40 \cdot 15\% + 23 \cdot 38\% + 19 \cdot 63\% + 11 \cdot 88\% / 150 = 25\%)$ ,

This requires that all observations have been made at the same equidistant intervals all over the lake. If relatively more observations have been made in dense near-shore macrophytes, the observations should be weighted before RPA is calculated.

### Laboratory work

Before filtering for chlorophyll *a* (Chl *a*), absolute conductivity (accuracy 1  $\mu$ S), temperature ( $^{\circ}$ C) and pH (accuracy 0.1 pH unit) is measured. This is done in the field. For chlorophyll measurements 2 x 1 litre from each lake is filtered (exact volume in ml is recorded) through two 47 mm GF/C filter. The filters are folded, placed in separate test tubes, wrapped in tinfoil, and analysed according to Dansk Standard, DS2201 (Appendix 12).

### Dissolved organic carbon (DOC)

In the laboratory, cover your “work space” / table with tinfoil to ensure a carbon-free environment. Prepare 2 test tubes (“burned” 6 hours at 450  $^{\circ}$ C in Muffle oven) labelled with lake ID, date and 150  $\mu$ l 2 M HCl. Use tweezers to place a 25 mm (also “burned” 6 hours at 450 $^{\circ}$ C in Muffle oven) Whatman GF/F filter in a filter bracket. A 60 ml plastic syringe is mounted with a 15 cm teflon tube. The syringe is filled with 60 ml of the water sample, which is then used to rinse the syringe. Afterwards the syringe is filled with min. 50 ml of water sample. The teflon tube is removed and the filter bracket is mounted on the syringe. Press the sample through the filter (for flushing of filter) until 40 ml is left. The test tubes are each filled with 15 ml of filtered sample (filtered through the same filter) and the lids are tightened.

The test tubes are wrapped in tinfoil and stored in the fridge until analysis. The filter is removed from the filter bracket and thrown away. The syringe, teflon tube and the filter bracket are rinsed in ELGA water and air dried. DOC is analysed at Department of Bioscience, Aarhus University, Silkeborg.

Water samples for water chemistry are frozen until analyses at Department of Bioscience, Aarhus University, Silkeborg.

Zooplankton and phytoplankton samples are stored in darkness – not frozen. These samples are sent to Department of Bioscience, Aarhus University, Silkeborg following the last sampling.

### 3.6.3 Parameters monitored every 5th year

- Fish (taxonomic groups, population and for isotopes)
- Macroinvertebrates (taxonomic groups, abundance and for isotopes)
- Phytoplankton and zooplankton for isotopes
- Sediment (paleo-analyses of chironomids, diatoms and cladocerans).

### Fish

In each lake a maximum of 9 (1.5 m deep) sinking Lundgren biological multi mesh gill nets are used for approximately 16-18 hours. Gill nets are set in the littoral and in the pelagic (in the middle of the water column) and at the bottom (benthic nets) in the late afternoon. Nets are gathered the following morning. The catch is treated per net and per net type (littoral, pelagic, benthic). Each fish is given a consecutive number, identified to species (Arctic char *Salvelinus alpinus* or Three-spined stickleback *Gasterosteus aculeatus*) and sex, length and weight is measured.

- a. Tissue samples (dorsal muscle) for isotope analyses: For Arctic char, samples are taken from approximately 20 fish per lake considering all size classes of the species. The tissue samples are frozen in plastic vials and marked with lake name, date, fish number (same as above) and content. Three-spined sticklebacks are sampled as whole fish.



- b. From Arctic char, stomach content is taken. Stomachs are preserved in 96 % ethanol in vials or 100, 200 or 300 ml jars, depending on the size of the stomach. The container is marked with lake name, date, fish number (same as above) and content.
- c. From Arctic char, otoliths are taken. These are kept in paper envelopes and marked with lake name, date, fish number (same as above) and content.

