Institute of Landscape Ecology and Resources Management
Division of Landscape Ecology and Landscape Planning
Justus-Liebig-University Giessen

Effects of natural variation in snow depth on growth, flowering phenology and clonal structure of the evergreen dwarf shrub *Empetrum hermaphroditum* Hagerup

Inaugural Dissertation submitted to the
Faculty 09
Agricultural Sciences, Nutritional Sciences and Environmental Management
Justus-Liebig-University Giessen

for the degree of
Doctor agriculturae (Dr. agr.)

submitted by

Miriam Judith Bienau, M.Sc.
born in Lich/Hessen

Giessen, 2016

With permission from the Faculty 09 Agricultural Science,
Nutritional Science and Environmental Management,
Justus-Liebig-University Giessen
Dean: Prof. Dr. Klaus Eder

Examining Committee

Chair: Prof. Dr. Bernd Honermeier

Supervisor: Prof. Dr. R. Lutz Eckstein

Second supervisor: Prof. Dr. Dr. habil. Dr. h.c. Annette Otte

Reviewer: Prof. Dr. Rolf-Alexander Düring

Reviewer: Prof. Dr. Thomas Wilke

Date of defence: 10.10.2016

Declaration in accordance with the Doctoral Examination Regulations of the Faculty of Agricultural Science, Nutritional Science, and Environmental Management, Justus-Liebig-University Giessen, 7. July 2004 § 17 (2)

"I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation.

I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me.

At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the 'Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice'."

(Miriam J. Bienau) Ort, Datum

“Indeed I was now, for the first time, upon the Alps!

Snowy mountains encompassed me on every side.

I walked in snow, as if it had beed the severest winter.

All the rare plants I had previously met with,

and which had from time to time afforded me so much pleasure ,

were here as in miniature, and new ones in such profusion,

that I was overcome with astonishment,

thinking I had now found more than I should know what to do with.”

Carl Linnaeus, A Tour in Lapland, 6th July 1732

Contents

Chapter 3 – Synchronous flowering despite differences in snowmelt among habitats of *Empetrum hermaphroditum*

Chapter 4 – Clonality increases with snow depth in an arctic dwarf shrub

List of publications

This thesis is based on the following papers:

1) Bienau M.J., Hattermann D., Kröncke M., Kretz L., Otte A., Eiserhardt W.L., Milbau A., Graae B.J., Durka W., Eckstein R.L. (2014) Snow cover consistently affects growth and reproduction o*f Empetrum hermaphroditum* across latitudinal and local climatic gradients.
   Alpine Botany, DOI: 10.1007/s00035-014-0137-8 *
2) Bienau M.J., Kröncke M., Eiserhardt W.L., Otte A., Graae B.J., Hagen D., Milbau A., Durka W., Eckstein R.L. (2015) Synchronous flowering despite differences in snowmelt among habitats of *Empetrum hermaphroditum*.
   Acta Oecologica, DOI: 10.1016/j.actao.2015.10.005 **
3) Bienau M.J., Eckstein R.L., Otte A., Durka W. Clonality increases with snow depth in an arctic dwarf shrub (manuscript)

Author's contribution:

In paper **1**, I had the main responsibility for the data analysis, literature survey and writing. The co-authors helped with fieldwork and gave invaluable ideas and suggestions for this study.

In case of paper **2,** I did the main field work, data analysis and paper writing. The co-authors were involved in design, fieldwork and result evaluation of these studies.

In paper **3**, I performed most of the realization, data analysis and writing of the paper. The co-authors were involved in design, laboratory work and result evaluation of the study.

---

* Reprinted with kind permission of Springer

** Reprinted with kind permission of Elsevier

CHAPTER 1

Effects of natural variation in snow depth on growth, flowering phenology and clonal structure of the evergreen dwarf shrub *Empetrum hermaphroditum* – A synthesis

1.1 General introduction

Ecosystems at high latitudes and altitudes are characterized by cold and relatively short growing seasons (Bliss 1971); and snow cover, may last for over 8 months. Topography and prevailing wind directions shape a heterogeneous snow distribution during winter (Molau et al. 2005). The alpine tundra represents a mosaic of early-melting habitats on wind-exposed ridges with shallow snow cover, and late-melting habitats in wind-sheltered depressions with deep snow cover. In sub-arctic birch forests, birch stems act as snow traps causing an accumulation of snow (Saarinen and Lundell 2010). Usually, habitats with different snow conditions are inhabited by plant communities with contrasting species composition, characterized by species preferring or avoiding winter snow cover (Jonasson 1981; Odland and Munkejord 2008). However, some species occupy a wide range of habitats, and intraspecific differences in responses to variation in snow depth and duration can then be found. Generally, individuals growing on wind-exposed ridges usually have smaller leaves (McGraw and Antonovics 1983) and a compact growth with shorter internodes (Lid and Lid 1994) compared to individuals on sites where snow accumulates. Hence, growth and morphology can show intraspecific variability along environmental gradients. But also phenological processes, like flowering and fruiting of plant species can be affected by snow depth and the associated timing of snow melt in spring (Bliss 1962). Furthermoe, flowering of plant species is controlled by temperature, photoperiod, irradiation, and precipitation (Hülber et al. 2010; Kudo 1991; Kudo and Hirao 2006; Molau et al. 2005). As a consequence, flowering of different populations of a single species along a natural snowmelt gradient may be highly asynchronous (Hirao and Kudo 2004). Asynchronous flowering and phenological isolation due to differences in snowmelt timing may restrict gene flow via airborne pollen in wind pollinated species (Hirao and Kudo 2004) and thus may promote adaptation of plants to local environmental conditions (Kawecki and Ebert 2004).

Besides growth patterns and phenological processes also the mode of reproduction plays an important role in species. In arctic-alpine environments sexual reproduction through seeds is not very common (McGraw and Shaver 1982; Boudreau et al. 2010; Graae et al. 2011). Instead, vegetative reproduction and spread through clonal growth play an important role in many species (Bliss 1971; Cook 1983; Callaghan et al. 1992;

Molau and Larsson 2000). Clonal growth, which occurs parallel to flowering and fruiting during the growing season might help species to persist in communities independently of reproductive success (Körner 2003). Clonal plants are able to rapidly occupy new habitats and space locally (Callaghan et al. 1992) through horizontal clonal growth (Cook 1983) and may therefore often dominate tundra ecosystems (Tybirk et al. 2000). The relation of clonal growth and sexual reproduction might be also influenced by environmental conditions and could vary along natural gradients (Szmidt et al. 2002). The extent of clonality within different populations does have a strong influence on spatial genetic structure with neighboring ramets being more closely related than more distant ones (Reusch et al. 1999; Pluess and Stöcklin 2004). In alpine tundra habitats, populations may tend to be genetically aggregated because suitable habitats are isolated from each other and recruitment within a habitat or from neighboring habitats is more likely than colonization from other habitats by long distance seed dispersal events, despite suitable dispersal mechanisms (Pluess and Stöcklin, 2004).

Besides local snow cover patterns, which influence species composition and intraspecific variation, there are also general changes in snow depth in Arctic ecosystems. Since the 1980s Arctic ecosystems face profound changes in snow conditions: While snow depth has increased (Kohler et al. 2006; AMAP 2012), the duration of snow cover has shortened with an earlier start of the growing season (Shabanov et al. 2002), probably owing to increasing winter and spring temperatures (Callaghan et al. 2011). This may also influence nutrient availability and water supply, since snow cover has cascading feedback effects on the conditions during spring and summer. Moreover, also the quantity and quality of solar radiation will co-vary with vegetation composition and structure along a snow cover gradient.

The expected temperature increase will alter the abiotic and biotic conditions of plants (e.g., ACIA 2004), which may respond through (a) shifts in phenology, (b) range shifts, and/or (c) in situ changes of morphological or physiological traits (Bellard et al. 2012). Particularly, Arctic clonal plant species and communities may be vulnerable to global warming (Sala et al. 2000) since such changes may force species either to adapt *in situ* or to colonize new sites to track their climatic niche. However, micro-habitats may partly buffer macroclimatic change (e.g. De Frenne et al. 2013; Lenoir et al. 2013).

A high degree of phenotypic trait variation within one species regarding contrasting environmental conditions broadens the range of habitats in which a species can survive (Crawford 2008). Due to the broader habitat range, we expect that these species may better cope with the on-going changes in the Arctic (Jonasson 1981). Furthermore, phenotypic plasticity is an essential component of plants response to changing environmental conditions and also might help individuals to buffer the effects of climate change (Jump and Peñuelas 2005).

The response of several species to climate change in terms of growth and reproduction has been monitored within the tundra biome worldwide (Walker et al. 2006). However, most studies investigated the effects of variation in snow depth and snow cover duration in single-site experimental snow cover manipulations (e.g., Wipf et al. 2006; Bokhorst et al. 2008, 2009; Wipf et al. 2009; Wipf 2010; Gerdol et al. 2013). The results of these artificial snow manipulation experiments have been summarized recently in a meta analysis by Wipf and Rixen (2010). The results show that morphological and phenological responses in snow manipulation experiments depend on plant growth form, habitat and the type and degree of snowmelt manipulation. In contrast, studies on intraspecific variation in growth and morphology to snow cover using natural gradients of snow depth are yet very scarce (e.g., McGraw and Antonovics 1983; Kudo et al. 1999). Along natural snow cover gradients, snowmelt timing may differ up to two months between the early and late melting habitats (Kudo and Hirao 2006). While there are differences in timing of snowmelt between years, the spatial distribution of snow is rather constant (Kudo and Hirao 2006). This allows analyses of morphological and phenological species responses to different environmental conditions along the full extent of natural gradients (e.g. Kameyama and Kudo 2009; Kudo and Hirao 2006) without manipulating site conditions. The assumption of this gradient approach is that plants will respond to temporal changes of environmental conditions in the same way that they now vary with different conditions over space (Dunne et al. 2004). Studies along environmental gradients, encompassing the range of climate change predictions, is more likely to give a realistic picture concerning the extent of intraspecific phenotypic trait variation. This could predict the long-term adaptive potential of plant species to climate change (Körner 2003; Dunne et al. 2004; Kudo and Hirao 2006).

The evergreen dwarf shrub *Empetrum hermaphroditum* Hagerup (*Empetrum nigrum* agg., Jäger and Rothmaler 2011) is a prominent species in subarctic heath and mountain birch forest communities (Sonesson and Lundberg 1974; Nilsson and Wardle 2005). Although the species mostly reproduces vegetatively and expands clonally, fruits may be abundant (Bell and Tallis 1973; Callaghan and Emanuelsson 1985). The species covers a broad range of habitats with various environmental conditions.

This thesis compares the intraspecific performance of *Empetrum hermaphroditum* in habitats with contrasting snow cover conditions during winter and different light conditions during the growing season among regions differing in continentality and latitude. Using natural site variation, this comparative multi-site approach allows (a) the analysis of the response of *Empetrum hermaphroditum* in terms of growth, morphology and reproductive traits to natural, long-term variation of conditions and (b) the evaluation of the relative importance of local habitat conditions versus regional drivers such as climate and latitude.

The general aims of this thesis were:

(i) To investigate shoot growth, morphology and fruit and seed production of *Empetrum hermaphroditum* in habitats with different snow regimes,

(ii) To assess the flowering phenology of *Empetrum hermaphroditum* between the habitats in context of snow melt timing, and

(iii) To analyse the clonal structure of *Empetrum hermaphroditum* in the different habitats.

1.2 Study areas and habitat description

1.2.1 Study areas

The study was conducted in four study areas in Scandinavia. Two of them are located at 68°N (Abisko and Vassijaure in northern Sweden) and two are situated at 62-63°N (Kongsvoll and Samsjøen in central Norway). Within each latitude, one study area represented sub-continental climate (Abisko and Kongsvoll) and one area sub-oceanic climate (Vassijaure and Samsjøen), i.e. relatively low or high winter

precipitation patterns, respectively (Figure 1; for further information see Table 1 in Chapter 2). Altitude varied between 420 and 720 m a.s.l. at higher latitudes (Abisko, Vassijaure) and between 590 and 1140 m a.s.l. at lower latitudes (Kongsvoll, Samsjøen) and thus covers most of the sub-alpine and low alpine zone, i.e. the forest-tundra ecotone.

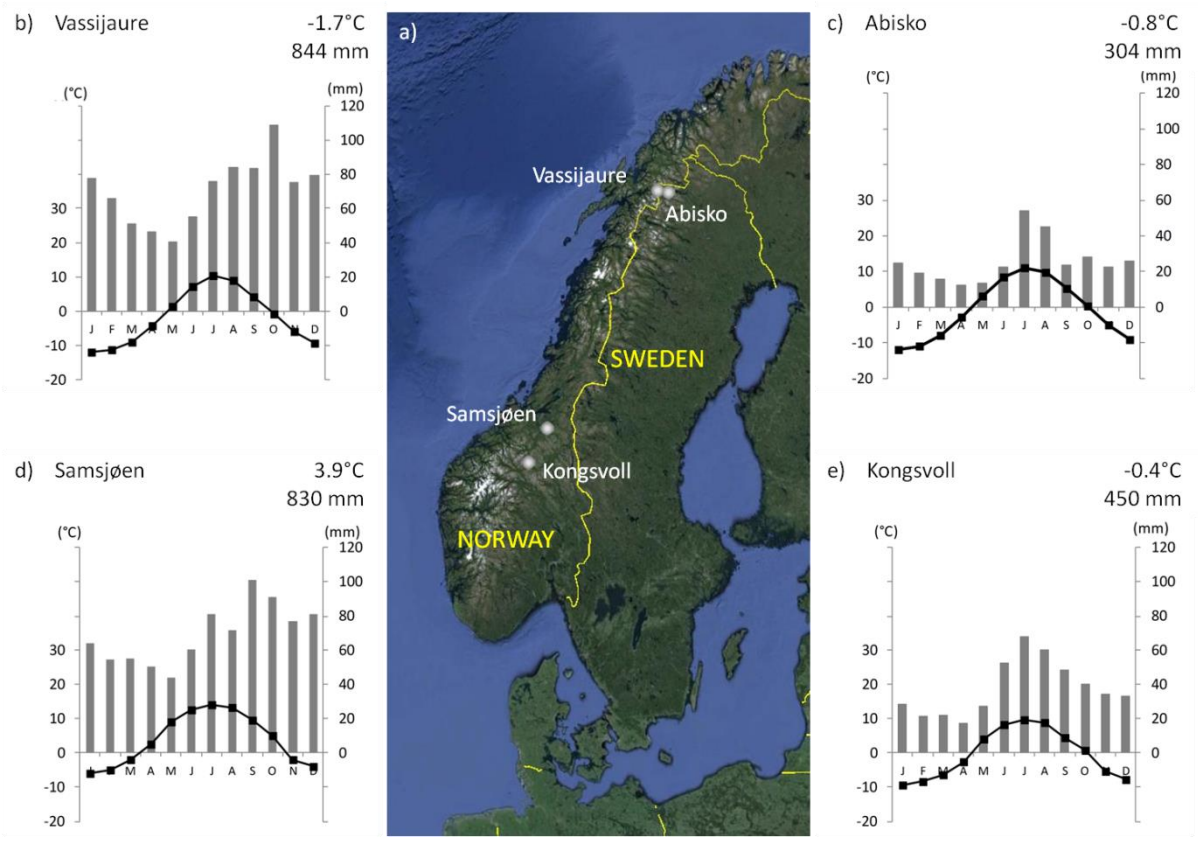


Figure 1: Map of Sweden and Norway with the study areas Vassijaure and Abisko in Sweden and Samsjøen and Kongsvoll in Norway (a) (Source: google earth 2016). Climate diagram for Vassijaure (closest station Katterjåkk) (b), Abisko (c), Samsjøen (closest station Melhus) (d), and Kongsvoll (e). Data for the period 1961-1990 from Sveriges meteorologiska och hydrologiska institut (SMHI, Sweden) and Norske Meteorologisk Institutt (NMI, Norway).

1.2.2 Habitat description

We distinguished three habitats differing in snow depth and co-varying abiotic factors (Table 1) based on topography, community type and indicator species of contrasting snow cover conditions (Jonasson 1981; Odland and Munkejord 2008): birch forest (***b***), sheltered depressions (***d***) and exposed ridges (***s***).

Table 1: Characteristics of the different habitats. In the ***b***-habitat, we used the height of *Parmelia olivacea* on birch stems to estimate the maximum winter snow depth (Sonesson et al. 1994), whereas in the alpine tundra (***d***- and ***s***-habitat), the height of the tallest but vital dwarf shrub or herb was used to estimate the minimum snow depth (Grogan and Jonasson 2006; Sturm et al. 2001). Data on estimated snow height and site openness from Bienau et al. (2014).

Habitat	***b***	***d***	***s***
Description	***b***irch forest with deep snow cover	alpine tundra with ***d***eep snow cover in wind-sheltered depressions	alpine tundra with ***s***hallow snow cover on wind-exposed elevated ridges
Indicator species for plot selection	*Betula pubescens* ssp. *czerepanovii*	snow-preferring species: *Betula nana, Vaccinium myrtillus*	snow-avoiding species: *Arctostaphylos alpina, Loiseleuria procumbens, Cetraria nivalis, Cetraria cucullata*
Snow depth	51-153 cm snow accumulation because vegetation acts as snow trap	29-44 cm snow accumulation because vegetation acts as snow trap	9-10 cm no snow accumulation because snow is blown away
Site openness	22-64 % lowest site openness through shading by trees	65-87 % higher site openness through shading by other dwarf shrubs	83-87 % highest site openness because of low vegetation height

Plot selection was done according to topography and indicator species for snow conditions on sites where *Empetrum hermaphroditum* was present. Distance between individual plots depends on habitat distribution and terrain structure in the natural landscape (Figure 2). The plots in Abisko and Vassijaure were arranged in south-north direction, with a mean distance between individual plots of 290 and 260 m,

respectively. Plot arrangement in Kongsvoll and Samsjøen was in west-east direction, with a mean distance between individual plots of 80 m and 60 m, respectively.

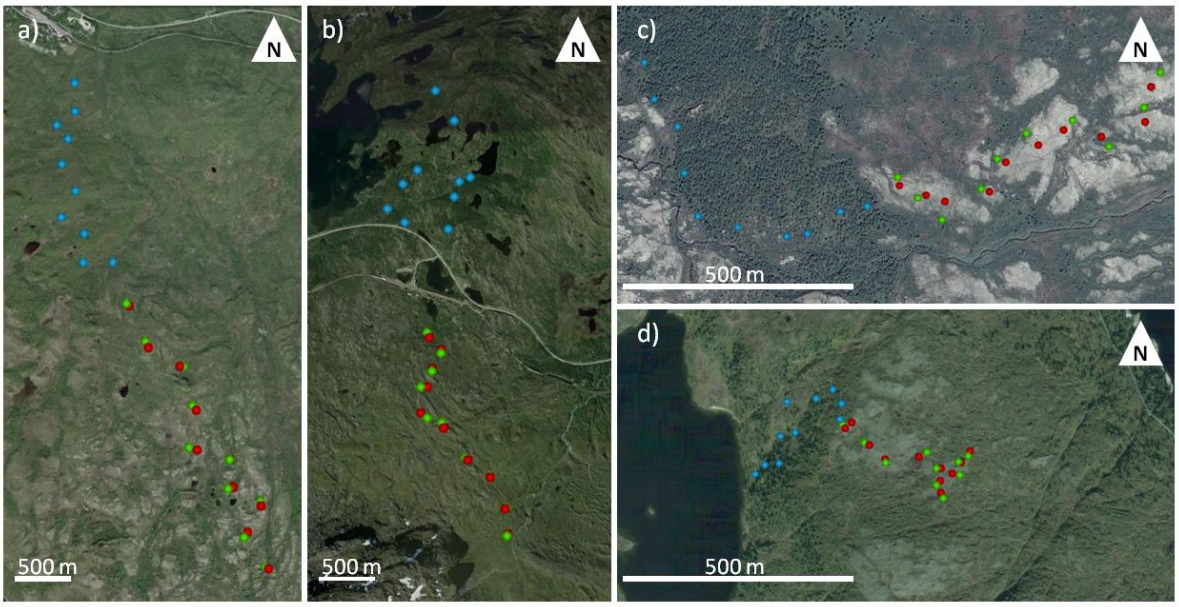


Figure 2: Plot locations in the four study areas Abisko (a), Vassijaure (b), Kongsvoll (c), and Samsjøen (d). Blue dots represent plots in the birch forest with deep snow cover, green dots represent plots in the alpine tundra with deep snow cover and red dots represent plots in the alpine tundra with shallow snow cover. (Source: google maps 2016)

Depending on snow depth, dates of snow melt timing in spring were different between the habitats. Snow melt occurred in the end of March and the beginning of April, respectively, in the ***s***-habitat and about 40 days later in ***d***- and ***b***-habitat (Bienau et al. 2015).

Thus, ***b*** + ***d*** habitats differ from ***s*** habitats with respect to winter snow cover and snow-melt timing, whereas in the growing season ***b*** differs from ***d*** with respect to canopy shade.

1.2.3 Study species

The evergreen dwarf shrub *Empetrum hermaphroditum* is one of the most abundant species in several subarctic heath and mountain birch forest communities (Sonesson and Lundberg 1974; Nilsson and Wardle 2005). Owing to its ability to build up dense mats through clonal growth and the release of allelochemicals (batatasin-III; Nilsson

and Wardle 2005), the species gains dominance in various habitats and controls community and ecosystem processes such as species recruitment, microbial activity, decomposition and nutrient cycling (Tybirk et al. 2000).

Empetrum hermaphroditum is considered to prefer either habitats with shallow (Jonasson 1981; Jonasson and Sköld 1983; Virtanen and Eurola 1997; Kudo et al. 1999; Odland and Munkejord 2008; Fletcher et al. 2010) or with relatively deep snow cover (Kudo et al. 1999; Tybirk et al. 2000; Fletcher et al. 2010), but it does not occur in the late-melting snowbed communities. It is abundant in our three habitat types: birch forest with deep snow cover (***b***), alpine tundra with deep snow cover in sheltered depressions (***d***) as well as in the alpine tundra with shallow snow cover on wind exposed ridges (***s***).

The species mostly reproduces through clonal growth, but fruit production also occurs by self- as well as crosspollination (Bell and Tallis 1973, Callaghan and Emanuelsson 1985). Flower buds are already produced in autumn (Bell and Tallis 1973), which enables this species to be one of the earliest flowering tundra species (Bell and Tallis 1973; Molau et al. 2005; Thórhallsdóttir 1998; Wipf et al. 2006), and flower buds break as soon as the conditions are favorable in the following spring (cf. Hülber et al. 2010).

In Europe the genus *Empetrum* is represented by two closely related taxa: the diploid, dioecious *Empetrum nigrum* L. s.str. and the tetraploid, hermaphrodite *Empetrum hermaphroditum* Hagerup, which can visually only be distinguished when flowering (Suda 2002). Both taxa show a circumpolar distribution, growing in boreal and subarctic zones (Meusel et al. 1978). *Empetrum hermaphroditum* is abundant in Scandinavia north of 60° latitude and only few scattered populations can be found in regions further south (Hultén and Fries 1986). *Empetrum nigrum* has a more southerly distribution ranging from the Alps to Scandinavia with a general limit of 60° latitude and it prefers a more oceanic climate (Bell and Tallis 1973). Both taxa co-occur in some areas (Suda 2002). In three of our study areas (Abisko, Vassijaure and Samsjøen) only *Empetrum hermaphroditum* (Suda, 2002) occurred, whereas, in the Dovrefjell National Park (study area Kongsvoll) we also found *Empetrum nigrum* (see also Suda, 2002).

1.3 Objectives

1.3.1 Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients (chapter 2)

In the first study, we investigated shoot growth and reproduction of *Empetrum hermaphroditum* across four study areas differing in latitude (62-63°N and about 68°N) and climate (sub-continental vs. sub-oceanic). In each study area three habitats were distinguished: birch-forest with deep snow cover, alpine tundra in wind-sheltered depressions with deep snow cover and alpine tundra on wind-exposed ridges with shallow snow cover. Different shoot growth and reproductive variables of *Empetrum hermaphroditum* were compared among the habitats to find possible trait variation between habitats.

The specific objectives were: (i) to investigate whether shoot growth of *Empetrum hermaphroditum* varied across habitats differing in winter snow cover regimes, and (ii) to determine the effect of habitat on fruit and seed production of *Empetrum hermaphroditum*.

1.3.2 Synchronous flowering despite differences in snowmelt among habitats of *Empetrum hermaphroditum* (chapter 3)

In the second study we investigated flowering phenology of *Empetrum hermaphroditum* across the three habitats in three of the four study areas during snow melt in late spring. The habitats differ in winter snow depth and thus also in snow melt timing.

The three main points of interest in this study were: (i) to investigate whether flowering of *Empetrum hermaphroditum* is synchronized with snow melt timing, (ii) to find out if there is a temporal overlap in flowering of *Empetrum hermaphroditum* between early and late melting habitats, and (iii) to determine possible differences in temperature and light conditions during snow melt and flowering of *Empetrum hermaphroditum* between the different habitats.

1.3.3 Clonality increases with snow depth in an artic dwarf shrub (chapter 4)

The third study dealt with the clonal structure of *Empetrum hermaphroditum*. Shoots were sampled within a special grid design to assess the clonal structure in each of the three habitats in all four study areas. The main objectives of this study were: (i) to find out if clonal diversity and clone size vary between habitats, and (ii) to investigate the small scale spatial genetic structure of *Empetrum hermaphroditum* in the different habitats.

1.4 Methods

1.4.1 Environmental variables – chapter 2, 3 and 4

To describe and quantitatively compare the environmental conditions of the different habitats for all further investigations, vegetation surveys, snow depth estimations and measurements of humus depth, site openness, vegetation cover, *Empetrum hermaphroditum* cover and near soil surface temperature were carried out for each of ten 1 x 1 m plots per habitat in each of the four study areas.

To characterize the vegetation in each habitat, total vegetation cover and *Empetrum hermaphroditum* cover, as well as the cover of all species in the tree-, shrub-, herb- and cryptogam layer were recorded. Furthermore, we measured the depth of the organic layer.

In the birch forest, the height of the lichen *Parmelia olivacea* on birch stems was used to estimate the maximum winter snow depth (Sonesson et al. 1994), whereas in the alpine tundra, the height of the tallest but vital dwarf shrub or herb was used to estimate the minimum snow depth (Sturm et al. 2001, Grogan and Jonasson 2006).

Hemispherical images were taken with a camera, equipped with a 180° fisheye lens, which were analysed with different software tools (Frazer et al. 1999, Nobis 2005) to evaluate site openness of each individual plot as an indicator of habitat light conditions (chapter 2 and 3).

Near soil surface temperature was measured with data loggers from September 2012 to July 2013 every 3 h to estimate the temperature which the plants experience from autumn to spring (chapter 3) and summer (chapter 2). The following temperature

variables were calculated for the morphological study (described in chapter 2): temperature sums from the 1st of April to the 27th of June 2013 for all habitats and study areas, daily mean temperatures for each plot monthly temperature sums for April, May and June. For observations on flowering phenology (chapter 3) snow melt date in each plot was estimated by daily temperature fluctuations. The following variables were calculated for the lag phase between snow melt and flowering of *Empetrum hermaphroditum*: growing degree days, frost days and temperature sum.

