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UNIS

Threat or opportunity? Landscape genetics in a coal mining area.

Bente Sved Skottvoll

BIO-3910 Master's thesis in Biology, 60 ETCS

May 2013

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Front cover photo: Tussock of *Luzula confusa* at Fuglefjellet in Bjørndalen.

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Abstract

The area of Sveagruva have experienced several periods of coal mining. This study aim to evaluating changes in vegetation composition, species distributions and genetic structure using a combination of field and molecular analyses, to identify whether or not coal mining have effected plant life in Svea. Investigations on species and gene level were done on two *Luzula* species, *L. confusa* Lindeb. and *L. nivalis* (Laest.) Spreng. No direct correlation was found, and the soil pH explains most of the seen variation. Though it was also discovered that *Luzula nivalis* in Sveagruva are composed of different gene lineages. Could this be a another cryptic species of Svalbard?

Keywords

Arctic, *Luzula* , Svalbard, coal dust, landscape genetics

Preface

This master project was part of a vegetation surveillance survey conducted for the coal company Store Norske Spitsbergen Kullkompani (SNSK) by P. B. Eidesen, UNIS, during the summer 2009. The master project was focused on evaluating changes in vegetation composition, species distributions and genetic structure using a combination of field and molecular analyses. Investigations on species and gene level were done on two *Luzula* species, *L. confusa* Lindeb. and *L. nivalis* (Laest.) Spreng.

1 Introduction

The Norwegian coal mining company SNSK is based in Svalbard, and the main operation is now located in Sveagruva (shortened to Svea). The study area Svea have experienced three coal-mining production periods since the start in 1917. The latest mining activity started in 2001 when the mine Svea Nord was opened. The question is; how are the mining activities affecting the vegetation in the surrounding area, when considering the changes in environment and landscape that industry and mining are introducing to an area considered being vulnerable and untouched?

Svea – History of coal mining and human impact on the environment

The Swedish coal mining company Nye Svenska Stenholsaktiesbolaget Spetsbergen were the first to claim the area of Svea in Braganzavågen fiord. They started mining the mine Svea Øst in 1917, and 454 602 tons of coal was extracted until a fire out break in 1925. In 1934 the mine and the Braganza coalfield was sold to the Norwegian mining company Store Norske Spitsbergen Kullkompani (SNSK), that started surveying for coal close to the Swedish mine and investigated the Braganza coalfield from 1934 to 1937. World War II interrupted further mining activity, but mining was resumed in 1946, this time in the western part of the same coal seam as the Swedes mined. The mine experienced many difficulties, and in 1949 this mining activity was suspended. After this, Svea was not considered for mining activity for 20 years. (Kvello, 2006; Westby, 2003)

New periods of surveying were done in the years 1970 to 1977 and 1980 to 1981. In 1979 simple production mining was initiated in the western area of the former Svea Øst coalfields. This coalfield was known as the Mid-alternative, or Svea Vest. Full-scale mining was initialized in 1984, but was in 1987 paused by Norwegian Parliament. From this point Svea Vest was used for research purposes only, including testing new mining equipment and mining methods. Mining activity was

resumed in 1997 and lasted till 2000, when plans for a new mine was in place. In 2001 the mine Svea Nord was portaled in the Sentral field. Here the coal reserves were great, with a coal seam up to 5 meters thick. Aside a fire outbreak in 2005, that implicated a production pause for 8 months, the mine is still running in 2013. (Kvello, 2004; Martinussen, 2005; SNSK, 2005)

Coal production and storage in Svea

During the first periods of mining activity in Svea, the coal was transported to a coal wharf close to the settlement. In the later periods, the wharf at Kapp Amsterdam was built. During the first years of mining in Svea Nord, the coal was transported from the mine entrance at Høganesbreen by lorry to Kapp Amsterdam, which is a distance of 12-13 km. Today the coal is transported 5-6 km by lorry from the stacker north of the settlement to the coal stockpiles at Kapp Amsterdam before shipping.

Mining at Svalbard have always been under ground, and the longwall mining method have enabled a higher coal production in Svea for the last decades. The coal production in Svea (Figure 1) rose significantly in 1997 and exceeded 1 million tons in 2001 when Svea Nord was set in full production. Though human activities have almost a century long history in this area of Svalbard, the mining activity had been minor until 1997, and hence the effect of coal dust on the vegetation before 1997 or 2001 can be evaluated as insignificant.

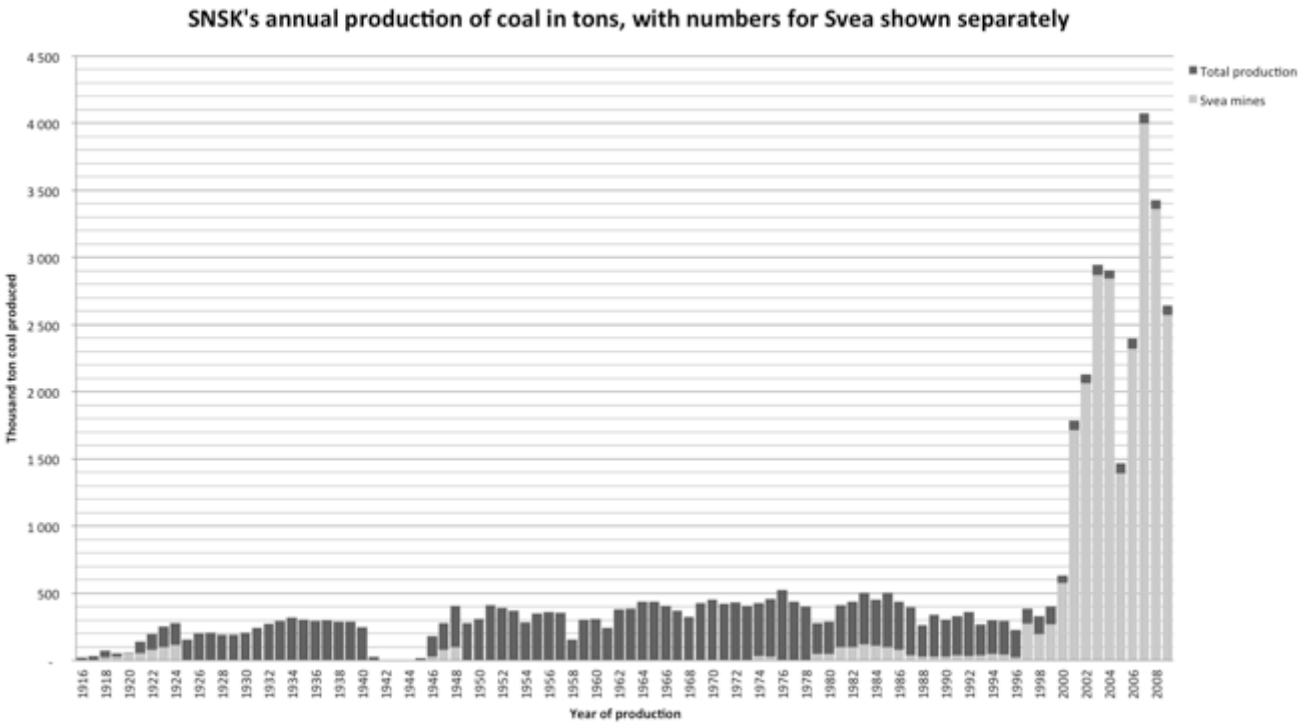

Figure 1: Annual coal production for Store Norske Spitsbergen Kullkompani (SNSK), where production in Svea mines is shown as a fraction of the totals. Since 2001, the coal production has exceeded 1 million tons a year, with a peak year in 2007 extracting almost 4 million tons of coal (SNSK, 2000-2001; StatistiskSentralbyrå, 1952, 2013).

The chemistry of coal

Layers of coal are organic sediment made in a long-lasting geological process, where thick layers of dead plant material are deposited in shallow water upon being exposed to high pressure. This implicates that coal also contains the same basic compounds and elements as the originating plant material, as carbon (C), nitrogen (N), sulphur (S), phosphorous (P) and different trace elements. One may thus presume that the addition and accumulation of coal to the soil alters the soil chemistry and pH. Spencer is among those who have measured lower soil pH on a coal dust plume than off (Spencer, 2001). As for the period 2006-2009, the coal assay for Svea Nord showed a S-content between 0.6 and 1.0 % [0.4 : 2.0], and P-content of 0.04 – 0.09 % (SNSK, 2009). This is low compared to other coalmines according to SNSK, but the levels might be high enough to alter soil pH of areas of coal dust pollution.

As a part of the mining process surrounding rocks low in coal content is removed and deposited in nearby waste piles. These rocks often contain iron sulphide minerals that oxidize in contact with air and water, and produce sulphuric acid and release heat (Elberling et al., 2007). As the pH is reduced, trace elements are leached from the oxidation of sulphides and other weathering processes

(e.g. (Blowes et al., 1994; Larsen et al., 2005). This is better known as acid mine drainage (AMD). AMD strongly affect vegetation close to the waste pile, because of the plants passive uptake of ions. Askaer et al. (2008) analysed the impact of AMD downstream of a mine waste pile in Bjørndalen, Svalbard, where the levels of the trace elements Al, Mn and As were found to accumulated to phyto-toxic levels, and Fe-oxide plaque covered the leaf surfaces during spring flush. Combined with low pH these are the main reasons for absence of plant life other than some lichen species in high AMD impact areas in Bjørndalen (Askaer et al., 2008).

Physical properties of coal and coal dust

Coal dust is spread by the wind from all unprotected coal sources. The wind erodes and transports particles of coal from the stacker, lorry transport and coal stockpiles at Kapp Amsterdam to the surrounding areas. In general 0.02% of all produced coal is lost as fugitive dust during loading, and an equal amount is lost during transportation (Sharma et al., 1992). Miller (2011) present an estimate of 0.05 to 1 % of coal lost during transit. For Svea, a 2 % loss of total coal production is estimated (SNSK, 2001), partly because of the lower humidity in arctic climate. Different actions minimize or prevent coal dust spreading, e.g. spraying water on roads, stockpiles and conveyor belts, and installation of dust-collecting systems.

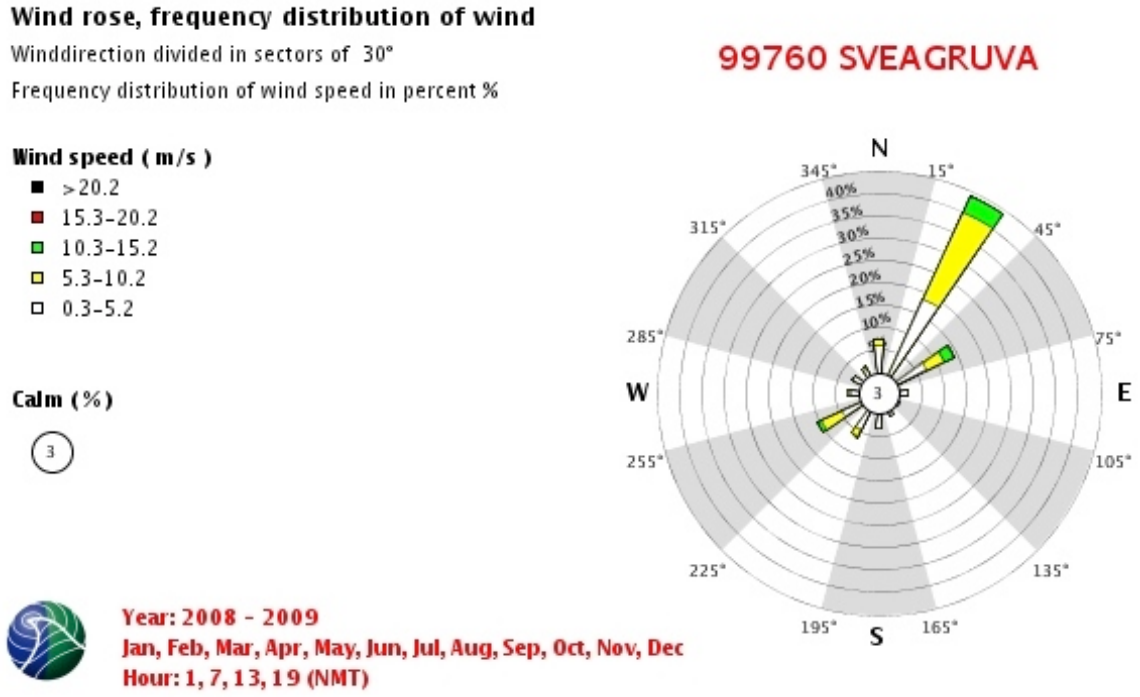

Figure 2 Windrose for weather station Svea, showing the dominant wind directions and wind speed in 2008 and 2009. This figure is made by the windrose-application available at eKlima, using chosen weather data collected by the weather station in Svea settlement for the period 01.01.2008 – 31.12.2009. (NorwegianMeteorologicalInstitute, 2011)

The mean wind speed at Svea varied between 3.1 and 6.1 m/s during the years 2008-2009, while the dominant wind direction was north-northeast (Figure 2).

During wintertime, transported coal dust on top snow layer is visible from satellite imagery. Black particles as coal, soot and dust, reduce the snow albedo (the snows ability to reflect solar radiation). The dark coal dust particles absorb solar radiation and accumulate heat. When deposited on snow, this causes the underlying snow to melt earlier than surrounding clean snow (Aamaas et al., 2011).

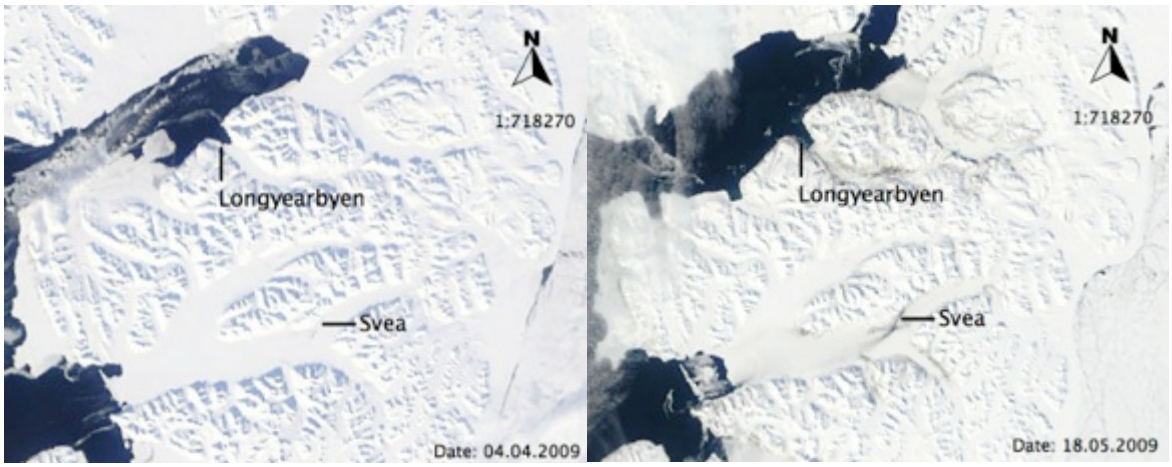

Figure 3 Satellite photos show traces of wind spread coal dust around the Svea transport road and coal stockpiles. The spread of coal dust is visible from satellites in low Earth orbit, in this case the Terra satellite orbiting at 705 km from the Earth's surface. The pictures were taken on 4th April and 18th May 2009. The picture to the right shows earlier snowmelt where the coal dust has been deposited. (NASA, 2011)

The possible effects of coal dust on the environment

The effect of coal dust on plant ecology could be several, since 1) the coal dust covers the vegetation and the ground (Farmer, 1993; Hirano et al., 1995; Naidoo, 2004; Naidoo et al., 2005), 2) are of dark colour and thus affect the local ground temperature (Brooker et al., 2003; Spencer, 2001; Spencer et al., 1997), and 3) has a small content of sulphur (S) and phosphorous (P), and compounds of these are well known to lower the pH (e.g. (Askaer et al., 2008)). In other words, coal can have a confounding effect on soil heat and pH.

Fugitive coal dust will at some point settle. Heavier coal dust particles will settle closer to the source, while lighter particles travel further (Smit, 1980). Aggregations of coal dust in the landscape can be compared to miniature coal stockpiles. Leakage from coal stockpiles or reject coal piles to the ground water have been measured to lower the pH and raise the salinity by Carlsonn (1990). The miniature stockpiles can be assumed to not have the same extent of impact as a normal scale stockpile. Rikard et al. (1990) identified a positive correlation between sulphate (SO_4) and

electroconductivity in coal mining-influenced streams, and suggested the use of electroconductivity as a measure of sulphate pollution in mining-influenced areas.

Early snowmelt has several effects on the underlying vegetation. As the snow melt, the vegetation loses an insulating layer and is exposed to the at present weather conditions (Sakai et al., 1987). This could have both negative and positive effects on the plant life (Wipf et al., 2006). Wind suspension of surface ice and snow crystal from surrounding area erode exposed evergreen plant parts as well as old dry plant material insulating the spring shoots. At low temperatures meristems and other susceptible plant tissue might freeze and get damaged, as plant tissue is only tolerant to . As sunlight start heating the ground, a microclimate suitable for early onset of photosynthesis is formed. Early spring growth onset might give the individual plant a head start in accumulation and storage of energy and other resources that are later needed for growth and reproduction. But this can be impeded if the ground water is still frozen and thus inaccessible, or the night temperatures lethal. (Jones, 2001; Marchand, 1996)

As the snow melts, the coal dust cover the ground and adds to the existing growth substrate as plants erupts from beneath the coal layer. During the summer, more coal dust is transported and deposited on the surface of the vegetation. The leaf morphology determines whether coal dust is deposited or not, and for how long (Naidoo et al., 2005). Coal dust is removed from glabrous leaves by rain and wind, while coal dust gets trapped on hairy leaves or leaves with glands. Coal dust covered leaves have a lowered photosynthetic performance, which in the longer term will reduce growth and reproduction (Naidoo et al., 2005). Other studies have found coal dust to block the stomata openings, stick to the stigmata and shade the leaves, and as a consequence the plants experience reduced photosynthetic activity, reduced growth and fruit set (Farmer, 1993).

Spencer and Tinnin (2001) did a study on vegetation changes in an arid environment, where the site had been accumulating coal dust for 15 years. They found that the annuals growing on the coal dust plume germinated and started flowering earlier, and had higher biomass than annuals growing off the plume. This was assumed to be due to elevated soil temperature early in the growing season. They did not have similar significant difference for perennial plants biomass on or off the plume, but this could be due to small sample size relative to high variance in the data set. Late flowering perennials and annuals on and off the plume also had synchronized flowering. They did not find any statistical significant differences or patterns regarding higher plant vegetation cover or frequency caused by the coal dust, but moss species composition was shifted, and the lichen cover was lower on the plume than off (Spencer, 2001; Spencer et al., 1997).

Vegetation mapping in combination with landscape genetics as a tool to assess the possible effects of mining activity

Both community and species diversity are essential ecosystem properties, and it is important to reveal the environmental factors that determine plant diversity in a given area. However there are a range of factors influencing vegetation composition and plant species diversity. Temperature, topography, bedrock, soil moisture, nutrients, and freeze-thaw events are all important factors influencing pattern of plant species diversity (Arnesen et al., 2007; Chapin et al., 1996; Gough et al., 2000; Tkach et al., 2008; Virtanen, 1996; Young et al., 1997).

From a management perspective, it is then important to decipher changes due to human activities from changes due to natural processes such as succession, natural disturbance and temporal variability. The last vegetation mapping in Svea was performed in 2002, just after the onset of more intense mining activity in the area (Figure 1). This mapping was based on investigation of aerial photographs and detailed vegetation analyses of 174 1x1 m plots, which later were assigned to ten different vegetation classes (Cooper and Nilsen, unpublished). Unfortunately, no abiotic factors were collected. However, as this "baseline" data existed, it was possible to revisit the investigated areas, and evaluate whether the mining had generated larger changes in vegetation composition. As part of the vegetation surveillance survey for SNSK, Eidesen (2010) concluded that there were no significant changes of vegetation types or species diversity in the area compared to the mapping performed in 2002, except in areas where there had been mechanical disturbance of the soil due to construction work etc. This physical disturbance were clearly a result of the mining activity; while the other smaller differences in vegetation types and species composition could not be directly linked to the mining activity (Eidesen, 2010).

Analysis of species composition of plant communities are usually performed in selected sample plot, where either species abundance, species cover in percentage or species frequencies within analysis frame or point frame are common and well established methods in vegetation ecology when investigating plant species distribution in vegetation types (Maarel, 2005). By collection of abiotic and/or biotic ecological variables, or also landscape structures, it is possible to describe the vegetation types and the species distribution over the sampled variables by statistical analysis. In this study canonical correspondence analysis (CCA) was chosen as the statistical method of analysis.

Vegetation analyses like this can however only detect rather larger scale changes. At more detailed scales, down on species to gene level, more fine-scale changes can be detected. Genetic analysis

have for the last five decades been used to investigate the patterns of genetic variation and adaption to the natural environment of the species (Lowe et al., 2004), and are now also utilized as a monitoring tool in management and conservation (Schwartz et al 2006). Several processes related to the mining activity might affect genetic diversity and genetic structure of species. For instance, processes like mechanical disturbance of the soil, such as the area used to dig out sand and gravel used in construction work in Svea, may lead to habitat fragmentation. Habitat fragmentation affects ecological processes like pollination and dispersal, which maintain genetic diversity. Maintaining genetic diversity is important, as it e.g. provides plant populations with the resources to adapt to changing environmental conditions, and prevent inbreeding depressions.

One way to investigate how mining activity in Svea might influence the genetic structure and diversity of species is through landscape genetics, which is the combination of landscape ecology, population genetics and spatial statistics (Holderegger et al., 2008). Landscape ecology investigates the relation between ecosystems, environment and the ecological variations, while population genetics investigate the changes in genetic composition due to neutral and adaptive selection. From stochastic events in a species history that leads to reproductive isolation or barriers, species can either diverge in sympatric, parapatric or allopatric manner. spatial, temporal

Neutral versus adaptive diversity

The genetic diversity within populations is defined as the genetic variation among individuals of populations compared to an expected mean level of heterozygosity. Genetic diversity can be further divided into neutral and adaptive genetic diversity. Neutral diversity arises from the neutral evolutionary forces as genetic drift, bottlenecking, mutations, migration or gene flow, and this genetic variation is not affected by selection and do not have consequences for individual fitness. Adaptive diversity arises directly from adaptive evolution due to natural selection (Bonin et al., 2007), but also linkage and null alleles are possible causes for non-concordant diversity (Lowe et al., 2004). DNA sequences and not just molecular markers e.g. AFLP-markers, are needed to distinguish between migration or separation as origin to diversity and divergence. Possible methods of investigating adaptive variation by AFLP-markers are mentioned below. Natural selection is the process where organisms better adapted to the environment, increase in frequency compared to organisms less adapted, but can only act on the present genetic diversity and do not give rise to new mutations. Coal dust as an ecological factor affecting the genetic material have only had a short time span (see Figure 1) to act as an ecological driver in Svea, and other factors acting on natural selection for a longer time span should be considered as important.

Amplified fragment length polymorphism (AFLP)

The genetic marker amplified fragmented length polymerism (AFLP) was presented in 1995 by Vos et al. (1995). The AFLP-method produces a genetic fingerprint by PCR amplification of selected restriction fragments. Evenly sized fragments are read as peaks, comparable to bands in an electrophoresis gel, and are denoted markers. AFLP-analysis produces a pattern of only present or absent fragments, representing mainly neutral, dominant markers. The pattern of present or absent of markers for each individual, also known as alleles, can be summarized in a binomial matrix of data. The method is fast, require minimal amounts of DNA from any organism, show low error levels, have a high resolution because of the nearly unlimited amounts of markers it can produce, and the markers segregate by mendelian fashion (Mueller et al., 1999). The method have been used for genome mapping, breeding studies, in ecologic genetics and phylogenetic and phylogeographic studies (Lowe et al., 2004).

As AFLPs are dominant markers, thorough population genetic calculations are not possible, but AFLPs provide a good description of genetic structure, levels of differentiation between genetic groups and measures of genetic diversity. Good estimation of genetic diversity using AFLPs is dependent on the resolution of the final dataset, and both a proper sample population size and a certain amount of scored alleles are preferred. Although AFLP mainly reflect neutral variation, it is possible to investigate loci under selection with AFLPs as well. By calculating marker frequencies in genetic clustered groups can point out cluster specific markers, or private alleles. Bonin et al. (2007) introduced a method to investigate the adaptive value of populations through the population adaptive index (PAI). The population adaptive index utilised the program DFDIST to investigate loci under selection by detecting loci with a higher F_{ST} than the expected average neutral genetic differetiation beetween populations under a neutral model of evolution. But (Pompanon et al., 2005) point out that selection signatures at given locus is particulary sensitiv to genotyping errors. Ford (2002) presents an approach to test for action of selection by comparing distribution of adaptive gene variation within the same individuals and populations using DNA sequencing data, and can not be applied in this study. Bonin et al. (2007) utilized 392 and 87 AFLP markers when detecting adaptive loci for the common frog and the Austrian dragon head, respectively. A large amount of markers are achieved by using several different primer combinations when using the AFLP-method for genetic analysis.

2 Aims

The history of mining activity in Svea stretches over almost a century, while intesive coal production has a short history of roughly a decade. The effects these pioneer human activities have had on the vegetation composition and species distribution have earlier just slightly been addressed, where genetic variation was not investigated. I wanted to investigate the effect of the ecological variables known to be important for species composition and assumed to be related to coal dust and human activities in the area of Svea. To address the effect of ecological variables and landscape barriers on species distribution and genetic diversity and structure, a combination of vegetation data and genetic analyses was performed. For genetic analyses, two species, *Luzula confusa* and *Luzula nivalis*, were chosen. A short generation time, implicating a rapid genetic turnover, is preferred as processes because founder effects or bottleneck events would be visible within a shorter timespan.

The frequency and ecological preferences of the species (and possible changes of these) could be assessed based on the vegetation analyses from 2002 and 2009, and genetic analysis could give a picture of present genetic variability within the Svea population of the study species *Luzula confusa* and *Luzula nivalis*. Reference populations for the genetic analysis was sampled in former coal mining areas (Bjørndalen) and clean areas (Engelskbukta, Danskøya, and Kapp Linné, all Svalbard, and Tromsø, Norway).

The main goal of the project is to evaluate whether coal dust or mining activities affect the landscape genetics of Luzula confusa and Luzula nivalis through changes in landscape ecology and/or genetic structure and diversity.