### **Sediment/paleo samples**

Four sediment cores are sampled in each of the two lakes using the kajak sampler. One from a depth of approx. 15m (in part of the lake with more or less low inclination/horizontal bottom) and three close to the shore (taken by hand). Be careful not to disturb the sediment cores when unscrewing the core from the sampler. When the cores are taken on board there must be a sharp threshold between the sediment and the water phase. If this is not the case it indicates that the core may have tipped over at the bottom and the sample must be replaced.

On shore, surface sediment (0-1 cm) from the three near-shore cores are pooled in a 250 ml plastic jar and marked with lake name, date, number of cores and depth (0-1 cm). Surface sediment from the core sampled at 15m is kept separate, but marked as above.

Samples are kept cool and dark.

Samples are analysed at Department of Bioscience, Aarhus University, Silkeborg.

### **Isotope sampling and analyses**

Samples of fish (see above).

- a. Benthic invertebrates: Pelagic and littoral invertebrates are kept separate. Surface sediment is sampled with a sweep net (littoral and in macrophytes) or an Ockelmann-sledge. Samples are rinsed as much as possible in the dinghy before being put into a bucket (several samples from one habitat can be pooled in one bucket as a qualitative sample). On shore, the samples are filtered through a 212  $\mu\text{m}$  mesh and invertebrates are sorted into groups in small glass jars (~5 ml) and marked with lake name, date.
- b. Zooplankton is sampled as two fractions (> 140  $\mu\text{m}$  and > 500  $\mu\text{m}$ ). Both nets are pulled after the dinghy until enough material has been collected. Samples are put into 20 ml vials.
- c. Phytoplankton: A large amount of water is filtered through first a 80  $\mu\text{m}$  mesh followed by a 11  $\mu\text{m}$  mesh and samples are put into 20 ml vials.
- d. Benthic algae: The top 5 mm from one sediment core is put into a vial. If animals are present they should be removed.
- e. Stones: First large stones are collected in the littoral zone and epiphytes are scraped into a vial.
- f. Periphyton: Plants are selected randomly. Plants are washed in a 1 litre jar and periphyton is scraped off. The water including the periphyton is filtered on a 20  $\mu\text{m}$  mesh. Animals are removed and the filtrate is put into a vial.
- g. Macrophytes: A sample is taken of the dominating taxa. Periphyton is removed and roots are avoided. The sample is put into a vial.

All samples are kept frozen in small glass jars. Keep enough material for 3 replicates of all samples.

### **Laboratory work**

#### *Identifying and counting of benthic invertebrates*

Samples are shipped to Department of Bioscience, Aarhus University, Silkeborg, together with the plankton samples. The samples are sorted, identified and counted to species or genus level.

### *Isotope sample*

All isotope samples (fish, invertebrates, plankton, periphytes, macrophytes) are prepared for analyses, i.e. each sample is freeze dried, homogenized and a 5 mg sub sample is weighed in a tin capsule (ready for analyses). Preparation for analyses is undertaken in Nuuk. For analysis, samples are shipped to: University of California, Davis Stable Isotope Facility, One Shields Avenue, Mailstop #1, Davis, CA 95616 (important to make an appointment in advance). The isotope samples can also be analysed at the AU Department of Bioscience Stable Isotope Analytic Center.

### **Equipment for every 5th year field work (done in 2008, 2013 and 2018)**

#### *Fish*

- Gillnets
- Floaters and robe
- Sinks and robe
- Poles for nets
- Buckets for fish
- Field schemes
- Ruler, balance
- Gear for the isotope sampling (see below)

#### *Sediment*

- Kajak sampler
- Sediment cores and rubber stoppers
- Equipment to process sediment cores

#### *Isotopes*

- Tweezers and scalpels
- Rubber gloves
- Ethanol and squeeze bottles
- Phytoplankton net (prefer 11  $\mu\text{m}$  net but 20  $\mu\text{m}$  is OK)
- Zooplankton net, 140  $\mu\text{m}$
- Vertical net for large zooplankton, 500  $\mu\text{m}$
- Sweep net for invertebrates, 500  $\mu\text{m}$
- Sorting trays
- Plastic vials for invertebrates
- Coolers

## 3.7 Random observations of birds and mammals

### **Species to be monitored**

All bird species and mammals (Arctic foxes, caribou and hares).