1.4.2 Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients (chapter 2)

In each of the 4 study areas ten 1 x 1 m plots per habitat were selected and permanently marked along elevation transects from the sub-alpine birch forest to the low alpine heath zone with wind-sheltered depressions or wind-exposed ridges. Plot selection depended on the criteria for habitat selection (chapter 1.2.2) and the presence of *Empetrum hermaphroditum*. Within each plot three randomly selected individual *Empetrum hermaphroditum* ramets were analysed in terms of height above ground, and after harvesting of ramets the following variables were measured: length of the main shoot, number of lateral shoots, number of living green leaves, dead brown leaves, leaf scars (shed leaves) and leaf density. After drying of shoots, total biomass, total leaf dry mass and total stem dry mass were analysed. Furthermore, leaf life expectancy of shoots for each plot was calculated.

Flower and fruit production of *Empetrum hermaphroditum* was analysed in close proximity to the ten plots per habitat in the study areas Abisko and Vassijaure in September 2012. Number of berries per *Empetrum hermaphroditum* shoot was counted using a special wooden frame with intersection points to ensure reproducibility of the results across all study plots. Furthermore, 20 berries per plot were randomly sampled to detect the mean number of seeds per berry and to measure seed mass. Additionally, we counted the number of flower buds on *Empetrum hermaphroditum* shoots that were collected for a common garden experiment. For each plot in Abisko and Vassijaure, one clone was sampled and ramets were cut and planted. After 8 weeks in a greenhouse, all visible flower buds were counted, and the percentage of flower buds for each clone (one plot) was

calculated. Since flower buds are fully developed before harvesting, we assume that greenhouse conditions did not influence number of flower buds.

To analyse the collected data, a hierarchical analysis of variance (ANOVA) with sequential sums of squares (Quinn and Keough 2002) was used to test the effect of latitude, climate and habitat on environmental variables and shoot growth of *Empetrum hermaphroditum*. To compare means between habitat types we employed two orthogonal planned contrasts: ***b*** + ***d*** vs. ***s***, and ***b*** vs. ***d*** (Quinn and Keough 2002). The vegetation survey data were analyzed by detrended correspondence analysis (DCA) to detect possible environmental gradients and compositional similarity between plots.

1.4.3 Synchronous flowering despite differences in snowmelt among habitats of *Empetrum hermaphroditum* (chapter 3)

Flowering phenology of *Empetrum hermaphroditum* during spring was investigated in the three study areas, Abisko (in the two consecutive years 2013 and 2014), Kongsvoll (in 2013) and Vassijaure (in 2014). Within five 2 x 2 m plots per habitat, 20 randomly sampled *Empetrum hermaphroditum* shoots were numbered and permanently tagged with wire. Phenophases were determined at intervals of one to seven days during the pre-flowering and flowering period and four different phenophases were differentiated: before flowering (***bf***), flowers opening (***fo***), full-flowering (***ff***) and end of flowering (***ef***). The mean date of full-flowering was calculated as the weighted mean of full-flowering. Furthermore, Primack's overlap index (Primack 1980) was calculated to estimate the degree of overlap in flowering of individuals during the whole observation period, whereas a Monte-Carlo simulation was used to simulate the probability for all pairs of plots that both plots had shoots in full-flowering at a given day (Kunnen 2012). Both methods helped to estimate the relationship between habitat and full-flowering of *Empetrum hermaphroditum* for all possible within (***b*******b***, ***d*******d***, ***s*******s***) and among habitat combinations (***b*******d***, ***b*******s***, ***d*******s***).

To test the effects of habitat on flowering phenology of *Empetrum hermaphroditum* and environmental variables non linear mixed effect models (NLME; Pinheiro et al. 2015) were used. Furthermore, we used the Bonferroni-method to test for significant differences among the habitats ***b*** vs. ***d***, ***b*** vs. ***s*** and ***d*** vs. ***s***.

1.4.4 Clonality increases with snow depth in an artic dwarf shrub (chapter 4)

In each of the 4 study areas one 14 x 14 m plot per habitat was established to test for general patterns of clonal structure of *Empetrum hermaphroditum* between habitats. Within each plot, we aimed to sample 36 individual *Empetrum hermaphroditum* shoots using a systematic grid (Figure 1b in chapter 4; cf. Szmidt et al., 2002). The different populations (N = 374 shoots; 27 to 36 per plot) of *Empetrum hermaphroditum* were analysed with amplified fragment length polymorphism (AFLP; Vos et al. 1995) resulting in 105 polymorphic AFLP markers. During the analyses it became apparent that in Kongsvoll the tetraploid *Empetrum hermaphroditum* and the diploid *Empetrum nigrum* occurred, which can be distinguished by their AFLP genotypes.

Analyses based on hierarchical clustering and different diversity indices were used to investigate clonal structure of *Empetrum hermaphroditum* in the different habitats. Two different threshold definitions of genotypic distance between two samples that are considered the same clone were used, to obtain robust results. Furthermore, spatial autocorrelation methods were used to examine the small-scale spatial genetic structure, which analyzes the genetic relatedness between pairs of individuals regarding to their spatial distance (Vekemans and Hardy, 2004).

1.5 Main results and discussion

The habitat conditions play an important role for morphology, phenology and clonal structure of *Empetrum hermaphroditum*.

Snow depth differed significantly among the three habitats across all study areas and increased from the ***s***-, to ***d***- and ***b***-habitat. Along the climatic gradient, there was a significantly higher snow depth in the sub-oceanic than the sub-continental study regions. However, the effect of habitat explained 83.7 % of the observed variation in snow depth, whereas climate explained only 1.8 %. The factor latitude had no significant effects on snow depth.

Contrary to snow depth, site openness as a proxy for light availability decreased from ***s***-, to ***d***- and ***b***-habitat. Along the climatic gradient, site openness was higher in the

sub-oceanic than in the sub-continental study regions. Furthermore, site openness decreased from North to South. Again, the factor habitat explained most of the total variation in site openness (77.0 %), whereas climate and latitude explained only 7.1 % and 6.5 %, respectively.
Furthermore, we observed significant earlier snow melt in the ***s***-habitat than in ***d*** and ***b*** across all study areas in both observation years. But not only the date of snow melt differed between habitats, the ***s***-habitat experienced significantly more growing degree days, frost days and a higher amount of solar energy than the late melting ***b***- and ***d***-habitats between snow melt and flowering of *Empetrum hermaphroditum*.

The results of **chapter 2** revealed a strong relationship of habitat conditions in terms of snow depth and light availability on shoot growth and reproduction of *Empetrum hermaphroditum* across all study areas. Furthermore, the effects of habitat were mostly larger than the effects of latitude and climate. In Addition, consistently different performance in terms of growth and reproduction between contrasting habitat types suggests that there may be local adaptation (Kawecki and Ebert 2004) to habitats with different winter snow cover and co-varying abiotic conditions during the growing season, despite more or less continuous populations.
In the birch forest habitat, ramet height, length of annual shoot segments, number of lateral shoots and total biomass were highest and decreased in the ***d***- and ***s***-habitat. In contrast, leaf density and relative leaf mass were highest in ***s***-habitats, intermediate in ***d***-and lowest in ***b***-habitats. Also leaf life expectancy of the C+1 shoot generation was higher in the ***s***-habitats than in the ***b***- and ***d***-habitats.
The explanations for the observed patterns in shoot growth of *Empetrum hermaphroditum* could be a consequence of physical protection from wind damage and ice abrasion in winter. Snow cover has an insulating effect, which protects shoots below the snow surface from extreme temperatures and reduces the potential damage of frost spells early in the season (Körner 2003). The more procumbent growth form of *Empetrum hermaphroditum* with lower ramet height and shorter shoot segments but higher leaf density protects *Empetrum hermaphroditum* from cold winter temperatures and prevailing strong winds and reduced freezing and desiccation during periods of unstable snow cover (Körner 2003). This growth form probably presents an adaptation to the harsh conditions in this habitat.

Furthermore, within the sheltered ***b***- and ***d***-habitats the surrounding vegetation (mainly *Betula pubescens* ssp. *czerepanovii* and other dwarf shrubs like *Betula nana* and *Vaccinium myrtillus*) acts as snow traps during winter and as protection against wind during the snow free period (Fletcher et al. 2010, Sturm et al. 2001). In arctic ecosystems, protecting effects of surrounding vegetation might be higher than negative effects of competition (Carlsson and Callaghan 1991; Shevtsova et al. 1995; Callaway et al. 2002; Wipf et al. 2006; Olofsson et al. 2011). Furthermore, high snow depth leads to higher water availability during snow melt (Billings and Bliss 1959; Hadley and Smith 1987; Sturm et al. 2001; Fletcher et al. 2010). A further positive effect of high snow depth is the storage of atmospherically deposited inorganic nitrogen in the snowpack, leading to greater nitrogen inputs during snowmelt (Bowman 1992; Weih 1998). In fertilizer experiments, *Empetrum hermaphroditum* responded to artificially increased nutrient availability with an increase in leaf number and leaf mass per shoot, a greater shoot mass, an increase in shoot extension growth and stem length, an increase in height and production of more lateral branches (Chapin and Shaver 1985; Wookey et al. 1993; Parsons et al. 1994; Campioli et al. 2012; but see Press et al. 1998).

Besides winter snow cover, the amount of solar radiation during the growing season is an important abiotic factor for plant growth. On wind exposed ridges, *Empetrum hermaphroditum* experiences almost full illumination due to topographic exposure and low vegetation height in this habitat. Therefore, higher relative allocation to leaves and higher leaf density might ensure sufficient assimilation and biomass production in the ***s***-habitats. However, the high light availability in ***s***-habitats can also induce water stress in spring and summer through stomatal limitation of photosynthesis. Hence, the photosynthetic capacity of plants might be higher in plots with deep snow cover and higher water availability during the late snow melt (Kudo et al. 1999; Fletcher et al. 2010). The lower light availability in ***b***- and ***d***-habitats promotes shade avoidance of plants by longer shoot length, because plants in darker environments show elongated stems to reach solar radiation (Schmitt and Wulff 1993; Stuefer and Huber 1998; McConnaughay and Coleman 1999; Callaway et al. 2003; Semchenko et al. 2012).

Although habitat explained the highest percentage of variation, most vegetative traits differed significantly among latitudes and climates. Along the latitudinal gradient

Empetrum hermaphroditum showed significantly higher ramet heights, longer lengths of annual shoot segments, produced more lateral shoots and total biomass at lower latitudes but significantly lower leaf density, relative leaf mass and leaf life expectancy in the C+1 shoot generation. This is probably related to the relatively milder climate at lower latitudes, which allowed prolonged growth (Jonas et al. 2008) and can be achieved through high assimilation rates, leading in turn to higher tissue turnover and lower leaf life expectancy (Karlsson 1992).

Along the climatic gradient, relative leaf mass and leaf life expectancy were lower and in contrast, number of lateral shoots and total biomass were significantly higher in the sub-oceanic than in the sub-continental study regions. This is consistent with the response of *Empetrum hermaphroditum* to different habitats and might be promoted by greater nitrogen inputs during snowmelt on sites with higher snow accumulation (Bowman 1992; Weih 1998) as well as higher physical protection from wind and ice abrasion in winter (Sonesson and Callaghan 1991; Callaghan et al. 2011).

Fruit and flower production differed significantly (except number of berries and seed mass in Abisko) among habitat types with increasing number of berries per shoot, seed mass and number of flower buds from ***b***- and ***d***- to ***s***-habitats. This might be an effect of the open habitat, where flowering, fruit maturation and seed quality will be promoted by a longer growing season with higher temperatures (Graae et al. 2008). Furthermore, a high amount of open soil in the ***s***-habitat might facilitate germination due to reduced competition by surrounding vegetation (Szmidt et al. 2002) and by soil disturbance, which removes the insulating cover over seeds and enables warm stratification (Baskin et al. 2002). Therefore, the ***s***-habitat seems to be the most favourable habitat for seed production and seedling establishment of *Empetrum hermaphroditum*. This pattern was also observed in our study on the clonal structure of *Empetrum hermaphroditum* where we detected significant higher clone number and clonal diversity in the ***s***-habitat than in ***b*** and ***d***. After establishment, *Empetrum hermaphroditum* reproduces mostly vegetative in the ***b***- and ***d***-habitat and reproduction by seeds seems to play a minor role in these habitats, different to the ***s***-habitat.

Significant effects along the climatic gradient were found. More seeds per berry and lighter seeds were produced in the sub-oceanic than in the sub-continental study region. This could be explained by the later start of the growing season in Vassijaure

than in Abisko due to later snow melt, which was revealed in the phenological study. Since the growing season started later, seeds had less time to ripen and were therefore smaller.

The results of **chapter 3** showed that the full-flowering of *Empetrum hermaphroditum* occurred nearly at the same time in early melting tundra habitats with shallow snow cover and in late melting tundra and birch forest habitats with deep snow cover, despite significant differences in snow melt timing. Consequently, flowering of *Empetrum hermaphroditum* is not synchronized with snowmelt in the early melting ***s***-habitat, but in the late melting ***b***- and ***d***-habitat. These results are in line with the observations on flowering of snow bed species (Hülber et al. 2006) that the earlier a plant becomes snow free, the longer the time until it flowers. Rapid growth and reproduction thus seems to be more important for survival and reproduction in late melting than in early melting habitats due to a shorter growing season (Totland and Alatalo 2002).

Synchronous flowering, i.e. a large overlap in full flowering within as well as between different habitats in all study areas and study years was found with Primack's flowering overlap index as well as with Monte-Carlo simulation. The results of Primack's overlap index demonstrated that overlap in full-flowering of *Empetrum hermaphroditum* occurred during the whole flowering period. No clear pattern of higher overlap within than between habitats could be detected. In contrast, Monte-Carlo simulation revealed higher overlap within than between habitats at given days and gave information on the chronological sequence of full-flowering in within and between habitat combinations. This might indicate that reproductive isolation and genetic differentiation among the habitats are rather unlikely. The environmental conditions during the lag-phase showed that the ***s***-habitat experienced a higher number of frost days, more growing degree days and a higher amount of solar energy but lower temperature sums than the ***b***- and ***d***-habitats. Consequently, snowmelt timing, the amount of solar energy and temperature are closely related and the single effects could not be distinguished easily from one another. To reduce the risk of frost damage in plants caused by too early flowering (Inouye 2008; Semenchuk et al. 2013), the development of plants often depends on photoperiodism, i.e. plants remain frost hardy until a certain day length is reached, especially in early melting habitats

(Hülber et al. 2010; Keller and Körner 2003; Wipf et al. 2009). Keller and Körner (2003) observed that temperature dependent flowering of high alpine species decreases with increasing photoperiod.

The results of **chapter 4** revealed an increase of clonal diversity of *Empetrum hermaphroditum* in conjunction with a decrease of snow cover. Accordingly, reproduction by seedlings might be more important under the conditions of the ***s***-habitat than in ***b*** and ***d*** (Eriksson, 1992). Within the ridge habitat, competition is lower and the proportion of open soil is higher, which likely facilitates recruitment from seeds (Callaghan et al., 1992; Szmidt et al., 2002). This was also confirmed by our observation that fruit and seed production is higher in the ***s***-habitat. Furthermore, besides the higher fruit and seed production in this habitat, the probability of seed dispersal to the ***s***-habitat by bird droppings might be high, because birds often roost on exposed, elevated rocks around which a lot of bird droppings can be found (pers. obs.). In addition to long distance seed dispersal also seedling recruitment in the surrounding of the mother plant might be important for clonal diversity of *Empetrum hermaphroditum*.

Clonal growth is expected to promote spatial autocorrelation (SGS), i.e. increasing genetic similarity with decreasing spatial distance (Reusch et al., 1999). We observed higher SGS in the ***b***-habitat than in the ***d***- and ***s***-habitat. As expected, SGS increased with decreasing clonal diversity and increasing clone size. Furthermore, we detected significant differences in SGS between all habitats, which could be explained by the clonal growth form. In the ***s***-habitat *Empetrum hermaphroditum* grows more compact with shorter internodes and higher leaf density, this growth form might facilitate a high density of flowers in one clone, whereby the possibility of selfing increases (cf. Handel, 1985). However, effective sexual propagation does not only depend on pollen flow, also seed dispersal, seed germination and seedling establishment are of high importance (Alberto et al., 2005). Thus, the ***s***-habitat might be more favorable for seedling establishment, due to seed dispersal in this habitat by bird droppings and a higher amount of open soil and less competition than in ***b*** and ***d***.

Implications for the response of Empetrum hermaphroditum to climate change & outlook

Arctic ecosystems face strong changes in snow conditions due to global warming (e.g., ACIA 2004). Climate change affects snowmelt twofold: First, Arctic ecosystems may experience changes in snow cover and earlier snowmelt in spring despite increasing precipitation during winter because much of the precipitation occurs as rain as a consequence of increasing temperature (ACIA 2004; Callaghan et al. 2011; Bokhorst et al. 2012). Second, winter and early spring thawing events may occur more often (Bokhorst et al. 2012), leading to a colder spring in a warmer world which increases the probability of frost damage on plant species by snowmelt in early spring, when temperature is often very low (Wipf et al. 2009).

The present thesis investigated the response of *Empetrum hermaphroditum* to natural variation of snow cover in the field across latitudinal and local climatic gradients. This comparative multi-site analysis along a steep natural environmental gradient, encompassing the range of climate change predictions, is more likely to give a realistic picture concerning the extent of intraspecific phenotypic trait variation, which may determine the long-term adaptive potential of *Empetrum hermaphroditum* to climate change (Körner 2003; Dunne et al. 2004; Kudo and Hirao 2006). We found consistent variation among habitat types across latitudes and climatic gradients underlining that snow cover potentially represents a strong force of selection.

Clear and consistent differences in growth, reproduction and clonal structure may suggest local adaptation of *Empetrum hermaphroditum* to habitats differing in snow depth (Kawecki and Ebert 2004; Gonzalo-Turpin and Hazard 2009). Earlier snow melt resulted in a longer growing season but may also lead to increased frost damage because of a high probability and frequency of frost spells early in the year. Flowering of *Empetrum hermaphroditum* might be slightly accelerated by advanced snow melt and soil warming (Anadon-Rosell et al. 2014; Wipf 2010; Wipf et al. 2006, 2009). Flowering of *Empetrum hermaphroditum* does not seem to be related to the time of snowmelt across all habitats, but to temperature conditions during the lag-phase between snowmelt and flowering. In this way small scale variation seems to matter less to flowering of *Empetrum hermaphroditum* than interannual differences in snowmelt timing. Changes in snow cover and temperature due to climate are not

likely to cause changes in flowering synchrony but general changes in flowering time, as demonstrated in the comparison of flowering time in our study area Abisko in the early melting year 2013 compared to the late melting year 2014.

This thesis demonstrates that *Empetrum hermaphroditum* has a broad ecological niche and shows a consistent match between its shoot growth, reproduction, clonal structure and flowering and the prevailing local habitat conditions. The high plasticity of *Empetrum hermaphroditum* supports findings of climate change experiments, and suggests that the species has the potential to cope with changing snow conditions in the course of climate change. Nevertheless, while phenotypic plasticity will allow individuals to adapt to changing conditions, locally adapted populations may locally go extinct. The latter will offer the possibility for seedling recruitment of adapted genotypes, but possibly also for replacement of *Empetrum hermaphroditum* by other species with cascading effects on ecosystem functioning.

As it is often the case, the findings of this study could answer some questions but in turn raised many new ones. Can the observed phenotypic plasticity of *Empetrum hermaphroditum* help populations to persist in a changing environment? How does the temporal progress of climate change influence adaptive mechanisms in *Empetrum hermaphroditum* populations? Is the response of the slow growing *Empetrum hermaphroditum* fast enough to persist?

Hence, further studies are needed that investigate the response of *Empetrum hermaphroditum* to changing snow cover conditions with increasing or decreasing snow depth during winter as well as earlier or later snow melt during the important phenological phases in spring, in existing populations. These further studys have to cover the whole spectrum of possible changes. The International Tundra Experiment (ITEX) provides a good basis to investigate the responses of arctic and alpine plants and ecosystems to climate change.

References

ACIA (2004). *Arctic Climate Impact Assessment: Impacts of a warming Arctic*. Cambridge University Press.

Alberto, F., Gouveia, L., Arnaud-Haond, S., Pérez-Lloréns, J.L., Duarte, C.M., and Serrão, E.A. (2005). Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. *Molecular Ecology*, *14*(9), 2669–2681.

AMAP (Arctic Monitoring and Assessment Programme) (2011). Snow, Water, Ice and Permafrost in the Arctic (SWIPA). Oslo.

Anadon-Rosell, A., Rixen, C., Cherubini, P., Wipf, S., Hagedorn, F., and Dawes, M.A. (2014). Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. *PLoS ONE*, *9*(6), e100577.

Baskin, C.C., Zackrisson, O., and Baskin, J.M. (2002). Role of warm stratification in promoting germination of seeds of *Empetrum hermaphroditum* (Empetraceae), a circumboreal species with a stony endocarp. *American Journal of Botany*, *89*(3), 486–493.

Bell, J.N.B., and Tallis, J.H. (1973). *Empetrum Nigrum* L. *Journal of Ecology*, *61*(1), 289–305.

Bellard, C., Bertelsmeier, C., Leadly, P., Thuiller, W., and Courchamp, R. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*, 365–377.

Bienau, M.J., Hattermann, D., Kröncke, M., Kretz, L., Otte, A., Eiserhardt, W.L., Milbau, A., Graae, B.J., Durka, W., and Eckstein, R.L. (2014). Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients. *Alpine Botany*, *124*(2), 115–129.

Bienau, M.J., Kröncke, M., Eiserhardt, W.L., Otte, A., Graae, B.J., Hagen, D., Milbau, A., Durka, W., and Eckstein, R.L. (2015). Synchronous flowering despite differences in snowmelt timing among habitats of *Empetrum hermaphroditum*. *Acta Oecologica*, (69), 129–136.

Billings, W.D., and Bliss, L.C. (1959). An Alpine Snowbank Environment and Its Effects on Vegetation, Plant Development, and Productivity. *Ecology*, *40*(3), 388–397.

Bliss, L.C. (1962). Adaptations of arctic and alpine plants to environmental conditions. *Arctic*, *15*(2), 117–144.

Bliss, L.C. (1971). Arctic and Alpine Plant Life Cycles. *Annual Review of Ecology and Systematics*, *2*, 405–438.

Bokhorst, S., Bjerke, J.W., Bowles, F.W., Melillo, J., Callaghan, T.V., and Phoenix, G.K. (2008). Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. *Global Change Biology*, *14*(11), 2603–2612.

Bokhorst, S., Bjerke, J.W., Tømmervik, H., Preece, C., and Phoenix, G.K. (2012). Ecosystem Response to Climatic Change: The Importance of the Cold Season. *AMBIO*, *41*(3), 246–255.

Bokhorst, S.F., Bjerke, J.W., Tømmervik, H., Callaghan, T.V., and Phoenix, G.K. (2009). Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. *Journal of Ecology*, *97*(6), 1408–1415.

Boudreau, S., Ropars, P., and Harper, K.A. (2010). Population dynamics of *Empetrum hermaphroditum* (Ericaceae) on a subarctic sand dune: Evidence of rapid colonization through efficient sexual reproduction. *American Journal of Botany*, *97*(5), 770–781.

Bowman, W.D. (1992). Inputs and Storage of Nitrogen in Winter Snowpack in an Alpine Ecosystem. *Arctic and Alpine Research*, *24*(3), 211.

Callaghan, T.V., Johansson, M., Brown, R.D., Groisman, P.Y., Labba, N., Radionov, V., Barry, R.G., Bulygina, O.N., Essery, R.L.H., Vladimir, D.M.F., Golubev, N., Grenfell, C.T., Petrushina, M.N., Razuvaev, V.N., Robinson, D.A., Romanov, P., Shindell, D., Shmakin, A.B., Sokratov, S.A., Warren, S., and Yang, D. (2011). The Changing Face of Arctic Snow Cover: A Synthesis of Observed and Projected Changes. *AMBIO*, *40*(1), 17–31.

Callaghan, T.V., Carlsson, B.Å., Jónsdóttir, I.S., Svensson, B.M., and Jonasson, S. (1992). Clonal Plants and Environmental Change: Introduction to the Proceedings and Summary. *Oikos*, *63*(3), 341–347.

Callaghan, T.V., and Emanuelsson, U. (1985). Population structure and processes of tundra plants and vegetation. In *The population structure of vegetation* (S. 399–439).

Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas C., Kikodze, D., and Cook, B.J. (2002). Positive interactions among alpine plants increase with stress. *Nature*, *417*(6891), 844–848.

Callaway, R.M., Pennings, S.C., and Richards, C.L. (2003). Phenotypic plasticity and interactions among plants. *Ecology*, *84*(5), 1115–1128.

Campioli, M., Leblans, N., and Michelsen, A. (2012). Twenty-Two Years of Warming, Fertilisation and Shading of Subarctic Heath Shrubs Promote Secondary Growth and Plasticity but Not Primary Growth. *PLoS ONE*, *7*(4), e34842.

Carlsson, B.A., and Callaghan, T.V. (1991). Positive Plant Interactions in Tundra Vegetation and the Importance of Shelter. *Journal of Ecology*, *79*(4), 973–983.

Chapin, F.S., and Shaver, G.R. (1985). Individualistic Growth Response of Tundra Plant Species to Environmental Manipulations in the Field. *Ecology*, *66*(2), 564–576.

Cook, R.E. (1983). Clonal Plant Populations: A knowledge of clonal structure can affect the interpretation of data in a broad range of ecological and evolutionary studies. *American Scientist*, *71*(3), 244–253.

Crawford, R.M.M. (2008). Cold climate plants in a warmer world. *Plant Ecology & Diversity*, *1*(2), 285–297.

De Frenne, P., Rodríguez-Sánchez, F., Coomes, D.A., Baethen, L., Verstraeten, G., Vellend, M., Bernhardt-Römermann, M., Brown, C.D., Brunet, J., Cornelis, J., Decocq, G.M., Dierschke, H., Eriksson, O., Gilliam, F.S., Hédl, R., Heinken, T., Hermy, M., Hommel, P., Jenkins, M.A., Kelly, D.L., Kirby, K.J., Mitchell, F.J., Naaf, T., Newman, M., Peterken, G., Petrik, P., Schultz, J., Sonnier, G., van Calster, H., Walther, G.R., White, P.S., Woods, K.D., Wulf, M., Graae, B.J., and Verheyen, K. (2013). Microclimate moderates plant responses to macroclimate warming. *Proceedings of the National Academy of Sciences*, *110*(46), 18561–18565.

Dunne, J.A., Saleska, S.R., Fischer, M.L., and Harte, J. (2004). Integrating Experimental and Gradient Methods in Ecological Climate Change Research. *Ecology*, *85*(4), 904–916.

Eriksson, O. (1992). Evolution of seed dispersal and recruitment in clonal plants. *Oikos*, *63*(3), 439–448.

Fletcher, B.J., Press, M.C., Baxter, R., and Phoenix, G.K. (2010). Transition zones between vegetation patches in a heterogeneous Arctic landscape: how plant

growth and photosynthesis change with abundance at small scales. *Oecologia*, *163*(1), 47–56.

Frazer, G., Canham, C., and Lertzman, K. (1999). *Gap Light Analyzer (GLA), Version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation.* Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York.

Gerdol, R., Siffi, C., Iacumin, P., Gualmini, M., and Tomaselli, M. (2013). Advanced snowmelt affects vegetative growth and sexual reproduction of *Vaccinium myrtillus* in a sub-alpine heath. *Journal of Vegetation Science*, *24*(3), 569–579.