I will approach this goal by answering the following questions:

- Which factors shape the landscape ecology in Svea? Are these factores related to abiotic/ biotic and/or human impact factors
- What are the preferred habitats of Luzula confusa and Luzula nivalis? How do mining activity affect their preferred habitats? (husk at du også kan bruke kartet ditt til å se på naturlige barrierer dannet av uegnet habitat)
- Do genetic groups within Luzula sp. relate to abiotic/ biotic and/or human impact factors?
- Do genetic diversity within Luzula sp. relate to abiotic/ biotic and/or human impact factors

3 Methods

3.1 Study area

The main study area was the surroundings of the settlement Sveaguva (77°53′ N 16°43′ Ø), Svalbard. Reference samples for the genetic material were gathered from other locations in Svalbard (Bjørndalen, Engelskbukta, Virgohamna and Kapp Linné), as well as from Tromsø, mainland Norway (Figure 4).

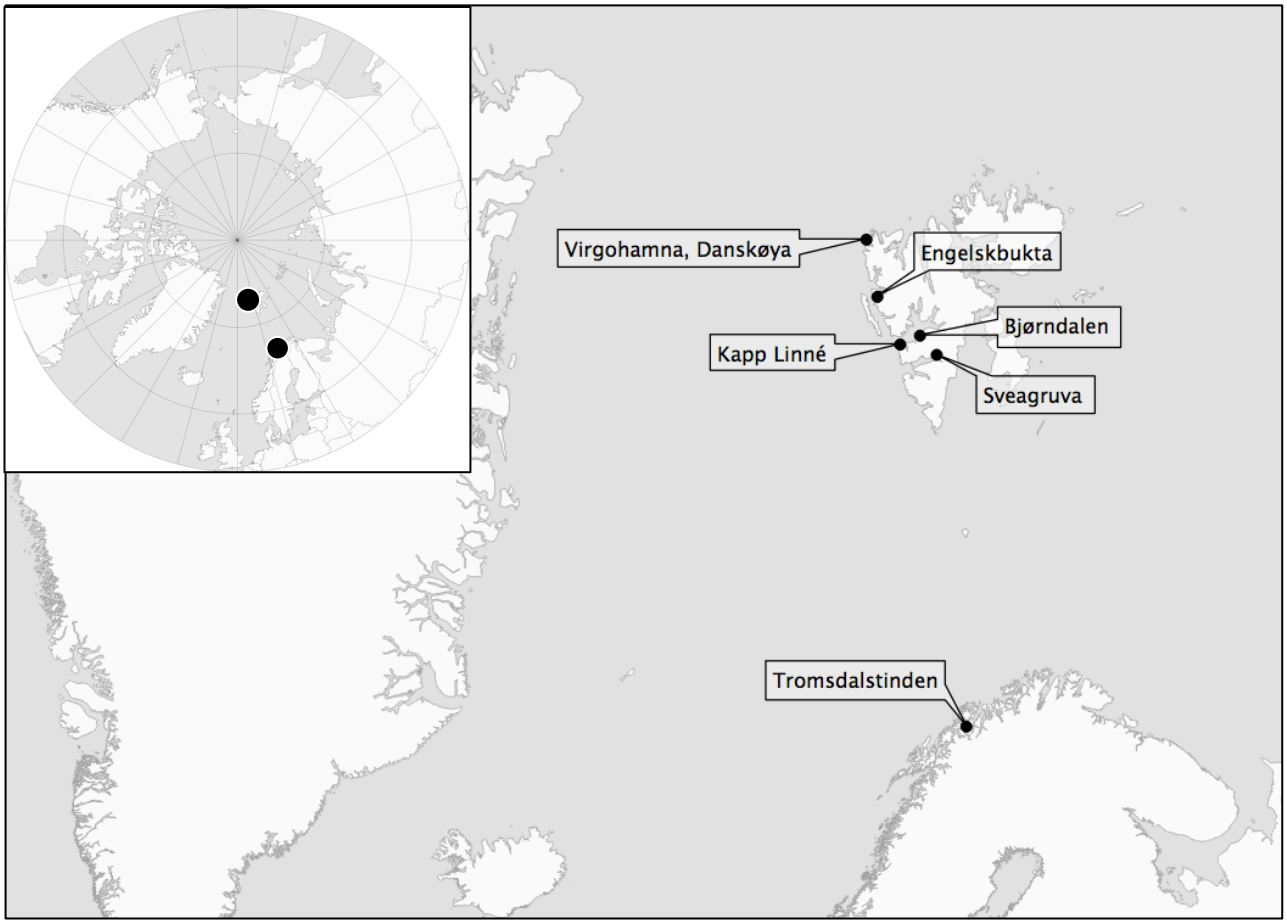

Figure 4 Overview of collection sites in this study. Map source: GSHHS (Wessel et al., 2013) and ("Arctic Ocean location map,"). Used under the licenses GNU Lesser General Public License and Creative Commons.

Svea is situated in the inner parts of the Van Mijen fiord in the Braganza bay. Here the climate is mid arctic in transition to low arctic because of the location in an inner fiord system. At altitudes 200 – 300 m above sea level the climate changes to high arctic. This area belongs to the weak continental vegetation section, and the mild oceanic conditions in the fiord in the western part of the archipelago results in somewhat higher precipitation than in the eastern part. Still, there is very little precipitation in Svea, off which the summer months is the driest period (Figure 5). The precipitation

usually comes as snow or drizzle, and events of fog, both contributing to lower the solar radiation and keep the temperatures down (Moen et al., 1999). The average temperature is 6 °C for July, which is well within the definition of Arctic areas using the 10 °C July-isotherm. This results in permafrost, which off only the top 30-150 cm thaws each year. The permafrost in Svea is circa 200 meters deep (Norwegian Polar Institute, 2000).

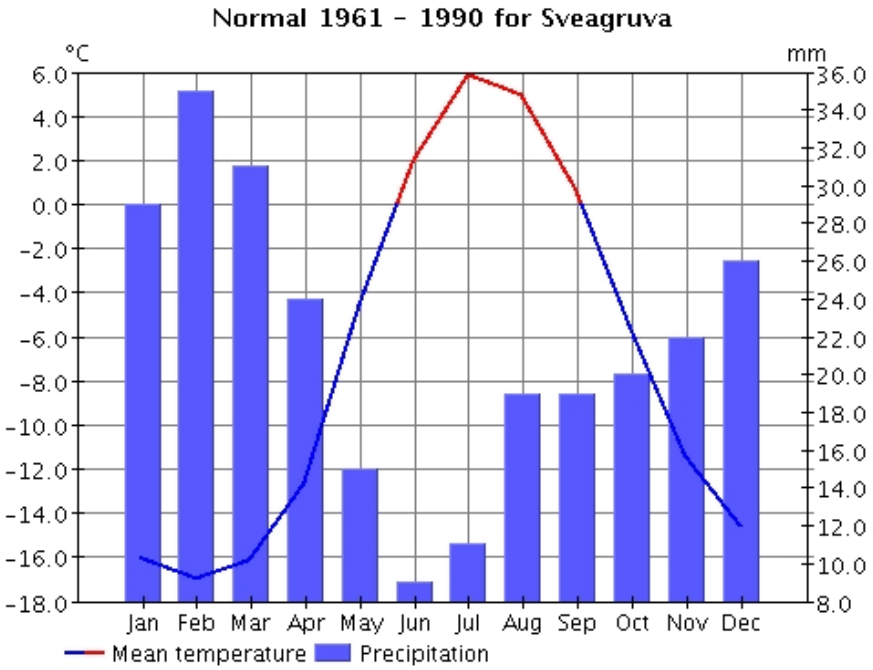

Figure 5 Climate normal from Svea weather station showing monthly temperature and precipitation. (NorwegianMeteorologicalInstitute, 2011)

3.2 Study species

The two species under study, *Luzula confusa* Lindeb. and *Luzula nivalis* (Laest.) Spreng belongs to the family *Juncuaceae*. As the flora describes them, they have quite similar ecology; both are found in wet and dry tundra vegetation, though *L .nivalis* is more abundant in more continental areas. Both have a circumpolar distribution, though *L. confusa* is more abundant than *L. nivalis*.

Luzula confusa (eng. Northern Wood-rush, no. vardefrytle) was formerly classified as a subspecies subspecies of *L. arcuata*, as these two species are closely related and difficult to tell apart. *Luzula. confusa* has a circumpolar arctic-alpine distribution, reaching the polar desert regions (Lid et al., 2005). It is widely distributed and common in Svalbard (Rønning et al., 1996). *Luzula confusa* often occurs in rather dry habitats, at ridges, moors, early snowbeds and tundra, and prefers acidic soil with low pH. It is a perennial species that normally sets seed every season. *Luzula confusa* is reported to be hexaploid (36=2n) (Brochmann et al., 1999).

The *Luzula nivalis* (eng. Arctic Wood-rush, no.: snøfrytle) was formerly known as *Luzula arctica* Blytt. *Luzula nivalis* has a more scattered arctic-alpine circumpolar distribution than *L. confusa*, but is also reaching the polar desert. It is bi-centric in Scandinavia (Lid et al., 2005), but widely distributed and common in Svalbard (Rønning et al., 1996). *Luzula nivalis* is a rather continental species compared to *L. confusa*. *Luzula nivalis* occurs only on alkali soil (Lid et al., 2005), and soil with high levels of electrolytes and circumneutral pH, avoiding pure marble lime (Engelskjøn, 1984). The literature is contradictory when describing moisture preferences, as Rønning et al. (1996) claim that the species is found in dry habitats, while Gjærevoll et al. (1990) describes it as hygrophilous and found where solifluction takes place, and that it prefers well developed moss carpets. According to Lid & Lid (2005), it is found in moist sward, early snow bed, and dry as well as moist tundra. *Luzula nivalis* is a perennial species that usually sets seed every season. A chromosome number of 24=2n makes it tetraploid (Brochmann et al., 1999).

3.3 Vegetation analysis and collection of ecological variables

Study design and vegetation analysis

The vegetation analysis was based on an analysis done by E. Cooper and L. Nilsen from the Norwegian Polar Institute (NP) in 2002. The area around Svea had been divided into 11 subareas named A to K (Figure 6). 176 plots were revisited in 2009 based on the coordinates taken in 2002, and given new and more accurate coordinates. For 60 of the revisited plots, a soil sample was taken, and air and soil temperature was measured. The revisited plots were evaluated for changes in vegetation type according to the vegetation type classification used in 2002. When changes were observed, a new vegetation analysis was done. Vegetation analysis was performed in a 1 x 1 meter frame marked by strings, and at least two persons opinion of percentage cover of each species was registered. For 16 of the revisited plots, a new vegetation analysis was performed. A vegetation analysis was also done where the paired temperature and moisture sensors were placed for each of the seven HOBO-loggers, in total 14 vegetation analyses. In addition a vegetation cover analysis was done for 17 new plots. Vegetation analyses were performed for a total of 47 plots during the summer 2009.

Two assumptions was therefore made **a)** the general vegetation types have not changed since 2002, and **b)** that the analysed plots were representative for the vegetation type and vegetation cover in the examined area.

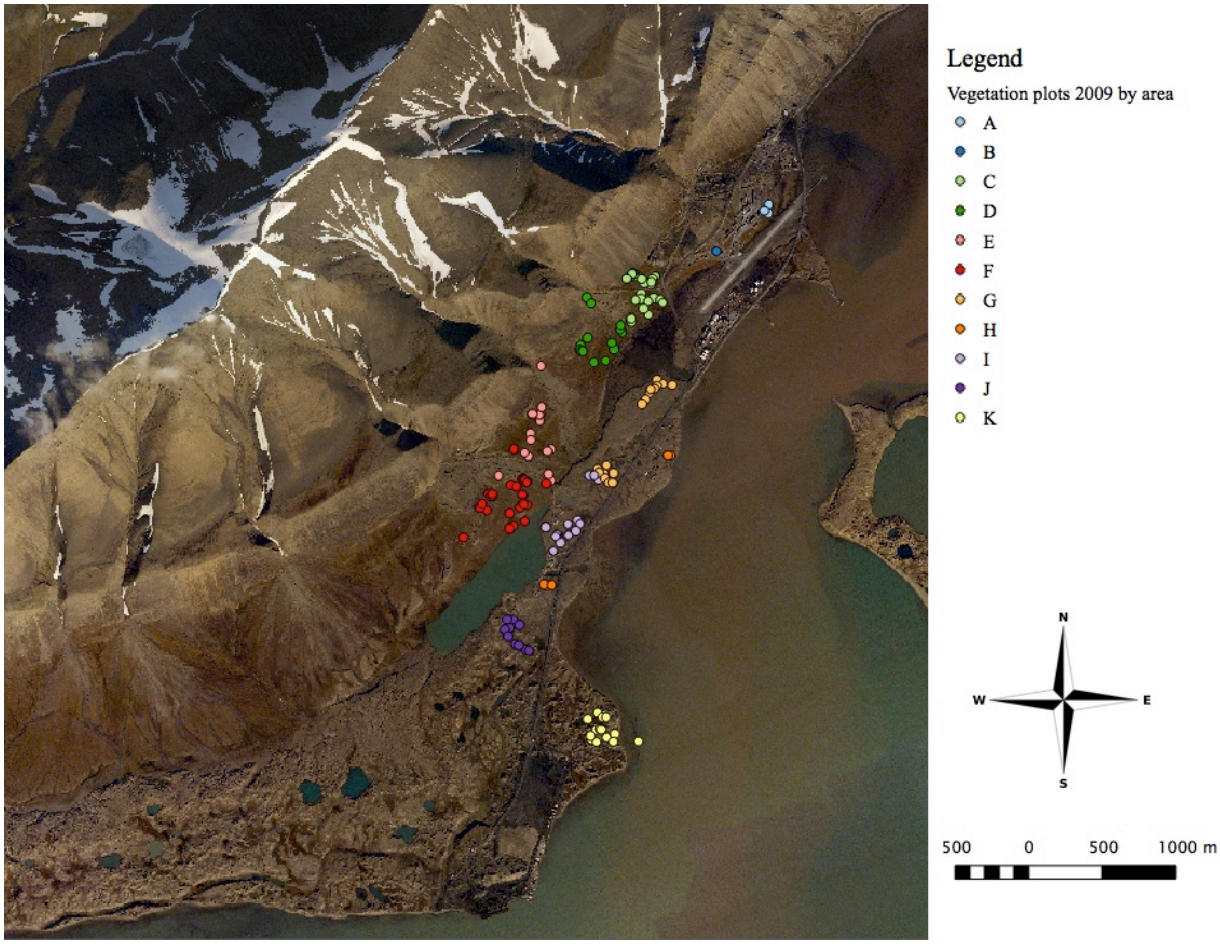

Figure 6 Vegetation plots from 2002 revisited in 2009 colour coded after subarea in Svea. Map source: SNSK.

HOBO®-logger sampling design

HOBO-loggers were placed in each of the 7 main sampling areas in Svea. The subareas for genetic sampling were established close to the HOBO. Each logger had 4 external channels, and was equipped with two temperature sensors and two moisture sensors. The loggers were programmed to start logging measurement data the 29.06.2009 at 9 pm, and the sensors were tested for proper function and logging before they were placed in the field. Not all loggers had been placed in the field at the start of logging. The sensory probes were placed pairwise (one temperature and one moisture measuring probe) and preferably in two different vegetation types in the vicinity of the logger. The pairwise probes were placed opposite to each other. The cords were dug approximately 10 cm down in the ground to shelter them from external damage (e.g. curious foxes and reindeers) and from having the sensors pulled out from the ground. The digging was done by cutting a slit in the ground using a knife, and pushing the cords into the slit. Small stones were used to cover the

sensors and the logger. It was assumed that the covering of the sensors would not have any effect on the local environment the sensor was measuring.

The loggers were revisited the 03.09.2009 and 09.09.2009. In three cases the HOBO-loggers had been damaged in one way or another (either by chewing from the foxes or pulled up electrical cords), and these HOBO-loggers were dug up and transported back to UNIS for repair and download of data. The data from the four loggers remaining in field were downloaded to the field computer, and the loggers were left to collect data through the winter.

Soil moisture, temperature measurements and coal dust estimations in field

Soil water content was measured in situ using the feel and appearance method as described in Raup (1969). The is four different categories where 1) soil is completely dry, 2) skin is moisturised when squeezing soil, 3) soil is giving off moisture when squeezed, and 4) soil is dripping wet. Soil moisture value was only noted where vegetation analysis was conducted

Temperature was measured using a thermometer with t °C ± 0,1 accuracy. Soil temperature was measured by sticking the measuring stick 5 cm into the soil (a pen mark was made 5 cm up from the tip of the stick to standardize measuring depth). By turning the thermometer up side down air temperature was measured at approximately 40 cm above ground. The Norwegian Institute of Meteorology does regular 2 m above ground air temperature measurements at Svea meteorological station.

No method for quantitative coal dust measurement had been evaluated as fitting for the fieldwork period. Instead a 4-step scale of coal dust cover estimates was used. Degree of coal dust at the sampling area was given a value from 1 to 4, where 1 represents no coal dust found on the vegetation in the area, 2 some coal dust particles were found, 3 coal dust was visible when viewing vegetation sitting or close to the ground, especially seen in the leaf corners of mosses, and coal dust was visible when hand was swept over vegetation. Value 4 was given when it was a visible coal dust cover at the ground (visible when standing), covering leaf surface and sticking to plant hairs. In field the dust covered vegetation was observed as green and thus believed to still be able to grow and survive. Coal dust estimates were set for whole sampling areas at a time, assuming that local observed differences in coal dust cover was a result of stochastic processes as wind distribution.

Soil sample collection and analysis

The top 5 cm of the soil sample was kept and stored in marked zip lock-bags. Parts from the vegetation cover were removed. The samples were frozen to prevent degradation or moulding.

The soil was placed in marked cups of folded aluminium foil and dried in an oven at 55°C for 48 hours. The samples were then grinded in a clean ceramic mortar, sieved in a 1mm meshed steel sieve and put on dram glass vials. The vials were marked with the soil sample ID-number and area.

To measure pH and conductivity a subsample of 3.0 ± 0.05 g soil was transferred to plastic scintillation vials, and dissolved in Milli-Q H_2O (1:5 mixture ratio) by shaking at 200 rpm for 60 min on a shaking board. The samples were let standing for >15 min to settle. Electroconductivity (EC) was measured first using portable pH- and EC meters from Mettler Toledo, and thereafter pH was measured. By measuring EC first we could bypass a possible error source of KCl from the pH sensor leaking into the sample and disturbing the conductivity. The measurements were done at 22 ± 0.7 °C and the same level above the vial bottom for all the vials. The sensor was stirred gently in the solution to make sure the contact was good and the readings stable. The sensor was rinsed with Milli-Q water and dried with a paper between measuring each sample.

An analysis of sample content of carbon (C; both organic and elemental) and nitrogen (N; reflects available soil nutritients) was done using a gas chromatograph. Because of the cost of analysing for soil N and C content, only selected samples from each area were analysed. A subsample of 5-10 mg soil was packed in tin capsules. The percentage content of elemental and organic carbon and nitrogen were analysed by thermal decomposition using a gas chromatograph (EA 1110 CHNS-O elemental analyser from CE (Carlo Ebra) instruments).

Table 1 Soil samples from the sampling spot of genetic samples used in this study. Counts shown are the total number of soil samples from the respective populations. One sample was lost in the lab during drying. pH and electro conductivity was measured for most of the samples as described above. Carbon(C) and nitrogen (N) content in soil was analysed for n sample from each population

			WGS 84 UTM 33X coordinates						Soil samples, *n* in total		
Location	**Population name**	**PopID**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**	**Comment**	**Collected**	**pH and electro-conductivity**	**CHN-content**
Svea, Svalbard	C, hill	1	0539675	8647340	3.7.09	EC, EM, GF, BSS, PBE	3		1	1	1
Svea, Svalbard		2	0539696	8647374	9.7.09	EM, GF, BSS	3	Snowbed to moss tundra	5	5	1
Svea, Svalbard		3	0539597	8647234	9.7.09	EM, GF, BSS	3	Salix heath to moss tundra	5	5	1
Svea, Svalbard		4	0539569	8647160	9.7.09	EM, GF, BSS	3	Grass dominated moss tundra	7	7	3
Svea, Svalbard	X, clean hill	5	0536368	8644946	5.7.09	EC, EM, GF, BSS	1	Moss tundra	1	1	1
Svea, Svalbard		6	0536302	8644932	5.7.09	EC, EM, GF, BSS	1	Moss tundra	1	1	-
Svea, Svalbard		7	0536277	8644880	5.7.09	EC, EM, GF, BSS	1	Moss tundra	1	1	-
Svea, Svalbard		8	0536418	8644832	5.7.09	EC, EM, GF, BSS	1	Moss tundra	1	1	1
Svea, Svalbard	E, hill	9	0538897	8646168	7.7.09	EM, GF, BSS	2	Moss tundra	1	1	1
Svea, Svalbard		10	0538902	8646202	7.7.09	EM, GF, BSS	2	Moss tundra	-	-	-

Table 1 Soil samples from the sampling spot of genetic samples used in this study. Counts shown are the total number of soil samples from the respective populations. One sample was lost in the lab

Table 1 (Cont.)

			coordinates								
Location	**Population name**	**PopID**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**	**Comment**	**Collected**	**pH and electro-conductivity**	**CHN-content**
Svea, Svalbard	A, by old mine entrance	13	0540796	8648628	8.7.09	EM, GF, BSS	4	Moderate snowbed?	6	6	1
Svea, Svalbard		14	0540810	8648646	8.7.09	EM, GF, BSS	4	Boulder field	7	7	2
Svea, Svalbard		15	0540747	8648666	8.7.09	EM, GF, BSS	4	Boulder field with transition to wet moss tundra	8	8	2
Svea, Svalbard		16	0540708	8648616	8.7.09	EM, GF, BSS	4	Inbetween boulderfields	7	7	1
Svea, Svalbard	K, moraine	17	0539213	8644190	8.7.09	EM, BSS, GF	4		6	6	1
Svea, Svalbard		18	0539243	8644192	8.7.09	EM, BSS, GF	4	Darker background	5	5	1
Svea, Svalbard		19	0539270	8644234	8.7.09	EM, BSS, GF	4	Darker background	5	5	-
Svea, Svalbard		20	0539294	8644222	8.7.09	EM, BSS, GF	4		5	5	1
Svea, Svalbard	J, moraine	21	0538494	8644630	16.7.09	EM, BSS	2	Darker background	5	5	1
Svea, Svalbard		22	0538543	8644606	16.7.09	EM, BSS	2	LN big and more abundant than LC in part of the area	6	6	-
Svea, Svalbard		23	0538592	8644602	16.7.09	EM, BSS	2		8	8	2
Svea, Svalbard		24	0538615	8644660	16.7.09	EM, BSS	2		6	6	1
Svea, Svalbard	X, moraine	25	0536267	8643617	20.7.09	EM, BSS, PBE	1		6	6	2

Table 1 Soil samples from the sampling spot of genetic samples used in this study. Counts shown are the total number of soil samples from the respective populations. One sample was lost in the lab

Table 1 (Cont.)

			coordinates								
Location	**Population name**	**PopID**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**	**Comment**	**Collected**	**pH and electro-conductivity**	**CHN-content**
Svea, Svalbard		26	0536257	8643637	20.7.09	EM, BSS, PBE	1		6	6	2
Svea, Svalbard		27	0536247	8643637	20.7.09	EM, BSS, PBE	1		5	5	1
Svea, Svalbard		28	0536237	8643637	20.7.09	EM, BSS, PBE	1		6	6	2
Bjørndalen, Svalbard	Bjørndalen	B1	0507399	8683074	11.7.09	BSS	Reference	Salix/Dryas-heath	5	5	-
Bjørndalen, Svalbard	Bjørndalen	B2	0507410	8683048	11.7.09	BSS	Reference	Moss tundra	5	4	-
Bjørndalen, Svalbard	Bjørndalen	B3	0507417	8683032	11.7.09	BSS	Reference	Wet moss	5	5	-
Bjørndalen, Svalbard	Bjørndalen	B4	0507427	8683016	11.7.09	BSS	Reference	Salix/Dryas-heath	5	5	-
Engelskbukta, Svalbard	Engelskbukta	100	0431802	8755151	10.7.09	PBE	Reference		2	2	-

3.4 Genetic analysis

Study design and sampling of genetic material

In total 360 leaf samples were collected from the six geographical regions (Figure 4). One sample consisted of ideally 3-5 fresh green leaves from the same individual, stored in marked jars or plastic bags prefilled with silica gel. The silica gel is an effective desiccator leaving only chemically bound water. By this silica gel preserves the leaf sample and minimizes the water-dependent DNA degradation. The samples were sampled at least 10 m apart to prevent sampling of closely related individuals (e.g. mother and offspring) and sampling of vegetative shots. A voucher was collected for the first individual sampled of each species in a sampling area, sometimes other individuals in the subarea as well. The importance of voucher collection is explained in Bates (2002). Vouchers marked with species name, sampling date, coordinates, project name and collector name were sent to the Botanical collections at Natural History Museum in Oslo for storage and enabling re-examination. Soil samples were collected from 4 of the 6 different sampling sites (Table 1).

At the main sampling site Svea, seven subareas were selected based on assumed coal dust coverage. Within each main area, four transects/subareas consisting of 5 sampling spots on a straight line were established. The exception was for transects 17, 19 and 20, which was sampled in a cross-like manner, with sample 2 in the centre. An aim of 20 samples from each main area was set to gain statistical power. A soil sample was taken in each of the subareas, soil and air temperature was measured and coordinates noted.

From Engelskbukta, Kapp Linné and Danskøya (only *L. confusa* present), 10 individuals of each at least 10 m apart. In Bjørndalen the sampling was done in 4 transects with 5 sampling spots, similar as was done in Svea. In Tromsø only seven individuals of *Luzula confusa* were sampled, and a voucher was taken for each individual to ascertain correct identification of the species. Based on morphology, the 10 individuals of *Luzula confusa* sampled at Danskøya (33X N8853321 E0419335) were regarded possible hybrids between *Luzula arcuata* and *L. confusa*.