### **Frequency of sampling**

Continuously during entire field season.

### **Equipment**

- Binoculars
- Spotting scope
- Notebook

- Marking of sampling plots
- Entire activity area.

### **Sampling method**

Keep watch for everything with feathers and fur. Record flock size, sex and age, special behaviour, geographical position etc.

### **3.8 Disturbance**

Parameters to be monitored:

'Person-days' spent in the area, aircraft activity over the area, boat trips to and from the area, discharges (burning of waste, human discharges into the fiord). All of these can only be monitored during periods with personnel at the station.

## 4 Storage of data

### 4.1 Data at GINR

Data collected during the season are downloaded/saved at the Greenland Institute of Natural Resources when returning to Nuuk. All data are typed into or transferred to the specific Excel files immediately. At GINR the data on the server are security copied every night along with the general server back up. Data is stored in the following folder: F:\40-59 PaFu\41 Vegetation\08 NuukBasic\_BioBasis. Each year has a corresponding folder (e.g. "BioBasis\_Nuuk\_2018") where each sub-programme has a folder of their own, containing their data files, where the collected data is entered. At the end of the season all data is sent to the Department of Bioscience, Aarhus University, Roskilde for storage (see 4.2 Data for the GEM database as different layout and formats may be used). All original data files are stored on the servers at GINR. Furthermore, written material is stored at GINR along with collected specimens until processed at the appropriate facilities in Denmark.

### 4.2 Data for the GEM database

Deadline for data delivery is 1 May. Before delivery, all data are quality checked and reformatted for the GEM database. Dates are given as YYYY-MM-DD and the file format is to be a text document (.txt). A table of all files to be sent is shown in table 5. Column and element descriptions can be found in the meta-data files "Biobasis\_Nuuk\_Column\_description" and "Biobasis\_Nuuk\_Element\_description" in the following folder: F:\40-59 PaFu\41 Vegetation\08 NuukBasic\_BioBasis\03\_GEM\_Database

Data can be downloaded from [www.g-e-m.dk](http://www.g-e-m.dk)

## 5 References

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## 7 Appendix

### Appendix 1 Example of annual sampling protocol

Date	May	June	July	August	September	October
1				NERO;	Phen; Art; K2 vegetationstransekt	
2	Phen; Art; NDVI; Birds			Phen; Art; NDVI		
3	opsætning ART, Phen, NDVI			UVB		
4			Birds; NERO; Phen; Art; NDVI			C-Flux; Phen; Art; NDVI; ½ NERO
5			Besøg MASTERCLASS	C-Flux;		
6		DRYAS; Tea Time; Mart				Birds
7			Besøg MASTERCLASS		C-Flux; Phen; Art; NDVI; Birds	
8		C-flux			Amerikanske stud; MART	
9		NERO; Phen; ½ Art; NDVI				
10		Birds; ½ art				
11	C-Flux; Phen; NDVI; DRYAS; ½ NERO		C-Flux; Phen; Art; NDVI			C-Flux; Art pakkes sammen
12	Art; opsætning; Mart; UV b		Kristian kommer			K1
13			Birds; Totaltælling		Birds; NERO; Art 3 + 4; NDVI;	
14		Birds; DRYAS			NDVI (ert1-emp2); ART 1+2; Jordprøver	
15		Sirius			Jordprøver	
16		C-Flux; Phen; Art; NDVI				
17	Birds; Phen; NDVI			Phen; Art; NDVI		
18						
19	Art			NERO		c-flux
20		Birds; DRYAS	C-Flux; Mart; Birds; NERO		C-Flux; Phen; Art; NDVI	c-flux
21				GU dag i Kobbefjord	Kamera Nordlandet	
22			Phen; Art; NDVI; UVB			
23	K1; Phen; NDVI; NERO; ITEX kh-NDVI			C-Flux; Phen; Art;		send prøver til Dk og Cflux Temp data
24		NERO; Phen; Art; NDVI; K1				
25				NERO; NDVI; Veg. trans ½ Badesø; K1		
26			Phen; NDVI; K1+K2	K2; ½ vegtrans Badesø	Birds;	
27	C-Flux; ; Art; Birds		C-flux; Art	Muslinger		
28		K2	C-flux; Birds; UVB; zooplankton;	Birds - GU i Kobbefjord	Phen; Art; NDVI; K2	
29		Phen; totaltælling; ART				
30		Birds; C-flux; NDVI		C-flux; Kommunal bestyrelse		
31			UVB			
31						