Gonzalo-Turpin, H., and Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species Festuca eskia. *Journal of Ecology*, *97*(4), 742–751.

Graae, B. J., Alsos, I. G., and Ejrnaes, R. (2008). The impact of temperature regimes on development, dormancy breaking and germination of dwarf shrub seeds from arctic, alpine and boreal sites. *Plant Ecology*, *198*(2), 275–284.

Graae, B.J., Ejrnaes, R., Lang, S.I., Meineri, E., Ibarra, P.T., and Bruun, H.H. (2011). Strong microsite control of seedling recruitment in tundra. *Oecologia*, *166*(2), 565–576.

Grogan, P., and Jonasson, S. (2006). Ecosystem CO_2 production during winter in a Swedish subarctic region: the relative importance of climate and vegetation type. *Global Change Biology*, *12*(8), 1479–1495.

Hadley, J.L., and Smith, W.K. (1987). Influence of krummholz mat microclimate on needle physiology and survival. *Oecologia*, *73*(1), 82–90.

Handel, S.N. (1985). The Intrusion of Clonal Growth Patterns on Plant Breeding Systems. *The American Naturalist*, *125*(3), 367–384.

Hirao, A.S., and Kudo, G. (2004). Landscape genetics of alpine-snowbed plants: comparisons along geographic and snowmelt gradients. *Heredity*, *93*, 290–298.

Hülber, K., Winkler, M., and Grabherr, G. (2010). Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. *Functional Ecology*, *24*(2), 245–252.

Hülber, K., Gottfried, M., Pauli, H., Reiter, K., Winkler, M., and Grabherr, G. (2006). Phenological responses of snowbed species to snow removal dates in the central

Alps: Implications for climate warming. *Arctic, Antarctic, and Alpine Research*, *38*(1), 99–103.

Hultén, E., and Fries, M. (1986). *Atlas of North European vascular plants, vol. 1-3.* Königstein: Koeltz.

Inouye, D.W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), 353–362.

Jäger, E.J., and Rothmaler, W. (2011). *Exkursionsflora von Deutschland Bd. 2, Bd. 2,*. Heidelberg [etc.]: Spektrum Akademischer Verlag.

Jonas, T., Rixen, C., Sturm, M., and Stoeckli, V. (2008). How alpine plant growth is linked to snow cover and climate variability. *Journal of Geophysical Research: Biogeosciences*, *113*(G3), n/a–n/a.

Jonasson, S. (1981). Plant communities and species distribution of low alpine *Betula nana* heaths in Northernmost Sweden. *Vegetatio*, *44*(1), 51–64.

Jonasson, S., and Sköld, S.E. (1983). Influences of frost-heaving on vegetation and nutrient regime of polygon-patterned ground. *Vegetatio*, *53*(2), 97–112.

Jump, A.S., and Peñuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*(9), 1010–1020.

Kameyama, Y., and Kudo, G. (2009). Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, Phyllodoce aleutica: effects of pollinators and self-incompatibility. *Annals of Botany*, *103*, 1385–1394.

Karlsson, P.S. (1992). Leaf longevity in evergreen shrubs: variation within and among European species. *Oecologia*, *91*(3), 346–349.

Kawecki, T.J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241.

Keller, F., and Körner, C. (2003). The role of photoperiodism in alpine plant development. *Arctic, Antarctic and Alpine Research*, *35*(3), 361–368.

Kohler, J., Brandt, O., Johansson, M., and Callaghan, T. (2006). A long-term Arctic snow depth record from Abisko, northern Sweden, 1913–2004. *Polar Research*, *25*(2), 91–113.

Körner, C. (2003). *Alpine plant life: functional plant ecology of high mountain ecosystems* (2nd Aufl.). Berlin, Heidelberg: Springer Verlag.

Kudo, G. (1991). Effects of snow-free period on the phenology of alpine plants inhabiting snow patches. *Arctic and Alpine Research*, *23*(4), 436.

Kudo, G., Nordenhäll, U., and Molau, U. (1999). Effects of snowmelt timing on leaf traits, leaf production, and shoot growth of alpine plants: Comparisons along a snowmelt gradient in northern Sweden. *Ecoscience*, *6*(3), 439–450.

Kudo, G., and Hirao, A.S. (2006). Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts - Springer. *Population Ecology*, *48*(1), 49–58.

Kunnen, S.E. (2012). *A dynamic systems approach of adolescent development*. Psychology Press.

Lenoir, J., Graae, B.J., Aarrestad, P.A., Alsos, I.G., Armbruster, W.S., Austrheim, G., Bergendorff, C., Birks, H.J.B., Bråthen, K.A., Brunet, J., Bruun, H.H., Dahlberg, C.J., Decocq, G., Diekmann, M., Dynesius, M., Ejrnaes, R., Grytnes, J.-A., Hylander, K., Klanderud, K., Luoto, M., Milbau, A., Moora, M., Nygaard, B., Odland, A., Ravolainen, V.T., Reinhardt, S., Sandvik, S.M., Schei, F.H., Speed, J.D.M., Tveraabak, L.U., Vandvik, V., Velle, L.G., Virtanen, R., Zobel, M., and Svenning, J.C. (2013). Local temperatures inferred from plant communities suggest strong spatial buffering of climate warming across Northern Europe. *Global Change Biology*, *19*(5), 1470–1481.

Lid, J., and Lid, D.T. (1994). *Norsk flora*. Oslo: Det Norske Samlaget.

McConnaughay, K.D.M., and Coleman, J.S. (1999). Biomass allocation in plants: Ontogeny or optimality? A test along three resource gradients. *Ecology*, *80*(8), 2581–2593.

McGraw, J.B., and Antonovics, J. (1983). Experimental Ecology of *Dryas Octopetala* Ecotypes: I. Ecotypic Differentiation and Life-Cycle Stages of Selection. *Journal of Ecology*, *71*(3), 879–897.

McGraw, J.B., and Shaver, G.R. (1982). Seedling density and seedling survival in Alaskan cotton grass tussock tundra. *Ecography*, *5*(2), 212–217.

Meusel, H., and Jäger, E.J. (1978). *Vergleichende Chorologie der zentraleuropäischen Flora, Karten* (1. Auflage, Bd. Band 2). Jena: VEB Gustav Fischer Verlag.

Molau, U., and Larsson, E.L. (2000). Seed rain and seed bank along an alpine altitudinal gradient in Swedish Lapland. *Canadian Journal of Botany*, *78*(6), 728–747.

Molau, U., Nordenhäll, U., and Eriksen, B. (2005). Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. *American Jurnal of Botany*, *92*(3), 422–431.

Nilsson, M.-C., and Wardle, D.A. (2005). Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Frontiers in Ecology and the Environment*, *3*(8), 421–428.

NMI (Norwegian Meteorological Institute). http://sharki.oslo.dnmi.no/portal /page?_pageid=73,39035,73_39049&_dad=portal&_schema=PORTAL

Nobis, M. (2005). SideLook 1.1. http://sidelook.software.informer.com/1.1/

Odland, A., and Munkejord, H.K. (2008). Plants as indicators of snow layer duration in southern Norwegian mountains. *Ecological Indicators*, *8*(1), 57–68.

Olofsson, J., Ericson, L., Torp, M., Stark, S., and Baxter, R. (2011). Carbon balance of Arctic tundra under increased snow cover mediated by a plant pathogen. *Nature Climate Change*, *1*(4), 220–223.

Parsons, A.N., Welker, J.M., Wookey, P.A., Press, M.C., Callaghan, T.V., and Lee, J.A. (1994). Growth Responses of Four Sub-Arctic Dwarf Shrubs to Simulated Environmental Change. *Journal of Ecology*, *82*(2), 307–318.

Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D. (2015). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-121.

Pluess, A.R., and Stöcklin, J. (2004). Population genetic diversity of the clonal plant Geum reptans (Rosaceae) in the Swiss Alps. *American Journal of Botany*, *91*(12), 2013–2021.

Press, M.C., Potter, J.A., Burke, M. J.W., Callaghan, T.V., and Lee, J.A. (1998). Responses of a subarctic dwarf shrub heath community to simulated environmental change. *Journal of Ecology*, *86*(2), 315–327.

Primack, R.B. (1980). Variation in the Phenology of Natural Populations of Montane Shrubs in New Zealand. *Journal of Ecology*, *68*(3), 849–862.

Quinn, G.P., and Keough, M.J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press.

Reusch, T.B.H., Hukriede, W., Stam, W.T., and Olsen, J.L. (1999). Differentiating between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity*, *83*(2), 120–126.

Saarinen, T., and Lundell, R. (2010). Overwintering of *Vaccinium vitis-idaea* in two sub-Arctic microhabitats: a reciprocal transplantation experiment. *Polar Research*, *29*(1), 38–45.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanawald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, L.N., Sykes, M.T., Walker, B.H., Walker, D. and Wall, D.H. (2000). Global Biodiversity Scenarios for the Year 2100. *Science*, *287*(5459), 1770–1774.

Schmitt, J., and Wulff, R.D. (1993). Light spectral quality, phytochrome and plant competition. *Trends in Ecology & Evolution*, *8*(2), 47–51.

Semchenko, M., Lepik, M., Götzenberger, L., and Zobel, K. (2012). Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate? *Journal of Ecology*, *100*(2), 459–466.

Semenchuk, P.R., Elberling, B., and Cooper, E.J. (2013). Snow cover and extreme winter warming events control flower abundance of some, but not all species in high arctic Svalbard. *Ecology and Evolution*, *3*(8), 2586–2599.

Shabanov, N.V., Zhou, L., Knyazikhin, Y., Myneni, R.B., and Tucker, C.J. (2002). Analysis of interannual changes in northern vegetation activity observed in AVHRR data from 1981 to 1994. *IEEE Trans. Geosci. Remote Sens.*, *40*(1), 115–130.

Shevtsova, A., Ojala, A., Neuvonen, S., Vieno, M., and Haukioja, E. (1995). Growth and Reproduction of Dwarf Shrubs in a Subarctic Plant Community: Annual Variation and Above-Ground Interactions with Neighbours. *Journal of Ecology*, *83*(2), 263–275.

SMHI (The Swedish Meteorological and Hydrological Institute). http://www.smhi.se/klimatdata/meteorologi/temperatur/dataserier-med-normalv%C3%A4rden-1.7354

Sonesson, M., and Callaghan, T.V. (1991). Strategies of Survival in Plants of the Fenoscandian Tundra. *ARCTIC*, *44*(2), 95–105.

Sonesson, M., and Lundberg, B. (1974). Late Quaternary Forest Development of the Torneträsk Area, North Sweden. 1. Structure of Modern Forest Ecosystems. *Oikos*, *25*(2), 121.

Sonesson, M., Osborne, C., and Sandberg, G. (1994). Epiphytic Lichens as Indicators of Snow Depth. *Arctic and Alpine Research*, *26*(2), 159.

Stuefer, J. F., and Huber, H. (1998). Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous Potentilla species. *Oecologia*, *117*(1–2), 1–8.

Sturm, M., Holmgren, J., McFadden, J.P., Liston, G.E., Chapin, F.S., and Racine, C.H. (2001). Snow–Shrub Interactions in Arctic Tundra: A Hypothesis with Climatic Implications. *Journal of Climate*, *14*(3), 336–344.

Suda, J. (2002). New DNA polidy level in *Empetrum* (Empetraceae) revealed by flow cytometry. *Annales Botanici Fennici*, *39*(2), 133–141.

Szmidt, A.E., Nilsson, M.-C., Briceño, E., Zackrisson, O., and Wang, X.-R. (2002). Establishment and genetic structure of *Empetrum* hermaphroditum populations in northern Sweden. *Journal of Vegetation Science*, *13*(5), 627–634.

Thórhallsdóttir, T.E. (1998). Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. *Oecologia*, *114*(1), 43–49.

Totland, Ø, and Alatalo, J.M. (2002). Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, Ranunculus glacialis - Springer. *Oecologia*, *133*(2), 168–175.

Tybirk, K., Nilsson, M.-C., Michelsen, A., Kristensen, H. L., Shevtsova, A., Strandberg, M.T., Johansson, M., Nielsen, K.E., Riis-Nilsen, T., Strandberg, B. and Johnsen, I. (2000). Nordic *Empetrum* dominated ecosystems: function and susceptibility to environmental changes. *AMBIO: A Journal of the Human Environment*, *29*(2), 90–97.

Vekemans, X., and Hardy, O.J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*(4), 921–935.

Virtanen, R., and Eurola, S. (1997). *Middle oroarctic vegetation in Finland and middle-northern arctic vegetation on Svalbard*. Svenska växtgeografiska sällsk.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*(21), 4407–4414.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, S., Calef, M.P., Callaghan, T.V., Carroll, A.B., Epstein, H.E., Jónsdóttir, I.S., Klein, J.A., Magnússon, B., Molau, U., Oberbauer, S.F., Rewa, S.P., Robinson, C.H., Shaver, G.R., Suding, K.N., Thompson, C.C., Tolvanen, A., Totland, Ø., Turner, P.L., Tweedie, C.E., Webber, P.J., and Wookey, P.A. (2006). Plant community

responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(5), 1342–1346.

Weih, M. (1998). Seasonality of Nutrient Availability in Soils of Subarctic Mountain Birch Woodlands, Swedish Lapland. *Arctic and Alpine Research*, *30*(1), 19.

Wipf, S. (2010). Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. *Plant Ecology*, *207*(1), 53–66.

Wipf, S., and Rixen, C. (2010). A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research*, *29*(1), 95–109.

Wipf, S., Rixen, C., and Mulder, C.P.H. (2006). Advanced snowmelt causes shift towards positive neighbour interactions in a subarctic tundra community. *Global Change Biology*, *12*(8), 1496–1506.

Wipf, S., Stoeckli, V., and Bebi, P. (2009). Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. *Climatic Change*, *94*(1–2), 105–121.

Wookey, P.A., Parsons, A.N., Welker, J.M., Potter, J.A., Callaghan, T.V., Lee, J.A., and Press, M.C. (1993). Comparative Responses of Phenology and Reproductive Development to Simulated Environmental Change in Sub-Arctic and High Arctic Plants. *Oikos*, *67*(3), 490.

CHAPTER 2

Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients

Miriam J. Bienau, Dirk Hattermann, Michael Kröncke, Lena Kretz, Annette Otte, Wolf L. Eiserhardt, Ann Milbau, Bente J. Graae, Walter Durka, R. Lutz Eckstein

Alpine Botany 124:2 (2014) 115-129

Alp Botany (2014) 124:115–129
DOI 10.1007/s00035-014-0137-8

VEGETATION IN COLD ENVIRONMENTS UNDER CLIMATE CHANGE

Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients

Miriam J. Bienau · Dirk Hattermann · Michael Kröncke · Lena Kretz · Annette Otte · Wolf L. Eiserhardt · Ann Milbau · Bente J. Graae · Walter Durka · R. Lutz Eckstein

Received: 29 April 2014 / Accepted: 16 August 2014 / Published online: 4 September 2014

Abstract Arctic ecosystems face strong changes in snow conditions due to global warming. In contrast to habitat specialists, species occupying a wide range of microhabitats under different snow conditions may better cope with such changes. We studied how growth and reproduction of the dominant dwarf shrub *Empetrum hermaphroditum* varied among three habitat types differing in winter snow depth and summer irradiation, and whether the observed patterns were consistent along a local climatic gradient (sub-continental vs. sub-oceanic climates) and along a latitudinal gradient (northern Sweden vs. central Norway). Habitat type explained most of the variation in growth and reproduction. Shoots from shallow snow cover and high summer irradiation habitats had higher numbers of flowers and fruits, lower ramet heights, shorter shoot segments, lower numbers of lateral shoots and total biomass but higher leaf density and higher relative leaf allocation than shoots from habitats with higher snow depth and lower summer irradiation. In addition, biomass, leaf allocation and leaf life expectancy were strongly affected by latitude, whereas local climate had strong effects on seed number and seed mass. *Empetrum* showed high phenotypic trait variation, with a consistent match between local habitat conditions and its growth and reproduction. Although study areas varied strongly with respect to latitude and local climatic conditions, response patterns of growth and reproduction to habitats with different environmental conditions were consistent. Large elasticity of traits suggests that *Empetrum* may have the potential to cope with changing snow conditions expected in the course of climate change.

This article is part of the special issue Vegetation in cold environments under climate change.

Electronic supplementary material The online version of this article (doi:10.1007/s00035-014-0137-8) contains supplementary material, which is available to authorized users.

M. J. Bienau (✉) · L. Kretz · A. Otte · R. L. Eckstein
Institute of Landscape Ecology and Resource Management, Research Centre for BioSystems, Land Use and Nutrition (IFZ), Justus-Liebig University Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany
e-mail: Miriam.J.Bienau@umwelt.uni-giessen.de

D. Hattermann
Faculty of Geography, University of Marburg, Deutschhausstraße 10, 35032 Marburg, Germany

M. Kröncke
Faculty of Nature and Technology (Faculty 5), University of Applied Sciences Bremen, Neustadtswall 30, 28199 Bremen, Germany

W. L. Eiserhardt · B. J. Graae
Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

A. Milbau
Department of Ecology and Environmental Science, Climate Impacts Research Centre, Umeå University, 98107 Abisko, Sweden

W. Durka
Helmholtz Centre for Environmental Research UFZ, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany

Present Address:
W. L. Eiserhardt
Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Ny Munkegade 116, Build 1540, 8000 Aarhus C, Denmark

Keywords *Empetrum hermaphroditum* · Snow cover gradient · Growth response

Introduction

Temperature increase due to climate change alters the abiotic and biotic conditions of plants (e.g., ACIA 2004; IPCC 2013), which may respond though (a) shifts in phenology (b) range shifts and/or (c) in situ changes of morphological or physiological traits (Bellard et al. 2012). To better understand and predict the potential responses of plant species to these rapidly changing conditions, we need more knowledge concerning the effects of driving environmental factors on plant growth, distribution and abundance.

Ecosystems at high latitudes and altitudes are characterized by a cold and relatively short growing season (Bliss 1971). However, the Arctic is experiencing an increase in temperature, most pronounced in winter and spring, causing an earlier onset of snowmelt (Callaghan et al. 2011) and an earlier start of the growing season (Shabanov et al. 2002). Snow cover, which may last for over 8 months, represents an especially strong selection factor (e.g., Haapasaari 1988; Tybirk et al. 2000; Körner 2003) with significant effects on the distribution and abundance of plant species and communities (e.g., Sandberg 1958; Virtanen and Eurola 1997). Spatial variation in snow depth in Arctic ecosystems, created by a combination of topography and wind, ranges from snow-free wind-exposed ridges to sheltered depressions with deep snow accumulation (Saarinen and Lundell 2010). Usually, these habitat types are inhabited by plant communities with contrasting species composition (e.g., Jonasson 1981; Haapasaari 1988; Odland and Munkejord 2008) characterized by chionophilous (species preferring winter snow cover; phytosociological alliance: *Phyllodoco-Vaccinion*, Dierßen 1996) or chionophobous species (snow-avoiding species; phytosociological alliance: *Arctostaphylo-Cetrarion*, Dierßen 1996). However, some species occupy a wide range of habitats, and intraspecific differences in responses to variation in snow depth and duration can then be found in terms of growth, phenology and reproduction (McGraw and Antonovics 1983; Kudo et al. 1999; Bokhorst et al. 2008; Crawford 2008; Bokhorst et al. 2009; Wipf et al. 2009; Saarinen and Lundell 2010; Wipf 2010). For instance, individuals growing on wind-exposed ridges usually have smaller leaves (McGraw and Antonovics 1983) and a compact growth with shorter internodes (Lid and Lid 1994) compared to individuals on sites where snow accumulates. Besides local snow cover patterns, which influence species composition (Sandberg 1958; Virtanen and Eurola 1997), there are also general changes in snow depth in Arctic ecosystems. While snow depth has increased since the 1980s (Kohler et al. 2006; AMAP 2012), the duration of snow cover has decreased, probably owing to increasing winter and spring temperatures (Callaghan et al. 2011). Plants in Arctic ecosystems thus face profound changes in winter snow conditions. These may also influence nutrient availability and water supply, since snow cover has cascading feedback effects on the conditions during spring and summer. Thus, also the quantity and quality of solar radiation will co-vary with vegetation composition and structure along a snow cover gradient.

A high degree of phenotypic trait variation within one species regarding contrasting environmental conditions broadens the range of habitats in which a species can survive (Crawford 2008). Due to the broader habitat range, we expect that these species may better cope with the on-going changes in the Arctic (Jonasson 1981). One such species is *E. hermaphroditum* Hagerup (*Empetrum nigrum* agg., Jäger and Rothmaler 2011; hereafter, denoted as *Empetrum*), a prominent evergreen dwarf shrub in several subarctic heath and mountain birch forest communities (Sonesson and Lundberg 1974; Nilsson and Wardle 2005). Owing to its ability to build up dense mats through clonal growth and by the release of allelochemicals (batatasin-III; Nilsson and Wardle 2005), the species gains dominance in various habitats and controls community and ecosystem processes such as species recruitment, microbial activity, decomposition and nutrient cycling (Tybirk et al. 2000). Although *Empetrum* mostly reproduces vegetatively and expands clonally, fruits may be abundant (Bell and Tallis 1973; Callaghan and Emanuelsson 1985). Despite its pivotal role in Arctic ecosystems its response to snow cover variation is equivocal. Thus, the species is considered to prefer either habitats with shallow (Jonasson 1981; Jonasson and Sköld 1983; Virtanen and Eurola 1997; Kudo et al. 1999; Odland and Munkejord 2008; Fletcher et al. 2010) or with relatively deep snow cover (Kudo et al. 1999; Tybirk et al. 2000; Fletcher et al. 2010), but *Empetrum* does not occur in the late-melting snowbed communities (phytosociological class *Salicetea-herbaceae*, Dierßen 1996).

In the context of the International Tundra Experiment (ITEX), the immediate response of circumpolar plant species to climate change in terms of growth and reproduction has been monitored within the tundra biome worldwide (Walker et al. 2006). The most important climate manipulation of ITEX is passive warming of small tundra plots using open top chambers (Walker et al. 2006), whereas the effects of variation in snow depth and snow cover duration are only examined in single-site experimental snow cover manipulations (e.g., Wipf et al. 2006; Bokhorst et al. 2008, 2009; Wipf et al. 2009; Wipf 2010; Gerdol et al. 2013). The results of these snow manipulation experiments, which artificially add or remove snow from local plots, have been summarized recently in a meta analysis (Wipf and Rixen 2010); the meta analysis shows that the growth response in snow

manipulation experiments depends on plant growth form, habitat type and the type and degree of snowmelt manipulation. In contrast, studies on intraspecific variation in growth and morphology to snow cover using natural gradients of snow depth are scarce (e.g., McGraw and Antonovics 1983; Kudo et al. 1999). The assumption of this gradient approach is that plants will respond to temporal changes of environmental conditions in the same way that they now vary with different conditions over space (Dunne et al. 2004). A comparative multi-site gradient approach using natural site variation (a) allows the analysis of the response of species in terms of growth, morphology and reproductive traits to natural, long-term variation of conditions and (b) allows the evaluation of the relative importance of local habitat conditions versus regional drivers such as climate and latitude. This analysis may thus shed new light on elasticity and phenotypic trait variation of *Empetrum*—a keystone species of boreal and arctic ecosystems—in response to snow cover changes in the course of global change.

Therefore, the present paper compares intraspecific performance of *Empetrum* in habitats with contrasting winter snow cover and growing season light availability among regions differing in climate (continentality) and latitude. Assuming that snow cover represents a strong selection force, we expect larger differences in terms of growth and reproduction among habitats, than between climates and latitudes.

The following questions were addressed.

Q1: Do shoot growth and morphology of *Empetrum* vary significantly among habitats defined according to their winter snow cover regimes? How large is the effect of habitat type in comparison with latitudinal and climatic variation?

Q2: Do fruit and seed production of *Empetrum* vary significantly among habitats differing in winter snow cover regimes? Which habitat type is most suitable for seed production of *Empetrum*? How large is the effect of habitat type in comparison with climatic variation?

Materials and methods

Study regions and habitats

The study is based on data from four regions, two of which are located at latitudes of about 68°N (abbreviated 'North-'; regions: Abisko and Vassijaure in northern Sweden) and two are situated at 62–63°N (abbreviated 'South-'; regions: Kongsvold and Samsjøen in central Norway). Within each latitude, one study region represented sub-continental climate (abbreviated 'SC'; regions: Abisko and Kongsvold) and one region sub-oceanic climate (abbreviated 'SO'; regions: Vassijaure and Samsjøen), i.e., relatively low or high winter precipitation patterns, respectively (Table 1). Altitudes varied between 420 and 720 m a.s.l. at higher latitudes (North-SC, North-SO) and between 590 and 1,140 m a.s.l. at lower latitudes (South-SC, South-SO) and thus cover the sub-alpine and low alpine zone, i.e., forest–tundra ecotone.

We distinguished three habitats differing in snow depth and co-varying abiotic factors based on topography, community type and indicator species of contrasting snow cover conditions (Jonasson 1981; Odland and Munkejord 2008).

- Birch forest with deep snow cover (abbreviated by ***b***): sub-alpine birch forest with *Betula pubescens* ssp. *czerepanovii*.
- Alpine tundra with deep snow cover (***d***): wind-sheltered depressions in low alpine heath with tall and dense *Betula nana* as characteristic chionophilous species (Jonasson 1981; Odland and Munkejord 2008).
- Alpine tundra with shallow snow cover (***s***): wind-exposed ridges on low alpine heath. Characteristic chionophobous species for identifying this habitat type were *Arctostaphylos alpina*, *Loiseleuria procumbens*, *Cetraria nivalis* and *Cetraria cucullata* (Jonasson 1981; Odland and Munkejord 2008).

Thus, $\boldsymbol{b} + \boldsymbol{d}$ habitats differ from ***s*** habitats with respect to winter snow cover, whereas, within the habitats with deep winter snow cover, ***b*** differs from ***d*** with respect to canopy shade during the growing season.

Plot selection

During summer 2012, we selected and permanently marked 10 1 × 1 m plots per habitat type in each of the 4 study regions for analyses of shoot growth and vegetation surveys along elevation transects from the sub-alpine birch forest to the low alpine heath zone with wind-sheltered depressions or wind-exposed ridges. The plots in North-SC and North-SO were located in south–north direction across a distance of 4,500 and 3,900 m, respectively. Plot arrangement in South-SC and South-SO was in west–east direction across a distance of 1,100 and 600 m, respectively. Plot selection was conditional on the criteria for habitat type selection above and the presence of *Empetrum*. Distance between individual plots depended on habitat affiliation and relief structures in the landscape.