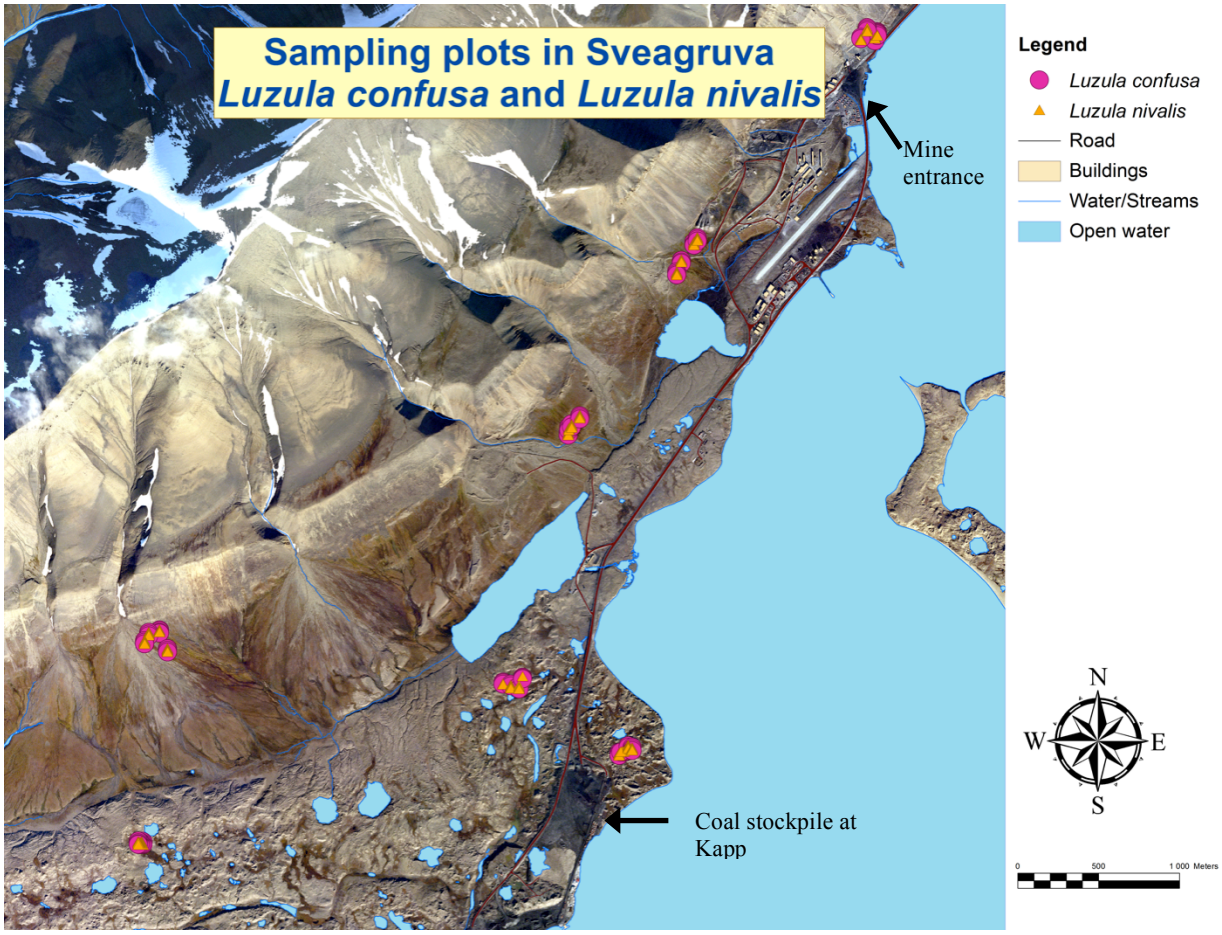

Figure 7 Sampling sites in Svea. The majority of sampled Luzula confusa and L. nivalis were sampled <1m apart, and thus have the same coordinates. Map source: SNSK

Table 2 Sampled Luzula material in this study. All samples are collected at Svalbard, except from the 7 reference samples from mainland Norway. N in analysis is the number of individuals with a successful AFLP analysis. Collectors: EC – Elisabeth J. Cooper, EM – Elke Morgner, GF – Gunn Frilund, PBE –Pernille B. Eidesen, BSS – Bente S. Skottvoll.

						WGS84 UTM 33X coordinates				
Location	**Population name**	**PopID**	**Species**	***n* collected**	***n* in analysis**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**
Svea, Svalbard	C, hill	1	*L. confusa*	5	4	0539675	8647340	3.7.09	EC, EM, GF, BSS, PBE	3
Svea, Svalbard	C, hill	2	*L. confusa*	5	5	0539696	8647374	9.7.09	EM, GF, BSS	3
Svea, Svalbard	C, hill	3	*L. confusa*	5	5	0539597	8647234	9.7.09	EM, GF, BSS	3
Svea, Svalbard	C, hill	4	*L. confusa*	5	5	0539569	8647160	9.7.09	EM, GF, BSS	3
Svea, Svalbard	X, clean hill	5	*L. confusa*	5	5	0536368	8644946	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	6	*L. confusa*	5	5	0536302	8644932	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	7	*L. confusa*	5	5	0536277	8644880	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	8	*L. confusa*	5	5	0536418	8644832	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	E, hill	9	*L. confusa*	5	5	0538897	8646168	7.7.09	EM, GF, BSS	2
Svea, Svalbard	E, hill	10	*L. confusa*	5	4	0538902	8646202	7.7.09	EM, GF, BSS	2
Svea, Svalbard	E, hill	11	*L. confusa*	5	2	0538918	8646218	7.7.09	EM, GF, BSS	2

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						WGS84 UTM 33X coordinates				
Location	**Population name**	**PopID**	**Species**	***n* collected**	***n* in analysis**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**
	entrance									
Svea, Svalbard	A, by old mine entrance	14	*L. confusa*	5	5	0540810	8648646	8.7.09	EM, GF, BSS	4
Svea, Svalbard	A, by old mine entrance	15	*L. confusa*	5	4	0540747	8648666	8.7.09	EM, GF, BSS	4
Svea, Svalbard	A, by old mine entrance	16	*L. confusa*	5	3	0540708	8648616	8.7.09	EM, GF, BSS	4
Svea, Svalbard	K, moraine	17	*L. confusa*	5	4	0539213	8644190	8.7.09	EM, BSS, GF	4
Svea, Svalbard	K, moraine	18	*L. confusa*	5	4	0539243	8644192	8.7.09	EM, BSS, GF	4
Svea, Svalbard	K, moraine	19	*L. confusa*	5	2	0539270	8644234	8.7.09	EM, BSS, GF	4
Svea, Svalbard	K, moraine	20	*L. confusa*	5	4	0539294	8644222	8.7.09	EM, BSS, GF	4
Svea, Svalbard	J, moraine	21	*L. confusa*	5	4	0538494	8644630	16.7.09	EM, BSS	2
Svea, Svalbard	J, moraine	22	*L. confusa*	5	3	0538543	8644606	16.7.09	EM, BSS	2
Svea, Svalbard	J, moraine	23	*L. confusa*	5	2	0538592	8644602	16.7.09	EM, BSS	2
Svea, Svalbard	J, moraine	24	*L. confusa*	5	3	0538615	8644660	16.7.09	EM, BSS	2
Svea, Svalbard	X, moraine	25	*L. confusa*	5	2	0536267	8643617	20.7.09	EM, BSS, PBE	1
Svea, Svalbard	X, moraine	26	*L. confusa*	5	4	0536257	8643637	20.7.09	EM, BSS, PBE	1

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Location	**Population name**	**PopID**	**Species**	***n* collected**	***n* in analysis**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**
Svea, Svalbard	X, moraine	27	*L. confusa*	5	5	0536247	8643637	20.7.09	EM, BSS, PBE	1
Svea, Svalbard	X, moraine	28	*L. confusa*	5	4	0536237	8643637	20.7.09	EM, BSS, PBE	1
Bjørndalen, Svalbard	Bjørndalen	B1	*L. confusa*	5	4	0507399	8683074	11.7.09	BSS	Reference
Bjørndalen, Svalbard	Bjørndalen	B2	*L. confusa*	5	3	0507410	8683048	11.7.09	BSS	Reference
Bjørndalen, Svalbard	Bjørndalen	B3	*L. confusa*	5	4	0507417	8683032	11.7.09	BSS	Reference
Bjørndalen, Svalbard	Bjørndalen	B4	*L. confusa*	3	3	0507427	8683016	11.7.09	BSS	Reference
Engelskbukta, Svalbard	Engelskbukta	100	*L. confusa*	10	9	0431802	8755151	10.7.09	PBE	Reference
Kapp Linné, Svalbard	Kapp Linné	301	*L. confusa*	10	10	0470178	8666792	10.8.09	PBE	Reference
Virgohamna, Danskøya, Svalbard	Danskøya	*L.confusa* x *arcuata*	*L. confusa*	10	8	0419335	8853321	21.7.09	Eike Müller	Reference
Tromsdalstinden, Tromsø, Norway	Tromsø	T (Tromsø)	*L. confusa*	7	7	34W 693615	190835	23.8.09	BSS	Reference

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Location	**Population name**	**PopID**	**Species**	***n* collected**	***n* in analysis**	**Easting**	**Northing**	**Date**	**Collector(s)**	**Coal dust level**
Svea, Svalbard	C, hill	1	*L. nivalis*	5	5	0539675	8647340	3.7.09	EC, EM, GF, BSS, PBE	3
Svea, Svalbard	C, hill	2	*L. nivalis*	5	5	0539696	8647374	9.7.09	EM, GF, BSS	3
Svea, Svalbard	C, hill	3	*L. nivalis*	5	4	0539597	8647234	9.7.09	EM, GF, BSS	3
Svea, Svalbard	C, hill	4	*L. nivalis*	5	5	0539569	8647160	9.7.09	EM, GF, BSS	3
Svea, Svalbard	X, clean hill	5	*L. nivalis*	4	4	0536368	8644946	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	6	*L. nivalis*	5	5	0536302	8644932	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	7	*L. nivalis*	5	5	0536277	8644880	5.7.09	EC, EM, GF, BSS	1
Svea, Svalbard	X, clean hill	8	*L. nivalis*	5	5	0536418	8644832	5.7.09	EC, EM, GF, BSS	1
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Svea, Svalbard	E, hill	11	*L. nivalis*	5	5	0538918	8646218	7.7.09	EM, GF, BSS	2
Svea, Svalbard	E, hill	12	*L. nivalis*	5	5	0538969	8646274	7.7.09	EM, GF, BSS	2
Svea, Svalbard	A, by old mine	13	*L. nivalis*	2	2	0540796	8648628	8.7.09	EM, GF, BSS	4

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Svea, Svalbard	J, moraine	23	*L. nivalis*	5	4	0538592	8644602	16.7.09	EM, BSS	2
Svea, Svalbard	J, moraine	24	*L. nivalis*	5	1	0538615	8644660	16.7.09	EM, BSS	2
Svea, Svalbard	X, moraine	25	*L. nivalis*	5	1	0536267	8643617	20.7.09	EM, BSS, PBE	1
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Bjørndalen, Svalbard	Bjørndalen	B2	*L. nivalis*	5	2	0507410	8683048	11.7.09	BSS	Reference
Bjørndalen, Svalbard	Bjørndalen	B3	*L. nivalis*	5	5	0507417	8683032	11.7.09	BSS	Reference
Bjørndalen, Svalbard	Bjørndalen	B4	*L. nivalis*	4	3	0507427	8683016	11.7.09	BSS	Reference
Engelskbukta, Svalbard	Engelskbukta	100	*L. nivalis*	10	7	0431802	8755151	10.7.09	PBE	Reference
Kapp Linné, Svalbard	Kapp Linné	301	*L. nivalis*	10	9	0470178	8666792	10.8.09	PBE	Reference

Primer test

A primer test was done to identify possible primers to use in the AFLP analysis. No scientific paper using members of the *Luzula* genus for AFLP fingerprinting had been published before the primer test was carried out in September 2009. Schönswetter (Peter Schönswetter et al., 2007) described selected primer combinations for AFLP-fingerprinting fitted to some members from the genus *Juncus*, which belongs to the same family as *Luzula*. The primer combinations found by Schönswetter were tested as possible primer combinations to use for the AFLP-analysis of *Luzula*. Samples from eight individuals (Table 4) were used in the test: four individuals from each species, all from Svea sampling area, and geographically separated by different coal dust cover classifications. The eight individuals were fingerprinted using the AFLP-method as described below, using 72 different primer combinations (Table 3).

Table 3 The different primer combinations tested for the two Luzula species marked by (-). Yellow squares marks the primer combinations used in the paper (Peter Schönswetter et al., 2007), while the squares marked in green are the primer combinations used in this project. The EcoRI primer ACA was tested using the green fluorescent dye named VIC, while in the project the primer was marked using the blue dye 6FAM.

		EcoRI											
		6Fam					Vic				Ned		
		ACT	AAG	ATG	AGT	AGA	ACG	ACA	AGG	ATC	AGC	AAC	ACC
MseI	CAA	-	-		-		-	-	-	-	-	-	-
	CAC		-								-	-	-
	CAG	-	-		-		-	-	-	-	-	-	-
	CAT	-	-		-		-	-	-	-	-	-	-
	CTA		-								-	-	-
	CTG	-	-		-		-	-	-	-	-	-	-
	CT	-	-	-	-	-	-	-	-	-	-	-	-
	CA	-	-	-	-	-	-	-	-	-	-	-	-

6 primer combinations from the primer test (green markings in Table 3) were used in the further genetic analysis. The resulting electropherograms from the primer test were visually examined in the program GeneMapper® Software ver. 3.7 (Applied Biosystems Inc.), and selected with regard to the amount of distortion, visible peaks, variability of the loci/bands, how easy the peaks were to tell apart and applicability to both species. For practical reasons with regard to the final capillary electrophoresis step where genetic samples from the same individual but different dye markers can be analysed in one run, the dye markers should be represented at two EcoRI-primers each. Thus the EcoRI primer ACA was purchased with the blue fluorescent dye 6FAM instead of the green fluorescent dye VIC.

Table 4 Individuals used in the primer test showing the distribution of chosen samples in geography and coal dust level.

Well	SampleID	Population name	PopID	Species	Coal dust level
1	LC-5-4	X, clean hill	5	*L.confusa*	1
2	LC-13-1	A, by old mine entrance	13	*L.confusa*	4
3	LC-17-1	K, moraine	17	*L.confusa*	4
4	LC-25-2	X, moraine	25	*L.confusa*	1
5	LN-8-2	X, clean hill	8	*L.nivalis*	1
6	LN-15-3	A, by old mine entrance	15	*L.nivalis*	4
7	LN-19-5	K, moraine	19	*L.nivalis*	4
8	LN-26-1	X, moraine	26	*L.nivalis*	1

DNA-isolation by fast CTAB

The 360 samples as listed in table 2 was used in isolation of DNA using the fast CTAB-method described in J. J. Doyle et al. (1987); J.J. Doyle et al. (1987) with modifications as described in P. Schönswetter et al. (2002) and in the text below. 30 – 70 mg of dried leaf material was grinded before adding any liquids by using two 3 mm Tungsten Carbide beads (Qiagen) in each 2.0 mL Eppendorf SafeLock tubes and placing the samples in a mixer mill (MM301, Retsch GmbH, Haan, Germany) for grinding at 22 Hz for 2x2 min, swapping the tube racks between the runs to minimize unequal grinding due to differences in placing. One tube was kept empty to use as a blank sample during the extraction procedure.

After adding CTAB-extraction-buffer (500:1 2% CTAB x mercaptoethanol), the samples were placed in a -80°C freezer for 15 min, before incubation at 65 °C for 30 min. DNA was extracted by adding 24:1 chloroform x isoamylalchohol, inverting and keeping the tubes at room temperature for 10 min. Centrifugation was done at 13 000 rpm for 4 min to separate the phases. After transferred the aqueous phase to clean tubes containing cold isopropanol, the tubes were inverted and stored at 4°C for at least 30 min, succeeded by centrifuging at 13 000 rpm for 10 min. At this step the DNA was precipitated and deposited in the tube. The DNA was washed using 70 % ethanol (EtOH), inverting the tubes and centrifuging at 13 000 rpm for 2 min, before heating at 60 °C for 15-30 min to evaporate the EtOH. The DNA-pellet was resuspended in 1xTE-buffer and put on a 60°C heating block for 30 sec. The samples were cooled and RNAase A was added before storing in the fridge overnight.

To test for DNA presence in the samples, as well as checking for successful DNA-restriction after each of the three main steps in the AFLP procedure, a gel-electrophoresis was run. Only a subset of the samples from the AFLP-procedure was run each time. A 0.7 % agarose gel with 1xTAE-buffer (Tris-acetate-EDTA), and GelRed™ as the fluorescent nucleic acid gel stain, was used. The ladder used was either MassRuler Express DNA ladder mix or LambdaMarker when the purpose was to check for DNA-extraction results, while FastRuler Low Range was used to check the PCR-runs.

The smaller gel was run on 90 V in 30 – 45 min, while the bigger gel was run at 160 V for 40 min.

Amplified Fragment Length Polymorphism (AFLP)

The AFLP-analysis (Vos et al., 1995) was carried out as described in Gaudeul et al. (2000) with the following modifications:

Adaptors were prepared for 100 samples by adding 50 µl each of MseI Ad 1 and MseI Ad 2 to a 0.2 ml PCR-tube. 50 µl each of EcoRI Ad 1 and EcoRI Ad 2 was added to another 0.2 ml PCR-tube. The tubes were heated in a PCR machine (GeneAmp® PCR system 9700, Applied Biosystems, Foster City, CA) for denaturation at 95°C for 5 min, and then let to cool slowly at room temperature for the annealing process. The master mix (MM) for the *digestion ligation process* was prepared by adding the ingredients (Table 5) to a 2 ml Eppendorf tube.

Table 5 Chemicals and enzymes used in the master mix (MM) for the digestion-ligation process.

Amount	Substance	Concentration	Final consentration	Function
20 µl	Milli-Q H2O			
110 µl	T4-buffer	10 X	1 X	Stabilize enzyme activity
110 µl	NaCl-solution	0.5 M	0.05 mM	Stabilize DNA
55 µl	BSA (Bovine Serum Albumide)	1mg/ml	0.05 mg/ml	Stabilize enzymes
100 µl	AdapterMseI	10 µM	0.90 µM	Adapter
100 µl	AdapterEcoRI	10 µM	0.90 µM	Adapter
10 µl	MseI	10 U/ µl	1 U/tube	Restriction enzyme
25 µl	EcoRI	20 U/ µl	5 U/tube	Restriction enzyme
20 µl	T4-ligase	5 U/ µl	1U/tube	DNA-ligase

A plate consists of 12 x 8 0,2ml PCR-tube strips (Axygen Inc) marked with sample names, null sample and references at every step in the protocol. 90 individuals were utilized at each plate. After adding master-mix and DNA-template to each tube, the plate was incubated in a PCR-machine (Applied Biosystems ^{TM}), and diluted 10x with Milli-Q H_2O. The samples were stored in a -18 °C freezer.

The master mix (MM) for the *pre-selective PCR* was prepared on ice by mixing the ingredients (Table 6) in a 2 ml Eppendorf tube. The MM was briefly vortexed and centrifuged upon adding of the Taq-enzyme.

Table 6 Chemicals and enzymes used in the master mix (MM) for the pre-selective PCR-step.

Amount	Substance	Concentration	Final concentration
740 µl	Milli-Q H_2O		
125 µl	PCR I buffer(red)	10 X	1.1 X
75 µl	$MgCl_2$	25 mM	1.7 mM
100 µl	dNTP	2 mM or 10 mM	0.2 or 0.9 mM
25 µl	EcoRI primer (pre-selective)	10 µM	0.23 µM
25 µl	MseI primer (pre-selective)	10 µM	0.23 µM
10 µl	Taq DNA polymerase (Red)	5 U/µl	0.5 U/tube

11 µl of the MM and 1.5 µl DigLig-product was added to the respectively marked strips while kept cold. The strips were briefly centrifuged on a mini centrifuge upon running the pre-selective strips in a PCR machine on a pre-selective program (30 cycles of 72°C for 2 min, 94°C for 30 sec, 56°C for 30 sec, 72°C for 2 min, and after the cycles 72 °C for 10 min, then kept cold at 4°C). The pre-selective samples were diluted 20 times by adding 150 µl Milli-Q H_2O.

The master mix (MM) for the *selective PCR* was prepared by adding the ingredients (Table 7) to a 2 ml Eppendorf tube. The primer combinations (Table 8) with the fluorescent marked 3-base EcoRI selective primer and the unmarked 3-base MseI selective, primer were used.

Table 7 Chemicals used in the master mix (MM) for the selective PCR.

Amount	Substance	Concentration
125 µl	GeneAmp® 10X PCR Gold Buffer	10X
125 µl	$MgCl_2$	25 mM
600 µl	Milli-Q H_2O	
100 µl	dNTP	2 mM or 10 mM
5 µl	EcoRI XXX (selective primer)	10 µM
10 µl	MseI XXX (selective primer)	10 µM
10 µl	BSA	1 mg/ ml
10 µl	AmpliTaq Gold® DNA Polymerase	5 U/ µl

10µl of the MM and 2.5 µl preselective PCR-product (DNA-template) was added to the correspondingly marked PCR-tube. The strips were run in the PCR-machine on the selective PCR-program (initiating heating at 95°C for 10 min to activate the Taq-enzyme, then 13 cycles with 94°C for 13 sec, 65°C lowered to 55.9°C for 1 min and 72°C for 2 min, then another 26 cycles of 94°C for 30 sec, 56°C for 1 min and 72°C for 1 min. After the cycles another 72°C for 10 min is held, before the samples are cooled to 4°C). The samples were stored in the freezer.

Table 8 Selective primers and their fluorescent dye colour used in the different master mixes (MM) for selective PCR runs.

Colour	EcoRI primer	MseI primer	Plate
FAM (blue)	AGT	CTG	1

VIC (green)	AGG	CAG	1
NED (yellow)	ACC	CTG	1
FAM (blue)	ACA	CAG	2
VIC (green)	ATC	CTG	2
NED (yellow)	ACC	CAT	2

The samples were transported to the DNA-lab of Naturhistorisk Museum (NMH), university of Oslo. The individual samples were originating from the same DigLig, but different selective PCR (different primer combinations). One sample of each fluorescent dye marking were added to a marked 96-well PCR microplate: 2 µl 6'FAM-sample, 2 µl VIC-sample and 3 µl NED-sample was added to each corresponding well on the microplate. All the samples were kept on ice to limit evaporation. 12 µl HiDi-mix (1:29 GeneScan™ 500 ROX™ Size Standard x Hi-Di™ Formamide) was added to each well. A silicone rubber septa mat was used to lock the 96-well microplate before heating at 95°C for 2 min and placed directly onto ice. The microplates were kept in the fridge for later capillary electrophoresis on an automated sequencer (ABI 3730 Genetic Analyzer).

Scoring of the AFLP-profiles

The electropherograms were read, scored and manually checked using the program GeneMapper® Software ver. 3.7 (Applied Biosystems Inc.), using the file type recognition option "AFLP-method". The default settings were used, except for the cases where peak detector for the red band was kept at a minimum peak height of 15. Panel and bins was generated using all samples, allele calling was done by using labels and deleting common alleles. Analysis range was set to be between 50 and 500 bp. The size standard setting used was GeneScan™ 500 ROX™ (GS500), which recognises the base pair lengths 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490 and 500. The program had difficulties reading some files where the DNA concentration differed greatly between the sample and the ladder used. To improve the reading of these profiles a new size standard similar to the GS500 was created in the program where the 490 bp peak was left out. The quality of the sizing was manually checked using the size match editor. If the sizing quality was not improved to above 50 % recognition of size standard peaks by using the new sizing standard,

the sample was deleted. Also when the profile was absent or partly unreadable in more than one of the six primer combinations, the sample was deleted (Appendix 4).

20 peaks (markers/alleles) were chosen from each of the six primer combination data sets. Only the markers in the range 75 - 500 bp was considered relevant, starting revising from the longest fragments first and choosing the 20 best out of these. In the range 250bp to 500bp, a cut-off at 40 fragments height was used, and for peaks in the range 75 to 250 bp, a cut-off at 100 bp was used. Common alleles were ignored (present in more than 95 % of the samples), as were alleles present in only one individual (singletons). Variable peaks were thought of as good alleles. A cut-off at 10% was used in most cases, but for some individuals with a weak profile in general, the peak would be evaluated to be included in the dataset as long as it was considered present. Each peak in the electropherogram gets a very precise position down to the first decimal place, and because of this only the average size of the fragments is used as the allele size name.

Each species matrix was subdivided into matrixes containing only individuals from Svea or all individuals (Svea and reference populations).

Error check

Error rate was calculated according to the guidelines given in Bonin et al. (2004). Briefly, sample controls scoring were compared to original samples scorings. Numbers of differing scorings were divided by total number of scorings for the control dataset, according to equation 1:

$$Error\ rate = \frac{N_{errors}}{N_{controls} \times N_{markers}}$$

Individuals where more than 6 of the total 120 alleles were variably expressed and scored differently in the control and the original DNA-profile had a high error rate and were removed. The individuals with a high total error scoring were removed before markers were removed. These individuals were also manually checked in GeneMapper for differences between the profiles. Alleles contributing to a high number of error scorings were then removed from the dataset.

The error rate checked dataset was grouped after diglig-run plate number to check for plate specific markers in R (R. C. Team, 2011) using the R package AFLP.dat (Ehrich, 2006). The datasets were also run in PAST (Hammer et al., 2001) for detection of clustering tendencies. In PAST, a principal coordinate analysis (PCO) and Neighbour joining clustering were used, using the Jaccard similarity index. The results were plotted and the clustering differences were visually reviewed to check for clear correlations between divisions in the dataset and the plate origin of samples.

As plate-specific markers were identified and removed, the error rate was revised. Markers of less than five present (1) or absent (0) scored alleles were removed from the dataset, as these were within the error rate of the dataset.

3.5 Statistical analysis

The data from the genetic analysis and from the soil sample analysis was gathered into an Excel file. Sampling spot coordinates, sampling area name, soil moisture and coal dust level were also included. Genetic sampling spot coordinates were calculated as described below under the GIS-analysis. Since soil moisture data was only sampled for the first genetic sample in each transect, it was assumed to be the same for all samples from the same transect.

Statistical analysis of the vegetation data

Canoco ver. 4.5 (Ter Braak, 1988) for Windows was used to perform Canonical correspondence analysis (CCA). CANOCO runs were performed using vegetation data from 2002 and 2009, with ecological data sampled and analysed in 2009. CCA analysis was performed, with no transformation of data. Ordination plots were drawn using the solution data from CCA-analysis in CanocoDraw.

GIS-analysis

ArcGIS ver. 10.0 (ESRI, 2011) was used to perform GIS-analysis and preparation of map graphics. The Quantum GIS Application (QGIS ver. 1.8, (Q. G. D. Team, 2013)) was also used in making map graphics. Bernt Holst from SNSK provided map databases and ortographic files of Svea. The Global Self-consistent, Hierarchical, High-resolution Geography Database (GSHHG) ver.2.2.2 (Wessel et al., 2013) released under the GNU Lesser General Public Licence, was used producing the world map overview.

The vegetation data for the vegetation analysis performed in 2002 and 2009 (hereafter named "Plot vegetation data") consist of area, transect number, plot ID, vegetation type classification based on plot cover estimations, coordinates of plot, and more. The plot vegetation data was imported to ArcGisMap, and stored as point layer. A buffer zone of 1 meter was calculated for each plot point, and the resulting buffer polygons were used as training sites when performing a supervised classification of the 3-band raster image from the Svea area (ortographic file).