Appendix 2 Data form for plant phenology monitoring

Date:				Observer:							Page 1/2		
Plot	Time	Photo	Cloud cover	#	Snow	Buds	Male flower	Female flower	Hair/ Scene.	Fungus	Larvae	Total	Remarks
ERI1				A									
				B									
				C									
				D									
EMP1				A									
				B									
				C									
				D									
ERI2				A									
				B									
				C									
				D									
EMP2				A									
				B									
				C									
				D									
ERI3				A									
				B									
				C									
				D									
EMP3				A									
				B									
				C									
				D									
LOI1				A									
				B									
				C									
				D									
SIL1				A									
				B									
				C									
				D									
SAL1				A									
				B									
				C									
				D									
SAL2				A									
				B									
				C									
				D									

Date:				Observer:							Page 2/2		
Plot	Time	Photo	Cloud cover	#	Snow	Buds	Male flower	Female flower	Hair/ Scene.	Fungus	Larvae	Total	Remarks
LOI2				A									
				B									
				C									
				D									
LOI3				A									
				B									
				C									
				D									
LOI4				A									
				B									
				C									
				D									
ERI4				A									
				B									
				C									
				D									
EMP4				A									
				B									
				C									
				D									
SAL3				A									
				B									
				C									
				D									
SIL4				A									
				B									
				C									
				D									
SIL2				A									
				B									
				C									
				D									
SIL3				A									
				B									
				C									
				D									
SAL4				A									
				B									
				C									
				D									



Appendix 3 Data form for CO<sub>2</sub>-flux monitoring

BioBasis Nuuk 2018		Date:		EGM-4: A/B				Observer:					
Time	Plot	Record nos.		Cloud cover	Photo	Soil_moisture				Remarks			
		Light	Dark			1	2	3	Chamber_height				
						1	2	3	1	2	3	4	
:	1C	-	-										
:	1SG	-	-										
:	1S	-	-										
:	1LG	-	-										
:	1T	-	-										
:	2SG	-	-										
:	2LG	-	-										
:	2S	-	-										
:	2C	-	-										
:	2T	-	-										
:	3C	-	-										
:	3T	-	-										
:	3SG	-	-										
:	3LG	-	-										
:	3S	-	-										
:	4LG	-	-										
:	4SG	-	-										
:	4S	-	-										
:	4T	-	-										
:	4C	-	-										
:	6S	-	-										
:	6SG	-	-										
:	6C	-	-										
:	6T	-	-										
:	6LG	-	-										
:	5T	-	-										
:	5S	-	-										
:	5LG	-	-										
:	5SG	-	-										
:	5C	-	-										

Appendix 4 Data form for plant phenology in CO<sub>2</sub>-flux plots

Plot no/ Snow	Date:		Observer:					Total	Remarks
	Buds	M flowers	F flowers	Hairs	Fungus	Larvae			
1C									
1SG									
1S									
1LG									
1T									
2SG									
2LG									
2S									
2C									
2T									
3C									
3T									
3SG									
3LG									
3S									
4LG									
4SG									
4S									
4T									
4C									
6S									
6SG									
6C									
6T									
6LG									
5T									
5S									
5LG									
5SG									
5C									