Site characteristics

To describe and quantitatively compare the environmental conditions of the different habitat types, we conducted vegetation surveys, estimated snow depth, and measured

Table 1 Climate data of the four study regions North-SC, North-SO, South-SC and South-SO (period 1961–1990; based on data by Swedish meteorological institute (SMHI www.smhi.se/klimatdata) and Norwegian meteorological Institute (MET www.eklima.met.no)

Region	Coordinates	Altitude of plots (m.a.s.l.)	Climate	Mean annual precipitation (mm)	Mean annual temperature (°C)	Monthly temperature maxima (°C)	JJA mean temperature (°C)	Monthly precipitation maxima (mm)	Monthly temperature minima (°C)	Precipitation as snow (mm) (Nov–Mar)	Growing season length (days) (mean 2009–2011)
Abisko North-SC	68°2′N18°49′E	420–720	Sub-continental	304	−0.8	Jul (11.0)	9.7	Jul (54)	Jan (−11.9)	107	140
Vassijaure North-SO	68°2′N18°10′E	480–680	Sub-oceanic	844	−1.7	Jul (10.4)	8.9	Oct (109)	Jan (−11.9)	349	128
Kongsvold South-SC	62°18′N09°36′E	980–1140	Sub-continental	450	−0.4	Jul (9.6)	8.9	Jul (68)	Jan (−9.4)	135	155
Samsjøen South-SO	63°05′N10°38′E	590–650	Oceanic	830	3.9	Jul (14.0)	13.2	Sep (101)	Jan (−6.0)	331	172

Climate data for the region around North-SO are available from Katterjåkk at a distance of 3 km to North-SO. Climate data for the region around South-SO are available from Melhus at a distance of 30 km to South-SO. Growing season starts when at least 5 consecutive days reached a threshold temperature of at least 5 °C and ends when the mean daily temperature of 5 consecutive days is below the 5 °C threshold. Calculation of growing season length in Norway is based on data from Dombås in 35 km distance to South-SC and Selbu in 25 km distance to South-SO

Jan January, *Mar* March, *Jul* July, *Sep* September, *Oct* October, *Nov* November, *JJA* June, July and August

humus depth, site openness, vegetation cover and *Empetrum* cover for each plot. Furthermore, we measured temperature at the soil surface with data loggers (micro-T, DS1922L; NexSens Technology, Alpha, Ohio, USA) from September 2012 to July 2013 every 3 h. We summed temperatures from the 1st of April to the 27th of June 2013 for all habitats and study regions. For statistical analyses, we calculated daily mean temperatures for each plot and summed up monthly temperature sums for April, May and June. The temperature curves (see Electronic Supplementary Material S1) of the habitat types showed almost the same patterns in all four regions during the analyzed time period. From early- to mid-April temperature curve of *s*-habitats fluctuated around −5 °C, whereas temperature of ***b***-habitats was around 0 °C; ***d***-habitats took an intermediate position. Only in South-SC, temperatures of all habitats were slightly higher, but not above 0 °C. From mid-April to early-May, temperature curves of all habitats fluctuated around 0 °C. From mid-May to late-June, the temperature curve of *s*-habitats was significantly higher than those of ***b***–and ***d***-habitats, which had nearly the same temperature. Higher temperatures in ***b***-than in *s*-habitats in North-SO might be due to lower canopy closure as a consequence of leaf damage due to a caterpillar outbreak. Independent of altitude, temperatures were very similar. Therefore, we assume that altitudinal differences between sites (especially high altitudes in South-SC) were compensated by a latitudinal effect.

Vegetation surveys were carried out in the northern study regions between the 19th of June and the 8th of July 2012 and in the southern study regions between the 9th and 17th of July 2013. To characterize the vegetation within the 1 × 1 m plots, we recorded the cover of all species in the tree-, shrub-, herb- and cryptogam-layer. Cover was estimated on an ordinal scale, ranging from 1 to 9: 1 = <5 % cover, only 1 individual, 2 = <5 % cover, 2–5 individuals, 3 = <5 % cover, 6–50 individuals, 4 = <5 %, >50 individuals, 5 = >5–12.5 %, 6 = >12.5–25 %, 7 = >25–50 %, 8 = >50–75 %, and 9 = 76–100 % cover (cf. Tremp 2005). Nomenclature follows Mossberg and Stenberg (2008) for vascular plants and Skytte Christiansen et al. (1996); Ursing (1953); Hallingbäck et al. (2006); Moberg and Holmasen (1999) for cryptogams.

In the birch forest, we used the height of *Parmelia olivacea* on birch stems to estimate the maximum winter snow depth (Sonesson et al. 1994), whereas in the alpine tundra, the height of the tallest but vital dwarf shrub or herb was used to estimate the minimum snow depth (Grogan and Jonasson 2006; Sturm et al. 2001).

Furthermore, at each plot, we measured the depth of the organic layer (from ground surface down to the mineral layer) and estimated total vegetation cover (proportion of vegetation-covered ground within the plot) and *Empetrum* cover (proportion of total plot area covered by crowberry, with 5 % accuracy).

To measure site openness, hemispherical images were taken with a Nikon Coolpix 4500 digital camera equipped with a 180° fisheye lens. The camera was installed on a tripod within the center of each plot at a height of 15 cm (due to technical errors during fieldwork at 30 cm in South-SO). Incidentally, in North-SC and North-SO, fisheye images were taken after the start of a caterpillar outbreak, which damaged birch leaves and thus influenced the estimates of site openness especially in birch forest plots. Color images were transformed into black and white images with the program Sidelook 1.1 (Nobis 2005). Afterwards, the software Gap light analyzer 2.0 (Frazer et al. 1999) was used to extract site openness of each individual plot as an indicator of habitat light conditions.

Shoot growth

To analyze shoot growth and morphology, three individual *Empetrum* ramets were randomly selected and harvested, in each plot in the northern and southern study regions in mid-June 2012 and September 2012, respectively. Before harvesting, ramet height was determined as height from soil surface to the top of the current year's shoot. The harvested ramets were stored in labeled plastic bags with a wet tissue in a cold room (5 °C) for a maximum of 3–4 days before further analysis.

Shoot morphology of *Empetrum* was measured according to Shevtsova et al. (1997) for the last four shoot generations (C = 2012, C_{+1} = 2011, C_{+2} = 2010, C_{+3} = 2009) of the main stem and of the lateral shoots. For further analyses, the current year's shoots were discarded because of different harvest periods at the two latitudes. For the three remaining shoot generations (C_{+1}, C_{+2}, C_{+3}), the following variables were recorded: length of the main shoot, number of lateral shoots, number of living green leaves, dead brown leaves, leaf scars (shed leaves) and leaves per mm stem length (hereafter, denoted as leaf density). We measured total biomass, total leaf dry mass and total stem dry mass after drying for 48 h at 65 °C. Furthermore, we calculated leaf life expectancy of shoots for each plot according to Krebs (1985), using the average number of vital leaves during the age interval C_{+1} to C_{+2} (=leaf life expectancy C_{+1}).

To obtain robust data integrated over years and to avoid pseudoreplication, for all statistical analyses, the three shoot generations C_{+1}, C_{+2} and C_{+3} were averaged per individual and data of the three selected ramets were averaged per plot. Thus, *N* equals 120 for data on environmental characteristics of plots and shoot growth.

Reproduction

In September 2012, we analyzed flower and fruit production of *Empetrum* shoots. For logistic reasons, this could only be done in the northern study regions (North-SC and North-SO; thus $N = 60$). We wanted to use the same plots like in the shoot growth study, but the caterpillar outbreak during the spring and summer of 2012 led to almost total defoliation of shoots in 5 plots in North-SO. Therefore, we replaced the previous 5 birch forest plots with 5 new plots with intact shoots at a distance of maximum 3.5 km away from the old ones, in birch forests with similar conditions. We used a wooden frame of 50 × 50 cm with a 7 × 7 grid of elastic threads, resulting in 49 intersection points. Within a maximum distance of 5 m of each study plot, we randomly selected five frame positions that contained *Empetrum*. At each intersection point within the frame, we recorded the presence of *Empetrum* shoots and counted the number of berries on that particular shoot. Furthermore, we randomly sampled 20 berries per plot. Seeds were extracted from berries and counted. We tested the floatability of seeds to separate filled (=alive; sinking) and empty (=dead; floating) seeds (Baskin et al. 2002) and measured the mass of sunken seeds after drying at 60 °C for 24 h.

Additionally, we counted the number of flower buds on *Empetrum* shoots that were collected for a common garden experiment. For each plot in North-SC and North-SO, one clone was sampled in autumn 2012 and from each clone between 30 and 60 ramets were cut and planted into a mixture of peat and sand. After 8 weeks in a greenhouse (day temperature 26 °C; night temperature 18 °C; air humidity 80 %), all visible flower buds were counted, and the percentage of flower buds for each clone (one plot) was calculated. Since flower buds are fully developed by September in the season before flowering (Bell and Tallis 1973), we assume that greenhouse conditions did not influence number of flower buds.

The following reproductive traits were recorded: number of berries per *Empetrum* shoot, mean number of seeds per berry, seed mass per filled (=sinking) seed (mg) (field data) and number of flower buds per shoot (data from clones in the greenhouse).

Statistical analysis

We used a hierarchical analysis of variance (ANOVA) with sequential sums of squares (Quinn and Keough 2002) to test the effect of latitude (factor levels [k] = 2: North, South), climate (k = 2: sub-atlantic (North-SO, South-SO), sub-continental (North-SC, South-SC), nested within latitude) and habitat (k = 3: ***b***, ***d*** and ***s***, nested within climate and latitude) on environmental variables and shoot growth of *Empetrum*. All factors were treated as fixed effects. ANOVA assumptions, such as normality, were visually checked using diagnostic plots and homogeneity of variances was tested by Cochran's test. Variables were log-, ln- or arcsine-transformed when necessary to improve homogeneity of

variances. As a simple measure of the relative effect of each factor on each of the dependent variables, we divided the sums of squares of each factor by the total sums of squares and expressed this ratio as a percentage (cf. Welden and Slauson 1986).

To compare means between habitat types, we employed two orthogonal planned contrasts (Quinn and Keough 2002). First, we tested whether plots with high winter snow accumulation (habitat types: ***b*** plus ***d***) differed from plots with shallow snow cover (habitat type: ***s***), and second, whether the two habitat types with high snow accumulation but contrasting canopy shade during the growing season differed from each other (i.e., ***b*** vs. ***d***).

The vegetation survey data were analyzed by detrended correspondence analysis (DCA) to analyze environmental gradients and compositional similarity between plots.

Ordinations were performed using PC-ORD 5.32 (McCune and Mefford 2006), all other analyses were done with STATISTICA 10.0 (StatSoft 2010).

Results

Site characteristics

Both environmental variables differed significantly among the three habitat types (Table 2; Fig. 1). As expected, snow depth decreased significantly within all study regions from the ***b***- (mean ± standard error 103.0 ± 8.5 cm) and ***d***- (36.3 ± 2.2 cm) to the ***s***-habitats (10.1 ± 0.7 cm). Along the climatic gradient, there was a significantly higher snow depth in the sub-oceanic (64.4 ± 8.4 cm) than the sub-continental study regions (35.1 ± 3.0). The factor habitat explained 83.7 % of the observed variation in snow depth, whereas climate explained only 1.8 %. The effect of latitude on snow depth was not significant.

We found highest site openness in the ***s***-habitats (86.0 ± 0.4 %), followed by ***d***-habitats and ***b***-habitats which were characterized by lower site openness (77.0 ± 1.7 and 49.2 ± 2.9 %, respectively). Along the climatic gradient, site openness (SC 67.0 ± 3.0; SO: 74.4 ± 2.0 %) was higher in the sub-oceanic study regions. Furthermore, along the latitudinal gradient, site openness decreased from North to South (North 75.7 ± 1.3; South 65.5 ± 3.3). The factor habitat explained 77.0 % of the total variation in site openness, whereas climate and latitude explained only 7.1 % and 6.5 %, respectively.

The ordination of the vegetation survey data showed a clear differentiation of habitats along the first axis (Fig. 2) with ***b***-habitat plots in the left part, ***d***-habitat plots in the center, and ***s***–habitat plots in the right part of the diagram. Environmental characteristics correlated with the first axis as follows: estimated snow depth ($r = -0.616$), temperature sum May ($r = 0.529$), leaf density ($r = 0.590$), humus depth ($r = -0.485$), vegetation cover ($r = -0.318$) and *Empetrum* cover ($r = -0.244$). Furthermore, there was a clear differentiation of the latitudes along the second axis, with North-SO in the lower part, North-SC and South-SC in the center and South-SO in the upper part. Environmental characteristics correlated with the second axis as follows: total biomass ($r = 0.676$), temperature sum April ($r = 0.577$), length of shoot segment ($r = 0.554$), leaf dry mass ($r = -0.508$), no. of lateral shoots ($r = 0.494$) and site openness ($r = -0.484$).

Table 2 The effect of habitat type, latitude and climatic region on site characteristics (hierarchical ANOVA)

Factor		Snow depth (log)		Site openness	
	df	SQ	% ev	SQ	% ev
Latitude	1	0.028^{ns}	0.1	3077.7***	6.5
Climate (latitude)	2	0.454**	1.8	3384.1***	7.1
Habitat [climate (latitude)]	8	21.279***	83.7	36468.4***	77.0
Contrasts					
b + ***d*** vs. ***s***	1	15.722***		13784.0***	
b vs. ***d***	1	3.346***		15478.0***	
Residuals	108	3.648	14.4	4454.9	9.4

ANOVA was performed on log-transformed data for estimated snow depth. Residual df was 107 for site openness

b birch forest, *d* deep snow cover sites, *ns* not significant, *s* shallow snow cover sites. *% ev* percent of explained variance

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Vegetative growth

For all vegetative traits, except leaf life expectancy, habitat explained the highest percentage of variation (38–80 %, Table 3; Fig. 3, Electronic Supplementary Material S2). Ramet height (***b***: 15.0 ± 0.8; ***d***: 11.7 ± 0.5; ***s***: 4.6 ± 0.2 cm), length of annual shoot segments (***b***: 32.3 ± 1.9; ***d***: 24.2 ± 1.5; ***s***: 10.4 ± 0.5 mm), number of lateral shoots (***b***: 3.9 ± 0.3; ***d***: 3.1 ± 0.2; ***s***: 1.8 ± 0.1) and total biomass (***b***: 32.3 ± 4.6; ***d***: 21.7 ± 3.2; ***s***: 5.7 ± 0.4 mg) showed lowest values in ***s***-habitats, intermediate values in ***d***-habitats and highest values in ***b***-habitats. In contrast, leaf density and relative leaf mass were highest in ***s***-habitats, intermediate in ***d***- and lowest in ***b***-habitats (leaf density ***b***: 1.4 ± 0.0; ***d***: 1.6 ± 0.1; ***s***: 2.5 ± 0.1 leaves per mm stem; relative leaf mass: ***b***: 6.0 ± 0.6; ***d***: 9.0 ± 0.8; ***s***: 19.0 ± 1.3 % of total biomass). Leaf life expectancy of the C_{+1} shoot generation was higher in the ***s***-habitats (1.2 ± 0.1 years) than in the ***b***- plus ***d***-habitats (***b***: 1.1 ± 0.1; ***d***: 1.0 ± 0.1 years).

Additionally, most vegetative traits differed significantly among latitudes (Fig. 3), although latitude mostly explained less of the total variation than habitat type. Relatively high

Alp Botany (2014) 124:115–129

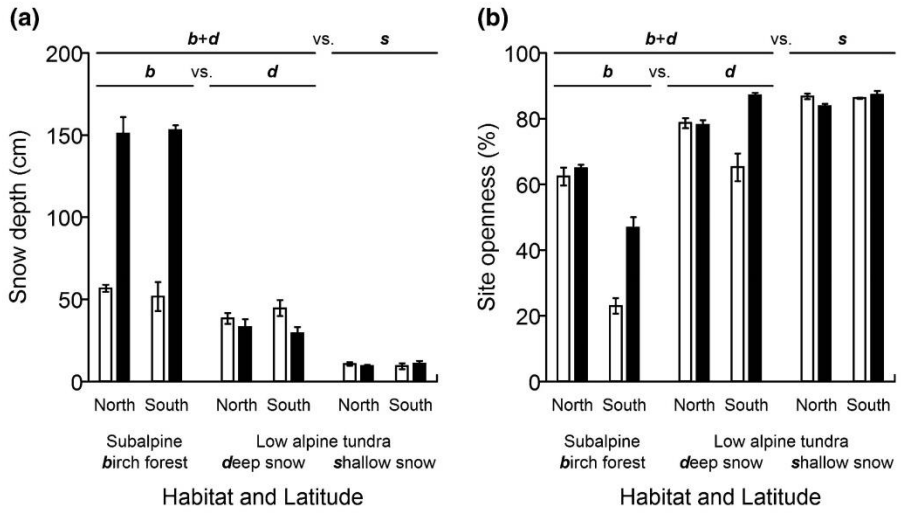


Fig. 1 Estimated snow depth (cm) (**a**), and site openness (%) (**b**) in different habitats with *Empetrum* along the climatic and latitudinal gradient. Values represent untransformed mean ± SE, $n = 10$. *White bars* represent sub-continental climate and *black bars* sub-oceanic climate. *Lines* above the *bars* depict the planned contrasts between $b + d$ vs. s and b vs. d, respectively. A break between the *lines* indicates significant differences between groups

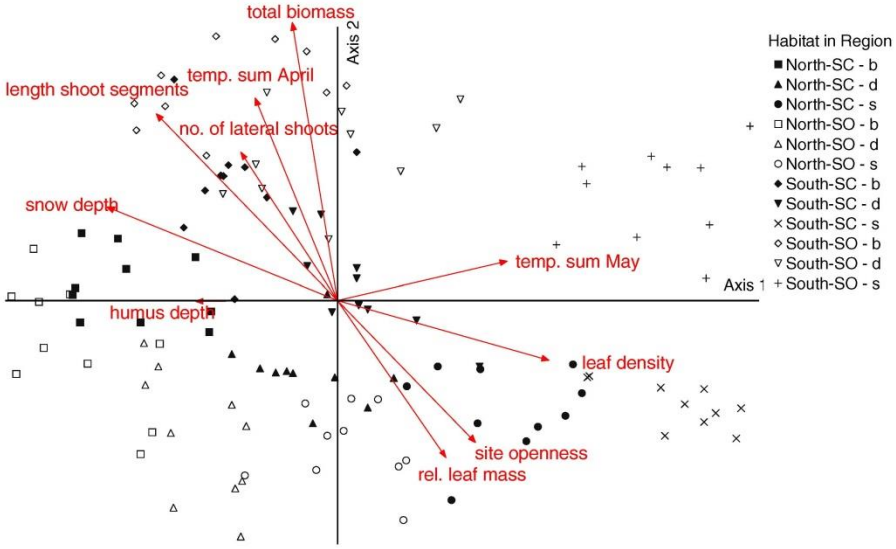


Fig. 2 DCA ordination of 120 vegetation surveys from habitats with deep snow cover and low (***b***) and intermediate site openness, respectively (***d***) and from habitats with shallow snow cover and higher site openness (***s***) in North and South with post hoc correlation of the ordination axes with environmental data and growth variables. Eigenvalue axis 1/axis 2: 0.44/0.23; length of gradient: 3.16/2.14

percentages of explained variation were found for leaf allocation (26.3 %) and total biomass (30.9 %), and the importance of latitude exceeded that of habitat type in the case of leaf life expectancy (31.2 %). Ramet height (North 9.1 ± 0.5; South 11.7 ± 0.9 cm), length of annual shoot segments (North 18.9 ± 0.10; South 25.5 ± 2.0 mm), number of lateral shoots (North 2.4 ± 0.1; South 3.4 ± 0.2) and total biomass (North 8.5 ± 0.6; South 31.0 ± 3.6 mg) were generally higher at lower latitudes. In contrast, leaf density (North 2.0 ± 0.1; South 1.7 ± 0.1 leaves per mm stem), relative leaf mass (North 15.1 ± 1.1; South 7.7 ± 0.8 % of total biomass) and leaf life expectancy of the C_{+1} shoot generation (North 1.3; South 0.9 years) were significantly lower at lower latitudes.

Although climate explained only between 1 and 14 % of the total variation, most traits differed significantly among climates within latitudes. Along the climatic gradient, relative leaf mass (SC 12.6 ± 1.1; SO 10.2 ± 1.0 % of total biomass) and leaf life expectancy were lower in the sub-oceanic study regions (SC 1.2 ± 0.0; SO 1.0 ± 0.0 years). In contrast, number of lateral shoots (SC 2.6 ± 0.2; SO 3.2 ± 0.2) and total biomass (SC 14.3 ± 1.6; SO 25.5 ± 3.8 mg) were significantly higher in the sub-oceanic

Table 3 The effect of habitat type, latitude and climatic region on shoot growth variables of *Empetrum* (hierarchical ANOVA)

Factor		Ramet height (log)		Shoot length (log)		# Lateral shoots		Total biomass (log)	
	df	SQ	% ev	SQ	% ev	SQ	% ev	SQ	% ev
Latitude	1	0.216***	3.0	0.213***	2.9	32.661***	11.5	6.149***	30.9
Climate (latitude)	2	0.090*	1.3	0.075^{ns}	1.0	13.354**	4.7	0.690***	3.5
Habitat [climate (latitude)]	8	5.722***	79.6	5.285***	71.2	108.798***	38.2	9.850***	49.4
Contrasts									
b + ***d*** vs. ***s***	1	5.345***		4.522***		75.561***		8.230***	
b vs. ***d***	1	0.181***		0.323***		10.691**		0.417***	
Residuals	107	1.160	16.1	1.846	24.9	130.131	45.7	3.238	16.3

Factor	*df*	Leaf density (log)		Relative leaf mass (log)		Leaf life exp. C_{+1}	
		SQ	% ev	SQ	% ev	SQ	% ev
Latitude	1	0.143***	6.3	4.254***	26.3	4.788***	31.2
Climate (latitude)	2	0.146***	6.4	0.693***	4.3	2.165***	14.1
Habitat [climate (latitude)]	8	1.349***	59.4	7.548***	46.5	1.990***	13.0
Contrasts							
b + ***d*** vs. ***s***	1	1.184***		5.671***		0.767***	
b vs. ***d***	1	0.071***		0.729***		0.213^{ns}	
Residuals	107	0.634	27.9	3.732	23.0	6.404	41.7

ANOVA was performed on log-transformed data for ramet height, shoot length, total biomass, leaf density and relative leaf mass. Residual df was 103 for leaf life exp. C_{+1}

b birch forest, *d* deep snow cover sites, *ns* not significant, *s* shallow snow cover sites. *% ev* percent of explained variance

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

than in the sub-continental regions. For ramet height and leaf density, there was no clear trend.

Reproduction

Fruit and flower production differed significantly (except number of berries and seed mass in North-SC) among habitat types (Table 4; Fig. 4). Number of berries per shoot (***b***: 0.02 ± 0.00; ***d***: 0.04 ± 0.01; ***s***: 0.07 ± 0.01), seed mass (***b***: 0.8 ± 0.0; ***d***: 0.9 ± 0.0 ***s***: 1.0 ± 0.0 mg) and number of flower buds (***b***: 6.3 ± 2.4; ***d***: 16.0 ± 4.7; ***s***: 26.9 ± 6.3) increased from ***b***- and ***d***- to ***s***-habitats. For the mean number of seeds per berry, there was no effect of habitat type. However, the number of seeds per berry (North-SC 7.5 ± 0.1; North-SO 8.0 ± 0.1) was significantly higher, and seed mass (North-SC 1.1 ± 0.0; North-SO 0.8 ± 0.0 mg) was significantly lower in the sub-continental study region. The number of berries and number of flower buds showed no significant effect of climate.

The factor habitat explained 31.4 and 24.9 % of variation in number of berries per shoot and number of flower buds per clone, whereas the factor climate had no significant influence on these two variables. In contrast, climate explained 34.6 and 39.1 % of the variation in number of seeds per berry and seed mass, respectively, whereas habitat explained only 5.6 and 25.0 %, respectively.

Discussion

Our data clearly show that vegetative growth and reproduction of *Empetrum* varied significantly among habitats defined according to winter snow depth. The relationship appears to be strong, as habitat effects were mostly larger than the effects of latitude and climate. This allows a novel multi-scale perspective on the geographic variation of morphological traits in *Empetrum*. Additionally, consistently different performance in terms of growth and reproduction between contrasting habitat types suggests that there may be local adaptation (Kawecki and Ebert 2004) to habitats with different winter snow cover (and co-varying abiotic conditions during the growing season) in this keystone species despite more or less continuous populations. Local adaptation, despite gene flow, has been recently demonstrated in the alpine grass *Festuca eskia* (Gonzalo-Turpin and Hazard 2009). For *Empetrum*, on-going landscape genetic studies will show whether observed

Alp Botany (2014) 124:115–129

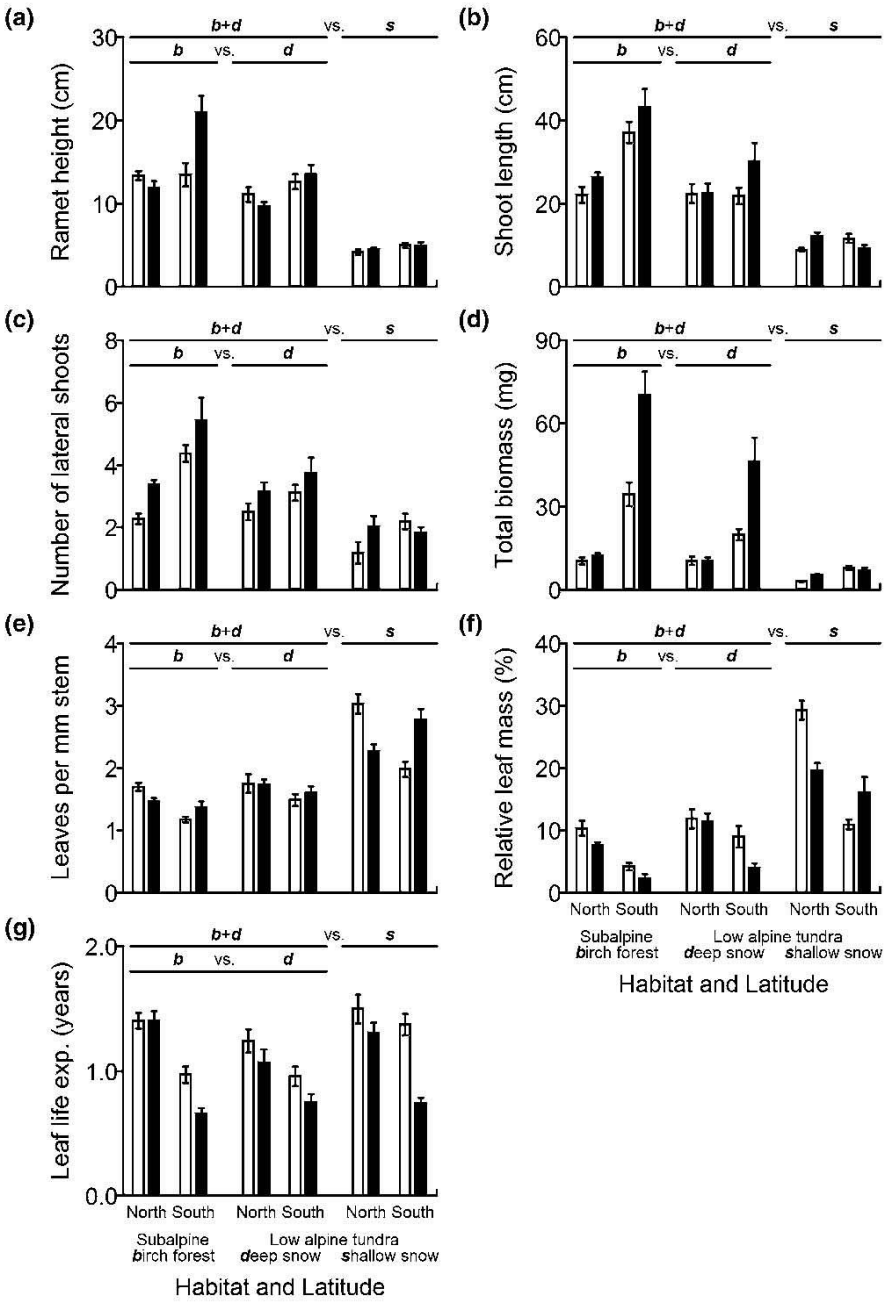


Fig. 3 Ramet height above ground (cm) (**a**), length of shoot segment (mm) (**b**), number of lateral shoots (**c**), total biomass (mg) (**d**), leaves per mm stem length (**e**), relative leaf dry mass (% of total biomass) (**f**), and leaf life expectancy (C_{+1}) (**g**) of *Empetrum* in different habitats along the climatic and latitudinal gradient. Values represent untransformed mean ± SE, $n = 10$. *White bars* represent sub-continental climate and *black bars* sub-oceanic climate. *Lines* above the *bars* depict the planned contrasts between ***b*** + ***d*** vs. ***s*** and ***b*** vs. ***d***, respectively. A break between the *lines* indicates significant differences between groups

phenotypic trait variation is genetically fixed or rather owing to phenotypic plasticity (Bienau et al. in progress).