The files with data for the *Luzula nivalis* (LN) and *Luzula* confusa (LC) population in Svea consists of individual IDs, coordinates, transect numbers, area names, coal dust cover estimate and more. The LN and LC data was imported to ArcGisMap and stored as two point vector layers named LN Svea and LC Svea, respectively. The two point vector layers were manually supervised. Since coordinates was only taken for the first sample in each transect, the remaining four coordinates for each sample was calculated by adding 5 meters distance per sampling point in a transect. These coordinates were revised and moved using the supervision from map notes taken in field (hand drawn maps), and in regard to the landscape features seen in the orthographic layer.

The tool "Extract values to points" in the Spatial analyst toolbox was used to extract the vegetation type data from the supervised classification file to the LN Svea and the LC Svea layers respectively. The values at the point location were not interpolated (point values is not calculated from the adjacent raster cells).

The revised coordinates was extracted by adding two columns to the attribute table of each layer, and calculate the geometry for x- and y-coordinates in the two columns. Data on abundance of the different *Luzula* species in the Svea area was calculated from the extracted simulated vegetation class value.

Statistical analysis of the genetic material

The R package AFLP.dat (Ehrich, 2006) was run in R (R. C. Team, 2011) to produce input files for the programs BAPS, Arlequin, and Structure. The genetic matrix was also run in AFLP.dat to estimate genetic diversity. The analysis for gene diversity was bootstrapped by a factor of 10.000.

BAPS (Corander et al., 2006; Corander, Marttinen, et al., 2008; Corander, Siren, et al., 2008; Corander et al., 2003) was run using non-spatial information, for datasets with and without reference samples.

Structure ver.2.2.3 is a model-based program, based on the method introduced by Pritchard et al. (2000) and extended by Falush et al. (2007). Structure analysis was carried out using the freely available Bioportal server (www.bioportal.uio.no). A burning period of 100,000 and 1,000,000 MCMC repetitions were used. Other settings were kept at default. The dataset including reference samples was run with a K spanning from 1 to 16, with 24 iterations. The dataset from Svea was run with a K from 1 to 20, with 20 iterations.

The R-package Structure-Sum-2009 (Ehrich et al., 2007) was used to sum the result files from the Structure runs. Two kinds of plots was made; plots where the probability measure LnP(D) was plotted against the respective K for the runs to find the proper K, and plots showing the deltaK (calculated as described in Evanno et al. (2005)). The programming strand for the plots was written as described in the user manual for Structure-Sum-2009.

To evaluate genetic differentiation between groups, analysis of molecular variance (AMOVA) was carried out in Arlequin ver. 3.5.1.2 (Excoffier et al., 2010). The dataset was grouped after coal gradient area, into groups as clustered by Structure and BAPS, and as a hierarchical combination of these. Standard AMOVA computation was done using haplotypic format, and the distance matrix was computed using pairwise differences.

Canoco ver. 4.5 (Ter Braak, 1988) for Windows was used to perform Canonical correspondence analysis (CCA) using the genetic data from Svea, where the markers from the dataset in the analysis functioned equal to what plant species in a vegetation analysis do. CCA analysis was performed, with $\log_{10}$ -transformation of electroconductivity data. Ordination plots were drawn using the solution data from CCA-analysis in CanocoDraw.

Wind rose and climate normal from eKlima

Norwegian Meteorological Institute offers free access to historical and real time weather- and climate data through the web-portal eKlima (NorwegianMeteorologicalInstitute, 2011). Here they also offer to do simple statistics, where the frequency report type wind rose was the option selected for this run. Hourly weather data from Svea (weather station nr. 99760) for the years 2008 throughout 2009 was selected as data source for the statistics. The default settings were kept for the remaining options.

4 Results

4.1 Ecological variables

Soil temperature and moisture logger data

Of the seven HOBO-loggers mounted in field, arctic foxes or reindeers disturbed four loggers during the 2 months time between visits. One logger was short circuited, and it was not possible to retrieve data from this logger. Logger HOBO-01 was damaged from chewing by foxes, but all data were available. The moisture sensor of sensor pair CD of HOBO-02 was damaged 28th august; but this did not heavily affect the average mean for this month. HOBO-09 had both moisture sensors damaged in August, first AB the 18th and then CD the 28th. The soil temperature was overall higher in July than in August, and trended to be higher in coal classes 3 and 4 than in coal classes 1 and 2, regardless of soil moisture, vegetation class or locality in hillside or moraine areas.

Table 9 Summary table of soil temperature and soil moisture data logged by the HOBO-loggers placed in each of the seven transects of Svea sampling site. Table copied from P. B. Eidesen (2010)

	Coordinates WGS 84 UTM 33X						Average soil temperature (°C)		Average soil moisture (m^3/m^3)		
Logger	East/ North	Sensor pair	Plot	Area	Vegetation class	Coal class	July	Aug	July	Aug	Comment
HOBO-01	0539675/ 8647344	AB-01	0946	C, hill	3	3	9,0	7,7	0.11	0.09	Moisture sensor eaten by polar fox
		CD-01	0947		4	3	9,1	7,8	0.29	0.19	
HOBO-02	0536368/ 8644946	AB-02	0924	X, hill	3	1	6,3	6,0	0.19	0.16	Moisture sensor eaten in August
		CD-02	0925		3	1	6,3	6,0	0.30	0.28	
HOBO-04	0538552/ 8644614	AB-04	0926	J, moraine	3	2	NN	NN	NN	NN	Sensors chewed and logger short circuited
		CD-04	0927		5	2	NN	NN	NN	NN	
HOBO-06	0538900/ 8646170	AB-06	0931	E, hill	5	2	6,8	6,1	0.25	0.23	
		CD-06	0932		3	2	6,1	5,5	0.21	0.20	
HOBO-07	0539214/ 8644194	AB-07	0928	K, moraine	3	4	6,2	5,2	0.28	0.26	
		CD-07	0929		4	4	7,1	5,8	0.25	0.25	
HOBO-08	0540808/ 8648612	AB-08	0933	A, hill	5	4	7,9	6,8	0.20	0.20	
		CD-08	0934		4	4	9,0	7,4	0.25	0.24	

HOBO-09	0536271/ 8643644	AB-09	0930	X, moraine	3	1	5,2	5,2	0.15	NN	Moisture sensor eaten in August
		CD-09	0944		5	1	6,7	6,3	0.25	0.25	

The second method of measuring soil moisture using Raup's scale (Raup, 1969) scale show that vegetation classes 6 open grassland and 9 Sparce *Puccinellia* fields were the driest. Vegetation class 1 pond and stream edge was the wettest, while moss tundra (3) was second moist. Moss tundra was moister than wet tundra (2). Vegetation class 5 (*Salix* heath), 7 (Vegetated patterned ground and boulder field) and 8 (Silt flats) were equally moist.

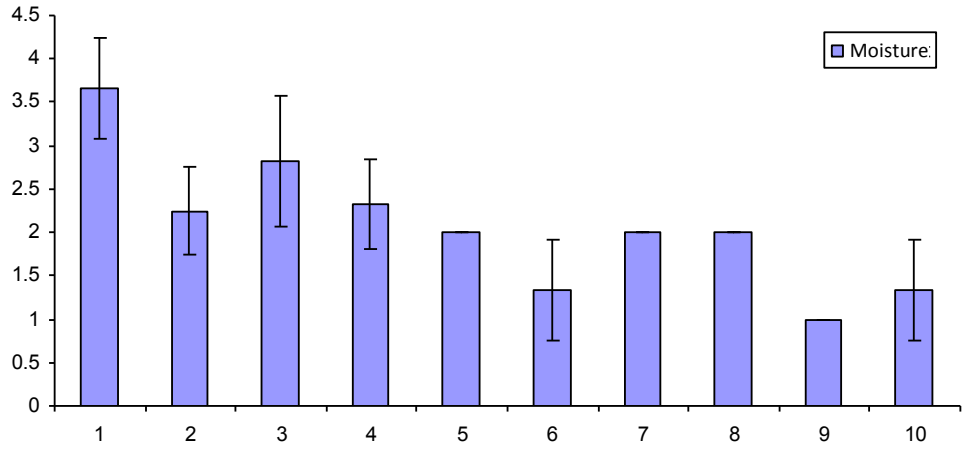


Figure 8 Average values of soil moisture in the vegetation plots based on feel and appearance method as described in The x-axis numbering corresponds to the following vegetation classes: 1 Pond and stream edge, 2 Wet tundra, 3 Moss tundra, 4 Moderate snowbed, 5 *Salix* heath, 6 Open grassland, 7 Vegetated patterned ground and boulder field, 8 Silt flats, 9 Sparse *Puccinellia* fields, 10 Pioneer vegetation, 11 Disturbed ground. Standard deviation is indicated. Figure is copied from P. B. Eidesen (2010)

Soil samples from vegetation analysis plots

A total of 99 soil samples were collected from the vegetation analysis plots. All 99 samples was successfully analysed for pH and electroconductivity (EC). A subset of 55 soil samples were successfully analysed for carbon content, while 53 of 55 soil samples were successfully analysed for nitrogen content (Table 10, Figure 9). Few soil samples were from area A/B and H, which had four samples each.

Table 10 Summary tables of averages and standard deviation (SD) for soil sample pH, electroconductivity, carbon content and nitrogen content between the different vegetation classes.

Vegetation class	Average pH ± SD	Average electroconductivity (µS/cm) ± SD	Average nitrogen content (%) ± SD	Average carbon content (%) ± SD	N samples
1 – pond and stream edge	5.67 ± 0.3	683.7 ± 884.3	0.10 ± 0.07	9.63 ± 9.05	9
2 – wet tundra	6.03 ± 0.4	162.1 ± 156.1	0.06 ± 0.05	7.01 ± 5.48	12
3 – moss tundra	5.73 ± 0.5	167.4 ± 74.5	0.06 ± 0.05	8.59 ± 8.59	19
4 – moderate snowbed	5.72 ± 0.3	128.2 ± 130.0	0.08 ± 0.04	11.76 ± 7.78	12
5 – *Salix* heath	5.66 ± 0.4	154.7 ± 50.9	0.08 ± 0.11	4.46 ± 1.43	10
6 – open grassland	6.04 ± 1.0	165.5 ± 96.6	0.03 ± 0.02	3.04 ± 2.08	5
7 – vegetated patterned ground and boulder field	5.77 ± 0.7	112.9 ± 62.4	0.08 ± 0.03	8.55 ± 3.94	13
8 –silt flats	6.84 ± 0.5	4145.0 ± 2403.9	0.06 ± 0.03	3.32 ± 1.43	7
9 – scarce *Puccinellia* fields	6.74 ± 0.6	2567.7 ± 2448.5	0.06 ± 0.01	6.85 ± 2.31	7
10 – pioneer ground	5.96 ± 0.1	5867.5 ± 5646.2			2
11 – disturbed ground	5.62	84.5	0.06	7.67	1
Not classified	5.16 ± 0.0	118.3 ± 2.8	0.07	5.37 ± 4.68	2
Total	5.92 ± 0.6	764.7 ± 1728.1	0.07 ± 0.05	7.46 ± 6.09	99

pH-measurements of vegetation plot soil samples showed an in general lower pH in hillside areas, and higher pH at the silt flats (Figure 9, Table 11). The values for EC show a similar pattern. Soil nitrogen (N) content and soil carbon (C) content were higher in area A, C and K and in the wetter vegetation types with denser plant cover (1-5) that also dominated these areas (Appendix 1).

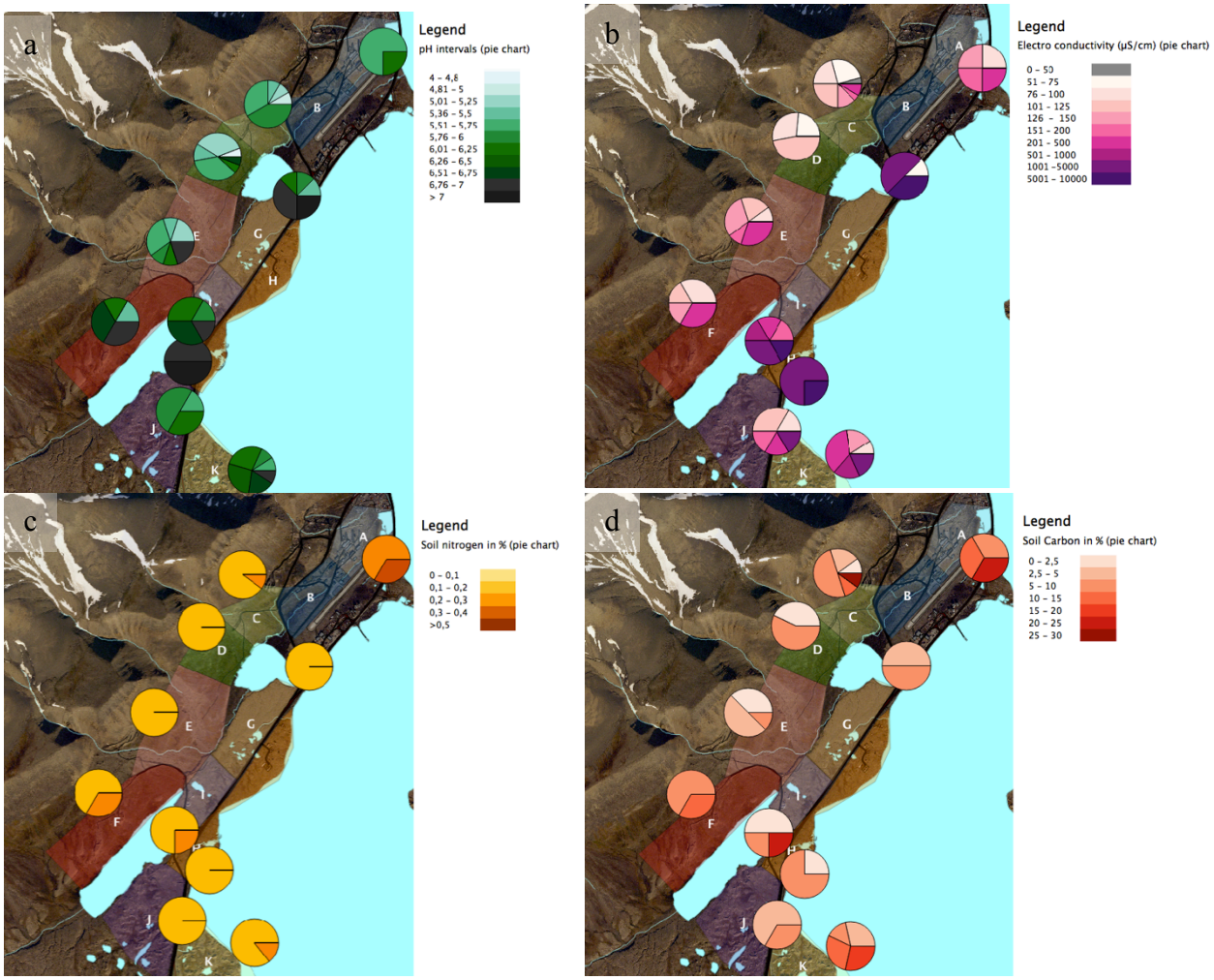


Figure 9 The frequency of measuring values within given intervals shown as pie charts for the four environmental variables pH, electro conductivity (in µS/cm), soil nitrogen content (%) and soil carbon content (%) (map a, b, c and d, respectively). The environmental variables were collected for the vegetation plot analysis. Map source: SNSK.

Soil samples from genetic sampling sites

All the 120 soil samples were successfully analysed for pH and electroconductivity (EC) (

Table 11, Figure 10 and table 2 in appendix 2). Few soil samples were from area 2 and 3, which had five and one sample respectively. Of the 31 samples analysed for soil nitrogen (N) and carbon (C) content, five gave no result for N content and three no result for C content.

Table 11 Summary table of averages and standard deviation (SD) of pH, electroconductivity, carbon content and nitrogen content of soil samples within different vegetation classes for the two species *Luzula confusa* and *Luzula nivalis*.

Luzula confusa

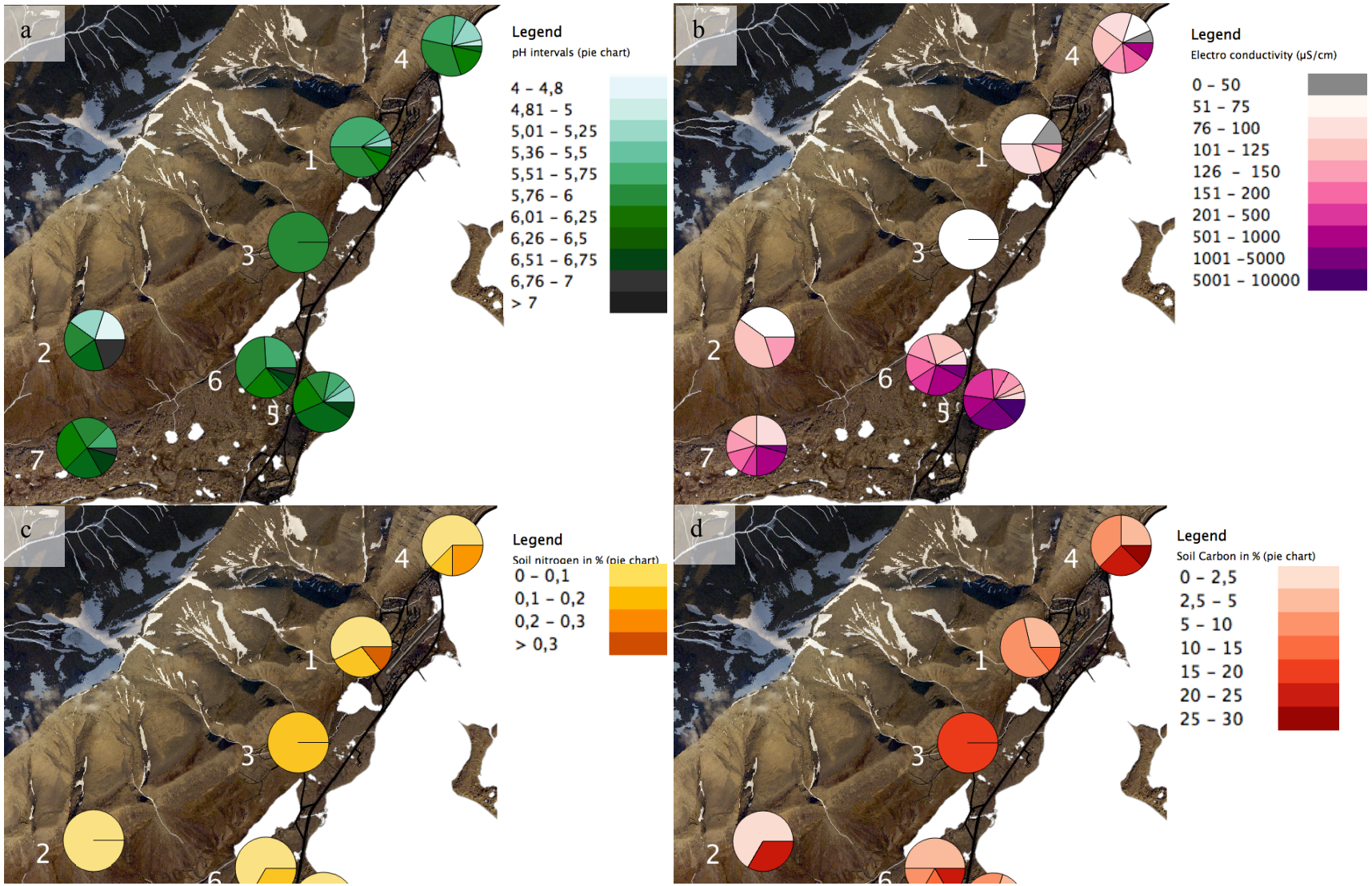
Transect	Average pH ± SD	Average electroconductivity (µS/cm) ± SD	Average nitrogen content (%) ± SD	Average carbon content (%) ± SD	Coal class	N soil samples
1	5.80±0.26	78.2±26.3	0.10±0.05	6.91±3.88	3	18
2	5.74±0.82	98.7±34.8	0.1	8.36±12.72	1	5
3	5.86	68.4	0.14	15.41	2	1
4	5.83±0.35	119.5±49.2	0.16±0.09	16.02±10.61	4	22
5	6.05±0.46	285.9±195.4	0.11±0.08	9.71±6.63	4	21
6	5.93±0.30	165.8±50.8	0.08±0.08	8.68±8.57	3	22
7	6.17±0.27	151.3±55.1	0.03±0.02	3.20±1.84	1	19
Sum average	5.94±0.39	158.6±116.7	0.10±0.07	8.82±7.84		108
Bjørndalen	6.86±0.43	85.1±25.6			Ref.	17
Engelsbukta	6,54	509			Ref.	1
Total average	6.1±0.50	151.4±115.8				126

Luzula nivalis

Transect	Average pH ± SD	Average electroconductivity (µS/cm) ± SD	Average nitrogen content (%) ± SD	Average carbon content (%) ± SD	Coal class	N soil samples
1	5.78±0.30	78.2±28.0	0.12±0.11	7.72±3.94	3	18
2	5.74±0.82	98.7±34.8	0.1	8.36±12.72	1	5
3	5.86	68.4	0.14	15.41	2	1
4	5.67±0.37	122.6±55.5	0.12±0.10	8.97±8.13	4	18
5	6.07±0.46	287.8±196.2	0.11±0.08	9.71±6.63	4	22
6	6.02±0.37	159.1±50.6	0.06±0.05	6.77±4.77	3	18
7	6.20±0.33	144.7±54.8	0.05±0.04	4.37±3.00	1	19
Total	5.94±0.44	159.6±123.0	0.10±0.08	8.03±6.30		101
Bjørndalen	6.84±0.40	96.5±32.3			Ref.	17

Engelsbukta	7,19	245	Ref.
Total average	6.08±0.54	151.3±116.3	

In transect area 2, large variation in pH was detected (from 4 to 7; Figure 10a) Transect 1, 4 and 5, which were grouped to coal gradient 3 and 4, all showed a shift to lowered pH when compared to transect 2, 3, 6 and 7, which are grouped to coal gradient 1 and 2. The electroconductivity was higher in moraine areas than in hillside areas (Figure 10b). Soil nitrogen content (Figure 10c) was higher in transect classified to coal gradients 2, 3 and 4, but these transects were also situated closer to the settlement, coal mining influence and human activities. Soil carbon content (Figure 10d) showed similar patterns as soil N content, but in addition soil C content had what looks like an outlier in transect 2 where one sample had a measured soil C content of 23,0 %.


a
4
1
3
2
6
5
7
Legend
pH intervals (pie chart)
4 – 4,8
4,81 – 5
5,01 – 5,25
5,36 – 5,5
5,51 – 5,75
5,76 – 6
6,01 – 6,25
6,26 – 6,5
6,51 – 6,75
6,76 – 7
> 7
b
4
1
3
2
6
5
7
Legend
Electro conductivity (µS/cm)
0 – 50
51 – 75
76 – 100
101 – 125
126 – 150
151 – 200
201 – 500
501 – 1000
1001 –5000
5001 – 10000
c
4
1
3
2
6
Legend
Soil nitrogen in % (pie chart)
0 – 0,1
0,1 – 0,2
0,2 – 0,3
> 0,3
d
4
1
3
2
6
Legend
Soil Carbon in % (pie chart)
0 – 2,5
2,5 – 5
5 – 10
10 – 15
15 – 20
20 – 25
25 – 30

4.2 Vegetation analysis

The vegetation classification system that was established and used during the survey of E. Cooper and L. Nilsen in 2002, was compressed to the classification system as shown in Table 12.

Table 12 The vegetation classification system used during registration in 2009.

Class	Abbreviated	Vegetation class name
1	Pon	Pond and stream edge
2	Wet	Wet tundra
3	Mos	Moss tundra
4	Sno	Moderate snowbed
5	Hea	Salix heath
6	Gra	Open grassland
7	Bou	Vegetated patterned ground and boulderfield
8	Sil	Silt flats
9	Puc	Sparce *Puccinellia* fields
10	Pio	Pioneer ground
11	D	Disturbed ground

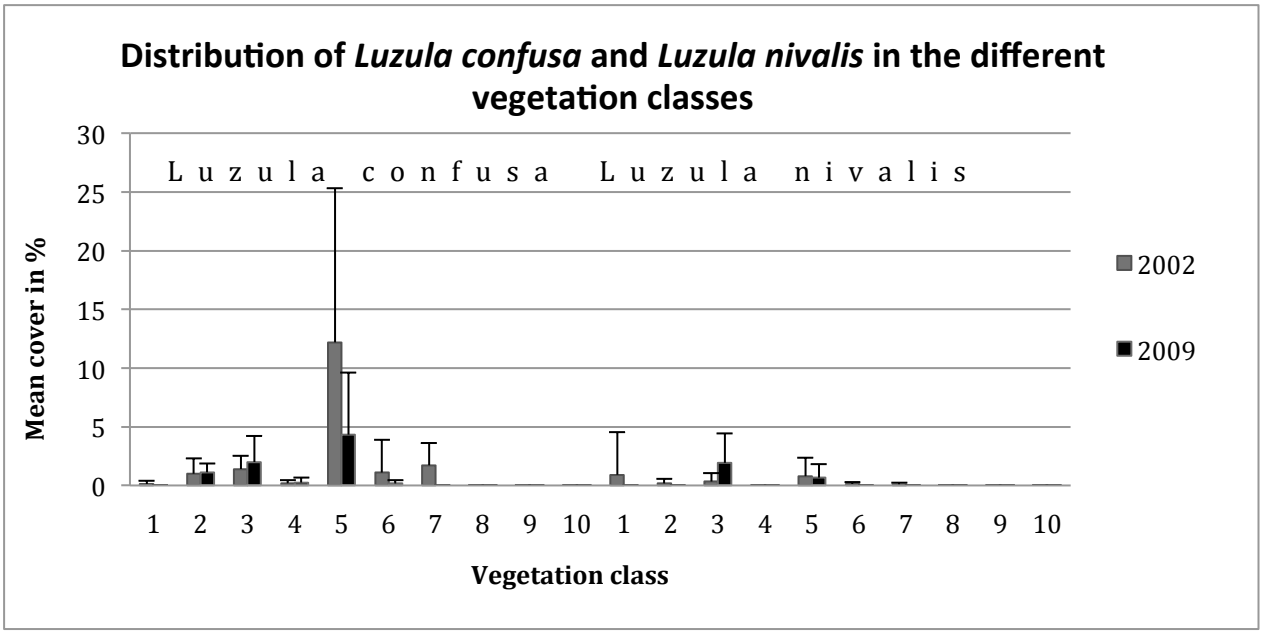


Figure 11 Distribution of the species *Luzula confusa* and *Luzula nivalis* in the different vegetation classes given in Table 13. Here the mean value and SD for each vegetation class was used.