Appendix 5 Data form for UV-B effects monitoring

BioBasis Nuuk 2018 Date: \_\_\_\_\_ Observer: \_\_\_\_\_

Betula				Vaccinium			
Block	Treatment	Rec. no.	Remarks (DOH)	Block	Treatment	Rec. no.	Remarks (DOH)
1	B (Mylar)	-		1	B (Mylar)	-	
1	C (kontrol)	-		1	C (kontrol)	-	
1	F (film)	-		1	F (film)	-	
2	B (Mylar)	-		2	B (Mylar)	-	
2	C (kontrol)	-		2	C (kontrol)	-	
2	F (film)	-		2	F (film)	-	
3	B (Mylar)	-		3	B (Mylar)	-	
3	C (kontrol)	-		3	C (kontrol)	-	
3	F (film)	-		3	F (film)	-	
4	B (Mylar)	-		4	B (Mylar)	-	
4	C (kontrol)	-		4	C (kontrol)	-	
4	F (film)	-		4	F (film)	-	
5	B (Mylar)	-		5	B (Mylar)	-	
5	C (kontrol)	-		5	C (kontrol)	-	
5	F (film)	-		5	F (film)	-	

Appendix 6 Data form for arthropod monitoring

BioBasis Nuuk 2018				Date:				Observer:				Windtraps			
Time	Plot no.	Sample	Snow	Pitfall opened	Remarks	Plot no.	Sample	Snow	Pitfall opened	Remarks	Plot no.	Sample	Snow	Pitfall opened	Remarks
:	1	A				1	A				1	A			
		B					B					B			
		C					C					C			
		D					D					D			
		E					E					E			
		F					F					F			
		G					G					G			
		H					H					H			
:	2	A				2	A				2	A			
		B					B					B			
		C					C					C			
		D					D					D			
		E					E					E			
		F					F					F			
		G					G					G			
		H					H					H			
:	3	A				3	A				3	A			
		B					B					B			
		C					C					C			
		D					D					D			
		E					E					E			
		F					F					F			
		G					G					G			
		H					H					H			
:	4	A				4	A				4	A			
		B					B					B			
		C					C					C			
		D					D					D			
		E					E					E			
		F					F					F			
		G					G					G			
		H					H					H			

Appendix 7 Data form for nest phenology

	Bird nests														

Appendix 8 Data form for passerine bird monitoring

BioBasis Nuuk 2018		Date:		Observer:													
Time	Site	State	Cloud	Snow bunting			Lapland bunting			Red poll			Northern wheatear			Remarks / Other birds	
				M	F	UK	M	F	UK	M	F	UK	M	F	UK		
:	A	Initial															
:	A	Obs															
:	B	Initial															
:	B	Obs															
:	C	Initial															
:	C	Obs															
:	D	Initial															
:	D	Obs															
:	E	Initial															
:	E	Obs															
:	F	Initial															
:	F	Obs															
:	G	Initial															
:	G	Obs															
:	H	Initial															
:	H	Obs															
:	I	Initial															
:	I	Obs															
:	J	Initial															
:	J	Obs															
:	K	Initial															
:	K	Obs															
:	L	Initial															
:	L	Obs															
:	M	Initial															
:	M	Obs															

When arriving at a new site, count the numbers you see within the first 5 min (Initial). Then start your observation (Obs) for 5 min.  
 If a bird is seen within the Initial period AND the Obs period let it only count in the Obs period



Appendix 10 Data form for lake monitoring

BioBasis Nuuk 2018		Lake:				Observer:				Remarks		
		Date	Time	Ice cover	Cloud cover	Wind speed	Secchi depth	Depth	Temperature, surface		pH	Conductivity/temp C

Dybbø (m)/Temp (°C)
/
/
/
/
/
/
/
/
/

App. 25 litres of pooled water from 0.5m below the surface to 0.5m above the bottom

Samples taken from pooled water to:

Water chemistry (ca. 100 ml. water in 250 ml bottle)

Chl a (2 litres, wrap in tin foil)

Phytoplankton (60ml preserved with 1 ml lugol)

Zooplankton (15 litre filtered through 20µm filter into 60ml bottle with 2.5ml lugol)

DOC (fill half a 250ml bottle and wrap with tin foil)

pH and conductivity are measured on the pooled water

**RINSE BOTTLES WELL WITH LAKE WATER BEFORE USE**



Appendix 11 Data form for submerged vegetation monitoring in lakes

Veg_Lake:		Dato		Side 1/
Waypoint	Dybde	Vandstjerne	Mos	Bemærkninger
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				

Veg_Lake:		Dato		Side 2/
Waypoint	Dybde	Vandstjerne	Mos	Bemærkninger
26				
27				
28				
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				

## Appendix 12 Measuring chlorophyll a in the laboratory

Remember that chlorophyll is sensitive to light, thus filtering and measurements must take place in dimmed lighting.