Shoot growth

Statistical analysis confirmed that habitat types across latitudes and climates differed significantly in snow depth and snow data are quantitatively in line with the long-term snow depth records for Abisko (North-SC) of 51.5 cm in March (Kohler et al. 2006). The performance of *Empetrum* in habitats with deep winter snow cover with higher ramets, longer shoot segments, more lateral shoots and higher total biomass could, first, be a consequence of physical protection from wind damage and ice abrasion in winter. Shoot height of most dwarf shrubs is probably controlled by snow depth since shoots protruding above the protective snow layer will

Table 4 The effect of habitat type, latitude and climatic region on reproduction variables of *Empetrum* (hierarchical ANOVA)

Factor		Berries per shoot (ln)		Seeds per berry		Seed mass		# Flower buds (arcsine)	
	df	SQ	% ev	SQ	% ev	SQ	% ev	SQ	% ev
Climate	1	0.001^{ns}	0.2	4.564***	34.6	0.818***	39.1	0.282^{ns}	4.4
Habitat (climate)	4	0.192***	31.4	0.743^{ns}	5.6	0.522***	25.0	1.581**	24.9
Contrasts									
b + ***d*** vs. ***s***	1	0.098***		0.341^{ns}		0.225***		0.795**	
b vs. ***d***	1	0.039*		0.121^{ns}		0.158**		0.231^{ns}	
Residuals	54	0.419	68.4	7.871	59.7	0.751	35.9	4.499	70.7

ANOVA was performed on ln-transformed data for berries per shoot and on arcsine-transformed data for number of flower buds

b birch forest, *d* deep snow cover sites, *ns* not significant, *s* shallow snow cover sites. *% ev* percent of explained variance

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

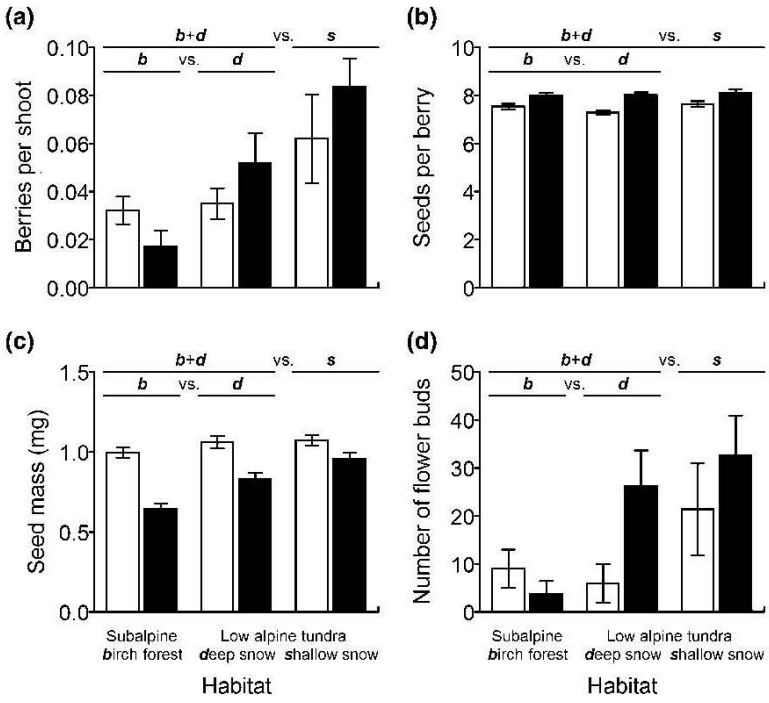


Fig. 4 Number of berries per shoot (**a**), mean number of seeds per berry (**b**), seed mass (mg) (**c**), and number of flower buds (**d**) of *Empetrum* in different habitats along the climatic and latitudinal gradient. Values represent untransformed mean ± SE, $n = 10$. *White bars* represent sub-continental climate and *black bars* sub-oceanic climate. *Lines* above the *bars* depict the planned contrasts between ***b*** + ***d*** vs. ***s*** and ***b*** vs. ***d***, respectively. A break between the *lines* indicates significant differences between groups

be damaged (Sonesson and Callaghan 1991; Callaghan et al. 2011). Second, snow has an insulating effect (Kelley and Weaver 1969). Thus, a snow layer of >20 cm will protect plant tissues from extreme temperatures and also reduce the potential damage of frost spells early in the season (Körner 2003). Finally, the performance of *Empetrum* in habitats with deep snow may be caused by facilitation through co-occurring erect shrubs such as *Betula nana* (Fletcher et al. 2010), which acts as a snow trap in winter, but also presents wind protection during the snow-free period (Sturm et al. 2001). Furthermore, water and nutrient availability during summer are higher in sheltered habitats (Billings and Bliss 1959; Hadley and Smith 1987; Sturm et al. 2001; Fletcher et al. 2010). In Arctic ecosystems with extreme abiotic conditions, facilitative effects of neighbors may be stronger than negative effects of competition (Carlsson and Callaghan 1991; Shevtsova et al. 1995; Callaway et al. 2002; Wipf et al. 2006; Olofsson et al. 2011).

In wind-exposed *s*-habitats, with low or lacking snow cover during winter, *Empetrum* has a more procumbent growth form with lower ramet height, shorter shoot segments and lower numbers of branches, but higher leaf density. As a result of an unstable, shallow snow cover during winter, soil temperature is lower and frost can penetrate more deeply into the soil than on sites with a protecting snow cover during winter (Sjögersten and

Wookey 2005). Consequently, a denser leaf packing probably presents an adaptation to cold winter temperatures and the prevailing strong winds, reducing freezing and desiccation (Körner 2003). Furthermore, the present study revealed significantly higher leaf life expectancy of the C_{+1} generation for *Empetrum* in the ***s***-than in ***b***-and ***d***-habitats. In the latter habitats with deep snow, leaf mortality may increase as a consequence of higher abundance of pathogens beneath the long-lasting snow cover (e.g., Olofsson et al. 2011). Specifically, *Arwidssonia empetri*, a host-specific fungal pathogen of *Empetrum*, may cause dramatic declines of its abundance (Olofsson et al. 2011). Deeper snow cover may also promote the development of other plant pathogens such as snow molds (fungi: Ascomycetes, Basidiomycetes, Zygomycetes; and fungi-like micro-organisms: Oomycetes), which damage plants at low temperatures under snow cover (Hoshino et al. 2009; Tojo and Newsham 2012). Generally, estimated leaf life expectancies are in line with observed leaf life spans of between 1 and 4 years for evergreen species, depending on species and habitat (Bliss 1971; Karlsson 1992).

Deeper snow cover may also lead to higher nutrient availability which might promote the growth of *Empetrum*. Generally, snowpack may act as a reservoir of atmospherically deposited inorganic nitrogen which leads to greater nitrogen inputs during snowmelt on sites with higher snow accumulation (Bowman 1992; Weih 1998) in ***b***-and ***d***-habitats as well as in sub-oceanic compared to sub-continental study sites. Furthermore, mineralization of organic matter as a source of soil inorganic nitrogen before and during snowmelt in spring is higher under deep snow packs (Brooks et al. 1996). In fertilizer experiments, *Empetrum* responded to artificially increased nutrient availability with an increase in leaf number and leaf mass per shoot, a greater shoot mass, an increase in shoot extension growth and stem length, an increase in height and production of more lateral branches (Chapin and Shaver 1985; Wookey et al. 1993; Parsons et al. 1994; Campioli et al. 2012; but see Press et al. 1998).

During the growing season, the amount of solar radiation is an important abiotic factor for *Empetrum* growth. Due to the low vegetation height, plants on wind-exposed ridges experience almost full illumination. In contrast, the ***b***-habitat showed the lowest site openness, caused by the presence of trees whose leaf canopies reduce solar radiation and light quality.

Higher relative allocation to leaves and higher leaf density might ensure sufficient assimilation and biomass production in the ***s***-habitats with high solar radiation and a long growing season, despite less favorable resource conditions. However, high solar radiation may lead to water stress in spring and summer through stomatal limitation of photosynthesis. Therefore, photosynthetic capacity of plants is higher in plots with late snow melt (Kudo et al. 1999; Fletcher et al. 2010). Furthermore, the longer shoot length in the more shaded ***b***- and ***d***-habitats may be related to shade avoidance. In general, plants show elongated stems and petioles and suppressed branching in darker environments to reach solar radiation (Schmitt and Wulff 1993; Stuefer and Huber 1998; McConnaughay and Coleman 1999; Callaway et al. 2003; Semchenko et al. 2012).

Owing to higher amounts of winter precipitation, our sub-oceanic study regions featured higher snow depths than sub-continental regions (Table 1). Also, *Empetrum* performance, in terms of shoot growth and morphology, varied significantly between climates, although the amount of variance explained by climate was relatively low.

The results showed higher relative leaf mass and leaf life expectancy and lower number of lateral shoots and total biomass in the sub-continental study regions. This is consistent with the response of *Empetrum* to different habitats and might be forced by greater nitrogen inputs during snowmelt on sites with higher snow accumulation (Bowman 1992; Weih 1998) as well as higher physical protection from wind and ice abrasion in winter (Sonesson and Callaghan 1991; Callaghan et al. 2011).

Furthermore, ramet height, length of annual shoot segments, number of lateral shoots and total biomass were higher at lower latitudes. This is probably related to relatively milder climate at more southern latitudes, e.g., indicated by c. 30 days (means of 2009–2011) longer growing season in the south, allowing prolonged growth (Jonas et al. 2008). Longer and more accelerated growth at southern latitudes can only be achieved through high assimilation rates, leading in turn to higher tissue turnover and lower leaf life expectancy. This is in line with Karlsson (1992), who found a positive relationship between leaf longevity and latitude.

Higher site openness in birch forest habitats in North-SC and North-SO than in South-SC and South-SO may be caused by the caterpillar outbreak during the summer of 2012 reducing the birch canopy in North-SC and North-SO. There is a 9- to 10-year cyclicity of caterpillar outbreaks (*Epirrita autumnata* and *Operophtera brumata*) in the Scandes (Tenow 1996; Bylund 1999; Ruohomäki et al. 2000). During these outbreaks, either limited areas might become totally defoliated or areas of hundreds of square kilometers might be damaged (Ruohomäki et al. 2000). The caterpillars do not only damage the birch leaves, but larvae dropping from the trees may defoliate the ground vegetation, in particular *Betula nana*, *Empetrum*, *Vaccinium myrtillus* and *V. vitis-idaea* (Tenow 1996).

Reproduction

Increasing numbers of berries per shoot, numbers of flower buds and seed mass from ***b***- and ***d***- to ***s***-habitats might be an

effect of the open habitat. Due to earlier snow melt, the growing season starts earlier which promotes flowering (Kudo and Suzuki 1999). Additionally, higher average temperatures in *s*-habitats during the growing season (Electronic Supplementary Material S1) will probably benefit fruit maturation and seed quality (Graae et al. 2008). *Empetrum* seeds need warm stratification after cold stratification to break dormancy (Baskin et al. 2002; Graae et al. 2008). Consequently, germination of *Empetrum* may be promoted by soil disturbance, which removes the insulating cover over seeds and enables warm stratification (Baskin et al. 2002) and by reduced competition from surrounding vegetation and other *Empetrum* individuals (Szmidt et al. 2002). A similar effect might be active in open habitats with earlier snowmelt. Therefore, the *s*-habitat seems to be the most favorable habitat for seed production and seedling establishment of *Empetrum.* Once *Empetrum* has established, clonal growth will be more important for determining site occupancy and population structure (Szmidt et al. 2002; Boudreau et al. 2010).

North-SC had lower seed numbers but heavier seeds than North-SO. Due to the later start of the growing season, seeds had less time to ripen and were therefore smaller. However, the significantly lower number of flower buds and berries and the production of lighter seeds in the ***b***-habitat of North-SO might partly be related to the effects of herbivory of caterpillars, which showed much higher abundances in the birch forest habitat in North-SO than in North-SC (personal observation). The caterpillars damaged *Empetrum* to a high degree which likely had a negative influence on reproductive variables of *Empetrum*.

Implications for the response of *Empetrum* to climate change

Expected changes in snow depth and timing of snow melt may have strong effects on Arctic ecosystems (Bokhorst et al. 2012). Snow manipulation experiments showed that earlier snowmelt resulted in a longer growing season (cf. Wipf et al. 2006). On the other hand, earlier snowmelt may also lead to increased frost damage because of a high probability and frequency of frost spells early in the year (Wipf et al. 2006, 2009). However, although higher elevation and earlier snow melt habitats had a higher risk of spring freezing exposure, spring freezing resistance of four shrub species did not differ significantly along elevational and snow melt gradients (Wheeler et al. 2014). Shoot growth, flower bud break and flowering in *Empetrum* were advanced when snowmelt occurred earlier (Wipf et al. 2009; Wipf 2010), whereas even short-term events like a 1-week episode of winter warming may have strong effects such as delayed bud burst in *Empetrum* and reduced shoot growth (Bokhorst et al. 2008, 2009).

The present study investigated the response of a plant species to natural variation of snow cover in the field across latitudinal and local climatic gradients. This comparative multi-site analysis along a steep natural environmental gradient, encompassing the range of climate change predictions, is more likely to give a realistic picture concerning extent of intraspecific phenotypic trait variation, which may determine the long-term adaptive potential of *Empetrum* to climate change (Körner 2003; Dunne et al. 2004; Kudo and Hirao 2006).

We found consistent variation among habitat types across latitudes and climatic gradients underlining that snow cover potentially represents a strong force of selection. Additionally, differences in the timing of snow melt may affect flowering phenology, restrict gene flow between habitats and lead to genetic isolation of microhabitats. Clear and consistent differences in growth and reproduction may suggest local adaptation of *Empetrum* to habitats differing in snow depth (Kawecki and Ebert 2004; Gonzalo-Turpin and Hazard 2009). However, shoots of *S. herbacea* from phenologically isolated microhabitats were not genetically differentiated (Cortés et al. 2014), but owing to asymmetric gene flow towards snow beds, these late-melting microhabitats were genetically more diverse than early melting ridge sites.

The present study demonstrates that *Empetrum* has a broad ecological niche and shows a consistent match between its growth and morphology and the prevailing local habitat conditions. The high morphological plasticity of *Empetrum* supports findings of climate change experiments, and suggests that the species has the potential to cope with changing snow conditions in the course of climate change. However, while phenotypic plasticity will allow individuals to immediately adapt to changing conditions, locally adapted populations may locally go extinct. The latter will offer the possibility for seedling recruitment of adapted genotypes, but possibly also for replacement of *Empetrum* by other species with cascading effects on ecosystem functioning. Therefore, it will be crucial to understand how much of the habitat-specific variation in growth and reproduction is driven by phenotypic plasticity or genetic variation before predictions concerning the effects of climate change on fitness and distribution of this ecosystem driver can be made.

Acknowledgments Field assistance was provided by Josef Scholz-vom Hofe, Sigrid Lindmo, Ingvil Kålås and Emmanuel Gardiner. We further thank Gabriel Schachtel for statistical advice, the director and staff of the Abisko Scientific Research Station for climate data, logistic support and accommodation. We are very grateful to Sonja Wipf and all anonymous reviewers for fruitful comments on an earlier draft of this manuscript, and to Darya Anderson and Christina Puzzolo for checking the English. Financial support was obtained from the Deutsche Forschungsgemeinschaft (DFG, grant EC209/9-1). All help is gratefully acknowledged.

References

ACIA (2004) Arctic climate impact assessment: impacts of a warming Arctic. Cambridge University Press

AMAP (2012) Arctic climate issues 2011: changes in Arctic snow, water, ice and permafrost. SWIPA 2011 overview report. Arctic monitoring and assessment programme (AMAP), Oslo

Baskin CC, Zackrisson O, Baskin JM (2002) Role of warm stratification in promoting germination of seeds of *Empetrum hermaphroditum* (Empetraceae), a circumboreal species with a stony endocarp. Am J Bot 89:486–493. doi:10.3732/ajb.89.3.486

Bell JNB, Tallis JH (1973) *Empetrum nigrum* L. J Ecol 61:289–305. doi:10.2307/2258934

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecol Lett 15:365–377. doi:10.1111/j.1461-0248.2011.01736.x

Billings WD, Bliss LC (1959) An alpine snowbank environment and its effects on vegetation, plant development, and productivity. Ecology 40:388–397. doi:10.2307/1929755

Bliss LC (1971) Arctic and alpine plant life cycles. Annu Rev Ecol Syst 2:405–438

Bokhorst S, Bjerke JW, Bowles FW et al (2008) Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. Glob Change Biol 14:2603–2612. doi:10.1111/j.1365-2486.2008.01689.x

Bokhorst SF, Bjerke JW, Tømmervik H et al (2009) Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. J Ecol 97:1408–1415. doi:10.1111/j.1365-2745.2009.01554.x

Bokhorst S, Bjerke JW, Tømmervik H et al (2012) Ecosystem response to climatic change: the importance of the cold season. Ambio 41:246–255. doi:10.1007/s13280-012-0310-5

Boudreau S, Ropars P, Harper KA (2010) Population dynamics of *Empetrum hermaphroditum* (Ericaceae) on a subarctic sand dune: evidence of rapid colonization through efficient sexual reproduction. Am J Bot 97:770–781. doi:10.3732/ajb.0900304

Bowman WD (1992) Inputs and storage of Nitrogen in winter snowpack in an alpine ecosystem. Arct Antarc Alp Res 24(3):211–215. doi:10.2307/1551659

Brooks PD, Williams MW, Schmidt SK (1996) Microbial activity under alpine snowpacks, Niwot Ridge, Colorado. Biogeochemistry 32:93–113. doi:10.1007/BF00000354

Bylund H (1999) Climate and the population dynamics of two insect outbreak species in the north. Ecol Bull 47:54–62

Callaghan T, Emanuelsson U (1985) Population structure and processes of tundra plants and vegetation. In: White J (ed) The population structure of vegetation. Springer, Netherlands, pp 399–439

Callaghan TV, Johansson M, Brown RD et al (2011) The changing face of Arctic snow cover: a synthesis of observed and projected changes. Ambio 40:17–31. doi:10.1007/s13280-011-0212-y

Callaway RM, Brooker RW, Choler P et al (2002) Positive interactions among alpine plants increase with stress. Nature 417:844–848. doi:10.1038/nature00812

Callaway RM, Pennings SC, Richards CL (2003) Phenotypic plasticity and interactions among plants. Ecology 84:1115–1128. doi:10.1890/0012-9658(2003)084[1115:PPAIAP]2.0.CO;2

Campioli M, Leblans N, Michelsen A (2012) Twenty-two years of warming, fertilisation and shading of subarctic heath shrubs promote secondary growth and plasticity but not primary growth. PLoS ONE 7:e34842. doi:10.1371/journal.pone.0034842

Carlsson BA, Callaghan TV (1991) Positive plant interactions in Tundra vegetation and the importance of shelter. J Ecol 79:973–983. doi:10.2307/2261092

Chapin FS, Shaver GR (1985) Individualistic growth response of Tundra plant species to environmental manipulations in the field. Ecology 66:564–576. doi:10.2307/1940405

Cortés AJ, Waeber S, Lexer C, Sedlacek J, Wheeler JA, van Kleunen M, Bossdorf O, Hoch G, Rixen C, Wipf S, Karrenberg S (2014) Small-scale patterns in snowmelt timing affect gene flow and the distribution of genetic diversity in the alpine dwarf shrub *Salix herbacea*. Heredity. doi:10.1038/hdy.2014.19

Crawford RMM (2008) Cold climate plants in a warmer world. Plant Ecol Divers 1:285–297. doi:10.1080/17550870802407332

Dierßen K (1996) Vegetation nordeuropas. Ulmer, Stuttgart

Dunne JA, Saleska SR, Fischer ML, Harte J (2004) Integrating experimental and gradient methods in ecological climate change research. Ecology 85:904–916. doi:10.1890/03-8003

Fletcher BJ, Press MC, Baxter R, Phoenix GK (2010) Transition zones between vegetation patches in a heterogeneous Arctic landscape: how plant growth and photosynthesis change with abundance at small scales. Oecologia 163:47–56. doi:10.1007/s00442-009-1532-5

Frazer G, Canham C, Lertzman K (1999) Gap light analyzer (GLA), Version 2.0: imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York

Gerdol R, Siffi C, Iacumin P et al (2013) Advanced snowmelt affects vegetative growth and sexual reproduction of *Vaccinium myrtillus* in a sub-alpine heath. J Veg Sci 24:569–579. doi:10.1111/j.1654-1103.2012.01472.x

Gonzalo-Turpin H, Hazard L (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. J Ecol 97:742–751. doi:10.1111/j.1365-2745.2009.01509.x

Graae BJ, Alsos IG, Ejrnaes R (2008) The impact of temperature regimes on development, dormancy breaking and germination of dwarf shrub seeds from arctic, alpine and boreal sites. Plant Ecol 198:275–284. doi:10.1007/s11258-008-9403-4

Grogan P, Jonasson S (2006) Ecosystem CO_2 production during winter in a Swedish subarctic region: the relative importance of climate and vegetation type. Glob Change Biol 12:1479–1495. doi:10.1111/j.1365-2486.2006.01184.x

Haapasaari M (1988) The oligotrophic heath vegetation of northern Fennoscandia and its zonation. Acta Bot Fenn 135:1–219

Hadley JL, Smith WK (1987) Influence of krummholz mat microclimate on needle physiology and survival. Oecologia 73:82–90. doi:10.1007/BF00376981

Hallingbäck T, Lönnell N, Weibull H, et al. (2006) Nationalnyckeln till Sveriges flora och fauna. Bladmossor: Sköldmossor—blåmossor. Bryophyta: Buxbaumia—Leucobryum. Artdatabanken, SLU, Uppsala

Hoshino T, Xiao N, Xiao N et al (2009) Cold adaptation in the phytopathogenic fungi causing snow molds. Mycoscience 50:26–38. doi:10.1007/S10267-008-0452-2

IPCC (2013) Climate change 2013: the physical science basis. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK et al (eds) Contribution of Working Group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

Jäger EJ, Rothmaler W (2011) Exkursionsflora von Deutschland Bd. 2, Spektrum Akademischer Verlag, Heidelberg

Jonas T, Rixen C, Sturm M, Stoeckli V (2008) How alpine plant growth is linked to snow cover and climate variability. J Geophys Res 113:1–10. doi:10.1029/2007JG000680

Jonasson S (1981) Plant communities and species distribution of low alpine *Betula nana* heaths in northernmost Sweden. Vegetatio 44:51–64. doi:10.1007/BF00119804

Jonasson S, Sköld SE (1983) Influences of frost-heaving on vegetation and nutrient regime of polygon-patterned ground. Vegetatio 53:97–112. doi:10.1007/BF00043030

Karlsson PS (1992) Leaf longevity in evergreen shrubs: variation within and among European species. Oecologia 91:346–349. doi:10.1007/BF00317622

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241. doi:10.1111/j.1461-0248.2004.00684.x

Kelley JJ, Weaver DF (1969) Physical processes at the surface of the Arctic tundra. Arctic 22:425–437. doi:10.14430/arctic3233

Kohler J, Brandt O, Johansson M, Callaghan T (2006) A long-term Arctic snow depth record from Abisko, northern Sweden, 1913–2004. Polar Res 25:91–113. doi:10.1111/j.1751-8369.2006.tb00026.x

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems, 2nd edn. Springer Verlag, Berlin

Krebs CJ (1985) Ecology—the experimental analysis of distribution and abundance, 3rd edn. Harper & Row, Publishers

Kudo G, Hirao AS (2006) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts. Popul Ecol 48:49–58. doi:10.1007/s10144-005-0242-z

Kudo G, Suzuki S (1999) Flowering phenology of alpine plant communities along a gradient of snowmelt timing. Polar Biosci 12:100–113

Kudo G, Nordenhall U, Molau U (1999) Effects of snowmelt timing on leaf traits, leaf production, and shoot growth of alpine plants: comparisons along a snowmelt gradient in northern Sweden. Ecoscience 6:439–450

Lid J, Lid DT (1994) Norsk flora. Det Norske Samlaget, Oslo

McConnaughay KDM, Coleman JS (1999) Biomass allocation in plants: ontogeny or Optimality? A test along three resource gradients. Ecology 80:2581–2593. doi:10.1890/0012-9658(1999)080[2581:BAIPOO]2.0.CO;2

McCune B, Mefford M (2006) PC-ORD. Multivariate analysis of ecological data. Version 5.32. MjM Software. Gleneden Beach, Oregon, USA.