At least three vegetation plots analyses were carried out in each vegetation type in 2009. While there was 173 vegetation plots analysed in 2002, only 45 was analysed in 2009. Many of the revisited plots in 2009 were noted as unchanged. *Luzula confusa* was more abundant and distributed in more vegetation types than *Luzula nivalis*, that was found in small amounts in the vegetation classes pond and stream edge (1), wet tundra (2), moss tundra (3) and *Salix* heath (5) (Figure 11), which correlated to what was observed in field. There was a change in percentage cover as well as distribution from 2002 to 2009. *Luzula confusa* was observed in vegetation types 1 to 7 during the 2002 survey, while it in 2009 it was less abundant in the same vegetation types and not registered in the veg types 1 and 7. *Luzula nivalis* was only found in vegetation type 3 and 5 during the 2009 survey, while it in 2002 it was additionally found in vegetation type 1 to 7 except for vegetation type 4. Neither of the species was found in the vegetation types 8, 9 or 10 during either of the vegetation surveys.

Supervised vegetation classification

The supervised vegetation classification resulted in the vegetation type map of Svea (Figure 12). In the underlying image used (Figure 6), most of the wet areas as the fiord and ponds had several pixel values identical or similar to the pixel values of vegetated areas. Similar pixel values were also caused by shadows and a mud-stream from a river falling into the fiord.

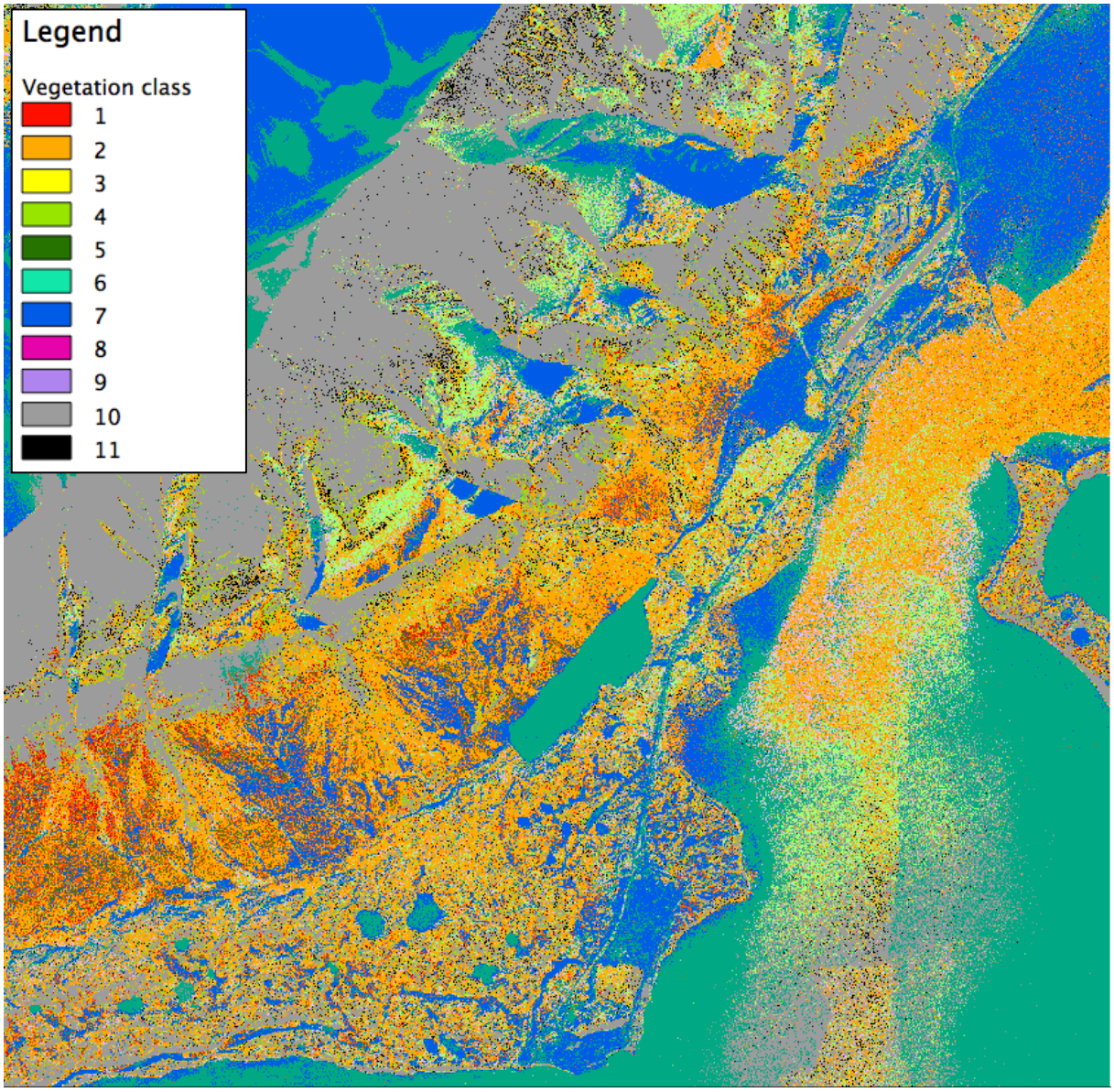


Figure 12 Supervised classification based on 1 m buffer zone polygons calculated around vegetation plots from the 2002-survey. A three-band raster image was used, No infrared (IR) data was available.

Using the frequency of assignment to the different vegetation types through extraction of vegetation data, the distribution of the *Luzula* species in the different vegetation types were extracted (Figure 13). Though this distribution relied on the quality of the underlying

supervised classification, the simulated data (Figure 13) showed a similar pattern as the pattern based on the original vegetation plots (Figure 11), where both species occur in vegetation type 1,2, 3 and 5, but in the simulated data the species also occurred in vegetation type 7 (vegetated patterned ground and boulder field). The simulated data was further used to assign genotyped individuals to vegetation type.

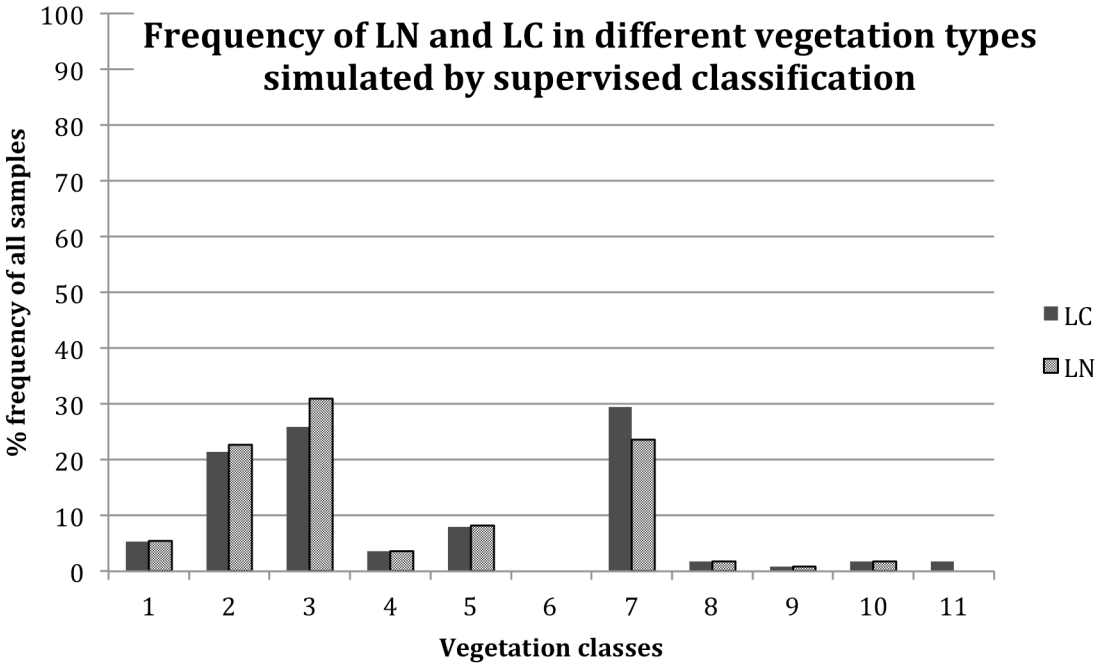


Figure 13 Frequency of assignments of the different vegetation types to the sampling coordinates of *Luzula confusa* and *L.nivalis* by performing a supervised classification in GIS.

Ordination of vegetation analysis plots and corresponding ecological variables

The correlation between electroconductivity (EC) of soil samples taken from the respective plots was strong, even though soil EC measurements were done for only 99 of the 218 plots investigated. The electroconductivity variable was excluded in subsequent analyses, as it was strongly correlated to only a few species and samples of the dataset and skewed the results.

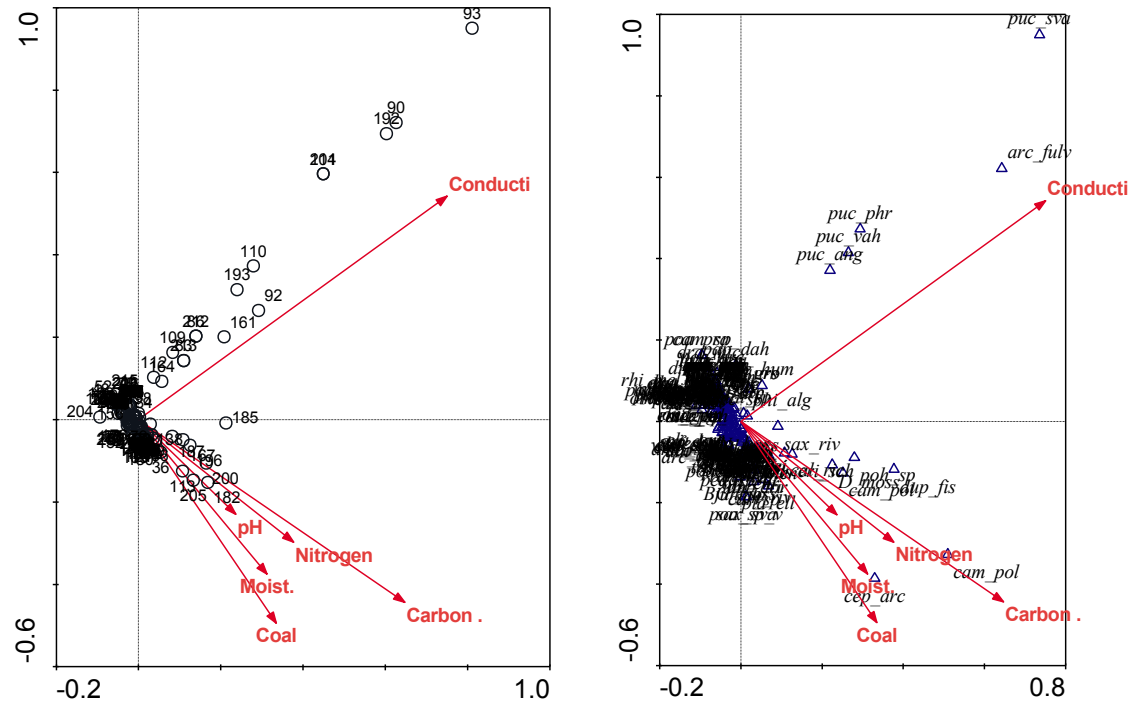


Figure 14 Ordination plot of vegetation analysis data from Svea (conducted in 2002 and 2009) correlated to different ecological variables sampled in 2009. The figure to the left shows the vegetation plots in correlation to the different ecological variables, while the figure to the right shows the species correlation.

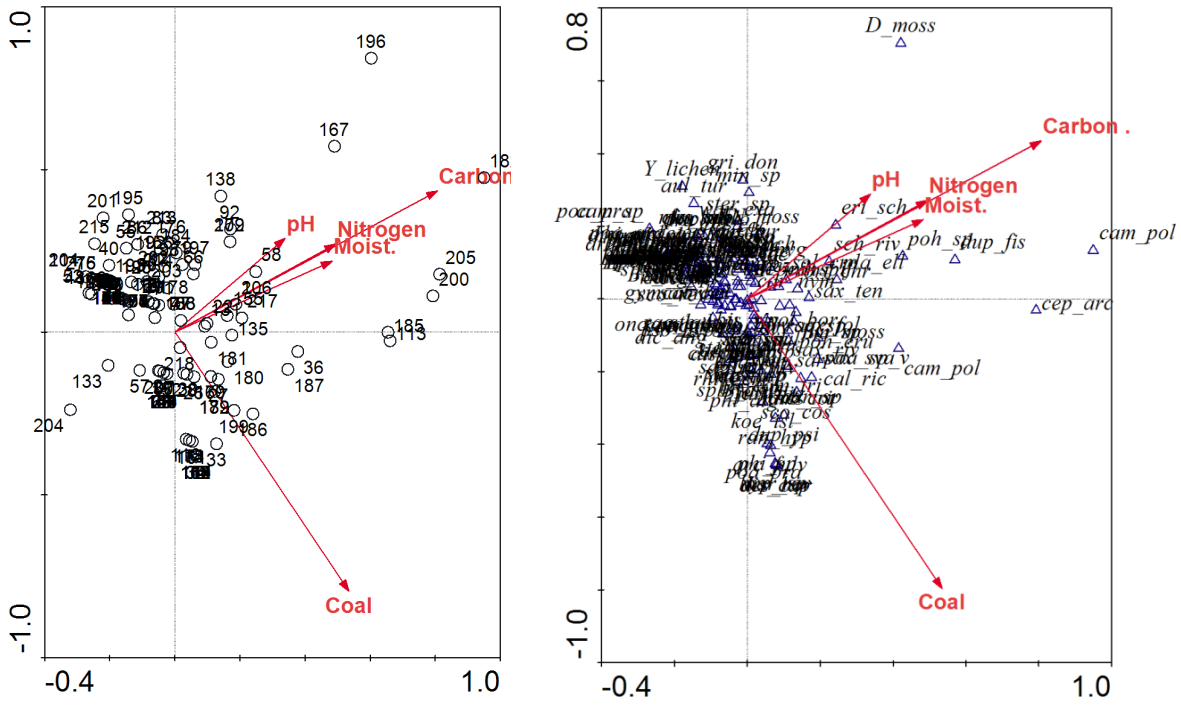


Figure 15 Ordination plot of vegetation analysis data from Svea correlated to different ecological variables sampled in 2009. Here the ecological variable electroconductivity was excluded from the CCA-analysis. The figure to the left shows the vegetation plots in correlation to the different ecological variables, while the figure to the right shows the species correlation to the variables.

By excluding EC, the ecological variables coal and carbon were the strongest explanatory axes (**Figure 15**).

4.3 Genetic analysis

Out of the 360 individuals collected, 305 individuals were used in the final AFLP matrix (162 for LC, and 141 for LN). Out of the 19 positive controls used (12 for LC, and 11 for LN) for the error check, 7 were discarded (5 for LC and 2 for LN).

Error rate

For the *Luzula confusa* (LC) samples, a good data matrix for use in the statistical analysis of the data was not achieved. An error rate for LC of 5.5 % was achieved when the dataset was discarded from further analysis, and an error rate below 5% were not achieved despite removal of obvious plate specific markers. Because of this, the LC-matrix was discarded, and further analysis of genetic structures and patterns was carried out using the *Luzula nivalis* dataset.

For *Luzula nivalis* (LN), originally 122 markers were scored using sampling from the automatic scoring done by GeneMapper 3.7. 11 individuals and their replicates were compared for allelic differences to achieve an error rate. Markers with a high error rate; a difference between original and replicated samples of two and more alleles, were discarded. So were markers present in the range of present in less than 4 individuals or more than 138 individuals. The check for plate specific markers (see appendix 4), found 9 markers that were deleted from the dataset. In total an error rate of 4.35 % was achieved. This error rate was still not optimal, but as the effect of plate specific markers was removed (Figure PCO in appendix 5), this dataset was regarded sufficient for analyses of genetic structure and diversity. Individuals with one or more unreadable or absent electropherograms of the six possible primer combinations, was also discarded. All removed markers and individuals are listed in table 3 in the appendix 4.

Investigation of genetic groups

The probability for number of genetic groups (K) in the dataset of all *Luzula nivalis* (LN) samples were highest at K= 5. However, the probability stopped being uniform at K=2. (**Figure 16**). This suggested that the dataset consisted of at least two genetic different groups, with some possible substructure.

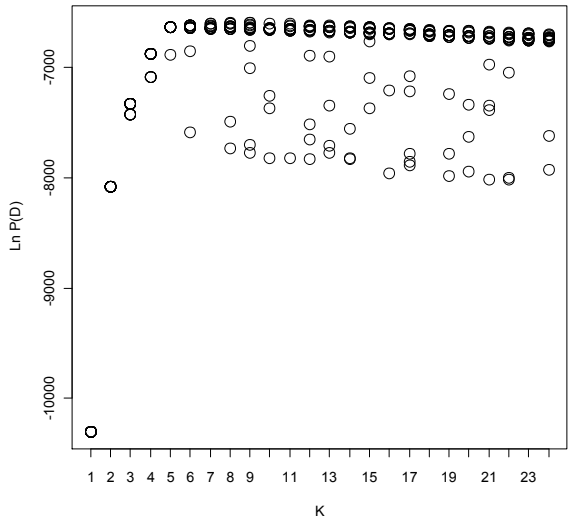


Figure 16 Logarithmic probability of data given a value of K(number of groups), with 16 replicates shown for each K. Results from Bayesian clustering analysis using Structure on a dataset with 141 individuals and 108 markers for the species Luzula nivalis.

The interpretation of K=2 for the whole LN dataset was supported calculations of delta K (Figure 17). By checking which individual was assigned to which group; the two groups were an intermixture of individuals from both the Svea transects and the reference populations.

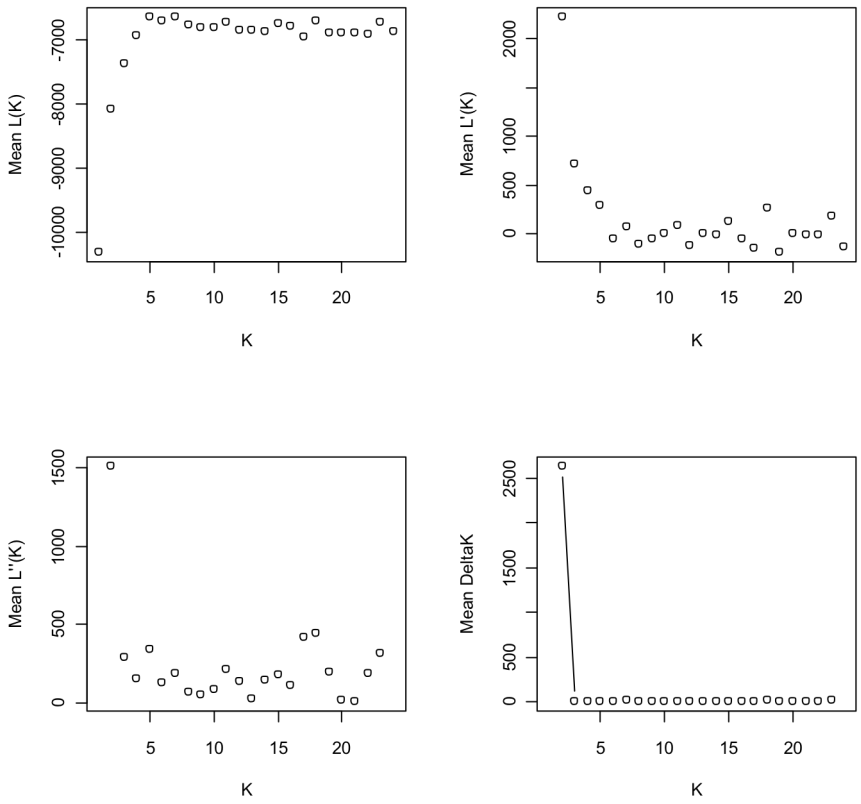


Figure 17 Delta K calculations as described in (Evanno et al., 2005) for all *Luzula nivalis* samples using the same Structure dataset as was used in plotting **Figure 16**.

The analysis of genetic clustering of LN samples only from Svea resulted in a probability for the given K (Figure 18) and ΔK (Figure 19) that gave a most probable K of 5 genetic clusters.

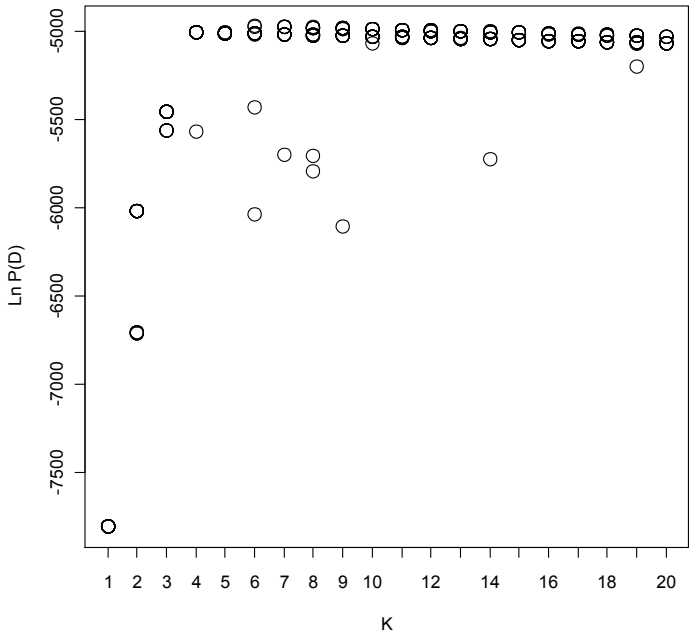


Figure 18 Logarithmic probability of data given a value of K(number of groups), with 20 replicates shown for each K.

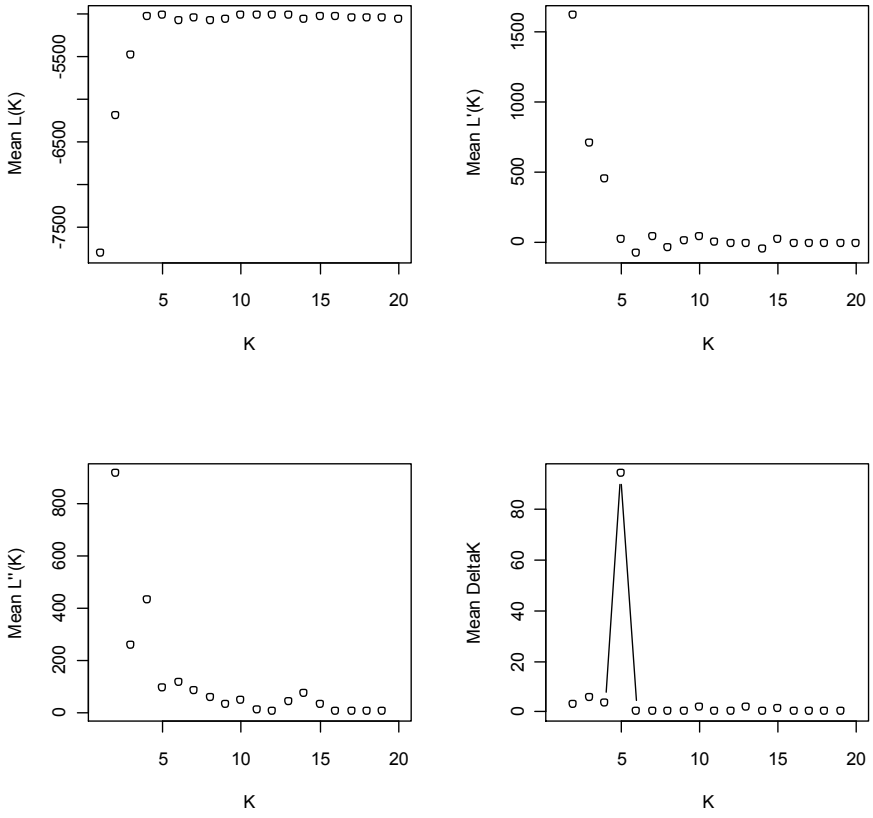


Figure 19 Delta K calculations as described in Evanno et al. (2005). Only individuals from Svea were used

By using BAPS (Corander et al., 2006; Corander, Marttinen, et al., 2008), another clustering program, but thought of the liberal counterpart to Structure, I found the most likely number of groups to be 4 when using individuals from Svea only. BAPS uses log(marginal likelihood) as a measure to find the most likely number of clusters. By running BAPS several times to check for number of groups, with maximum number of groups spanning from 1 up to 20, the best log(ml) was found for 4 groups (when using Svea LN individuals only). When using all LN individuals, I identified the number of groups to be five (which also where the number of groups with highest probability in Structure (Figure 20). Genetic group 2 was unique for Svea, while genetic group 5 had its main occurrence in Engelskbukta. The last three groups were occurring in Svea, Kapp Linne and Bjørndalen, though group 3 was most abundant in Bjørndalen and at Kapp Linné (Figure 20). When comparing the result from assigning individuals to different groups in Structure and in BAPS, the group composition did not differ. Thus, I chose to use the genetic structure supported by both programs, resulting in overall five genetic groups, whereof four were present in Svea (Figure 20).

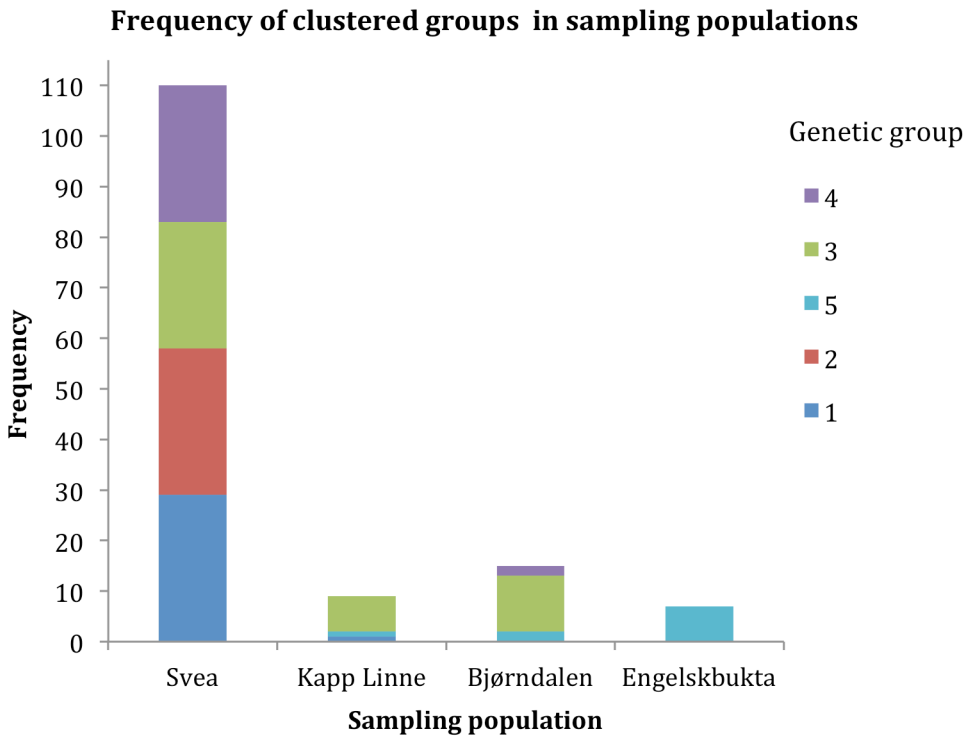


Figure 20 Frequency of the distribution of the different individuals from the different sampling populations to the groups assigned in BAPS and Structure clustering analysis.