### 1. Method: fluorometric measurement with ethanol extract

#### *Filtering and extraction*

- A known volume of water sample (preferably 2 x 1 litres) is vacuum filtered using GF/C filters. The pressure is not to exceed 0.3 bars or phytoplankton might be destroyed. Filtering time should not exceed ½ h. If so a smaller volume is to be filtered. Flush the filtering funnel in ELGA-water at the end of filtration.
- Fold the filter and insert in test tube with tweezers.
- Ad 10 ml of ethanol and seal with a lid.
- Shake the sample gently (large air bubbles should be avoided) and extract for 18-24 hours in darkness. If the samples can not be analysed the following day they may be frozen after addition of ethanol. After thawing the samples are extracted in darkness for 18-24 hours. Do not leave the samples in the freezer for longer periods of time since the chlorophyll will gradually break down.

#### *Measurements using a fluorometer*

- Turn the fluorometer on
- Press the filter to the bottom of the test tube using a tweezer
- Spin samples in the centrifuge at 2000 rpm for 10 minutes
- Carefully transfer the samples to a cuvette (ca. 2 cm from the upper edge) and measure in the fluorometer
- Ad 200 µl (3 drops) of 1 M HCl and turn the cuvette upside down a few times
- Measure again after 2 minutes

### 2. Reagents

Ethanol, 96% (v/v) density = 0,808

Hydrochloric acid, HCl, 1M

### 3. Waster and cleaning up

Samples can be poured into the sink when flushing with tap water. The plastic test tubes with filter paper are thrown in the regular dustbin. The 2 litre bottles, filtering funnels, tweezers and cuvettes are rinsed 3 times in ELGA-water.

### 4. Reference

Modified from Dansk Standard, DS2201 using a fluorometer instead of a spectrophotometer





Appendix 15 List with random numbers for microarthropod sampling

Year	Collection	Sample	X	Y
2019	1	A	1.5	5.5
2019	1	B	3	4
2019	1	C	5	2
2019	1	D	1	5
2019	2	A	2	1
2019	2	B	1	2.5
2019	2	C	3	7
2019	2	D	3.5	10
2019	3	A	4.5	7.5
2019	3	B	6	3.5
2019	3	C	4.5	6.5
2019	3	D	5	3.5
2020	1	A	1.5	4.5
2020	1	B	6.5	7.5
2020	1	C	5.5	7.5
2020	1	D	6.5	8.5
2020	2	A	10	6.5
2020	2	B	9.5	8
2020	2	C	6.5	2.5
2020	2	D	8.5	7
2020	3	A	9	5.5
2020	3	B	2.5	1
2020	3	C	1	7.5
2020	3	D	9	5
2021	1	A	2	9.5
2021	1	B	6	10
2021	1	C	0.5	4.5
2021	1	D	1.5	4
2021	2	A	1	5.5
2021	2	B	8.5	1
2021	2	C	0.5	6.5
2021	2	D	5	8.5
2021	3	A	2.5	1.5
2021	3	B	8.5	10
2021	3	C	1	4.5
2021	3	D	2.5	2
2022	1	A	0.5	3
2022	1	B	3	4.5
2022	1	C	2	4
2022	1	D	0.5	3.5
2022	2	A	6.5	1
2022	2	B	1	3.5
2022	2	C	6.5	0.5
2022	2	D	5.5	4
2022	3	A	4	9.5
2022	3	B	9.5	1
2022	3	C	3	8
2022	3	D	1.5	1