McGraw JB, Antonovics J (1983) Experimental ecology of *Dryas octopetala*. Ecotypes: I. ecotypic differentiation and life-cycle stages of selection. J Ecol 71:879–897. doi:10.2307/2259599

MET (Norwegian Meteorological Institute). http://sharki.oslo.dnmi.no/portal/page?_pageid=73,39035,73_39049&_dad=portal&_schema=PORTAL. Accessed 26 November 2013

Moberg R, Holmasen I (1999) Flechten von Nord- und Mitteleuropa. Ein Bestimmungsbuch, 1. Auflage. Spektrum Akademischer Verlag, Heidelberg

Mossberg B, Stenberg L (2008) Fjällflora. Wahlström & Widstrand, Stockholm

Nilsson MC, Wardle DA (2005) Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. Front Ecol Environ 3:421–428. doi:10.1890/1540-9295(2005)003[0421:UVAAFE]2.0.CO;2

Nobis M (2005) SideLook 1.1. In: Software Informer. http://sidelook.software.informer.com/1.1/. Accessed 16 October 2013

Odland A, Munkejord HK (2008) Plants as indicators of snow layer duration in southern Norwegian mountains. Ecol Indic 8:57–68. doi:10.1016/j.ecolind.2006.12.005

Olofsson J, Ericson L, Torp M et al (2011) Carbon balance of Arctic tundra under increased snow cover mediated by a plant pathogen. Nat Clim Change 1:220–223. doi:10.1038/nclimate1142

Parsons AN, Welker JM, Wookey PA et al (1994) Growth responses of four sub-Arctic dwarf shrubs to simulated environmental change. J Ecol 82:307–318. doi:10.2307/2261298

Press MC, Potter JA, Burke MJW et al (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. J Ecol 86:315–327. doi:10.1046/j.1365-2745.1998.00261.x

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press

Ruohomäki K, Tanhuanpää M, Ayres MP et al (2000) Causes of cyclicity of *Epirrita autumnata* (Lepidoptera, Geometridae): grandiose theory and tedious practice. Popul Ecol 42:211–223. doi:10.1007/PL00012000

Saarinen T, Lundell R (2010) Overwintering of *Vaccinium vitis-idaea* in two sub-Arctic microhabitats: a reciprocal transplantation experiment. Polar Res 29:38–45. doi:10.1111/j.1751-8369.2010.00152.x

Sandberg G (1958) Fjällens vegetationsregioner, vegetationsserier och viktigaste växtekologiska faktorer. In: Skunke F (ed) Renbeten och deras gradering. Lappväsendet—Renforskningen. Meddelande 4 36–60

Schmitt J, Wulff RD (1993) Light spectral quality, phytochrome and plant competition. Trends Ecol Evol 8:47–51. doi:10.1016/0169-5347(93)90157-K

Semchenko M, Lepik M, Götzenberger L, Zobel K (2012) Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate? J Ecol 100:459–466. doi:10.1111/j.1365-2745.2011.01936.x

Shabanov NV, Zhou L, Knyazikhin Y et al (2002) Analysis of interannual changes in northern vegetation activity observed in AVHRR data from 1981 to 1994. IEEE Trans Geosci Remote Sens 40:115–130. doi:10.1109/36.981354

Shevtsova A, Ojala A, Neuvonen S et al (1995) Growth and reproduction of dwarf shrubs in a subarctic plant community: annual variation and above-ground interactions with neighbours. J Ecol 83:263–275. doi:10.2307/2261565

Shevtsova A, Haukioja E, Ojala A (1997) Growth response of subarctic dwarf shrubs, *Empetrum nigrum* and *Vaccinium vitis-idaea*, to manipulated environmental conditions and species removal. Oikos 78:440–458. doi:10.2307/3545606

Sjögersten S, Wookey PA (2005) The role of soil organic matter quality and physical environment for Nitrogen mineralization at the forest-tundra ecotone in Fennoscandia. Arct Antarc Alp Res 37:118–126. doi:10.1657/1523-0430(2005)037[0118:TROSOM]2.0.CO;2

Skytte Christiansen M, von Krusenstjerna E, Waern M (1996) Vår flora i färg. Norsted & Söners, Stockholm

SMHI (The Swedish Meteorological and Hydrological Institute). http://www.smhi.se/klimatdata/meteorologi/temperatur/dataserier-med-normalv%C3%A4rden-1.7354. Accessed 30 August 2013

Sonesson M, Callaghan TV (1991) Strategies of survival in plants of the Fenoscandian tundra. Arctic 44:95–105. doi:10.14430/arctic1525

Sonesson M, Lundberg B (1974) Late quaternary forest development of the Torneträsk area, North Sweden. 1. Structure of modern forest ecosystems. Oikos 25:121–133. doi:10.2307/3543633

Sonesson M, Osborne C, Sandberg G (1994) Epiphytic lichens as indicators of snow depth. Arct Antarc Alp Res 26:159–165. doi:10.2307/1551779

StatSoft (2010) STATISTICA Version 10.0. Statistics and analytics software package

Stuefer JF, Huber H (1998) Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. Oecologia 117:1–8. doi:10.1007/s004420050624

Sturm M, Holmgren J, McFadden JP et al (2001) Snow–shrub interactions in Arctic tundra: a hypothesis with climatic implications. J Clim 14:336–344. doi:10.1175/1520-0442(2001)014<0336:SSIIAT>2.0.CO;2

Szmidt AE, Nilsson MC, Briceño E et al (2002) Establishment and genetic structure of *Empetrum hermaphroditum* populations in northern Sweden. J Veg Sci 13:627–634. doi:10.1111/j.1654-1103.2002.tb02090.x

Tenow O (1996) Hazards to a mountain birch forest—Abisko in perspective. Ecol Bull 45:104–114

Tojo M, Newsham KK (2012) Snow moulds in polar environments. Fungal Ecol 5:395–402. doi:10.1016/j.funeco.2012.01.003

Tremp H (2005) Aufnahme und Analyse vegetationsökologischer Daten: 41 Tabellen. Ulmer, Stuttgart (Hohenheim)

Tybirk K, Nilsson MC, Michelsen A et al (2000) Nordic *Empetrum* dominated ecosystems: function and susceptibility to environmental changes. Ambio 29:90–97. doi:10.1579/0044-7447-29.2.90

Ursing B (1953) Svenska växter i text och cild. Cryptogamer. Nordisk rotogravyr, Stockholm

Virtanen R, Eurola S (1997) Middle oroarctic vegetation in Finland and middle-northern arctic vegetation on Svalbard. Svenska växtgeografiska sällsk

Walker MD, Wahren CH, Hollister RD et al (2006) Plant community responses to experimental warming across the tundra biome. PNAS 103:1342–1346. doi:10.1073/pnas.0503198103

Weih M (1998) Seasonality of nutrient availability in soils of subarctic mountain birch woodlands, Swedish Lapland. Arct Antarc Alp Res 30:19–25. doi:10.2307/1551741

Welden C, Slauson W (1986) The intensity of competition versus its importance—an overlooked distinction and some implications. Q Rev Biol 61:23–44. doi:10.1086/414724

Wheeler JA, Hoch G, Cortés AJ, Sedlacek J, Wipf S, Rixen C (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. Oecologia 175:219–229. doi:10.1007/s00442-013-2872-8

Wipf S (2010) Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. Plant Ecol 207:53–66. doi:10.1007/s11258-009-9653-9

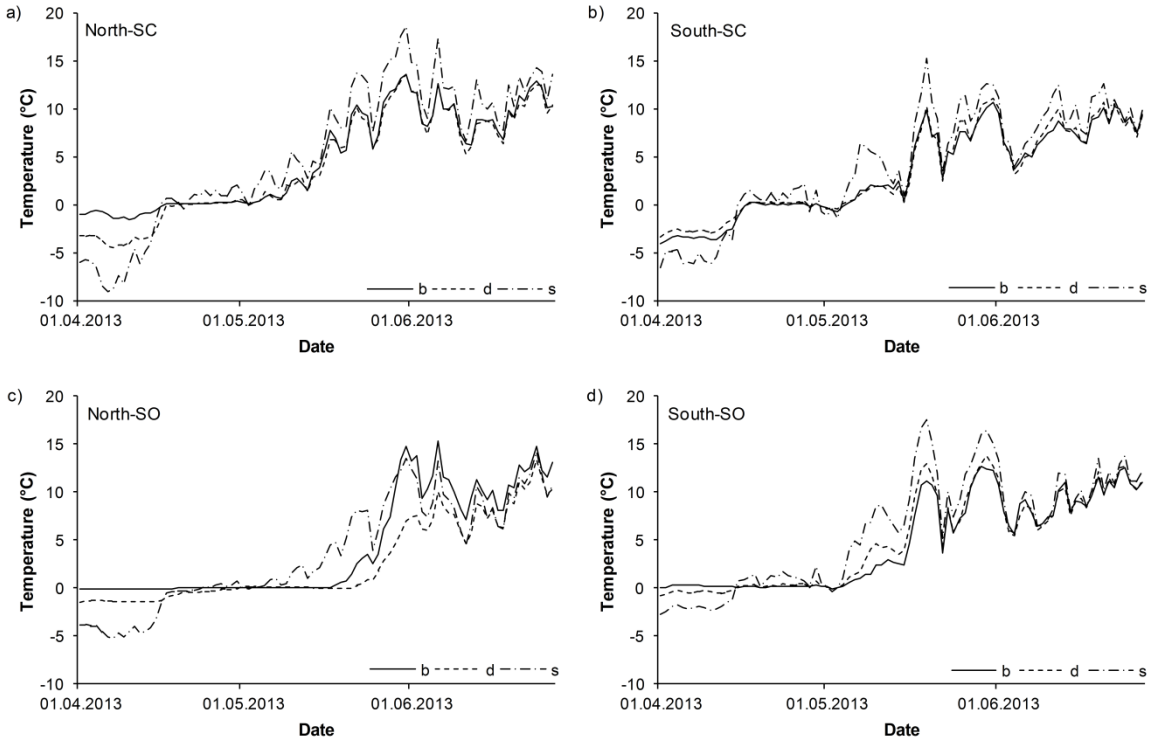
Wipf S, Rixen C (2010) A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. Polar Res 29:95–109. doi:10.3402/polar.v29i1.6054

Wipf S, Rixen C, Mulder CPH (2006) Advanced snowmelt causes shift towards positive neighbour interactions in a subarctic tundra community. Glob Change Biol 12:1496–1506. doi:10.1111/j.1365-2486.2006.01185.x

Wipf S, Stoeckli V, Bebi P (2009) Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. Clim Change 94:105–121. doi:10.1007/s10584-009-9546-x

Wookey PA, Parsons AN, Welker JM et al (1993) Comparative responses of phenology and reproductive development to simulated environmental change in sub-Arctic and high Arctic plants. Oikos 67:490–502. doi:10.2307/3545361

Supplementary Material



Supplementary Material S1: Graphs show temperature curves of North-SC, South-SC, North-SO and South-SO from 1st of April to 27th of June 2013. Solid line represents birch forest, dashed line alpine tundra with deep snow cover and dot-dashed line alpine tundra with shallow snow cover.

Supplementary Material S2: Photographs showing *Empetrum* shoots from the subalpine birch forest (left), low alpine tundra with deep snow cover (middle) and low alpine tundra with shallow snow cover (right).

CHAPTER 3

Synchronous flowering despite differences in snowmelt among habitats of *Empetrum hermaphroditum*

Miriam J. Bienau, Michael Kröncke, Wolf L. Eiserhardt, Annette Otte, Bente J. Graae, Dagmar Hagen, Ann Milbau, Walter Durka and R. Lutz Eckstein

Acta Oecologica 69 (2015) 129-136

Acta Oecologica 69 (2015) 129–136

Contents lists available at ScienceDirect

Acta Oecologica

journal homepage: www.elsevier.com/locate/actoec

Original article

Synchronous flowering despite differences in snowmelt timing among habitats of *Empetrum hermaphroditum*

Miriam J. Bienau [a, *], Michael Kröncke [b], Wolf L. Eiserhardt [c, 1], Annette Otte [a], Bente J. Graae [c], Dagmar Hagen [d], Ann Milbau [e], Walter Durka [f], R.Lutz Eckstein [a, 2]

[a] Institute of Landscape Ecology and Resource Management, Research Centre for BioSystems, Land Use and Nutrition (IFZ), Justus-Liebig University Giessen, Heinrich-Buff-Ring 26-32, DE-35392 Giessen, Germany
[b] Faculty of Nature and Technology (Faculty 5), University of Applied Sciences Bremen, Neustadtswall 30, DE-28199 Bremen, Germany
[c] Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, NO-7491 Trondheim, Norway
[d] Norwegian Institute for Nature Research, Department of Terrestrial Ecology, P.O. Box 5685, Sluppen, NO-7485 Trondheim, Norway
[e] Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University, SE-98107 Abisko, Sweden
[f] Helmholtz Centre for Environmental Research UFZ, Theodor-Lieser-Str. 4, DE-06120 Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 18 August 2015
Received in revised form
9 October 2015
Accepted 10 October 2015
Available online xxx

Keywords:
Sub-arctic
Birch forest
Tundra
Evergreen dwarf-shrub

ABSTRACT

The topography within arctic-alpine landscapes is very heterogeneous, resulting in diverse snow distribution patterns, with different snowmelt timing in spring. This may influence the phenological development of arctic and alpine plant species and asynchronous flowering may promote adaptation of plants to their local environments.

We studied how flowering phenology of the dominant dwarf shrub *Empetrum hermaphroditum* varied among three habitats (exposed ridges, sheltered depressions and birch forest) differing in winter snow depth and thus snowmelt timing in spring, and whether the observed patterns were consistent across three different study areas.

Despite significant differences in snowmelt timing between habitats, full flowering of *E. hermaphroditum* was nearly synchronous between the habitats, and implies a high flowering overlap. Our data show that exposed ridges, which had a long lag phase between snowmelt and flowering, experienced different temperature and light conditions than the two late melting habitats between snowmelt and flowering.

Our study demonstrates that small scale variation seems matter less to flowering of *Empetrum* than interannual differences in snowmelt timing.

1. Introduction

Within arctic-alpine landscapes topography and prevailing wind directions shape a heterogeneous snow distribution during winter (Molau et al., 2005). While there are differences in timing of snowmelt between years, the spatial distribution of snow is rather constant (Kudo and Hirao, 2006). The alpine tundra represents a mosaic of early-melting habitats on wind-exposed ridges with shallow snow cover, and late-melting habitats in wind-sheltered depressions with deep snow cover. Also in sub-arctic birch forest, birch stems act as snow traps, leading to accumulation of snow that then melts later than in more open habitats. Differences in snow cover across small spatial scales might promote local variation in the timing of flowering and fruiting (Bliss, 1962), which is controlled by temperature, photoperiod, irradiation, and precipitation (Hülber et al., 2010; Kudo, 1991; Kudo and Hirao, 2006; Molau et al., 2005). As a consequence, flowering of different populations of a single species along a snowmelt gradient may be highly asynchronous (Hirao and Kudo, 2004). Asynchronous flowering and phenological isolation as a result of differences in snowmelt timing may restrict gene flow via airborne pollen in wind pollinated species (Hirao and Kudo, 2004) and thus promote adaptation of plants to their local environmental conditions (Kawecki and Ebert, 2004). Flowering phenology is closely related to plant fitness. Late flowering may

* Corresponding author.
E-mail address: Miriam.J.Bienau@umwelt.uni-giessen.de (M.J. Bienau).
[1] Present address: Comparative Plant and Fungal Biology Dept., Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK.
[2] Present address: Department of Environmental and Life Sciences, Biology, Karlstad University, SE 651 88 Karlstad, Sweden.

http://dx.doi.org/10.1016/j.actao.2015.10.005
1146-609X/

lead to failure of seed-maturation when the growing season is short (Kawai and Kudo, 2011; Kudo, 1991; Thórhallsdóttir, 1998). However, very early flowering increases the risk of frost damage and may also reduce reproductive success (Inouye, 2008; Semenchuk et al., 2013; Wheeler et al., 2014). The relationship between date of snowmelt and onset of flowering is generally stronger for early flowering species than for later flowering species (Dunne et al., 2003). Flowering phenology may be influenced by climate, directly by altered thermal or precipitation conditions during the present and previous growing season, and indirectly through variation in snowmelt timing (Kudo and Hirao, 2006). Snow manipulation experiments as well as studies along natural snow gradients have shown that snowmelt timing affects phenology, with reduced flowering and reproductive success due to a shorter growing season after late snowmelt (Cooper et al., 2011; Kudo and Hirao, 2006). Wipf et al. (2009) demonstrated that manipulated snow cover in winter and experimentally advanced snowmelt in spring influenced flowering phenology in four abundant tundra species (*Empetrum hermaphroditum*, *Vaccinium myrtillus*, *Vaccinium uliginosum* and *Loiseleuria procumbens*). However, differences between snowmelt treatments in this study (6–11 days) were small as compared to those along natural snow cover gradients, where snowmelt timing may differ up to two months between the early and late melting habitats (Kudo and Hirao, 2006). Hence, it is not clear how flowering phenology varies among plants in habitats strongly varying in snowmelt timing along natural snow cover gradients. Studying natural instead of manipulated site conditions allows the analysis of phenological responses of species along the full extent of natural gradients (e.g. Kameyama and Kudo, 2009; Kudo and Hirao, 2006), and may shed new light on the impact of snowmelt timing on flowering phenology.

The wind-pollinated evergreen dwarf shrub *E. hermaphroditum* (*Empetrum nigrum* agg., Jäger and Rothmaler, 2011; hereafter denoted as *Empetrum*) is a keystone species of boreal, alpine and arctic ecosystems. The species mostly reproduces through clonal growth, but fruit production also occurs by self-as well as cross-pollination (Bell and Tallis, 1973). Flower buds are produced during autumn of the year before flowering (Bell and Tallis, 1973), which enables this species to start flowering as soon as the conditions are favorable (cf. Hülber et al., 2010). Hence, *Empetrum* is one of the earliest flowering tundra species, besides species like *Saxifraga oppositifolia*, *Carex rupestris* and *Salix herbacea* (Bell and Tallis, 1973; Molau et al., 2005; Thórhallsdóttir, 1998; Wipf et al., 2006).

The present study compares flowering phenology of *Empetrum* along a natural snow cover gradient with early (alpine tundra on wind-exposed ridges) and late melting habitats (birch forest and alpine tundra in wind-sheltered depressions). The study was conducted in three study areas. In the year 2013 two study areas at different latitudes were examined. In 2014 one of them was resampled together with a second one at the same latitude but along a local climatic gradient. Assuming that snowmelt is the main driver of phenological development of *Empetrum* (Anadon-Rosell et al., 2014; Wipf, 2010; Wipf et al., 2006) we expect differences in the timing of flowering of *Empetrum* in early and late melting habitats.

The following questions were addressed:

Q1: Is flowering of *Empetrum* synchronized with snowmelt timing across habitats with different snow cover? If so, we expect earlier flowering in early-than in late-melting habitats.
Q2: Is there a temporal overlap in the full flowering phenophase of *Empetrum* within and between early- and late-melting habitats? If full flowering is controlled by snowmelt, timing differences in snowmelt date among habitats will result in strongly asynchronous flowering.
Q3: Is the lag phase between snowmelt and flowering related to the prevailing temperature and light conditions across the three habitats?

2. Material and methods

2.1. Study regions and habitat description

Study regions and habitats have been described in detail by Bienau et al. (2014). In short: three study areas were selected at two different latitudes, i.e. northern Sweden (North: Abisko [68°2′N 18°49′E; mean annual precipitation: 304 mm; mean annual temperature: −0.8 °C] and Vassijaure [68°2′N 18°10′E; 844 mm; −1.7 °C]) and Central Norway (South: Kongsvoll [62°18′N 09°36′E; 450 mm; −0.4 °C]).

The sites in Sweden represent a steep gradient between sub-continental (SC; Abisko) and sub oceanic climate (SO; Vassijaure), with low and high winter precipitation, respectively. The study region Kongsvoll in Norway has sub-continental climate like Abisko in Northern Sweden. Study regions are abbreviated as North-SC (Abisko), North-SO (Vassijaure) and South-SC (Kongsvoll).

At each of the three sites we distinguished three habitat types differing in snow depth and co-varying abiotic factors (Table 1) based on topography, community type and indicator species of contrasting snow cover conditions (Jonasson, 1981; Odland and Munkejord, 2008): exposed ridges (***s***), sheltered depressions (***d***) and birch forest (***b***).

We established five 2 × 2 m plots per habitat in each of the three study sites in summer 2012. Plot selection was done according to topography and indicator species for snow conditions on sites where *Empetrum* was present. Distance between individual plots depended on habitat distribution and terrain structure in the natural landscape. The plots in North-SC and North-SO were arranged in south–north direction, with a mean distance of 500 and 350 m, respectively, between individual plots. The altitude of the plots was 420–720 m a.s.l. for North-SC and 480–680 m a.s.l. for North-SO. Plot arrangement in South-SC was in west–east direction, with a mean distance of 150 m between individual plots at altitudes of 980–1140 m a.s.l.

2.2. Observation of flowering phenology

Within each 2 × 2 m plot, 20 randomly sampled *Empetrum* shoots were numbered and permanently tagged with wire. The spatial distance between the ramets was at minimum 30 cm, to reduce the risk of sampling the same genetic individual. However, we could not exclude the effect of genetic relatedness on flowering of *Empetrum*.

Phenophases were determined at intervals of one to seven days during the pre-flowering and flowering period. Observations were made in spring 2013 in North-SC (2nd to 22nd of May; Day of the year since 1st January = DOY 122–142) and South-SC (19th of May to 7th of June; DOY 139–158) and in spring 2014 in North-SC (23rd to 29th of May; DOY 143–149) and North-SO (14th to 24th of June; DOY 165–175).

Four flowering phenophases were differentiated:

1 ***bf*** – before flowering: All buds of the shoot (formed in the previous autumn) are still completely closed.
2 ***fo*** – flowers opening: At least one bud of the shoot is beginning to swell and open (stamens still inside the bud), but no flower is fully opened yet.

Table 1
Characteristics of the different habitats. In the ***b***-habitat, we used the height of *Parmelia olivacea* on birch stems to estimate the maximum winter snow depth (Sonesson et al., 1994), whereas in the alpine tundra (***d***- and ***s***-habitat), the height of the tallest but vital dwarf shrub or herb was used to estimate the minimum snow depth (Grogan and Jonasson, 2006; Sturm et al., 2001). Data on estimated snow height and site openness from Bienau et al. (2014).

Habitat	***b***	***d***	***s***
Description	***b***irch forest with deep snow cover	Alpine tundra with ***d***eep snow cover in wind-sheltered depressions	Alpine tundra with ***s***hallow snow cover on wind-exposed elevated ridges
Indicator species	*Betula pubescens* ssp. *czerepanovii*	Snow-preferring species: *Betula nana*, *Vaccinium myrtillus*	Snow-avoiding species: *Arctostaphylos alpina, Loiseleuria procumbens*, *Cetraria nivalis*, *Cetraria cucullata*
Snow height	51–153 cm Snow accumulation because vegetation acts as snow trap	29–44 cm Snow accumulation because vegetation acts as snow trap	9–10 cm No snow accumulation because snow is blown away
Site openness	22–64% Lowest site openness through shading by trees	65–87% Higher site openness through shading by other dwarf shrubs	83–87% Highest site openness because of low vegetation height

3 ***ff*** – full flowering: At least one flower of the shoot is fully open (stamens exposed and anthers open) but not wilted yet.
4 ***ef*** – end of flowering: All flowers of the shoot are in senescence.

We calculated the mean date of full flowering as the weighted mean of ***ff*** (as DOY), abbreviated as ***ff*** day. Observation dates (x) at which the ***ff*** phenophase occurred were summed, weighted by the number of individuals which were in full-flower at respective dates, using the following equation:

$$ff\ day = \frac{\sum_{i-1}^{x} DOY \times shoots\ with\ flowers\ in\ ff}{\sum_{i-1}^{x} shoots\ with\ flowers\ in\ ff} \quad \text{(Eq. 1)}$$

In the birch-forest habitat in North-SO *Empetrum* was damaged by a caterpillar outbreak after plot selection during summer 2012. Consequently, only one plot with 11 flowers could be sampled in 2014; in the remaining four plots *Empetrum* produced no flowers.

Furthermore, we calculated the degree of overlap in flowering among individuals using Primack's phenological overlap index (Primack, 1980). First, $c = a/b$ was calculated for all possible pairs of plant individuals, where a is the number of observation dates where two individuals were in ***ff*** phenophase synchronously and b is the number of flowering days of the individual that had the shortest flowering duration. The index of overlap in flowering (Z) for the individuals within a population was calculated as: $Z = \Sigma\, c/N$ where N is the number of comparisons ($N = n * (n - 1)/2$). Z is a number between 0 and 1, where 0 denotes no overlap and 1 complete overlap. Z was calculated for pairs of all individuals within a habitat (***b**b**, ***d**d**, ***s**s***) and for pairs of all individuals between the different habitats (***b**d**, ***b**s**, ***d**s***). Furthermore, plot affiliation (for all individuals of the same plot) was randomly permuted 100 times to obtain a null distribution of random Z-values. The six Z-values within and between plots were compared to this null distribution (= random overlap) through one sided t-tests. We hypothesized that observed overlap would be greater than random overlap. Moreover, we plotted the values of observed overlap within and between habitat as well as random overlap for between habitats for all possible combinations (***b*** vs. ***b**d***, ***b*** vs. ***b**s***, ***d*** vs. ***b**d***, ***d*** vs. ***d**s***, ***s*** vs. ***b**s***, ***s*** vs. ***d**s***). All analyses were performed with R version 3.1.2 (R Core Team R, 2014) and the figures were made in Excel 2007.

In addition to Primack's phenological overlap index, we used a Monte-Carlo simulation to calculate the probability for all pairs of plots that both plots had shoots in ***ff*** at a given day. Hence, we estimated the probability to randomly draw individuals in ***ff*** within a habitat and between the different habitats in each study area and the different study years. For each sampling date and plot, we made random draws of n individuals (where n = number of individuals per plot) with replacement (Kunnen, 2012) to obtain the probability (p) of drawing at least one individual in the ***ff*** phenophase from that plot. Afterward, we performed a Monte-Carlo simulation with 3000 repetitions to estimate the probability to randomly draw one individual in ***ff*** phenophase (p^2) in two separate plots for each combination of plots within and between habitats. Finally, we averaged the p^2 values of all plot pairs within and among the different habitats for each study area and year. The resampling method and Monte-Carlo simulation were performed in Microsoft Excel 2007 using the add-in PopTools 3.2.5 (Hood, 2011).

We used two different methods to estimate the relationship between habitat and flowering phenology of *Empetrum* across all study areas. Primack's overlap index calculated the degree of overlap in flowering among individuals for all possible pairs of plant individuals during the whole observation-period within and between habitats. In contrast, the Monte-Carlo-Simulation calculated the probability for all pairs of plots that both plots had shoots in ***ff***-phenophase at a given day. This method helps us to estimate the probability of overlap in ***ff*** within and between habitats over time and to assess if the probability of overlap is higher and lower, respectively, for different within and between habitat-combinations at individual days.

2.3. Environmental variables

Temperature at the soil surface was measured in intervals of 3 h with data loggers (micro-T, DS1922L; NexSens Technology, Alpha, Ohio, USA) in each plot from September in the year before observation to the last date of the phenological observations. Temperature data loggers were put near the soil surface to simulate the temperature which the plants experience and to determine the snowmelt timing. Under deep snow cover, temperatures near the soil surface are characterized by very low amplitudes around zero (Hülber et al., 2010; Taras et al., 2002), and time of snowmelt therefore coincides with the start of diurnal temperature oscillations (Lundquist and Lott, 2008; Taras et al., 2002). In contrast, on sites with shallow snow cover (<20–30 cm) near-soil surface temperatures track air-temperatures, but with a damped signal (Lundquist and Lott, 2008). We calculated the following temperature variables for each plot in each habitat:

- snowmelt date: 3rd consecutive day with ≥3 °C difference between daily minimum and daily maximum temperatures, which indicates the date when insulating and protecting snow cover disappeared. In 2013 we did a visual calibration of our definition of snowmelt date by comparing the observed snowmelt date with the estimated snowmelt date.
- lag phase: number of days from snowmelt date to ***ff***-day

- growing degree days (GDD): accumulated degree sum above the threshold temperature of 1 °C (Førland et al., 2004; Körner, 2003) between snowmelt and ***ff***
- frost-days: number of days with at least one temperature-measurement below 0 °C between snowmelt and ***ff***
- temperature sum: sum of positive and negative temperature recordings between snowmelt and ***ff***

To determine the habitat light conditions, hemispherical images were taken with a Nikon Coolpix 4500 digital camera equipped with a 180° fisheye lens. One image was taken in each phenological plot at DOY 149 in North-SC and at DOY 173 in North-SO before birch foliation in 2014. The camera was installed on a tripod within the center of each plot at a height of 10 cm. Color images were transformed into black and white images with the program Sidelook 1.1 (Nobis, 2005). Afterward, the software Gap light analyzer 2.0 (GLA; Frazer et al., 1999) was used to extract the amount of direct and diffuse solar radiation transmitted by the canopy and the topographic mask of each individual plot, as an indicator of habitat light conditions. GLA includes a solar radiation model which takes into account the influences of topography to calculate different parameters like the amount of above- and below-canopy direct, diffuse and total solar radiation at different sites. Our calculated output variable is the absolute amount of total radiation (trans total; mol photons m^{-2} d^{-1}) below the canopy and topographic mask (Frazer et al., 1999) for the given day between snowmelt and ***ff***-day for each plot. To compare the amount of solar energy between habitats, we summed the amount of solar energy for the photosynthetic active phase of *Empetrum*, i.e. during daytime and at minimum temperatures of 1 °C (cf. Körner, 2003).