Figure 21 Distribution of genetic groups of *Luzula nivalis* within the sampling transects of Svea sampling site. Transects are labelled with transect number.

The compositions of genetic groups were differing between the geographic areas (Figure 21). Group 4 was not present in the northernmost area (transect 4). Group 3 was dominating in the southern moraine area (transect 7), but was absent from the moraine area closer to the sea with higher coal dust levels (transect 4). Three transects were identified with representatives from three of the four found in Svea, but the composition of genetic groups did differ between the transects, and there was no congruence to what genetic group that was not represented. The genetic structure represented by the four genetic groups found in Svea did not correspond to level of coal dust nor geography.

The genetic diversity differed between genetic groups, but the diversity was only significantly different between genetic group 2 and 3 This was more closely investigated by performing an AMOVA in Arlequin (see Table 14). As expected, the groups clustered by Structure and

BAPS analyses showed a lower variation in gene diversity between groups, since both programs cluster individuals to groups by using similarities in the input data.

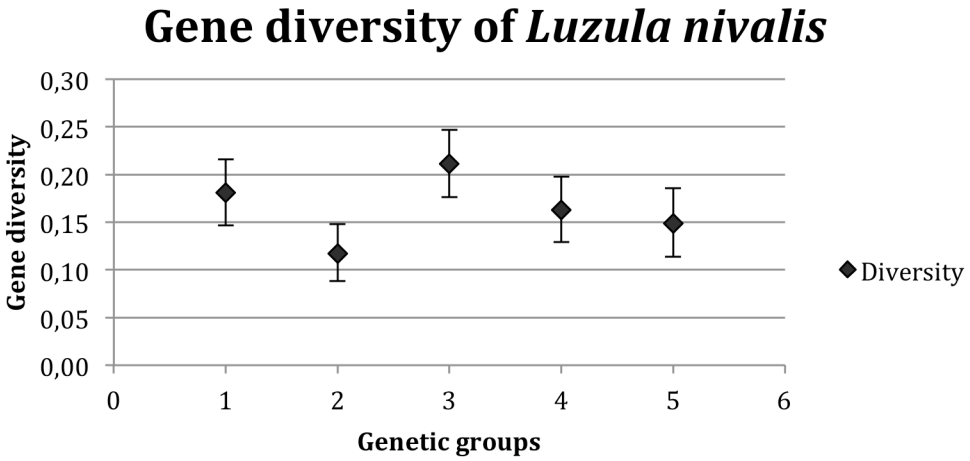


Figure 22 Gene diversities of all *Luzula nivalis* sampled, with a K=5 according to genetic clustering programs. A 95% confidence interval (CI) is shown.

In appendix 6, the diversity for all LN individuals was calculated when grouped after coal gradient. No significant difference could be detected between the groups.

A higher diversity was found when the dataset was grouped after sampling population instead of according to the genetic groups found by the clustering programs (Figure 23). Diversity levels increased according to the number of genetic groups present in the location. The sampling population in Engelskbukta, where only one genetic group was present, had significantly lower genetic diversity compared to the other sampling sites.

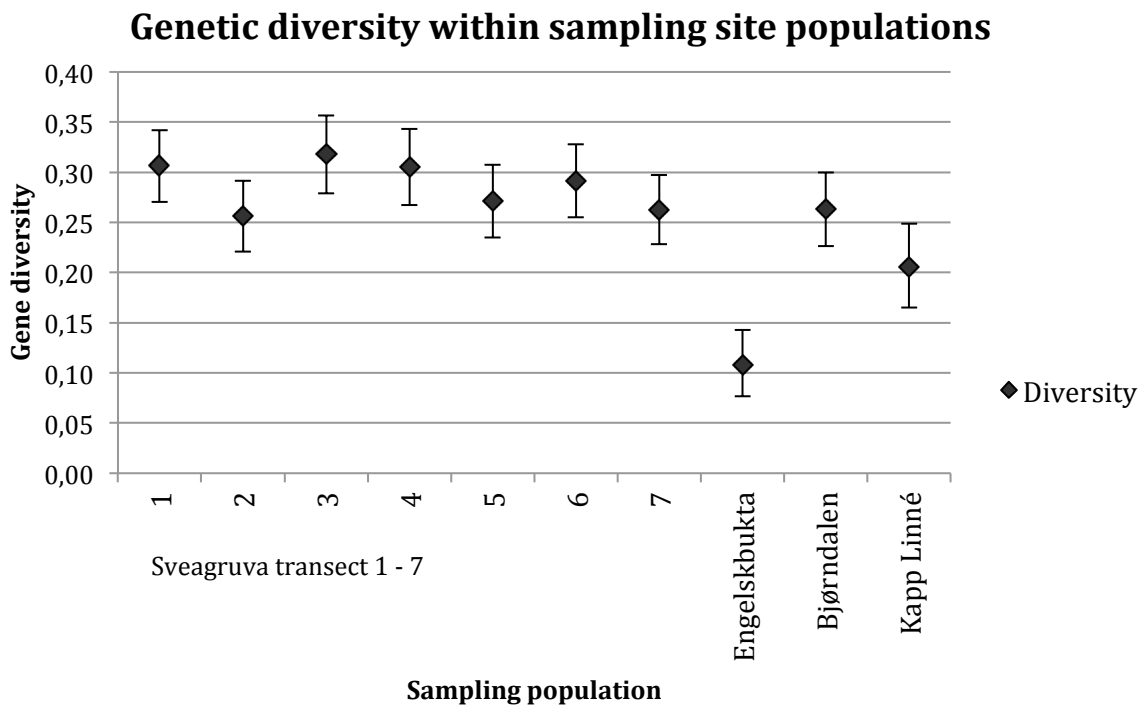


Figure 23 Gene diversities within the sampling populations. A 95 % CI is given as error bars.

The genetic groups present in Svea showed differences in average soil pH, EC and carbon content (Table 13), though the standard deviations were overlapping. Average N-content was highest and varied most in group 2. Group 2 had the lowest average pH combined with highest N and C-content.

Table 13 Summary table of averages and standard deviation (SD) for soil sample pH, electroconductivity, carbon content and nitrogen content between the different assigned genetic groups by Structure and BAPS in Svea..

Genetic group	Average pH ± SD	Average electroconductivity (µS/cm) ± SD	Average nitrogen (N) content (%) ± SD	Average carbon (C) content (%) ± SD	N samples	N sample of N and C
1	5.91±0.46	183.1±212	0.10±0.10	6.80±6.09	23	3
2	5.77±0.48	145.6±76	0.15±0.12	13.02±7.52	20	4
3	6.22±0.38	132.3±63	0.11±0.03	9.91±3.39	18	3
4	6.04±0.44	168.9±93	0.10±0.10	3.32±2.88	13	3
Total	5.97±0.46	158.1±133	0.12±0.09	8.63±6.19	74	13

Table 14 Summarised table of AMOVA analysis performed on different groupings of either the whole *Luzula nivalis* genetic matrix or the Svea sampling site.

Analysis performed	Source of variation	d.f.	Sum of squares	Variance	% variation	Fixation index: FST	Significance of Va and FST	
Svea sampling population grouped after coal gradient	Among pop.	3	112.533	0.77528 Va	4.50	0.04503	P(rand. value > obs. value)	0.00198
	Within pop.	106	1742.867	16.44214 Vb	95.50		P(rand. value = obs. value)	0.00000
	Total	109	1855.400	17.21742			P-value	0.00198 + -0.00095
Structure groups for K=4 for the Svea groups	Among pop.	3	927.109	10.93248 Va	55.52	0.55523	P(rand. value > obs. value)	0.00000
	Within pop.	106	928.291	8.75746 Vb	44.48		P(rand. value = obs. value)	0.00000
	Total	109	1855.400	19.68994			P-value	0.00000 + -0.00000
Structure groups for K=5 for all of the *Luzula nivalis* - groups	Among pop.	4	1211,819	10.79148 Va	53.84	0,53844	P(rand. value > obs. value)	0.00000
	Within pop.	136	1258,082	9.25060 Vb	46.16		P(rand. value = obs. value)	0.00000
	Total	140	2469,901	20,04208			P-value	0.00000 + -0.00000
All *Luzula nivalis* grouped by sampling populations	Among pop.	9	551,975	3.34176 Va	18.58	0,18583	P(rand. value > obs. value)	0.00000
	Within pop.	131	1917,926	14.64066 Vb	81.42		P(rand. value = obs. value)	0.00000
	Total	140	2469,901	17,98242			P-value	0.00000 + -0.00000

The AMOVA showed that low amounts of the variation in the dataset could be explained by the coal gradient (Table 13 Summary table of averages and standard deviation (SD) for soil sample pH, electroconductivity, carbon content and nitrogen content between the different assigned genetic groups by Structure and BAPS in Svea..

Genetic group	Average pH ± SD	Average electroconductivity (µS/cm) ± SD	Average nitrogen (N) content (%) ± SD	Average carbon (C) content (%) ± SD	N samples	N sample of N and C
1	5.91±0.46	183.1±212	0.10±0.10	6.80±6.09	23	3
2	5.77±0.48	145.6±76	0.15±0.12	13.02±7.52	20	4
3	6.22±0.38	132.3±63	0.11±0.03	9.91±3.39	18	3
4	6.04±0.44	168.9±93	0.10±0.10	3.32±2.88	13	3
Total	5.97±0.46	158.1±133	0.12±0.09	8.63±6.19	74	13

Table 14). The variation was better explained when the whole dataset was grouped according to sampling populations, but but more than half of the variation could be explained when the genetic groups assigned by clustering programs were used.

By dividing the datasets into groups according to vegetation type as classified using supervised classification in ArcGIS, no significant results were achieved from running the AMOVA. Both the FST and the variation were negative, so the result could not be trusted.

Ordination of genetic matrix and corresponding ecological variables

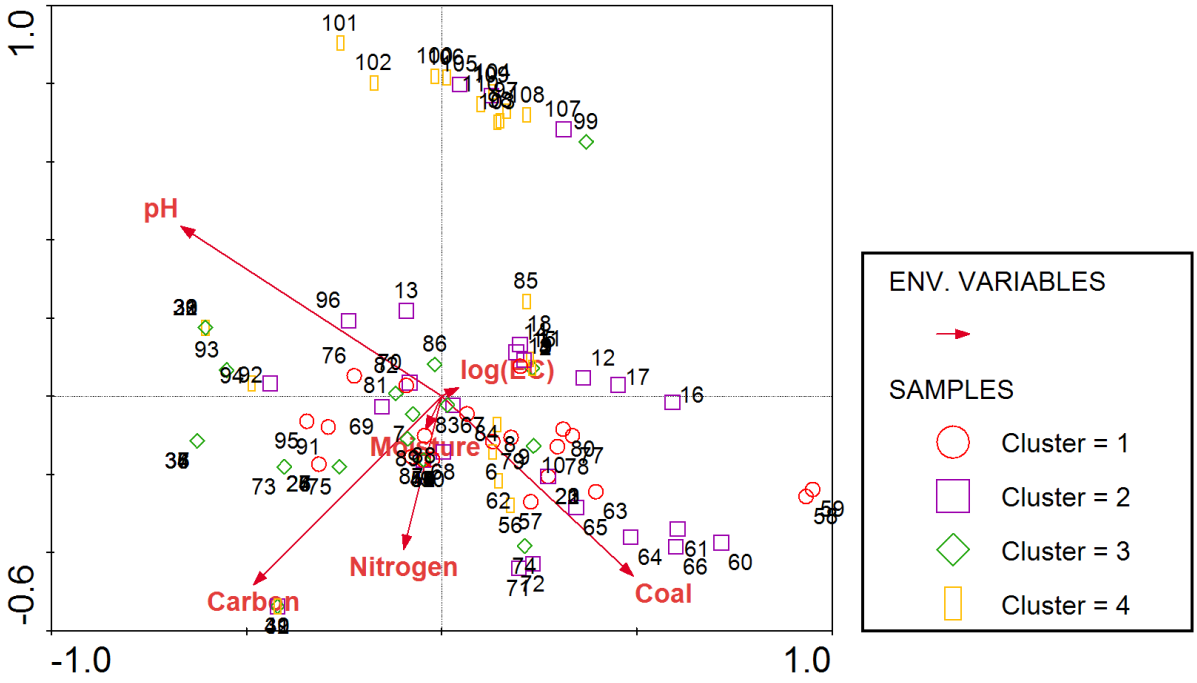


Figure 24 Ordination plot based on CCA analysis of Svea population matrix to investigate the correlation between individual sample and environmental variables. Structure groups are colored. Both semi-quantitative and quantitative ecological variables were used in the CCA, and averages was added to all individuals within the transect that the soil C and N content were measured for.

Both Figure 24 and Figure 25 suggest that the strongest correlation were between the dataset and pH, while soil nitrogen content claim the second or third explanatory axis.

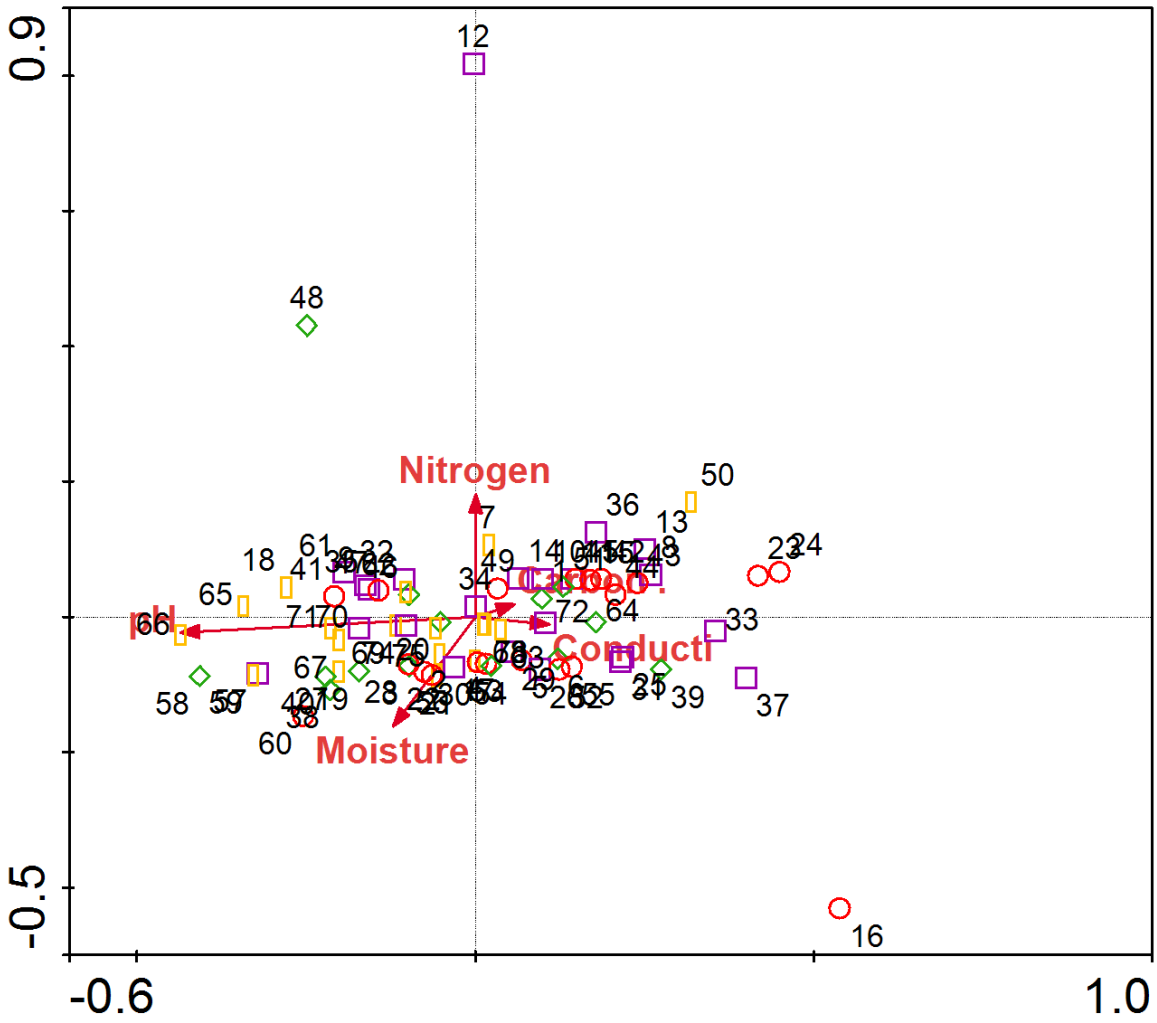


Figure 25 Ordination plot based on CCA analysis of Svea population matrix where only the ecological variables pH, moisture, electroconductivity, carbon and nitrogen content were used. Each genetic group clustered by Structure is colored distinctively. Nitrogen and carbon content was available only for 26 and 28 samples of the 110 analysed.

5 Discussion

5.1 Vegetation analysis

The canonical correspondence analysis (**Figure 15**) suggests that electroconductivity (EC) is the most important ecological variable that explains the species composition in vegetation plots or species distribution. This is mainly due to the extreme habitat shift between the areas dominated by mineral soils (silt flats and moraines) and more developed soil with denser vegetation. Thus, some of the observed variation in ecological variables can be seen in relation to landscape features. A clear example are the elevated values of EC in vegetation type 8 Silt flats, 9 Scarce *Puccinellia* fields and 10 pioneer ground. These vegetation types are only registered at or close to a salt plain that originated when a glacier across the fiord surged for approximately 800 yrs ago, shovelling the seabed up in open air. The plain is still salty, and salt crystals are even precipitated on the ground in dry periods. The strong correlation of electroconductivity to vegetation plots and selected species in the CCA (Figure 14), and especially four *Puccinellia*-species, is explained by the salty conditions at the sampling site in question resulting in elevated electroconductivity. The soil carbon content in these vegetation types was also in the lower range, as expected in the mineral rich sandy soil, which is still in an early successional stage. The area is dominated by mineral soil with low soil carbon content, as soil development is a really slow process in the Arctic (Kabala et al., 2012). Carbon and nitrogen is accumulated over time in e.g. recently de-glaciated terrain, but this process is dependent on both water availability and temperature, which both are limiting factors in the Arctic (Kabala et al., 2012). pH levels are usually not noticeable lowered when with lower carbon content (Spencer, 2001; Spencer et al., 1997), which can explain the higher level of pH in these vegetation types. It is also shown that pH is one of the main factors determining arctic-alpine vegetation (Arnesen et al., 2007; Elvebakk, 1982). Although the silt flats are in the main wind rose from the coal heap at Kapp Amsterdam, and had high coal dust influence, the coal dust do not seem to affect the C content in the soil. The coal dust might be more likely to be resuspended at the salt plains, at least in the dry areas.

When leaving EC out of the ordination analysis (Figure 15), soil C-content and coal class are strong explanatory variables, but they do not describe any species distribution or explain any of the variation in the same direction. While pH, soil moisture and soil N-content explains the vegetation cover and species distribution in the same direction as soil C-content, coal dust cover goes in a approximately 90 degree opposite direction. This could be due to the coal

cover classification, as this value was roughly distributed across the vegetation classes and landscape variations, and not performed by robust measures. Alternatively, the explanation factor might be linked to increased temperature rather than C-content. The temperature was higher in highly coal dust influenced areas than in less influenced areas (Table 9). However, this difference have to be seen in relation to the placing of the loggers in either hillside or moraine areas, as the moister moraine areas could experience an temperature buffer because of the retained water's heat capacity. An analysis using GML to investigate the correlation between coal dust and soil carbon content should ideally have been performed.

The high soil carbon content of moderate snowbed vegetation (4) may be explained by the aggregation of snow that could lead to lower biodegradation rates, or maybe aggregation of coal dust during wintertime. Though Smit (1980) modelled that most coal dust do not travel further than 400 m from the source, and most of the snowbed vegetation in Svea are situated a moderate distance from the coal dispersal sources. Moderate snow bed also has a low pH, low EC and moderate soil N-content. Vegetation type 7 (Vegetated patterned ground and boulderfield) and 3 (moss tundra) were high in soil C-content and similar had a low soil pH. Both vegetation types are characterised by an uneven ground surface, as the many pits or tussocks with the stones intermixed. The soil N-content and EC differs between vegetation type 7 and 3, where vegetation type 3 have the higher EC and lower N-content. Higher EC implicates easier uptake and travel of nitrogen compounds, and might lead to a more effective N-uptake in the moss tundra (Dong et al., 2001; Gavito et al., 2001). Vegetation type 1 (Pond and stream edge) have similar ecological variables as vegetation type 7 and 3, the exception is the higher moisture level and the highly elevated EC-variable in vegetation type 1. Pond and stream edge might also be a vegetation type that aggregates and retains coal dust in winter time as well as the coal dust transported from surrounding areas by water flow in spring and early summertime. In undisturbed areas of Svalbard, the bed rock is the main factor affecting pH (Arnesen et al., 2007; Elvebakk, 1982), and pH and climate are the factors best describing the distribution of vegetation types (Elvebakk, 1982, 1997).

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P. B. Eidesen (2010) show a figure where the mean carbon content related to coal dust classification have are trending, but this is far from significant. The C values might correspond to distance from Kapp Amsterdam and the mine entrance and stacker north of the settlement, as area E and D have the lowest C values and are the furthest away from the coal sources. But this does not fit with the wind direction in Svea. The elevated C levels in some vegetation types might be natural consequences of the landscape, like the origin of the substrate where more developed soil in areas with older substrate might coincide with more developed vegetation cover that have reached a stable community structure, and are not in succession from pioneer to stable vegetation. More vegetation produces more C through degradation of plant material. Adding N to this process, increase the degradation. Old sea bed and moraines are mainly composed of mineral soil, and might still be pioneer areas not experience the same stable vegetation cover.

Thus, all in all, none of the measured abiotic factors could be related directly to coal dust seem to influence

The *Luzula* species have a skewed distribution and frequency between the vegetation types. Although some variation between 2002 and 2009 was detected, neither of the species has been found in the vegetation types 8, 9 or 10 during either of the vegetation surveys (Silt flats, Scarce *Puccinellia* fields and Pioneer ground, respectively). These vegetation types are clearly not preferred habitats for Luzula, and this means that these vegetation types represent landscape barriers for *Luzula*, and may add to the genetic structuring of Luzula in Svea.

Luzula confusa is the most abundant species of the two *Luzula* species studied, and is especially frequent in vegetation type 5 *Salix* heath. *Salix* heath is also the vegetation type with the lowest pH range and moderate soil moisture. These observations fits well to how the species ecological preferences are described in the literature. *Salix* heath (5) and open grassland (6) are, as with the silt flats, the vegetation types that had the lowest soil C-content. *Salix* heath and open grassland are in the wintertime a smooth surface where the coal dust can travel past. Though probably some coal dust are captured in summertime in the vegetation.

Luzula nivalis is less abundant than *Luzula confusa*, and are registered in vegetation type 1 Pond and stream edge and 3 Moss tundra. These vegetation types all have a slightly higher pH-value, and are also in general moister and have a lower soil N-content than vegetation type 5. The high levels of electroconductivity as was described in the literature fits with the high EC-values of vegetation type 1. The pH values found in Sveagruva might be influenced

by carbon content or coal, but does not fit with the description of alkali soil, as is mentioned as a ecological preference for *Luzula nivalis*.

Local differences in elevation sampling on tussocks or in pits, temperature, sun light/shadow, snow

5.2 Do genetic groups and diversity within Luzula sp. relate to abiotic/ biotic and/or human impact factors?

The genetic groups detected by clustering analyses were well genetically differentiated (AMOVA), but overall rather intermixed. The high level of differentiation suggests that some reproduction barriers exist between the groups. Thus, calculating genetic diversity based on geography rather reflected presence of different genetic groups and diversity as a result of dispersal, while populations as inferred by the clustering analyses, probably gave a better measure of genetic diversity as a result of gene flow.

The genetic groups were however not equally wide spread. Sveagruva lies in the Van Mijen fiord, which is connected to the Isfiord where the sampling sites Kapp Linné and Bjørndalen lies. Engelskbukta is situated further north (Figure 4), and is separated by a longer mountain range and the Isfiord. The Engelskbukta population consist of one single genetic group (group 5), while several genetic groups are present in Svea. Bjørndalen and Kapp Linné have genetic elements from both Engelskbukta and Sveagruva (Figure 20). Group 3 is the most abundant genetic group in both Bjørndalen and Kapp Linné, and are fairly abundant in Svea too. The resolution of the genetics of the sampling populations is shifted due to large differences in sampling size. The Svea dataset consists of 110 samples, while Engelskbukta only consists of 7. Here lies a possible explanation to the low level of genetic diversity and presence of only one genetic group in Engelskbukta. Especially presence of other genetic groups might have been overlooked due to lower sampling intensity in Engelskbukta.

However, the presence of several genetic groups in Svea does not seem to only rely on sampling intensity, as all transects contained at least three different genetic groups. Thus, it seems that Svea has an elevated number of genetic groups present.

Genetic diversities were high, and did not differ between sampling transects within Svea, Kapp Linné or Bjørndalen, but were significantly lower in Engelskbukta where only one

group was present. Kapp Linné and Engelskbukta have similar sample size, but do still differ in genetic diversity. This suggests that the low gene diversity in Engelskbukta is caused by other factors than statistical sample size. As Engelskbukta seem to consist of one single genetic lineage, while the other sampling sites consists of several genetic lineages, may suggest higher isolation of the population in Engelskbukta, and less dispersal and/or establishment in this area (Alsos et al., 2007)

Alleles with different optimum environments reflect the niches of individuals of a species. Could the clusters in Sveagruva be due to one or more of the investigated ecological variables? The canonical correspondence analysis (CCA, Figure 24) point out soil pH as the best explanatory variable investigated correlating to the variation within the Sveagruva population, as cluster 1 and 2 are distributed in the ranges of lower pH-values, and cluster 3 and 4 are distributed in ranges of higher pH-values. Though a clear division of induviduals related to genetic groups due to pH levels does not stand out, and suggest that there could be other and stronger factors not investigated here that would better explain the genetic clustering of *Luzula nivalis.* Carbon/coal and nitrogen holds the second and third explanatory axis, and likely also affect the diversity. Soil ph have been show to correlate with coal (Spencer, 2001; Spencer et al., 1997), and the correlation between ecological variables should have been calculated by using generalized linear models (GML).

Genetic group 2 is only found in Svea, and show a significant lower diversity than the other genetic groups. This could implicate that the individuals of group 2 have established in an ecological niche only available in Sveagruva. Group 2 are characterized by the lowest pH average and highest average soil C-content of the genetic lineages in Svea, and absent only in transect 7, which is one of the transect with a higher pH and lower soil C-content. Transect 7 is also characteristic as it are in one of the moraine areas, and quite far from the coal stockpiles at Kapp Amsterdam both with regard to distance and wind direction. In a species preferred vegetation type(s), where the species are abundant, a higher genetic diversity is expected as these vegetation types meet the ecological optimums of many more individuals compared to marginal vegetation types. If the preferred habitat for group 2 is restricted or fragmented, the low genetic diversity can be explained by low habitat availability and low efficient population size.

Alternatively, if this group recently arrived in Svea, it might have experienced founder effect leading to lower diversity, or some disturbance that have effected this group in particular, leading to a bottle neck within this group.

The deviation of a genetic lineages from the Hardy-Weinberg equilibrium are supported by F_{ST} = 0.53 for all *Luzula nivalis* groups and F_{ST}= 0.55 for *Luzula nivalis* groups within Sveagruva (AMOVA; Table 14) and describe the genetic lineages as well separated. Some lineages are more widespread than others; group 3 are identified both in the Van Mijen fiord and the Isfjorden. This separation into lineages of *Luzula nivalis* suggests the presence of one or more reproductive barriers that obstruct the gene flow between lineages. Peter Schönswetter et al. (2007) identified three genetic lineages in the alpine-arctic species *Juncus biglumis*, and the three lineages was supported with data on differences in genome size and genome level, suggesting the lineages to act as cryptic species. Záveská Drábková et al. (2010) found hybridization (which is often seen in young and incompletely isolated species) within the sect. Thyrsanochlamydeae where *Luzula nivalis* and *Luzula confusa* are members. Bozek et al. (2012) found high variation in chromosome number (2n= 6- 66) and genome size (2C = 0.55 – 8.55 pg) in six *Luzula* species.

Self-pollination can produce reproductively separated lineages by inbreeding and limiting influx of genes from other populations within the species. *Luzula nivalis* have not yet been found to self-pollinate, and Brochmann et al. (1999) suggest the Svalbard *Luzula* species to be sexually reproducing. Several *Luzula* species are identified as dichogamous, having flowers where the pistils and stamens mature at different times (Molau, 1993).This promotes a low selfing rate and higher degrees of outcrossing , as is also related to high pollen:ovule ratio(P:O) (Cruden, 1977). Michalski et al. (2010) measured high P:O in five *Luzula* species, and low P:O ratio in 19 *Juncus* species studied. Molau (1993) suggests that the reproductive strategy in arctic and alpine plants are strongly correlated to flowering phenology and snow cover duration, after investigating reproductive strategies in 137 tundra plant species where he found arctic *Luzula*-species sampled in northern Sweden to have a relative reproductive success differing between the 0.098 (early summer flowering *Luzula arcuata*) and the 0.895 (late summer flowering *Luzula parviflora*). Fryxell (1957) described *Luzula campestris* as cross-breeding and dichogamous, and *Luzula purpurea* as self-fertilizing.

The division into lineages may have occurred due to historical events, as suggested by the geographically inter-mixing of lineages. Svalbard was almost fully ice covered during the last

glaciation (Landvik et al., 2003), and was recolonized from different refugia where species survived the last glaciation (Alsos et al., 2007). Fragmentation into separate refugia during periods of glaciation leads to genetic structure due to reduced gene flow between refugial areas, and genetic drift. *Luzula nivalis* might have colonized Svalbrad from different source areas after the last glaciation, and the lineages I observed might therefor be due long time separation in different refugia. Similar pattern with several lineages in Svalbard due to different source areas is e.g. shown in *Vaccinium uliginosum* (P. B. Eidesen et al., 2007).

Local migration and colonization between the genetic populations are likely to take place along the coastline, as the mountains partly function as a landscape barrier. Seeds of *Luzula* species are wind distributed; commonly in wintertime when the snow covered landscape and frozen fiords contribute to the seed spreading. Pollen grains are also wind spread, but might be slightly more challenged when crossing open water. The route of migration from Engelskbukta would have to go by Bjørndalen and then Kapp Linné to reach Sveagruva. This route of migration or re-colonization might explain parts of the distribution of the genetic groups, and maybe also the absence of group 5 in Sveagruva, as it might just have not colonized that area yet.

The four genetic groups present in Sveagruva could also have originated from human mediated dispersal, as there have been human activities in the area for a century, which is enough time for establishing generations of individuals of possible genetic foreign populations.

The AFLP-method produces mostly neutral markers, which also lead to neutral variation in the dataset. Neutral variation due to neutral selection as migration, bottleneck events or Stochastic events wiping out whole population in an area as when building roads or flooding in springtime may have erased parts of the genetic

Cryptic species are species segregated into groups incapable of breeding with each other and are in that sense undergoing speciation. Cryptic species groups cannot yet be distinguished by morphological traits and are thus still considered the same species. Reproductive isolated groups have recently been discovered in the Arctic species *Saxifraga oppositifolia* L. (P. B. Eidesen et al., in press). P. B. Eidesen et al. (in press) identified the correlation of growth form, pH optimum range and ridge/ snowbed preferences to differences in levels of polyploidy. This might also explain the lineages seen in *Luzula nivalis*. The genetic groups present in Sveagruva show differences in pH levels, soil nitrogen and carbon content (Table

13) that seem to correlate. Engelskbukta (two soil sampling points) and Bjørndalen (17 soil sampling points) show a higher soil pH-level than Sveagruva, and we could speculate if differences in pH optimum could be reflected in the observed genetic differences. Identification of adaptive alleles might have helped in identifying ecological variables likely to influence the genetic lineages, though the variation produced using AFLP-markers are predominantly neutral.

No clear relation between genetic groups and landscape structures can be identified at first glance. Though pH is related to vegetation class, and could probably aid in explaining the assumed differences in ecological optimums between the genetic lineages.

5.3 Methodical errors

There are several sources to error during execution of genetic analysis (Pompanon et al., 2005). During the CTAB DNA-extraction, one methodical error is known as the samples after addition of isoamylalchohol was kept at room temperature. This step is crucial in deposition of DNA, and is more effective when the samples are kept cold.

When opening PCR-strips there are always chances of sample contamination if drops from the lid spill into other samples. Sample contamination can also happen if the content of the pipette is ejected too fast causing the sample or drops of the sample to splash over to the neighbouring samples.

Another methodical error is known, as the digestion-ligation product was kept in the fridge for one to four days upon being used in the pre-selective PCR. At this temperature the restriction enzymes are still active and natural DNA degradation is possible, and storage at -18°C would have been ideal. This methodical error is the assumed origin of plate specific markers, as the digestion-ligation procedure was partitioned between four plates conducted at four differing dates.

The averages of soil temperature loggings for July 2009 have not been calculated with regard to which dates in July 2009 the HOBO-loggers were placed in the field. The temperatures measured in July are at least a little higher than the actual soil temperature, but does reflect the air temperature at the time as the loggers were kept outside upon placing in field.

5.4 How could this study be improved?

The supervised classification was performed using red, green and blue bands. In the image used (see figure 3 in methods section), most of the wet areas as the fiord and ponds had several pixel values identical or similar to the pixel values of vegetated areas. Similar pixel values were also caused by shadows and a mud-stream from a river falling into the fiord. The distribution of vegetation in the landscape is better distinguished from water or bedrock through application of a band of near infrared (IR) imagery data to produce a Normalized Difference Vegetation Index (NDVI) (Jackson et al., 1983; Tucker et al., 1979; Tucker et al., 1991), and a fourth band would have been ideal when classifying vegetation in Svea and simulating this data for the *Luzula* samples. A higher number of training sites could also have resulted in a more precise classification. During supervised classification choices concerning which groups to combine, is another factor contributing to the uncertainties of the final vegetation class map. Though it is plausible that the use of different methods and classification systems may explain parts of the observed differences.

6 References

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Alsos, Inger Greve, Eidesen, Pernille Bronken, Ehrich, Dorothee, Skrede, Inger, Westergaard, Kristine, Jacobsen, Gro Hilde, . . . Brochmann, Christian. (2007). Frequent Long-Distance Plant Colonization in the Changing Arctic. *Science, 316*(5831), 1606-1609. doi: 10.1126/science.1139178

Arctic Ocean location map. 27.01.2012). Retrieved 14.03.2013, 2013, from http://commons.wikimedia.org/wiki/File:Arctic_Ocean_location_map.svg?uselang=nb

Arnesen, Geir, Beck, Pieter S. A., Engelskj, oslash, & n, Torstein. (2007). Soil Acidity, Content of Carbonates, and Available Phosphorus Are the Soil Factors Best Correlated with Alpine Vegetation: Evidence from Troms, North Norway. *aare Arctic, Antarctic, and Alpine Research, 39*(2), 189-199.

Askaer, Louise, Schmidt, Lea, Elberling, Bo, Asmund, Gert, & Jónsdóttir, Ingibjörg. (2008). Environmental Impact on an Arctic Soil–Plant System Resulting from Metals Released from Coal Mine Waste in Svalbard (78° N). *Water, Air, & Soil Pollution, 195*(1), 99-114. doi: 10.1007/s11270-008-9730-z

Bates, Carole S. Griffiths; John M. (2002). Morphology, genetics and the value of voucher specimens: An example with Cathartes vultures. *Journal of Raptor Research, 36*(3), 183-187.

Blowes, David W., Jambor, J. L., & Alpers, Charles N. (1994). *The environmental geochemistry of sulfide mine-wastes*. Nepean, Ont., Canada: Mineralogical Association of Canada.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Mol Ecol, 13*(11), 3261-3273. doi: MEC2346 [pii]

10.1111/j.1365-294X.2004.02346.x

Bonin, A., Nicole, F., Pompanon, F., Miaud, C., & Taberlet, P. (2007). Population Adaptive Index: a New Method to Help Measure Intraspecific Genetic Diversity and Prioritize Populations for Conservation. *Conservation Biology, 21*(3), 697-708. doi: 10.1111/j.1523-1739.2007.00685.x

Bozek, M., Kuta, E., Leitch, A. R., Leitch, I. J., & Zaveska Drabkova, L. (2012). Chromosome and genome size variation in Luzula (Juncaceae), a genus with holocentric chromosomes. *Bot. J. Linn. Soc. Botanical Journal of the Linnean Society, 170*(4), 529-541.

Brochmann, Christian, & Steen, Snorre W. (1999). Sex and genes in the flora of Svalbard. from http://www.binran.ru/projects/paf/papers/sex%26genes/tabl.htm

Brooker, Rob, & van der Wal, RenÈ. (2003). Can soil temperature direct the composition of high arctic plant communities? *J Veg Sci Journal of Vegetation Science, 14*(4).

Carlsonn, Catherine A. (1990). Subsurface leachate migration from a reject coal pile in South Carolina. *Water, Air, and Soil Pollution, 53*(3-4), 345-366. doi: 10.1007/BF00170748

Chapin, F. S., & Koerner, C. (1996). Arctic and Alpine Biodiversity: Its Patterns, Causes and Ecosystem Consequences. *SCOPE report., 55*, 7.

Corander, J., Marttinen, P., & Mantyniemi, S. (2006). A Bayesian method for identification of stock mixtures from molecular marker data. *Fishery Bulletin, 104*(4), 550-558.

Corander, J., Marttinen, P., Siren, J., & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *Bmc Bioinformatics, 9*, 539. doi: 1471-2105-9-539 [pii]

10.1186/1471-2105-9-539

Corander, J., Siren, J., & Arjas, E. (2008). Bayesian spatial modeling of genetic population structure. *Computational Statistics, 23*(1), 111-129. doi: DOI 10.1007/s00180-007-0072-x

Corander, J., Waldmann, P., & Sillanpaa, M. J. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics, 163*(1), 367-374.

Cruden, R. W. (1977). Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution, 31*, 32-46.

Dong, S., Scagel, C. F., Cheng, L., Fuchigami, L. H., & Rygiewicz, P. T. (2001). Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. *Tree Physiology Tree Physiology, 21*(8), 541-547.

Doyle, J. J., & Dickson, E. E. . (1987). Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon, 36*, 715-722.

Doyle, J.J., & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, 19*, 11-15.

Ehrich, D. (2006). AFLPDAT: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes, 6*(3), 603-604. doi: DOI 10.1111/j.1471-8286.2006.01380.x

Ehrich, D., Gaudeul, M., Assefa, A., Koch, M. A., Mummenhoff, K., Nemomissa, S., & Brochmann, C. (2007). Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. *Mol Ecol, 16*(12), 2542-2559. doi: MEC3299 [pii]

10.1111/j.1365-294X.2007.03299.x

Eidesen, P. B. (2010). Vegetasjonskartlegging og overvåking i Svea - Påvirker gruvedriften vegetasjonen? (pp. 51): UNIS.

Eidesen, P. B. , Müller, E., Lettner, C., Alsos, I. G., Bender, M., Kristiansen, M., . . . Verweij, K. F. (in press). Tetraploids do not form cushions: association of ploidy level, growth form and ecology in the High Arctic Saxifraga oppositifolia L. s. lat. (Saxifragaceae) in Svalbard. *Polar Research*.

Eidesen, P. B., Alsos, I. G., Popp, M., Stensrud, ÿ, Suda, J., & Brochmann, C. (2007). Nuclear vs. plastid data: complex Pleistocene history of a circumpolar key species. *Molecular Ecology, 16*(18), 3902-3925.

Elberling, B. , Soendergaard, J., Jensen, L.A. , Schmidt, L.B., Hansen, B.U., Asmund, Gert, . . . Friborg, Thomas (2007). Arctic vegetation damage by winter-generated coal mining pollution released upon thawing. *Environmental Science and Technology, 41*(7), 2407-2413.

Elvebakk, Arve. (1982). Geological preferences among svalbard plants. *INTER-NORD, 16*, 11-31.

Elvebakk, Arve. (1997). Tundra diversitiy and ecological characteristics of Svalbard

Engelskjøn, Torstein. (1984). *Barduvassdraget : flora og vegetasjon i Barduvassdraget ovenfor Altevatn* (Vol. 36). Tromsø: Universitetet i Tromsø, Institutt for museumsvirksomhet.

ESRI. (2011). ArcGIS Environmental Systems Resource Institute. Redlands, CA.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology, 14*(8), 2611-2620. doi: 10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources, 10*(3), 564-567. doi: Doi 10.1111/J.1755-0998.2010.02847.X

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes, 7*(4), 574-578. doi: 10.1111/j.1471-8286.2007.01758.x

Farmer, A. M. (1993). The Effects of Dust on Vegetation - a Review. *Environmental Pollution, 79*(1), 63-75. doi: Doi 10.1016/0269-7491(93)90179-R

Ford, Michael J. (2002). Applications of selective neutrality tests to molecular ecology. *Mol Ecol Molecular Ecology, 11*(8), 1245-1262.

Fryxell, PaulA. (1957). Mode of reproduction of higher plants. *The Botanical Review, 23*(3), 135-233. doi: 10.1007/BF02869758

Gaudeul, M., Taberlet, P., & Till-Bottraud, I. (2000). Genetic diversity in an endangered alpine plant, Eryngium alpinum L.. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology, 9*(10), 1625-1637. doi: Doi 10.1046/J.1365-294x.2000.01063.X

Gavito, Mayra E., Curtis, Peter S., Mikkelsen, Teis N., & Jakobsen, Iver. (2001). Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *Journal of Experimental Botany, 52*(362), 1913-1923. doi: 10.1093/jexbot/52.362.1913

Gjærevoll, Olav, & Det Kongelige norske videnskabers selskab. (1990). *Alpine plants*. Trondheim: Tapir : The Royal Norwegian Society of Sciences and Letters.

Gough, Laura, Shaver, Gaius R., Carroll, Jenny, Royer, Dana L., & Laundre, James A. (2000). Vascular Plant Species Richness in Alaskan Arctic Tundra: The Importance of Soil pH. *Journal of Ecology, 88*(1), 54-66. doi: 10.2307/2648485

Hammer, Øyvind, Harper, David A. T., & Ryan, Paul D. (2001). Past paleontological statistics software package for educaton and data anlysis.

Hirano, T., Kiyota, M., & Aiga, I. (1995). Physical effects of dust on leaf physiology of cucumber and kidney bean plants. *Environmental Pollution, 89*(3), 255-261.

Holderegger, R., & Wagner, H. H. (2008). Landscape genetics. *Bioscience, 58*(3), 199-207. doi: Doi 10.1641/B580306

Jackson, R. D., Slater, P. N., & Pinter, P. J. (1983). Discrimination of growth and water stress in wheat by various vegetation indices through clear and turbid atmospheres. *Remote Sensing of Environment Remote Sensing of Environment, 13*(3), 187-208.

Jones, H. G. (2001). *Snow ecology : an interdisciplinary examination of snow-covered ecosystems*. Cambridge: Cambridge University Press.

Kabala, C., Waroszewski, J., Bogacz, A., & Labaz, B. (2012). On the Specifics of Podzols in Mountain Areas. *Soil Science Annual, LXIII*(2), 55-64.

Kvello, Jan. (2004). *Om å arbeide i en politisk bedrift på Svalbard 1987 - 2005*.

Kvello, Jan. (2006). *Fra privat til statlig eierskap 1945 - 1975*.

Landvik, Jon Y., Brook, Edward J., Gualtieri, Lyn, Raisbeck, Grant, Salvigsen, Otto, & Yiou, Francoise. (2003). Northwest Svalbard during the last glaciation: Ice-free areas existed. *Geology., 31*(10), 905.

Larsen, Daniel, & Mann, Richard. (2005). Origin of high manganese concentrations in coal mine drainage, eastern Tennessee. *Journal of Geochemical Exploration Journal of Geochemical Exploration, 86*(3), 143-163.

Lid, Johannes, & Lid, Dagny Tande. (2005). *Norsk flora*. Oslo: Samlaget.

Lowe, Andrew, Ashton, Paul, & Harris, Stephen. (2004). *Ecological genetics : design, analysis, and application*. Malden, Mass.: Blackwell Publ.

Marchand, Peter J. (1996). *Life in the cold : an introduction to winter ecology* (3rd ed.). Hanover: University Press of New England.

Martinussen, Berit. (2005). *Et Arktisk omstillingseventyr 1990 - 2002*.

Michalski, StefanG, & Durka, Walter. (2010). Pollen and ovule production in wind-pollinated species with special reference to Juncus. *Plant Systematics and Evolution, 286*(3-4), 191-197. doi: 10.1007/s00606-010-0299-9

Miller, Bruce G. (2011). 4 - The Effect of Coal Usage on Human Health and the Environment *Clean Coal Engineering Technology* (pp. 85-132). Boston: Butterworth-Heinemann.

Moen, Asbjørn, Lillethun, Arvid, & Odland, Arvid. (1999). *Vegetation*. Hønefoss: Norwegian Mapping Authority.

Molau, Ulf. (1993). Relationships between Flowering Phenology and Life History Strategies in Tundra Plants. *Arctic and Alpine Research, 25*(4), 391-402. doi: 10.2307/1551922

Mueller, U. G., & Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. *Trends in Ecology & Evolution, 14*(10), 389-394.

Maarel, E. van der. (2005). *Vegetation ecology*. Malden, MA: Blackwell Pub.

Naidoo, G. (2004). The effects of coal dust on photosynthetic performance of the mangrove, Avicennia marina in Richards Bay, South Africa. *Environmental Pollution, 127*(3), 359-366. doi: 10.1016/j.envpol.2003.08.018

Naidoo, G., & Naidoo, Y. (2005). Coal Dust Pollution Effects on Wetland Tree Species in Richards Bay,South Africa. *Wetlands Ecology and Management, 13*(5), 509-515. doi: 10.1007/s11273-004-3939-4

NASA, AERONET Hornsund Subsets. (2011). Terra 250m True Color. 2011, from http://lance-modis.eosdis.nasa.gov/imagery/subsets/?project=arctic&subset=AERONET_Hornsund

Norwegian Polar Institute. (2000). Landskapsbeskrivelse for området Bellsund – Braganzavågen. Notat. Norsk polarinstitutt. .

NorwegianMeteorologicalInstitute. (2011). eKlima@met.no. Retrieved 21.5, from http://sharki.oslo.dnmi.no/portal/page?_pageid=73,39035,73_39049&_dad=portal&_schema=PORTAL

Pompanon, F., Bonin, A., Bellemain, E., & Taberlet, P. (2005). Genotyping errors: causes, consequences and solutions. *Nat Rev Genet, 6*(11), 847-859. doi: 10.1038/nrg1707

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Raup, Hugh M. (1969). *The relation of the vascular flora to some factors of site in the Mesters Vig district, Northeast Greenland*. K¯benhavn: C. A. Reitzel.

Rikard, Michael, & Kunkle, Sam. (1990). Sulfate and conductivity as field indicators for detecting coal-mining pollution. *Environmental Monitoring and Assessment, 15*(1), 49-58. doi: 10.1007/BF00454748

Rønning, Olaf I., Brekke, Annemor, & Lid, Dagny Tande. (1996). *The flora of Svalbard*. Oslo: Norsk polarinstitutt.

Sakai, A., & Larcher, W. (1987). *Frost survival of plants : responses and adaptation to freezing stress*. Berlin; New York: Springer-Verlag.

Schönswetter, P., Tribsch, A., Barfuss, M., & Niklfeld, H. (2002). Several Pleistocene refugia detected in the high alpine plant Phyteuma globulariifolium sternb & hoppe (Campanulaceae) in the European Alps. *Mol Ecol, 11*(12), 2637-2647.

Schönswetter, Peter, Suda, Jan, Popp, Magnus, Weiss-Schneeweiss, Hanna, & Brochmann, Christian. (2007). Circumpolar phylogeography of Juncus biglumis (Juncaceae) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. *Molecular Phylogenetics and Evolution, 42*(1), 92-103. doi: 10.1016/j.ympev.2006.06.016

Sharma, Parveen Kumar, & Singh, Guardeep. (1992). Air Pollution In Coal Mining Areas - An Appraisal. *Minetech, 13*(3), 21-28.

Smit, P. P. A. (1980). Dust concentrations around coal stockpiles. *Science of The Total Environment Science of The Total Environment, 15*(3), 207-216.

SNSK. (2000-2001). Annual report.

SNSK. (2001). Svearapporten - Konsekvensutredning for Svea Nord (pp. 142).

SNSK. (2005). Annual report.

SNSK. (2009). Spesifikasjon energikull 2006-2009 (pp. 2): SNSK.

Spencer, S. (2001). Effects of coal dust on species composition of mosses and lichens in an arid environment. *Journal of Arid Environments, 49*(4), 843-853. doi: 10.1006/jare.2001.0816

Spencer, S., & Tinnin, R. (1997). Effects of coal dust on plant growth and species composition in an arid environment. *Journal of Arid Environments*(37), 475-485.

StatistiskSentralbyrå. (1952). Norges Bergverksdrift 1950 - Oppgaver over bergverksdriften på Svalbard i året 1950. *Norges offisielle statistikk, XI*(105), 41.

StatistiskSentralbyrå. (2013). Statistisk årbok (flere år) Kulldriften. Svalbard. from http://www.ssb.no

Team, Quantum GIS Development. (2013). Quantum GIS Geographic Information System: Open Source Geospatial Foundation. Retrieved from http://qgis.osgeo.org

Team, R Core. (2011). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Ter Braak, C.J.F. (1988). CANOCO – a FORTRAN program for canonical community ordination by canonical correspondence analysis (Version 3.0). *Tech. Rpt, LWA-88-02*(Agricultural Mathematics Group, Wageningen, The Netherlands), 95 pp.

Tkach, N., Roser, M., & Hoffmann, M. (2008). Range size variation and diversity distribution in the vascular plant flora of the Eurasian Arctic. *Organisms Diversity & Evolution Organisms Diversity & Evolution, 8*(4), 251-266.

Tucker, C. J., & Nasa, Goddard Space Flight Center Earth Resources Branch Greenbelt Md. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sensing Environ., 8*, 127-150.

Tucker, C. J., Newcomb, W. W., Los, S. O., & Prince, S. D. (1991). Mean and inter-year variation of growing-season normalized difference vegetation index for the Sahel 1981-1989. *International Journal of Remote Sensing International Journal of Remote Sensing, 12*(6), 1133-1135.

Virtanen, Risto. (1996). *Arctic and oroarctic vegetation patterns in Northern Europe as a consequence of topography, climate, bedrock conditions and grazing.* University of Oulu, Oulu, Finland. Available from http://worldcat.org /z-wcorg/ database.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., . . . et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res, 23*(21), 4407-4414. doi: 5w0130 [pii]

Wessel, P. , & Smith, W. H. F. (Cartographer). (2013). Global Self-consistent Hierarchical High-resolution Geography Database (GSHHG).

Westby, Sigurd. (2003). *Store Norske Spitsbergen Kullkompani 1916 - 1945*.

Wipf, Sonja, Rixen, Christian, & Mulder, Christa P. H. (2006). Advanced snowmelt causes shift towards positive neighbour interactions in a subarctic tundra community. *Global Change Biology, 12*(8), 1496-1506. doi: 10.1111/j.1365-2486.2006.01185.x

http://www.bioportal.uio.no. (2011). Bioportal. Retrieved April-May, 2011

Young, Kathy L., Woo, Ming-ko, & Edlund, Sylvia A. (1997). Influence of Local Topography, Soils, and Vegetation on Microclimate and Hydrology at a High Arctic Site, Ellesmere Island, Canada. *Arctic and alpine research., 29*(3), 270.

Záveská Drábková, L., & Vlček, Č. (2010). Molecular phylogeny of the genus Luzula DC. (Juncaceae, Monocotyledones) based on plastome and nuclear ribosomal regions: a case of incongruence, incomplete lineage sorting and hybridisation. *Molecular Phylogenetics and Evolution, 57*(2), 536-551.

Aamaas, B., Boggild, C. E., Stordal, F., Berntsen, T., Holmen, K., & Strom, J. (2011). Elemental carbon deposition to Svalbard snow from Norwegian settlements and long-range transport. *Tellus Series B-Chemical and Physical Meteorology, 63*(3), 340-351. doi: Doi 10.1111/J.1600-0889.2011.00531.X

Appendix

Appendix 1

Table 15 Results from analysis of the soil samples sampled close to the vegetation analysis plots or the revisited plots from 2002. Temperature of soil solution when measuring conductivity (in Siemens, µS/cm) and pH was 22.1±0.7°C.