2.4. Statistical analyses

We used nonlinear mixed effects models (NLME) to test the effects of habitat on flowering phenology of *Empetrum* and environmental variables. Year, locality and plots were treated as nested random effects. For this analysis the R-package NLME version 3.1-103 (Pinheiro et al., 2015) was used. Furthermore, we used the Bonferroni-method to test for significant differences among the habitats ***b*** vs. ***d***, ***b*** vs. ***s*** and ***d*** vs. ***s***.

3. Results

3.1. Timing of snowmelt

Snowmelt occurred significantly later in the ***b***- and ***d***-habitats than in the ***s***-habitats across all study areas and years (Fig. 1; Table 2). Furthermore, the date of snowmelt was very similar in North-SC and South-SC 2013. The comparison of DOY of snowmelt in North-SC between both years showed that snowmelt occurred later (mean = 12.8 days across all habitats) in 2014 than in 2013. In 2014, snowmelt occurred later in the sub-oceanic than in the sub-continental site across all habitats.

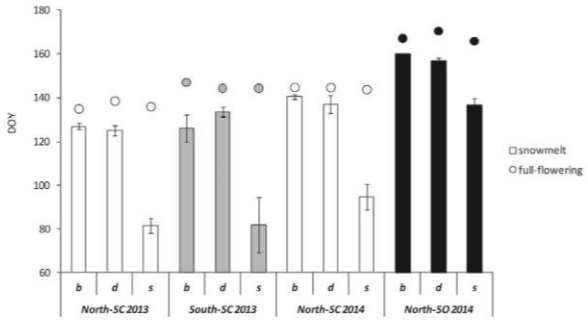


Fig. 1. Day of snowmelt and weighted mean of full flowering for all study regions. Values represent untransformed means ± SE. Bars represent snowmelt day (as day of year) in North-SC 2013 and 2014 (white bars), and South-SC 2013 (grey bars) and North-SO 2014 (black bars), respectively. Circles represent the weighted mean of full flowering day within each study area and year.

3.2. Flowering time

In contrast to the consistent patterns of snowmelt among habitats, differences in the date of full flowering among habitats were small (Fig. 1; Table 2). The mean range in ***ff***-day among the different habitats across all areas was only 0.7–3.2 days.

As a consequence of clear differences in snowmelt but small variation in ***ff***-day between habitats, the time span between snowmelt and ***ff***-day (lag-phase) was significantly longer in the ***s***-habitat (8–9 weeks) than in the ***b***- and ***d***-habitats (1–3 weeks; Fig. 1, Table 2).

3.3. Environmental conditions between snowmelt and full flowering

The early melting ***s***-habitat experienced significantly more (about two-fold to four-fold) growing degree days than the late melting ***b***- and ***d***-habitats (Fig. 2a; Table 2). On the other hand, the number of frost-days was significantly higher in the ***s***-habitat than in the ***b***- and ***d***-habitats (Fig. 2b; Table 2). For temperature sum no significant differences among the habitats could be found (Fig. 2c; Table 2). In the ***s***-habitat *Empetrum* received significantly higher amounts of solar energy than the d-habitat (Fig. 3; Table 2). The amount of solar energy in the ***d***-habitat was only 26% and 23% of the amounts in the ***s***-habitats in North-SC and North-SO, respectively. The ***b***-habitat received only 19% and 48% of the ***s***-habitat in North-SC and North-SO, respectively.

3.4. ***ff***-overlap

Primack's flowering overlap index among individuals (Primack, 1980, Fig. 4) ranged from 0.17 to 0.82 within habitats and from 0.23 to 0.78 between habitats. The comparison of the *Z*-value for within and between habitats showed no consistent pattern. In only half of the cases the overlap between habitats was lower than within habitats, in 7 out of 24 cases the overlap between habitats was even higher than within habitats, and in 5 cases overlap within and between habitats was very similar. Furthermore, the results of the t-test showed no clear pattern, only half of comparisons showed significantly lower random than observed overlap.

The results of Monte-Carlo simulations (Table 3) for North-SC 2013 showed that the probability of drawing each one individual in ***ff*** phenophase in two plots was highest for the ***s***-habitat (***s*******s*** 18.9%) on day 132, whereas maximum values for ***d*******d*** and ***b*******b*** (10.2%; 28.9%, respectively) were found on day 136. Although the probability of phenological overlap is earlier in ***s*******s*** than in ***b*******b*** and ***d*******d*** the highest probability of overlap between different habitats occurred on DOY 136 when the late melting habitats reached its maximum overlap. At lower latitude (South-SC 2013) the probability of ***ff***-overlap was generally lower. Maximum values within habitats were observed on DOY 141 in ***s*******s*** (10.5%) and ***d*******d*** (4.1%), and at DOY 145 in ***b*******b*** (2.1%). Between habitats maximum probabilities were found on DOY 141, when the tundra habitats reached its maximum overlap within habitats. In 2014 the highest probability of overlap within and

Table 2
The effect of habitat type on ***ff***-day of *Empetrum* and environmental variables (nonlinear mixed effects models) across all study areas. ***b*** birch forest, ***d*** deep snow cover sites, ***s*** shallow snow cover sites. Lag phase means the number of days between snowmelt and full-flowering, GDD are the number of growing degree days during the lag phase and temp. sum is the sum of positive and negative temperature recordings during the lag phase. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ^{ns} not significant.^{x} denominator df for solar energy was 13.

	Df	Full-flowering	Snowmelt	Lag phase	GDD	Frost-days	Temp. sum	Solar energy
Intercept	31^{x}	371.720^{***}	88.503^{***}	22.532^{***}	60.703^{***}	3.687^{ns}	78.657^{***}	56.207^{***}
Habitat	31^{x}	7.722^{**}	70.881^{***}	61.149^{***}	49.518^{***}	29.122^{***}	1.578^{ns}	10.692^{*}
t-test Bonferroni								
b vs. ***d***		ns	ns	ns	ns	ns	ns	ns
b vs. ***s***		ns	***	***	***	***	ns	ns
d vs. ***s***		ns	***	***	***	***	ns	**

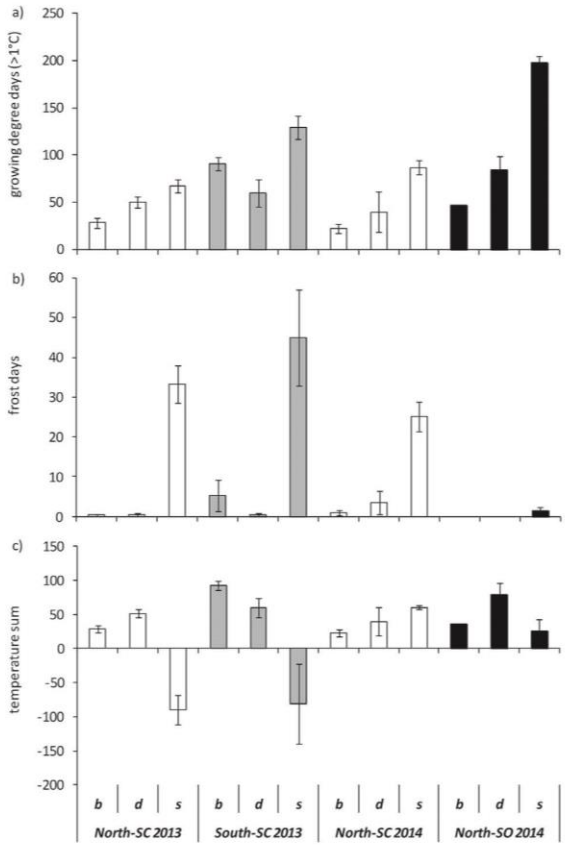


Fig. 2. Growing degree days (GDD) (a), frost days (b), and temperature sum (c) in North-SC 2013 and 2014 (white bars), and South-SC 2013 (grey bars) and North-SO 2014 (black bars), respectively. Growing degree days were the number of days with accumulated degree sum above 1 °C (cf. Körner, 2003) between snowmelt and ***ff***-day. Frost days were days between snowmelt and ***ff***-day with at least one temperature-measurement below 0 °C. Temperature sum were the summed daily mean temperatures from snowmelt date to ***ff***-day. Values represent untransformed means ± SE.

between all three habitats in North-SC occurred on DOY 143. In North-SO 2014, the probability of drawing one individual in ***ff*** phenophase in two plots within habitats was highest in ***s*****s*** on DOY 165 (63.9%). At the same day the highest probability of overlap between habitats was found for the both habitat-combinations with the early melting tundra habitat (***b*****s*** and ***d*****s***). At DOY 170, ***d*****d*** and ***b*****d*** experienced the highest probability of overlap.

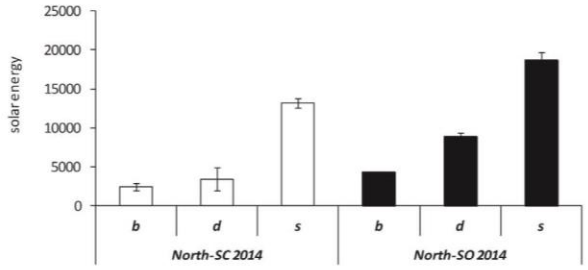


Fig. 3. Amount of solar energy (mol m^{-2} d^{-1}) in the different habitats in North-SC (white bars) and North-SO 2014 (black bars). Values represented untransformed means ± SE.

4. Discussion

4.1. Effect of habitat on flowering of Empetrum

Our data showed that the full flowering phenophase occurred nearly at the same time in early melting tundra habitats with shallow snow cover and in late melting tundra and birch forest habitats with deep snow cover. Consequently, there was a significantly longer lag phase between snowmelt and flowering in shallow snow cover habitats compared to deep snow cover habitats. With respect to our first study question, we can conclude that flowering of *Empetrum* is not synchronized with snowmelt in the early melting ***s***-habitat, but in the late melting ***b***- and ***d***-habitat. These results are in line with the observations on flowering of snow bed species (Hülber et al., 2006) that the earlier a plant becomes snow free, the longer the time until it flowers. However, previous studies on *Empetrum* had found that flowering always occurred within a few days after snowmelt (Anadon-Rosell et al., 2014; Wipf, 2010; Wipf et al., 2006, 2009). Our results are thus consistent with these observations only for habitats with late melting deep winter snow cover. Similarly, Kawai and Kudo (2011) found that flowering in *Gentiana nipponica* was less strongly correlated with snowmelt time in early-melting plots than in late melting-plots because plants in the latter are less temperature limited when snow disappears and thus flower immediately after snowmelt. This is in line with our results that the pre-flowering period of *Empetrum* was longer in early melting habitats than in late melting habitats. Rapid growth and reproduction thus seems to be more important for survival and reproduction in late melting than in early melting habitats due to a shorter growing season (Totland and Alatalo, 2002).

4.2. Flowering overlap

Synchronous flowering, i.e. a large overlap in full flowering within as well as between different habitats in all study areas and

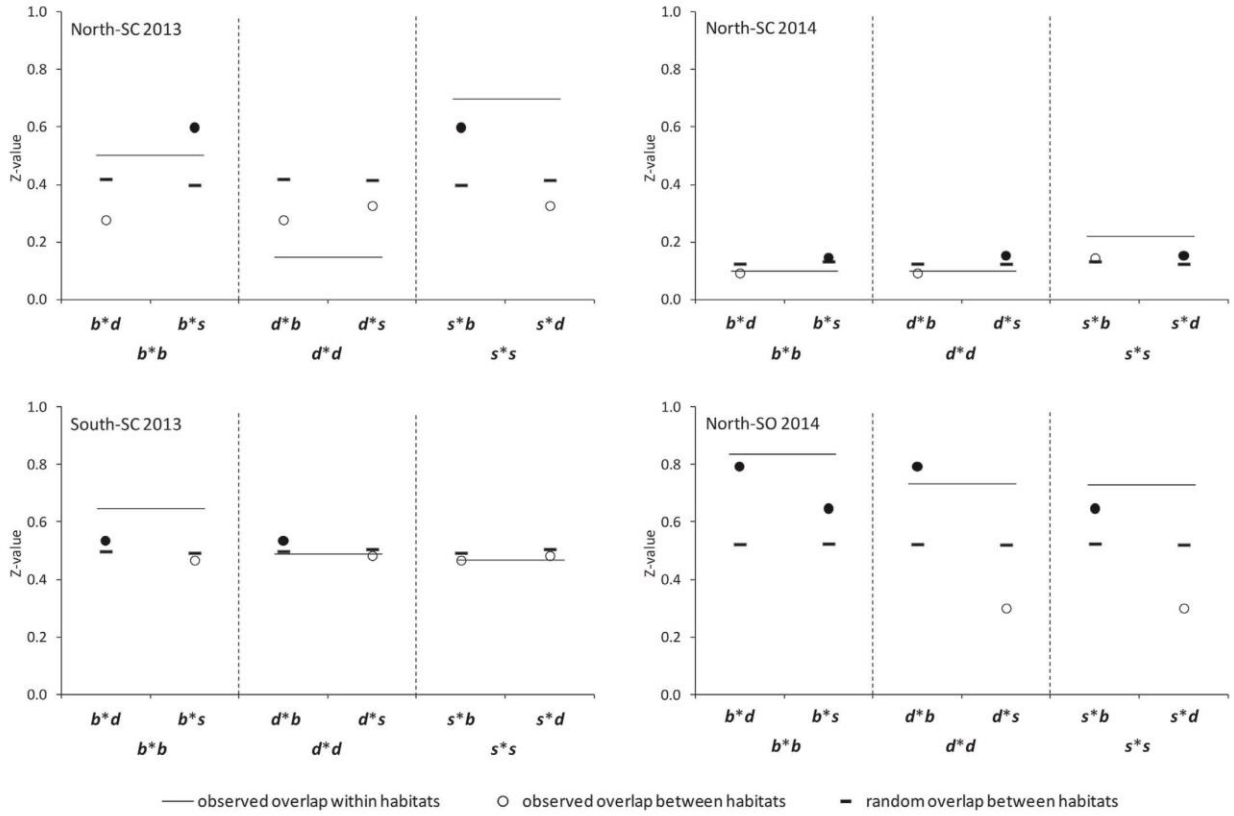


Fig. 4. Primack's flowering overlap index in North-SC 2013, South-SC 2013, North-SC 2014 and North-SO 2014. The thin line represent calculated observed overlap within habitats (***b*b***, ***d*d***, ***s*s***). Circles represent calculated observed overlap between habitats (***b*d***, ***b*s***, ***d*s***). Black circles represent significantly higher observed overlap than random overlap (t-test). Flat rectangles depict random overlap for between habitats, thus the calculated null distribution of Z-values.

Table 3
Results of Monte-Carlo simulation for the study areas North-SC and South-SC in 2013 and North-SC and North-SO in 2014. This shows the probability [%] of drawing at least one individual in the ***ff*** phenophase from two different plots within or between habitats. Empty cells indicate a value of 0.0; these results were removed from the table for clear arrangement. There were no values for ***b*b*** in North-SO, because there was only one plot with flowering *Empetrum* in the birch forest in North-SO.

Study region	North-SC 2013				South-SC 2013					North-SC 2014			North-SO 2014			
Day of year	129	132	136	142	138	141	145	151	158	143	146	149	165	170	173	175
Within habitats																
b*b		1.3	28.9			1.2	2.1	0.1		32.2	7.3	1.4	no data			
d*d		0.2	10.2	6.3		4.1	1.1			34.8	3.9	0.4	1.0	32.2	6.5	
s*s	0.9	18.9	8.7	0.2	0.1	10.5	2.4			41.7	3.5		63.9	1.4		
Among habitats																
b*d		0.6	17.9			3.0	1.9			29.9	6.2	1.0	7.0	10.4	4.9	0.5
b*s	0.3	5.3	16.2			4.3	2.6	0.1		32.3	5.7	0.3	50.9	2.2		
d*s	0.1	2.2	9.9	1.3	0.1	8.2	2.0			39.0	4.0	0.1	8.8	6.9		

study years was detected with Primack's flowering overlap index as well as with Monte-Carlo simulation. The results of Primack's overlap index demonstrated that overlap in full-flowering of *Empetrum* occurred during the whole study period. Contrary to our expectations, no clear pattern of higher overlap within than between habitats could be detected with Primack's flowering overlap index. In contrary, Monte-Carlo simulation revealed higher overlap within than between habitats at given days and gives information on the chronological sequence of ***ff*** in within and between habitat-combinations. This demonstrates that both methods were helpful to interpret the pattern of ***ff***-overlap in *Empetrum* along a snowmelt gradient.

According to our second study question we can conclude that both, within the same and between different habitats there is a temporal overlap in ***ff***-phenophase of *Empetrum* despite significant differences in snowmelt timing. This might indicate that reproductive isolation and genetic differentiation among the habitats is rather unlikely. The degree of phenological overlap along a snowmelt gradient may affect the spatial genetic structure of tundra species (Kudo and Hirao, 2006), leading to e.g. high genetic differentiation between neighboring populations (Hirao and Kudo, 2004). However, current studies are unequivocal. In *S. herbacea* no significant genetic differentiation was observed despite a large phenological lag between microhabitats differing in timing of

snowmelt, however, gene flow is asymmetric toward the late melting snowbeds, which are genetically more diverse than early melting sites (Cortès et al., 2014). In the alpine grass *Festuca eskia* local adaptation despite gene flow has recently been demonstrated along an altitudinal gradient (Gonzalo-Turpin and Hazard, 2009), indicating that in principle genetic differentiation may develop despite phenological overlap.

4.3. Effects of temperature and light conditions during the lag phase

Temperature is the main driver of flowering phenology in high alpine species which are expected to respond directly and quickly to increasing temperatures (Hülber et al., 2010). Flowers of many arctic species have been shown to open at air temperatures of 3–8 °C and to start mass flowering at 5–12 °C (Shamurin, 1958; cited in Bliss, 1962). This is in accordance with our results of temperatures at ***ff*** day (***b***: 5.7 °C ± 0.7; ***d***: 6.0 °C ± 0.6; ***s***: 8.4 °C ± 0.5; Bienau et al. unpublished data), even if we measured temperature near the soil surface. Some temperature based indices such as growing degree days (GDD), temperature sum and number of frost days, as well as the amount of solar energy, might be closely related to the onset of flowering of arctic plant species (Körner, 2003). Our data show that the ***s***-habitat with long lag phase between snowmelt and flowering experienced a higher number of frost days and more growing degree days but lower temperature sums than the ***b***- and ***d***-habitats. Thus, concerning our third study question these results show that temperature related environmental variables, in particular GDD, may be responsible for differences in the lag phase between snowmelt and flowering, resulting in synchronous flowering of *Empetrum* across different habitats. However, the amount of solar energy between snowmelt and flowering was higher in the ***s***-than in the ***b***- and ***d***-habitats. This trend is similar to the pattern in snowmelt timing and indicates that snowmelt timing and the amount of solar energy is closely related and the single effects could not be distinguished easily from one another. To reduce the risk of frost damage in plants caused by too early flowering (Inouye, 2008; Semenchuk et al., 2013, Wheeler et al., 2014), development of plants often depends on photoperiodism, i.e. plants remain frost hardy until a certain day length is reached, especially in early melting habitats (Hülber et al., 2010; Keller and Körner, 2003; Wipf et al., 2009). Keller and Körner (2003) observed that temperature dependent flowering of high alpine species decreases with increasing photoperiod. Furthermore, photoperiod interacts with other environmental factors like temperature (Keller and Körner, 2003). During the lag phase between snowmelt and full flowering of *Empetrum* the daily photoperiod increased by 8.5 h in South-SC, 10 h in North-SC and 5 h in North-SO (Solartopo, 2015). After 26th of May (DOY 146), the northern study areas experienced midnight sun. Therefore, photoperiod might affect flowering of *Empetrum* across the study areas and could reduce the risk of frost damage by flowering too early in the ***s***-habitats especially in South-SC, but also in the North.

4.4. Impact of climate change

Climate change affects snowmelt twofold: First, Arctic ecosystems may experience changes in snow cover and earlier snowmelt in spring despite increasing precipitation during winter because much of the precipitation occurs as rain as a consequence of increasing temperature (ACIA, 2004; Bokhorst et al., 2012). Advanced snowmelt and soil warming might slightly accelerate the flowering of *Empetrum* (Anadon-Rosell et al., 2014; Wipf, 2010; Wipf et al., 2006, 2009). In contrast, other studies could not show effects of natural winter warming events and warming by open top chambers on flowering phenophases of *Empetrum* (Bokhorst et al., 2011; Buizer et al., 2012). In general, *Empetrum* seems to be relatively stable against altered snow conditions as a consequence of climate change. Second, winter and early spring thawing events may occur more often (Bokhorst et al., 2012), leading to a colder spring in a warmer world which increases the probability of frost damage on *Empetrum* by snowmelt in early spring, when temperature is often very low (Wipf et al., 2009). However, it is rather difficult to predict the potential effects of climate change on timing of flowering of *Empetrum* in habitats along a snowmelt gradient.

5. Conclusion

Our study demonstrates that *Empetrum* flowers about the same time in early and late melting habitats, independent of snowmelt timing. Therefore, the plants in the exposed and sunny, early melting ***s***-habitat have a longer time-lag which might increase the risk of frost damage (Inouye, 2008; Semenchuk et al., 2013; Wheeler et al., 2014), whereas plants in the late melting habitats flower directly after snowmelt when temperatures are higher.

In contrast to previous theories that *Empetrum* as an early flowering species, flowers directly after snowmelt and flowering will therefore be asynchronous along a snowmelt gradient (Bliss, 1962; Dunne et al., 2003; Hirao and Kudo, 2004; Wipf et al., 2006, 2009), our results rebut this idea. Flowering of *Empetrum* does not seem to be related to snowmelt time across all habitats, but to temperature conditions during the lag-phase between snowmelt and flowering. In this way small scale variation seems to matter less to flowering of *Empetrum* than interannual differences in snowmelt timing. Changes in snow cover and temperature due to climate are not likely to cause changes in flowering synchrony but general changes in flowering time, as demonstrated in the comparison of flowering time in our study area North-SC in the early melting year 2013 compared to the late melting year 2014.

Author contributions

Conceived and designed the experiments: RLE WD MJB AO DH. Performed the experiments: MJB MK. Analyzed the data: MJB MK RLE WLE. Wrote the paper: MJB (main responsibility), RLE WD AM MK WLE BJG AO DH.

Acknowledgments

Financial support was obtained from the Deutsche Forschungsgemeinschaft (DFG, grant EC209/9-1). Additionally, the research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement n° 262693 [INTERACT]. Field assistance was provided by Tobias Donath and Adrian Bienau. We further thank Carsten Dormann and Eva Herzog for statistical advice, the director and staff of the Abisko Scientific Research Station for climate data, logistic support and accommodation. We are very grateful to Jacqui Shykoff and two anonymous reviewers for fruitful comments on an earlier draft of this manuscript. All help is gratefully acknowledged.

References

ACIA, 2004. Arctic Climate Impact Assessment: Impacts of a Warming Arctic.
Anadon-Rosell, A., Rixen, C., Cherubini, P., et al., 2014. Growth and phenology of three dwarf shrub species in a six-year soil warming experiment at the alpine treeline. PLoS ONE 9, e100577. http://dx.doi.org/10.1371/journal.pone.0100577.
Bell, J.N.B., Tallis, J.H., 1973. *Empetrum nigrum* L. J. Ecol. 61, 289–305. http://dx.doi.org/10.2307/2258934.
Bienau, M.J., Hattermann, D., Kröncke, M., et al., 2014. Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients. Alp. Bot. 124, 115–129. http://dx.doi.org/10.1007/s00035-014-0137-8.

Bliss, L.C., 1962. Adaptations of arctic and alpine plants to environmental conditions. Arctic 15, 117–144.

Bokhorst, S., Bjerke, J.W., Street, L.E., et al., 2011. Impacts of multiple extreme winter warming events on sub-Arctic heathland: phenology, reproduction, growth, and CO_2 flux responses. Glob. Change Biol. 17, 2817–2830.

Bokhorst, S., Bjerke, J.W., Tømmervik, H., et al., 2012. Ecosystem response to climatic change: the importance of the cold season. AMBIO 41, 246–255.

Buizer, B., Weijers, S., van Bodegom, P.M., et al., 2012. Range shifts and global warming: ecological responses of *Empetrum nigrum* L. to experimental warming at its northern (high Arctic) and southern (Atlantic) geographical range margin. Environ. Res. Lett. 7, 025501. http://dx.doi.org/10.1088/1748-9326/7/2/025501.

Cooper, E.J., Dullinger, S., Semenchuk, P., 2011. Late snowmelt delays plant development and results in lower reproductive success in the High Arctic. Plant Sci. 180, 157–167. http://dx.doi.org/10.1016/j.plantsci.2010.09.005.

Cortès, A.J., Waeber, S., Lexer, C., et al., 2014. Small-scale patterns in snowmelt timing affect gene flow and the distribution of genetic diversity in the alpine dwarf shrub *Salix herbacea*. Heredity 113, 233–239.

Dunne, J.A., Harte, J., Taylor, K.J., 2003. Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. Ecol. Monogr. 73, 69–86. http://dx.doi.org/10.1890/0012-9615(2003)073[0069:SMFPRT]2.0.CO;2.

Førland, E.J., Skaugen, T.E., Benestad, R.E., et al., 2004. Variations in thermal growing, heating, and freezing indices in the Nordic Arctic, 1900–2050. Arct. Antarct. Alp. Res. 36, 347–356. http://dx.doi.org/10.1657/1523-0430(2004)036[0347:VITGHA]2.0.CO;2.

Frazer, G.W., Canham, C.D., Lertzman, K.P., 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging Software to Extract Canopy Structure and Gap Light Transmission Indices from True-colour Fisheye Photographs, Users Manual and Program Documentation.

Gonzalo-Turpin, H., Hazard, L., 2009. Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. J. Ecol. 97, 742–751. http://dx.doi.org/10.1111/j.1365-2745.2009.01509.x.

Grogan, P., Jonasson, S., 2006. Ecosystem CO_2 production during winter in a Swedish subarctic region: the relative importance of climate and vegetation type. Glob. Change Biol. 12, 1479–1495. http://dx.doi.org/10.1111/j.1365-2486.2006.01184.x.

Hirao, A.S., Kudo, G., 2004. Landscape genetics of alpine-snowbed plants: comparisons along geographic and snowmelt gradients. Heredity 93, 290–298.

Hood, G.M., 2011. PopTools Version 3.2.5.

Hülber, K., Gottfried, M., Pauli, H., et al., 2006. Phenological responses of snowbed species to snow removal dates in the central Alps: implications for climate warming. Arct. Antarct. Alp. Res. 38, 99–103. http://dx.doi.org/10.1657/1523-0430(2006)038[0099:PROSST]2.0.CO;2.