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
09C2	53.5	5.8	-	-	4	C	3	2
09C3/0936	109.3	5.7	-	-	5	C	3	2
09D4/0919	109.1	5.6	0.0295	2.3079	2	D	2	2
09D5	97.9	5.6	-	-	3	D	2	3
09K7	126.2	6.6	0.0203	12.9677	2	K	4	3
09E12	191.5	6.0	-	-	5	E	2	3
09E14/0943	139.5	5.0	0.0340	3.5674	5	E	2	2
09E16	134.0	6.0	-	-	5	E	2	2
09D22	98.2	5.6	-	-	4	D	2	3
09D23	106.7	5.6	-	-	4	D	2	3
09D24	79.4	5.7	-	-	4	D	2	2

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
09E31/0945	270.0	6.8	0.0180	1.8363	6	E	2	1
09H36/0942	1475.0	6.9	0.0541	7.8587	9	H	2	1
09G39/0941	2000.0	6.8	0.0684	6.8646	9	G	2	1
09D43/0937	73.7	5.4	-	-	6	D	2	2
09D43	117.4	4.6	0.0450	5.4449	6	D	2	2
09H51	7690.0	7.0	0.0363	2.4711	9	H	2	3
09H53	3260.0	7.3	0.0547	9.1677	9	H	2	3
09D55	60.4	5.2	-	-	7	D	2	2
09D56	52.3	6.5	-	-	7	D	2	1
09K58	236.0	6.0	-	-	3	K	4	3
09K66	2610.0	5.8	-	-	1	K	4	2
09K68	79.1	6.5	-	-	2	K	4	2
09K70	316.0	6.1	-	-	2	K	4	2
09K72	128.2	6.6	0.0808	11.8309	3	K	4	2
09J75	110.4	6.3	-	-	3	J	2	2
09J77	91.9	5.9	-	-	2	J	2	2

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
09J80	182.9	5.6	0.0327	4.0375	3	J	2	
09J82	1101.0	6.1	-	-	1	J	2	3
09I85	429.0	5.9	0.1727	21.4418	1	I	2	3
09I86	164.2	6.2	0.0615	0.9505	2	I	2	3
09C94	74.7	5.8	-	-	1	C	3	4
09C96	116.5	5.8	-	-	1	C	3	4
09C98	100.1	5.9	-	-	2	C	3	3
09C99	197.1	4.9	-	-	3	C	3	3
09C100	216.0	5.0	-	-	3	C	3	4
09C101	93.6	5.9	0.0579	6.1756	2	C	3	2
09C102	87.6	5.6	-	-	3	C	3	3
09C104	58.2	5.6	-	-	2	C	3	3
09C106	119.2	5.3	-	-	3	C	3	2
09D107	121.2	5.0	n.a.	0.0346	1	D	2	3
09D108/0938	116.3	5.2	0.0716	8.6755	1	D	2	3
09A110	87.6	5.7	0.1574	14.3618	2	A	4	

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
09F113	238.0	7.0	0.1250	13.7816	7	F	2	2
09F116	79.0	5.3	0.0804	6.1310	7	F	2	2
09G124	9860.0	5.9	-	-	10	G	2	2
09G130	1875.0	6.0	-	-	10	G	2	2
09G131/0940	6040.0	7.5	0.0850	2.9906	8	G	2	2
09G136	1585.0	7.0	-	-	8	G	2	2
09I137	3800.0	6.5	-	-	8	I	2	2
09I139	980.0	6.2	-	-	8	I	2	2
09F147	224.0	6.1	-	-	7	F	2	2
09F148	135.6	6.8	-	-	7	F	2	1
09D154	77.0	5.2	0.0649	6.6605	7	E	2	
09E157	110.2	5.3	0.0306	4.1505	7	E	2	3
C160/0935	59.6	5.9	0.0732	9.0739	4	C	3	2
09C161	60.8	4.9	-	-	7	C	3	2
09C164	84.5	5.6	0.0606	7.6669	11	C	3	2
09C171	142.9	5.1	-	-	7	C	3	1

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
09C172	135.3	5.3	-	-	7	C	3	2
09C173	40.1	5.8	0.0920	13.0614	7	C	3	1
0901	220.0	6.1	-	-	2	A	4	2
0903	112.0	6.5	0.0741	7.5215	7	F	2	2
0904	329.0	6.1	0.0076	1.6592	7	F	2	2
0906	120.1	6.1	-	-	3	D	2	4
0908	1805.0	5.8	0.0320	3.4345	7	C	3	2
0909	102.2	5.7	0.0199	2.1264	1	C	3	4
0910	94.6	5.8	0.0516	4.4914	3	C	3	3
0911	214.0	5.7	0.1652	26.8465	3	C	3	4
0913	209.0	5.7	0.0602	6.5522	4	E	2	2
0914	1482.0	5.6	0.1215	15.8549	1	K	4	4
0915	358.0	6.3	0.0248	3.8930	3	K	4	3
0916	610.0	6.8	0.0895	9.9644	2	K	4	3
0919/09D4	109.1	5.6	0.0295	2.3079	2	D	2	2
0920	65.8	5.2	-	-	4	D	2	2

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
0921	7310.0	6.6	0.0588	5.3321	8	I	2	2
0922	3260.0	6.8	0.0171	1.9646	8	I	2	2
0924	120.3	5.1	n.a.	2.0631	3	X	1	2
0925	132.9	4.7	0.0957	23.0054	3	X	1	2
0926	117.5	6.0	0.0391	5.1164	3	J	2	3
0927	252.0	5.8	0.0364	4.5802	5	J	2	2
0928	295.0	6.3	0.0159	3.8988	3	K	4	2
0929	518.0	6.5	0.0888	19.3697	4	K	4	3
0930	222.0	5.7	0.0144	1.5398	3	X	1	3
0931	98.3	5.6	0.0352	3.6768	5	E	2	2
0932	104.5	5.7	0.0215	2.1521	3	E	2	3
0933	153.1	5.7	0.2834	6.9015	5	A (ØST)	4	2
0934	130.0	5.7	0.1630	23.2288	4	A (ØST)	4	3
0935/C160	59.6	5.9	0.0732	9.0739	4	C	3	2
0936/09C3	109.3	5.7	-	-	5	C	3	2
0937/09D43	73.7	5.4	-	-	6	D	2	2

Table 1 Continued

Sample name: veg. plot	Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Vegetation type	Area	Coal dust cover	Moisture
0938/D108	116.3	5.2	0.0716	8.6755	1	D	2	3
0940/09G131	6040.0	7.5	0.0850	2.9906	8	G	2	2
0941/09G39	2000.0	6.8	0.0684	6.8646	9	G	2	1
0942/09H36	1475.0	6.9	0.0541	7.8587	9	H	2	1
0943/09E14	139.5	5.0	0.0340	3.5674	5	E	2	2
0945/09E31	270.0	6.8	0.0180	1.8363	6	E	2	1
0946	147.1	5.9	0.0733	7.7142	3	C	3	3
0947	78.3	5.7	0.0357	3.2851	4	C	3	2

Appendix 2

Table 16 Results from analysis of the soil samples sampled close to or at the site of the genetic transect for the species Luzula confuse and L. nivalis. Temperature of soil solution when measuring conductivity (in Siemens, µS/cm) and pH was 22.1±0.7°C. Comments column is abbreviated as: ph – pH measured one day later than conductivity, Neg. CHN – Negative peak for the CHN analysis.

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
1-1/0946	LC	LN	147.1	5.85	0.0733	7.7142		C	3	
1-4/0947	LC	LN	78.3	5.73	0.0357	3.2851		C	3	
1-1	LC	LN	102.6	5.73	-	-	7	C	3	
2-1	LC	LN	97.0	5.57	0.1501	13.8137	7	C	3	
2-2	LC	LN	74.4	6.07	-	-	3	C	3	
2-3	LC	LN	36.0	5.83	-	-	7	C	3	pH
2-4	LC	LN	46.6	5.68	-	-	5	C	3	
2-5	LC	LN	46.6	5.56	-	-	3	C	3	
3-1	LC	LN	77.9	5.82	0.0975	7.7846	3	C	3	
3-2	LC	LN	74.5	5.55	-	-	3	C	3	
3-3	LC	LN	72.9	6.50	-	-	3	C	3	pH
3-4	LC	LN	73.6	5.91	-	-	3	C	3	
3-5	LC	LN	112.5	6.16	-	-	7	C	3	pH
4-1	LC	LN	87.2	5.82	-	-	7	C	3	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
4-2 LC	LC		77.3	5.71	0.1673	5.1317	10	C	3	
4-3	LC	LN	61.0	5.42	0.0505	3.7514	7	C	3	
4-4	LC	LN	54.0	5.98	-	-	7	C	3	
4-5 LC	LC		88.4	5.53	-	-	7	C	3	
4-5 LN		LN	111.0	5.77	-	-	7	C	3	
4-6 LN		LN	55.1	5.16	0.3289	9.9558		C	3	
5-8/0924	LC	LN	120.3	5.12	n.a.	2.0631		X	1	
5-1 LC = 0925	LC	LN	132.9	4.74	0.0957	23.0054	5	X	1	
6-1	LC	LN	54.6	5.83	-	-	3	X	1	pH
7-1	LC	LN	68.4	6.76	-	-	2	X	1	pH
8-1	LC	LN	117.2	6.26	n.a.	0.023	3	X	1	
9-1	LC	LN	68.4	5.86	0.1444	15.4077	2	E	2	
13-16/0933	LC	LN	153.1	5.70	0.2834	6.9015		A (East)	4	pH
13-16/0934	LC	LN	130.0	5.65	0.163	23.2288		A (East)	4	
13-1 LC	LC		68.4	6.16	0.0647	2.671	7	A	4	
13-1 LN		LN	233.0	5.70	-	-		A	4	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
13-2 LC	LC		174.0	5.92	-	-	7	A	4	
13-3 LC	LC		219.0	5.90	-	-	7	A	4	
13-4	LC	LN	246.0	6.07	-	-	2	A	4	
13-5 LC+LN	LC	LN	168.3	6.07	-	-	3	A	4	
14-1 LC	LC		81.6	5.98	0.219	26.8583	7	A	4	pH
14-1 LN		LN	108.2	5.08	-	-		A	4	
14-2 LC	LC		98.5	5.18	-	-	7	A	4	
14-3 LC	LC		102.7	5.92	-	-	11	A	4	
14-4 LC	LC		133.7	4.92	-	-	7	A	4	
14-4 LN		LN	127.4	5.07	0.0551	5.2751	7	A	4	
14-5	LC	LN	91.5	5.13	-	-	8	A	4	
15-1 LC	LC		48.6	5.55	0.0902	20.4365	7	A	4	
15-1 LN		LN	60.0	5.35	-	-	7	A	4	
15-2 LC	LC		114.7	5.99	-	-	7	A	4	
15-2 LN		LN	44.6	5.56	-	-	7	A	4	
15-3 LC	LC		83.0	6.08	-	-	7	A	4	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
15-3 LN		LN	64.9	5.56	0.0259	2.8334	7	A	4	
15-4	LC	LN	123.7	6.33	-	-	7	A	4	
15-5	LC	LN	52.0	6.06	-	-	7	A	4	
16-1	LC	LN	88.4	5.90	-	-		A	4	pH
16-2 LC	LC		96.2	5.99	-	-	4	A	4	pH; ;Neg. CHN
16-3 LC	LC		115.8	5.77	-	-		A	4	
16-4/27-2	LC	LN	126.5	5.98	-	-	8	A	4	
16-5	LC	LN	114.0	5.94	-	-	10	A	4	
16-6 LN		LN	120.8	5.39	-	-	3	A	4	
16-7 LN		LN	154.5	5.56	0.0764	6.6297		A	4	
17-20/0928	LC	LN	295.0	6.25	0.0159	3.8988		K	4	
17-20/0929	LC	LN	518.0	6.47	0.0888	19.3697		K	4	
17-1/HOBO-07	LC	LN	164.9	6.23	0.069	6.1252	2	K	4	
17-2	LC	LN	137.2	6.36	-	-	2	K	4	
17-3	LC	LN	914.0	5.79	-	-	2	K	4	
17-4 LC	LC		247.0	6.27	-	-	2	K	4	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
17-4 LN		LN	439.0	6.33	-	-	2	K	4	
17-5 LN		LN	134.9	6.52	-	-	2	K	4	
18-1	LC	LN	596.0	5.12	0.2159	13.8153	2	K	4	
18-2	LC	LN	429.0	5.14	-	-	2	K	4	
18-3	LC	LN	383.0	6.50	-	-	2	K	4	
18-4	LC	LN	258.0	5.33	-	-	3	K	4	
18-5	LC	LN	188.8	6.41	-	-	1	K	4	
19-1	LC	LN	216.0	6.68	n.a.	n.a.	3	K	4	Neg. CHN
19-2	LC	LN	102.4	5.73	-	-	2	K	4	
19-3	LC	LN	179.1	5.65	-	-	7	K	4	
19-4	LC	LN	340.0	5.81	-	-	2	K	4	
19-5	LC	LN	92.3	5.81	-	-	2	K	4	
20-1	LC	LN	194.5	6.14	-	-	2	K	4	pH
20-2	LC	LN	231.0	6.44	-	-	2	K	4	
20-3	LC	LN	154.3	6.50	-	-	2	K	4	
20-4 LC+LN	LC	LN	177.1	6.24	0.1731	5.3545	2	K	4	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
20-5	LC	LN	186.6	6.12	-	-	1	K	4	
21-24/0926	LC	LN	117.5	5.95	0.0391	5.1164		J	2	
21-24/0927	LC	LN	252.0	5.78	0.0364	4.5802		J	2	
21-2 LC	LC		161.1	5.54	0.0409	3.5318	7	J	2	pH
21-2 LC	LC		82.4	5.81	-	-		J	2	
21-3 LC	LC		176.5	5.70	-	-	1	J	2	
21-4	LC	LN	224.0	5.78	-	-	7	J	2	
21-5 LC	LC		202.0	5.91	-	-	2	J	2	
22-1	LC	LN	204.0	5.64	-	-	5	J	2	
22-2 LC	LC		131.6	6.03	n.a.	n.a.	7	J	2	pH; Neg. CHN
22-2 LN		LN	86.4	5.82	-	-	7	J	2	
22-3	LC	LN	118.1	5.82	-	-	3	J	2	
22-4	LC	LN	171.5	5.58	-	-	3	J	2	pH
22-5	LC	LN	216.0	6.07	-	-	2	J	2	pH
23-1	LC	LN	103.4	6.59	-	-	7	J	2	
23-2 LC	LC		239.0	6.09	0.1957	21.4958		J	2	pH

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
23-2 LN		LN	190.8	6.19	-	-		J	2	
23-3 LC	LC		255.0	5.84	-	-		J	2	
23-3 LN		LN	116.2	6.79	-	-	1	J	2	
23-4	LC	LN	131.7	6.62	-	-	2	J	2	
23-5 LC	LC		190.3	6.03	-	-	1	J	2	
23-5 LN		LN	235.0	6.16	0.1301	13.8581	1	J	2	
24-1	LC	LN	137.9	6.48	-	-	2	J	2	
24-2	LC	LN	157.4	5.73	-	-	2	J	2	
24-3	LC	LN	122.5	5.85	-	-	3	J	2	pH
24-4	LC	LN	111.7	5.69	-	-	2	J	2	
24-5 LC	LC		142.3	5.89	n.a.	n.a.		J	2	Neg. CHN
24-5 LN		LN	166.9	5.75	0.045	3.5397		J	2	
25-28/0930	LC	LN	222.0	5.71	0.0144	1.5398		X	1	pH
25-1 0930			222.0	5.71	-	-		X	1	pH
25-2 LC	LC		163.1	6.20	0.0173	5.3578		X	1	
25-2 LN		LN	229.0	6.17	0.0726	5.4068		X	1	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
25-3	LC	LN	122.5	6.15	-	-		X	1	
25-4	LC	LN	163.2	6.48	-	-	2	X	1	
25-5	LC	LN	127.8	6.05	-	-	1	X	1	
26-1	LC	LN	190.4	5.97	-	-	3	X	1	
26-2	LC	LN	130.1	5.62	-	-	3	X	1	
26-3 LN		LN	97.5	6.51	0.0967	8.118	2	X	1	
26-4 LC	LC		236.0	6.60	0.0221	2.1002	2	X	1	
26-4 LN		LN	106.0	6.99	-	-	2	X	1	
26-5	LC	LN	264.0	6.55	-	-	7	X	1	
27-1	LC	LN	208.0	5.96	0.0285	2.4096	2	X	1	
27-2/16-4	LC	LN	170.0	6.28	-	-	3	X	1	
27-3	LC	LN	79.7	6.26	-	-	3	X	1	
27-4	LC	LN	93.8	6.39	-	-	3	X	1	
27-5	LC	LN	87.7	6.48	-	-	2	X	1	
28-1 LC	LC		182.1	5.86	0.0584	5.7254	9	X	1	
28-1 LN		LN	113.9	5.78	-	-	9	X	1	

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
28-2	LC	LN	112.0	5.98	-	-		X	1	
28-3	LC	LN	90.8	6.23	-	-	2	X	1	
28-4	LC	LN	140.9	6.16	-	-	2	X	1	
28-5 LC	LC		90.2	6.25	0.0181	2.0489	1	X	1	
B1-1 LC	LC		102.2	7.3	-	-		*Ref.*		pH
B1-2	LC	LN	117.1	7.28	-	-		*Ref.*		
B1-3	LC	LN	84.4	6.32	-	-		*Ref.*		pH
B1-4	LC	LN	69.4	7.16	-	-		*Ref.*		
B1-5	LC	LN	134.6	7.65	-	-		*Ref.*		
B2-1	LC	LN	64.5	6.85	-	-		*Ref.*		pH
B2-2	LC	LN	114.2	6.59	-	-		*Ref.*		pH
B2-3	LC	LN	65.8	6.74	-	-		*Ref.*		
B2-4	LC	LN	91.6	6.8	-	-		*Ref.*		
B3-1	LC	LN	104.8	6.27	-	-		*Ref.*		
B3-2	LC	LN	87.5	6.33	-	-		*Ref.*		pH

Table 16 Continued

Sample name: genetic/veg. plot	Taken close to species:		Conductivity (µS/cm)	pH	Nitrogen %	Carbon %	Simulated veg.type (GIS-data)	Area	Coal dust cover	Comments
B3-3	LC	LN	43.6	6.68	-	-		*Ref.*		
B3-4	LC	LN	52	6.59	-	-		*Ref.*		
B3-5	LC	LN	93.2	6.53	-	-		*Ref.*		
B4-1	LC	LN	98.4	6.95	-	-		*Ref.*		
B4-2 LC	LC		50.2	7.63	-	-		*Ref.*		
B4-3 LC	LC		72.8	6.89	-	-		*Ref.*		
B4-3 LN		LN	135.9	6.87	-	-		*Ref.*		
B4-4 LN		LN	119.4	7.19	-	-		*Ref.*		
Bjørndalen hemmelig	LC	LN	164.1	7.45	-	-		*Ref.*		
Engelsbukta LC	LC		509	6.54	-	-		*Ref.*		pH
Engelsbukta LN		LN	245	7.19	-	-		*Ref.*		

Appendix 3

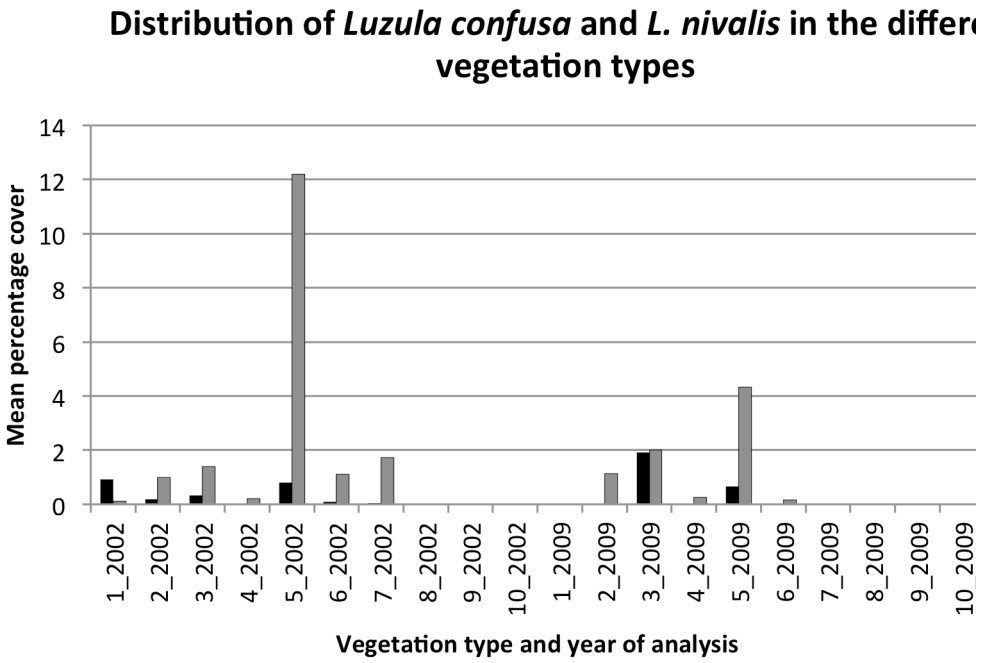

Figure 26 Distribution of the species L.confusa and L. nivalis in the different vegetation types, the mean percentage cover for each vegetation type. Data from the survey in 2002 and 2009 is u

Appendix 4

Table 17a) Markers deleted from the L. nivalis matrix due to high overall error rate.

Marker name	Cause of removal
2 LN B 275.3	High error rate marker
2 LN B 290.6	High error rate marker
2 LN G 493.1	High error rate marker
1LN B 197.6	High error rate marker
2 LN B 273.0	High error rate marker
1LN_B_112.5	Plate specific marker
1LN_B_174.6	Plate specific marker
1LN_G_149.5	Plate specific marker
1LN_G_252.6	Plate specific marker
1LN_G_257.6	Plate specific marker
1LN_G_290.7	Plate specific marker
1LN_Y_114.2	Plate specific marker
1LN_Y_473.5	Plate specific marker
2LN_B_279.2	Plate specific marker

Table 17b) Samples deleted from the matrix due to high error rate or bad sample run.

Individual	Cause of removal
LN3-5_2	Bad electropherogram
LN15-2	Bad sizing quality (SQ)
LN17-1	Bad sizing quality (SQ)
LN20-1	Bad electropherogram
LN24-2	Bad electropherogram
LN24-3	Bad electropherogram
LN24-4	Bad electropherogram
LN24-5	Bad electropherogram
LN25-1	Bad electropherogram
LN25-2	Bad electropherogram
LN25-3	Bad electropherogram
LN25-4	Bad electropherogram
LN27-1	Bad electropherogram
LN28-4	Bad electropherogram
LNB2-2	Bad electropherogram
LNB2-3	Displaced peaks
LNB2-4	Displaced peaks
LN100-2	Displaced peaks
LN100-3	Displaced peaks
LN100-4	Displaced peaks

Appendix 5

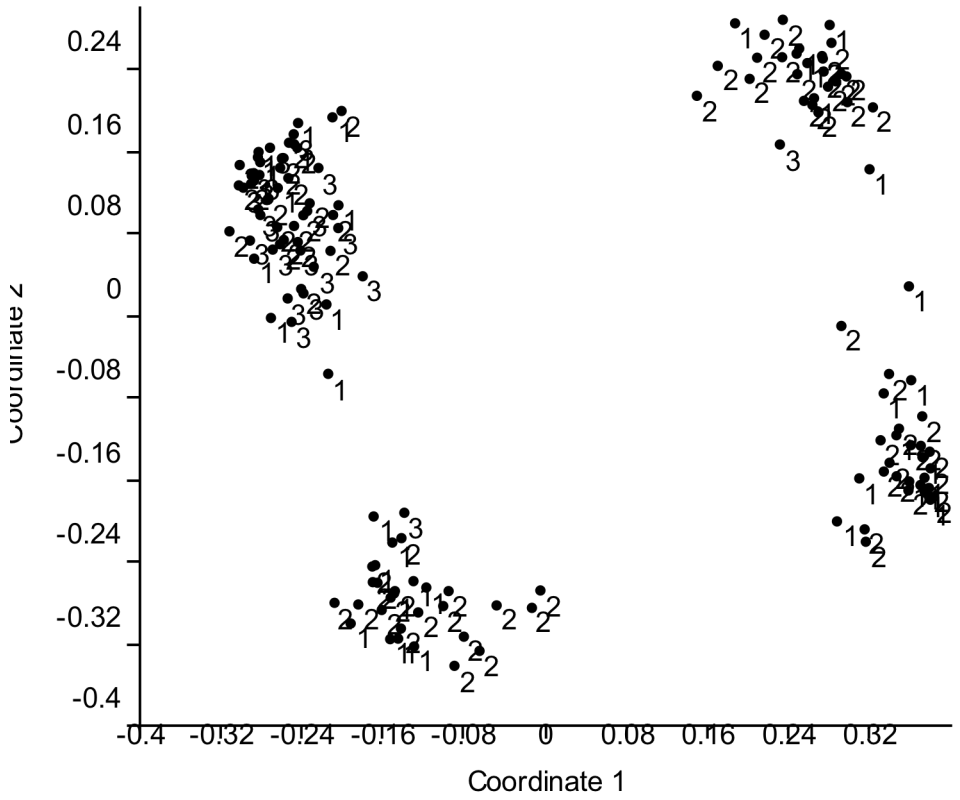

Figure 27 A principal coordinate (PCO) scatter plot showing the inter-mixing of individuals from different plates, as well as a quite clear division into four groups. Plates are numbered plate 1, 2 and 3. X-axis represents the first axis, while the Y-axis is the second axis of the analysis. All LN samples were used.

Appendix 6

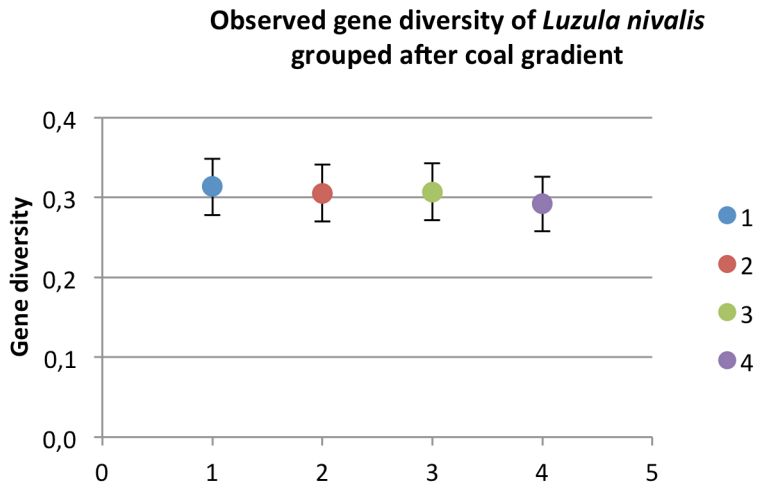

Figure 28 Gene diversities for all Luzula nivalis sampled, where group 1 – 4 consists of all individuals sampled in Svea, while the Reference population consists of all individuals sampled outside of Svea. A 95% confidence interval is given.