Hülber, K., Winkler, M., Grabherr, G., 2010. Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. Funct. Ecol. 24, 245–252. http://dx.doi.org/10.1111/j.1365-2435.2009.01645.x.

Inouye, D.W., 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89, 353–362. http://dx.doi.org/10.1890/06-2128.1.

Jäger, E.J., Rothmaler, W., 2011. Exkursionsflora von Deutschland Bd. 2, Bd. 2. Spektrum Akademischer Verlag, Heidelberg.

Jonasson, S., 1981. Plant communities and species distribution of low alpine *Betula nana* heaths in Northernmost Sweden. Vegetatio 44, 51–64. http://dx.doi.org/10.1007/BF00119804.

Kameyama, Y., Kudo, G., 2009. Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: effects of pollinators and self-incompatibility. Ann. Bot. 103, 1385–1394.

Kawai, Y., Kudo, G., 2011. Local differentiation of flowering phenology in an alpine-snowbed herb *Gentiana nipponica*. Botany 89, 361–367. http://dx.doi.org/10.1139/b11-024.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241.

Keller, F., Körner, C., 2003. The role of photoperiodism in alpine plant development. Arct. Antarct. Alp. Res. 35, 361–368.

Körner, C., 2003. Alpine plant Life: Functional Plant Ecology of High Mountain Ecosystems, second ed. Springer.

Kudo, G., Hirao, A.S., 2006. Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts – Springer. Popul. Ecol. 48, 49–58.

Kudo, G., 1991. Effects of snow-free period on the phenology of alpine plants inhabiting snow patches. Arct. Alp. Res. 23, 436–443. http://dx.doi.org/10.2307/1551685.

Kunnen, S.E., 2012. A Dynamic Systems Approach of Adolescent Development. Psychology Press.

Lundquist, J.D., Lott, F., 2008. Using inexpensive temperature sensors to monitor the duration and heterogeneity of snow-covered areas. Water Resour. Res. 44 (4) http://dx.doi.org/10.1029/2008WR007035.

Molau, Ulf, Nordenhäll, Urban, Eriksen, Bente, 2005. Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. Am. J. Bot. 92, 422–431.

Nobis, M., 2005. SideLook 1.1-Imaging Software for the Analysis of Vegetation Structure with True-colour Photographs.

Odland, A., Munkejord, H.K., 2008. Plants as indicators of snow layer duration in southern Norwegian mountains. Ecol. Indic. 8, 57–68. http://dx.doi.org/10.1016/j.ecolind.2006.12.005.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2015. nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-121.

Primack, R.B., 1980. Variation in the phenology of natural populations of montane shrubs in New Zealand. J. Ecol. 68, 849–862. http://dx.doi.org/10.2307/2259460.

R Core Team R, 2014. The R Project for Statistical Computing (accessed 11.03.15.). http://www.r-project.org/.

Semenchuk, P.R., Elberling, B., Cooper, E.J., 2013. Snow cover and extreme winter warming events control flower abundance of some, but not all species in high arctic Svalbard. Ecol. Evol. 3, 2586–2599. http://dx.doi.org/10.1002/ece3.648.

Shamurin, V.F., 1958. On the diurnal rhythm and the ecology of flowers in some arctic plants. Botanicheskii Zhurnal – Russ. 43, 1183–1191.

Solartopo Day Length Calculator. http://www.solartopo.com/daylength.htm. (accessed 23.02;.15.).

Sonesson, M., Osborne, C., Sandberg, G., 1994. Epiphytic lichens as indicators of snow depth. Arct. Alp. Res. 26, 159–165.

Sturm, M., Holmgren, J., McFadden, J.P., et al., 2001. Snow–shrub interactions in Arctic tundra: a hypothesis with climatic implications. J. Clim. 14, 336–344. http://dx.doi.org/10.1175/1520-0442(2001)014<0336:SSIIAT>2.0.CO;2.

Taras, B., Sturm, M., Liston, G.E., 2002. Snow–ground interface temperatures in the Kuparuk river basin, Arctic Alaska: measurements and model. J. Hydrometeorol. 3, 377–394. http://dx.doi.org/10.1175/1525-7541(2002)003<0377:SGITIT>2.0.CO;2.

Thórhallsdóttir, T.E., 1998. Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. Oecologia 114, 43–49. http://dx.doi.org/10.1007/s004420050418.

Totland, Ø., Alatalo, J.M., 2002. Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis* – Springer. Oecologia 133, 168–175.

Wheeler, J.A., Hoch, G., Cortés, A.J., Sedlacek, J., Wipf, S., Rixen, C., 2014. Increased spring freezing vulnerability for alpine shrubs under early snowmelt. Oecologia 175, 219–229. http://dx.doi.org/10.1007/s00442-013-2872-8.

Wipf, S., Rixen, C., Mulder, C.P.H., 2006. Advanced snowmelt causes shift towards positive neighbour interactions in a subarctic tundra community. Glob. Change Biol. 12, 1496–1506. http://dx.doi.org/10.1111/j.1365-2486.2006.01185.x.

Wipf, S., Stoeckli, V., Bebi, P., 2009. Winter climate change in alpine tundra: plant responses to changes in snow depth and snowmelt timing. Clim. Change 94, 105–121. http://dx.doi.org/10.1007/s10584-009-9546-x.

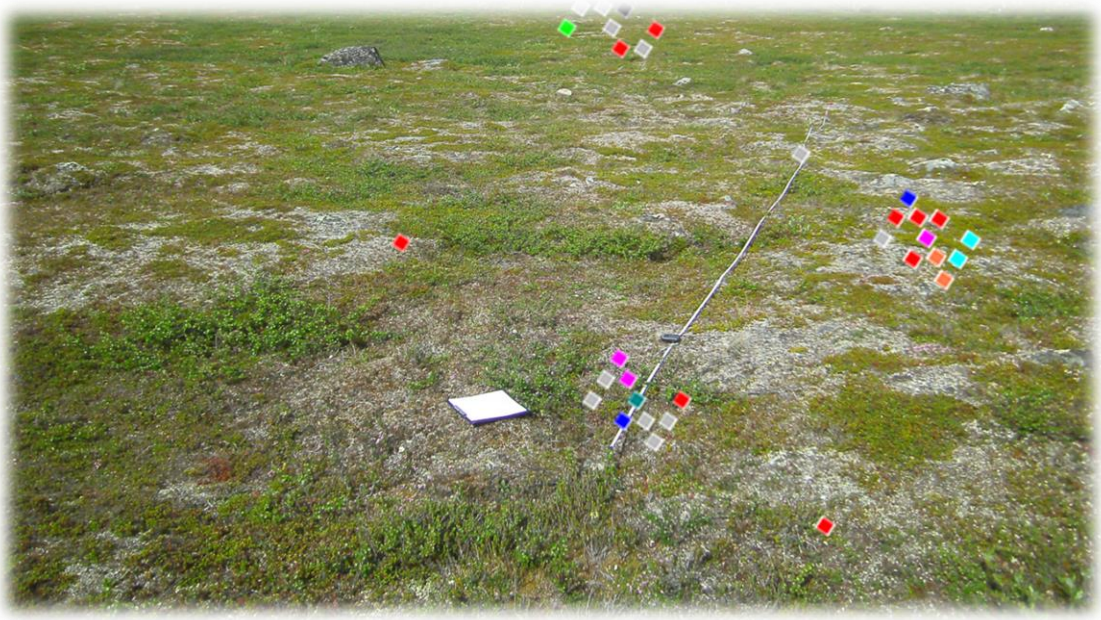
Wipf, S., 2010. Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. Plant Ecol. 207, 53–66. http://dx.doi.org/10.1007/s11258-009-9653-9.

CHAPTER 4

Clonality increases with snow depth in an arctic dwarf shrub

Miriam J. Bienau, R. Lutz Eckstein, Annette Otte and Walter Durka

manuscript



Abstract

Premise of the study: Vegetative reproduction and spread through clonal growth plays an important role in arctic-alpine ecosystems with short cool growing seasons. Local variation in winter snow accumulation leads to discrete habitat types which may provide divergent conditions for sexual *vs*. vegetative reproduction. Therefore, we studied variation in clonal structure of a dominant evergreen dwarf shrub (*Empetrum nigrum* s.l.) along a snow cover gradient and compared clonal diversity and spatial genetic struture between habitats.

Methods: We used 105 polymorphic AFLP markers and performed analyses based on hierarchical clustering, clonal diversity indices and small scale spatial genetic structure with pairwise kinship coefficient on 374 individual shoots. We used two different approaches to define a threshold of genotypic distance between two samples that are considered the same clone. Clonality was examined among three habitats (exposed ridges, sheltered depressions and birch forest) differing in snow conditions replicated in four study regions in Norway and Sweden.

Key results: In three study regions *E. hermaphroditum* was the only species, whereas in the southernmost region also *E. nigrum* s.str. occurred which was largely confined to the exposed ridge habitat. Clonality of *E. hermaphroditum* differed between habitats with an increase of clonal diversity with decreasing snow depth. Furthermore, we observed increasing small scale spatial genetic structure with decreasing clonal diversity and increasing clone size.

Conclusion: Our results demonstrated that snow cover in conjunction with associated habitat conditions plays an important role for the mode of propagation of the dwarf shrub *E. hermaphroditum*.

Keywords: sub-arctic, evergreen dwarf-shrub, snow cover gradient, clonal structure, small scale spatial genetic structure

4.1 Introduction

In arctic-alpine environments with cool growing seasons and cold long winters, sexual reproduction through seeds is not very common (McGraw and Shaver, 1982; Boudreau et al., 2010; Graae et al., 2011). Instead vegetative reproduction and spread through clonal growth play an important role in many species (Bliss, 1971; Cook, 1983; Callaghan et al., 1992; Molau and Larsson, 2000). Flowering and seed production can be a risky mode of propagation at high altitudes with the danger of recruitment failure in some years (Körner, 2003). Thus, clonal growth, which occurs parallel to flowering and fruiting during the growing season might help species to persist in communities independently of reproductive success (Körner, 2003). Clonal plants are able to rapidly occupy new habitats and space locally (Callaghan et al., 1992) through horizontal clonal growth (Cook, 1983) and may therefore often dominate tundra ecosystems (Tybirk et al., 2000). Benefits of clonal growth can be a better utilization of resources and a reduced risk of genet mortality. Disadvantages of clonal propagation include reduction of resources available for flowering and seed production, the lack of sexual reproduction and the dispersal of diseases between ramets (Klimeš et al., 1997).

Arctic clonal plant species and communities may be particularly vulnerable to global warming (Sala et al., 2000) since environmental change may force species either to adapt in situ or to colonize new sites to track their climatic niche. Although micro-habitats may partly buffer macroclimatic change (e.g. De Frenne et al., 2013; Lenoir et al., 2013), sexual reproduction and long distance dispersal of fruits and seeds for colonization of new habitats may then become crucial (Eriksson, 1989; Callaghan et al., 1992; Szmidt et al., 2002). Thus, studies addressing local and regional patterns of sexual vs. clonal reproduction among habitats are particularly needed in tundra ecosystems.

Spatial variation in snow distribution in Arctic ecosystems, created by a combination of topography and wind, ranges from snow-free wind-exposed ridges to sheltered depressions with deep snow accumulation (Saarinen and Lundell, 2010). Snow cover is a strong selection gradient within arctic and alpine landscapes affecting growing season length and winter conditions (Jonas et al., 2008). Usually, habitats with different snow conditions are inhabited by plant communities with contrasting species composition, characterized by species preferring and avoiding winter snow cover, respectively (Jonasson, 1981; Odland and Munkejord, 2008). However, some species occupy a wide

range of habitats and intraspecific differences in responses to variation in snow depth and duration can affect phenology and growth habit.

The clonal evergreen dwarf shrub *Empetrum* nigrum s.l. with its segregates *E. nigrum* s.str. and *E. hermaphroditum* is widely distributed and highly dominant in arctic-alpine ecosystems across the northern hemisphere (Bell and Tallis, 1973). It occurs in several subarctic heath and mountain birch forest communities (Nilsson and Wardle, 2005), ranging from habitats with shallow (Jonasson, 1981; Odland and Munkejord, 2008) to relatively deep snow cover (Jonasson, 1981; Tybirk et al., 2000). It reproduces both through clonal growth and by seed.

The extent of clonality within populations does have a strong influence on spatial genetic structure (SGS) with neighboring ramets being more closely related than more distant ones (Reusch et al., 1999; Pluess and Stöcklin, 2004). In alpine tundra habitats, populations may tend to be genetically aggregated because suitable habitats are isolated from each other and recruitment within a habitat or from neighboring habitats is more likely than colonization from other habitats by long distance seed dispersal events, despite suitable dispersal mechanisms (Pluess and Stöcklin, 2004). In clonal plant species the spatial distribution of clones will also depend on the clonal growth strategy (Escaravage et al., 1998). Two clonal growth forms can be distinguished, the guerilla and the phalanx strategy, in which the first have longer internodes and more widely spaced ramets which can infiltrate the surrounding vegetation and the latter have tight-packed ramets which exclude other plants from their growing space (Lovett Doust, 1981). The growth habit of *Empetrum* varies between different habitats with a more guerilla-type growth on sites with deep snow cover and a more phalanx-type growth on sites with shallow snow cover.

Using a molecular marker approach, the present study compares patterns of clonal structure of *Empetrum* along a natural snow cover gradient with early melting (alpine tundra on wind-exposed ridges) and late melting habitats (sub-alpine birch forest and alpine tundra in wind-sheltered depressions). The aims of this study were to analyze the patterns of clonal structure of *Empetrum* in three habitats differing in snow cover and snow melt timing.

The following questions were addressed:

Q1 Does clonal diversity and clone size vary between habitats? We expected higher clonal diversity and smaller clones in ridge-habitats than in depression-habitats in the alpine tundra and birch forest, due to the growth habit and better conditions for seedling recruitment.

Q2 Because the identification of clones may be problematic, we ask whether small scale spatial genetic structure of ramets differs between habitats. We expect higher genetic autocorrelation in the depression habitat in the alpine tundra and birch forest, due to better conditions for vegetative growth with longer internodes and therefore more widely spaced ramets.

4.2 Material and Methods

Study regions and habitats

To test for general patterns between habitats, we selected four study regions with steep local climatic gradients described in detail by Bienau et al. (2014). In short, the study regions were situated at two different latitudes, i.e. northern Sweden (Abisko [68°2'N 18°49'E; mean annual precipitation: 304 mm; mean annual temperature: -0.8 °C] and Vassijaure [68°2'N 18°10'E; 844 mm; -1.7 °C]) and Central Norway (Kongsvoll [62°18'N 09°36'E; 450 mm; -0.4 °C] and Samsjøen [63°05'N 10°38'E; 830 mm; 3.9 °C]). The regions at each latitude represent a steep gradient between sub continental (Abisko and Kongsvoll) and sub oceanic climate (Vassijaure and Samsjøen), with low and high winter precipitation, respectively. At each of the four regions we distinguished three habitat types differing in snow depth and co-varying abiotic factors (for details see Bienau et al., 2014) based on topography, community type and indicator species of contrasting snow cover conditions (Jonasson, 1981; Odland and Munkejord, 2008): birch forest (***b***), alpine tundra with deep snow cover in sheltered depressions (***d***) and alpine tundra with shallow snow cover on exposed ridges (***s***). In ***b*** and ***d*** snow height is > 50 cm and 30 – 50 cm, respectively, because vegetation acts as snow trap, whereas in ***s*** with sparse vegetation cover snow height is only 5 – 10 cm (Bienau et al., 2014). Depending on snow depth, dates of snow melt timing in spring are different between the habitats. Snow melt

occurred in the end of March and the beginning of April, respectively, in the ***s***-habitat and about 40 days later in ***d***- and ***b***-habitat (Bienau et al., 2015).

Study species

Within *Empetrum nigrum* s.l. two taxa are commonly distinguished: the diploid, dioecious *E. nigrum* L. s.str. and the tetraploid, hermaphrodite *E. hermaphroditum* Hagerup, which can only be distinguished visually when flowering. They are evergreen dwarf shrubs able to expand vegetatively by rooting of horizontal above ground and epigeogenous stems (Klimešová and de Bello, 2009). In both species fruit and seed production by wind pollinated flowers may be frequent (Bell and Tallis, 1973). Whereas *E. nigrum* is outcrossing, *E. hermaphroditum* seems to be predominantly self-pollinating as evidenced by an autodeposition efficiency of 0.9 (Tikhmenev, 1984, cited in the supplement of Alsos et al., 2012; see also Bell and Tallis, 1973; Callaghan and Emanuelsson, 1985). Seeds of *Empetrum* are dispersed by birds (Bell and Tallis, 1973) and the arctic fox (Graae et al., 2004).

Growth form varies according to habitat as on wind-exposed ridges with low snow depth and earlier snow melt timing, *Empetrum* is creeping and grows patchily within larger areas of open soil and gravelly subsoil, whereas in the wind-sheltered depressions and in birch forest it grows more elongated and wide spread within the surrounding vegetation (Bienau et al., 2014). This suggests that habitat types may vary with respect to their suitability for seedling establishment, shoot growth and vegetative propagation. Additionally, fruit production was significantly higher in ridge habitats than in sheltered depressions and birch forest (Bienau et al., 2014). Szmidt et al. (2002) observed higher plant establishment by seeds on open forest sites where the mineral soil had been exposed.

In three of our study regions (Abisko, Vassijaure and Samsjøen) only *E. hermaphroditum* (Suda, 2002) occurred, whereas, in the Dovrefjell National Park (study region Kongsvoll) we also found *E. nigrum* in expectedly high abundance (see also Suda, 2002). We distinguished both taxa *post-hoc* based on their AFLP profiles (see below).

Sampling design

We established one 14 x 14 m plot per habitat in each of the four study regions in summer 2012. Plots were selected according to topography and indicator species for snow conditions on sites where *Empetrum* was present. The distance between the different habitats ranged between 218 and 2383 m. However, the distance between the birch forest habitat and the two alpine tundra habitats (***d*** and ***s***) was larger than between the ***d***- and ***s***-habitat (Figure 1a), because the habitats represent an altitudinal gradient with birch forest habitats below the treeline and alpine tundra habitats above the treeline. Within each plot, we aimed to sample 36 individual *Empetrum* shoots using a systematic grid (Figure 1b) (cf. Szmidt et al., 2002).

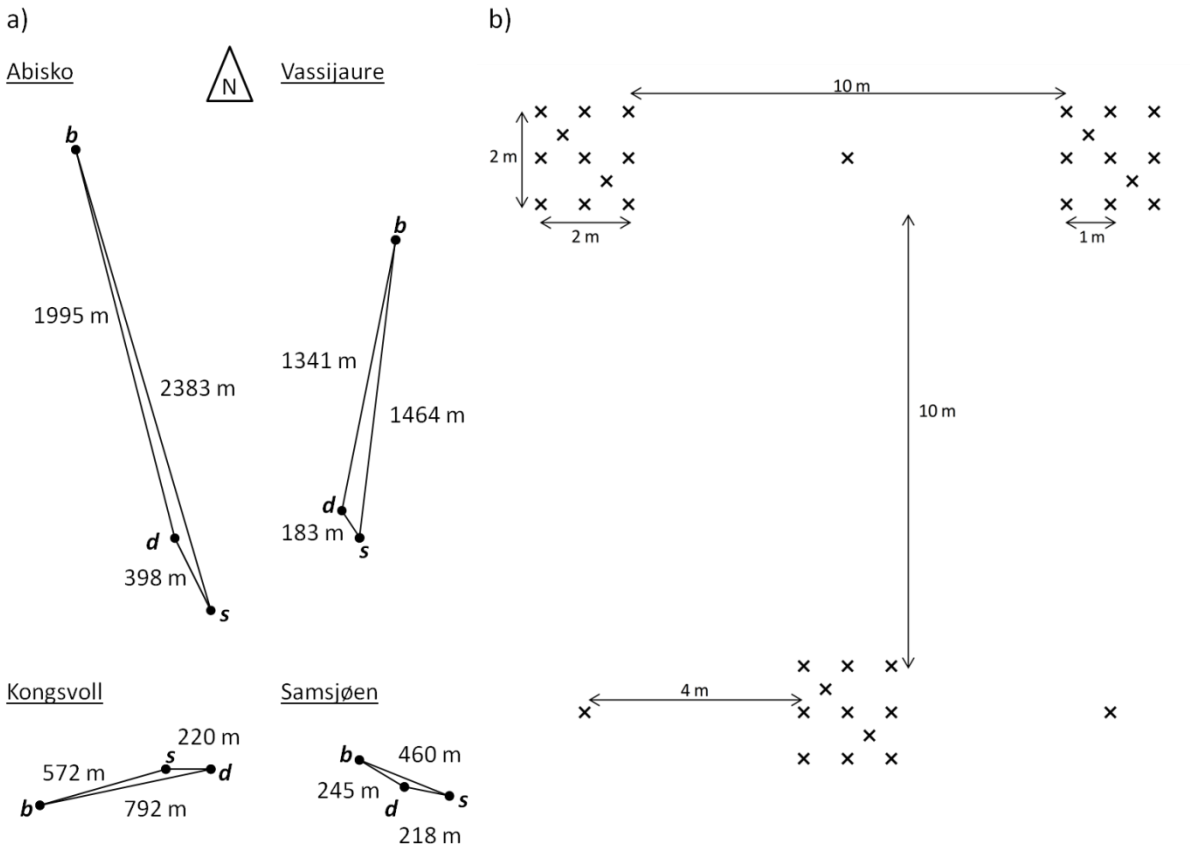


Figure 1: Schematic representation of a) distances between the three habitats birch forest (***b***), alpine tundra with deep snow cover (***d***), and alpine tundra with shallow snow cover (***s***) in the four study regions and b) sampling scheme of *Empetrum* shoots (N = 36) within each plot for analysis of clonal structure.

If there was no shoot at a predefined sampling point, we sampled a shoot and recorded exact coordinates within a distance of maximum 1 m, or omitted that sampling point.

Shoots were stored in teabags and within 3 to 4 hours, the samples were brought in a cooling room (5°C) to keep the material fresh. They were stored not longer than three days until they were freeze dried for 48 hours.

Molecular Analysis

We investigated a total of 374 shoots (27 to 36 per plot) for clonal structure of *Empetrum* with AFLP markers (Vos et al., 1995). Genomic DNA was extracted from 10 mg freeze dried leaf-material from each sample and from 20 randomly chosen replicate samples with the *Qiagen-DNeasy96 Plant Kit* (Qiagen, Hilden, Germany) following the manufacturer's manual. Details of the AFLP protocol are given in supplement 1. After an initial screening of 16 primer combinations, four primer pairs (ACT-CAG, ACA-CTC, AAG-CAG, AGC-CAC) were chosen for AFLP analyses of 374 samples, 20 DNA-extraction duplicates and another 90 duplicates starting from the same DNA. Fragment analysis was done on an *ABI 3130xx genetic analyzer* (Applied Biosystems, Foster City, USA) with Genescan 500 LIZ size standard. We used *GeneMapper* version 5.0 (Applied Biosystems) to analyze the AFLP profiles. We manually binned the fragments of all samples in one batch using peak height thresholds of 5 rfu. These data were exported and afterwards for each fragment a specific peak height threshold was manually determined based on the peak height distribution to score fragment presence and absence. Monomorphic or bands with an individual error rate > 5% were discarded, resulting in 105 polymorphic AFLP loci. Preliminary analyses had shown that a number of samples had strongly divergent AFLP genotypes, which proved to be diploid *E. nigrum* (unpublished flow cytometry data). We distinguished *E. nigrum* from *E. hermaphroditum* based on the total number of bands per individual which was <34 (mean: 25, range 21-33) for *E. nigrum* and >34 (mean: 46, range: 35-59) for *E. hermaphroditum*. *E. nigrum* was confined to region Kongsvoll where a single individual occurred in the birch forest, and where it was the only cytotype in the ***s***-habitat. *E. nigrum* was treated separately in the definition of clones.

Data analysis

Defining clones using molecular markers is notoriously difficult because of somatic mutations (Douhovnikoff and Dodd, 2003) and genotyping errors (Bonin et al., 2004),

which necessitates to define a threshold of genotypic distance between two samples that are considered the same clone. Two different approaches have been taken. The first method (henceforward called *mean threshold*) uses the mean error rate or mean genotypic distance of replicate samples as threshold (e.g. Vonlanthen et al., 2010), assuming that on average clonal samples differ that much. However, the observed number of differences between replicate samples follows a Poisson-distribution (Figure 2a, b), and the maximum observed error is larger than the mean. Thus using the *mean threshold* might falsely split clones and thus overestimate the number of clones. The second method, henceforward called *bimodal threshold,* defines the threshold using the minimum of the bimodal frequency distribution of pairwise differences between all samples, including clones and replicates (de Witte et al., 2012). Ideally, this threshold is derived by comparing pairwise differences between replicate samples with those between fullsibs, which represent the most closely related sexually derived genotypes that need to be distinguished from clones (Schleuning et al., 2001; Douhovnikoff and Dodd, 2003). The *bimodal threshold* approach potentially underestimates clone number, in particular when distributions of replicates and non-clonal genotypes overlap. In line with reports of very low germination rate of *E. hermaphroditum* (Graae et al., 2011), we were not able to grow fullsib plants from seeds and thus cannot quantify their genetic distance. We therefore used both the *mean threshold* and the *bimodal threshold* method for identification of clones thus setting lower and upper levels of clonality. Mean error rate was 0.78%, based on 78 errors in 95 replicate samples across 105 loci for *E. hermaphroditum* and 0.32% (3 errors in 9 replicate samples across 105 loci) for *E. nigrum*. Consequently, 1 and 0 difference was used as *mean threshold* for *E. hermaphroditum* and *E. nigrum*, respectively. Frequency distributions of pairwise differences were used to define the *bimodal thresholds*, which was 7 for *E. hermaphroditum* and 1 for *E. nigrum* (Figure 2c, d).

Definition of individual clones was performed by hierarchical clustering using the hclust function in R based on the number of differences between AFLP genotypes (manhattan distance) and using complete linkage agglomeration. Complete linkage assures that within a clone the maximal distance does not exceed the threshold. Clones were defined using the cutree function with the respective threshold values. Using the identified clones, we calculated six indices to estimate the clonal diversity: (1) clone number as number of different multilocus genotypes (2) clonal diversity (*R*) as $R = (G-1)/(N-1)$,

where *N* is the sample size and *G* is the number of genotypes detected in the sample (Ellstrand and Roose, 1987; Dorken and Eckert, 2001; Arnaud-Haond, 2007). (3) Simpson's index of diversity (*Sd*; complement) modified for finite population size (Pielou, 1969) as $Sd = 1-\Sigma[(G*(G-1))/(N*(N-1))]$ and (4) Simpson's evenness (*Se*) index as $Se = (Sd-min(Sd))/(max(Sd)-min(Sd))$. *Sd* expresses the probability that two randomly selected individuals from a sample will belong to the same clone and *Se* the relative abundance of clones (Arnaud-Haond, 2007). To allow comparison with (Szmidt et al., 2002) we also calculated (5) Shannon's diversity index (*Hs*) as $Hs = sum [p_i * ln(p_i) / ln(N]$ where p_i is the proportion of genotypes and (6) Clonal fraction (*Cf*) as $Cf = (N-G)/N$, which is close to 1-*R*. Furthermore, we calculated clone size as the maximum Euclidian distance among all sampling coordinates of a clone per plot. All analyses were done in R 3.2.3 (R Core Team, 2015), using custom R scripts, see supplement 2.

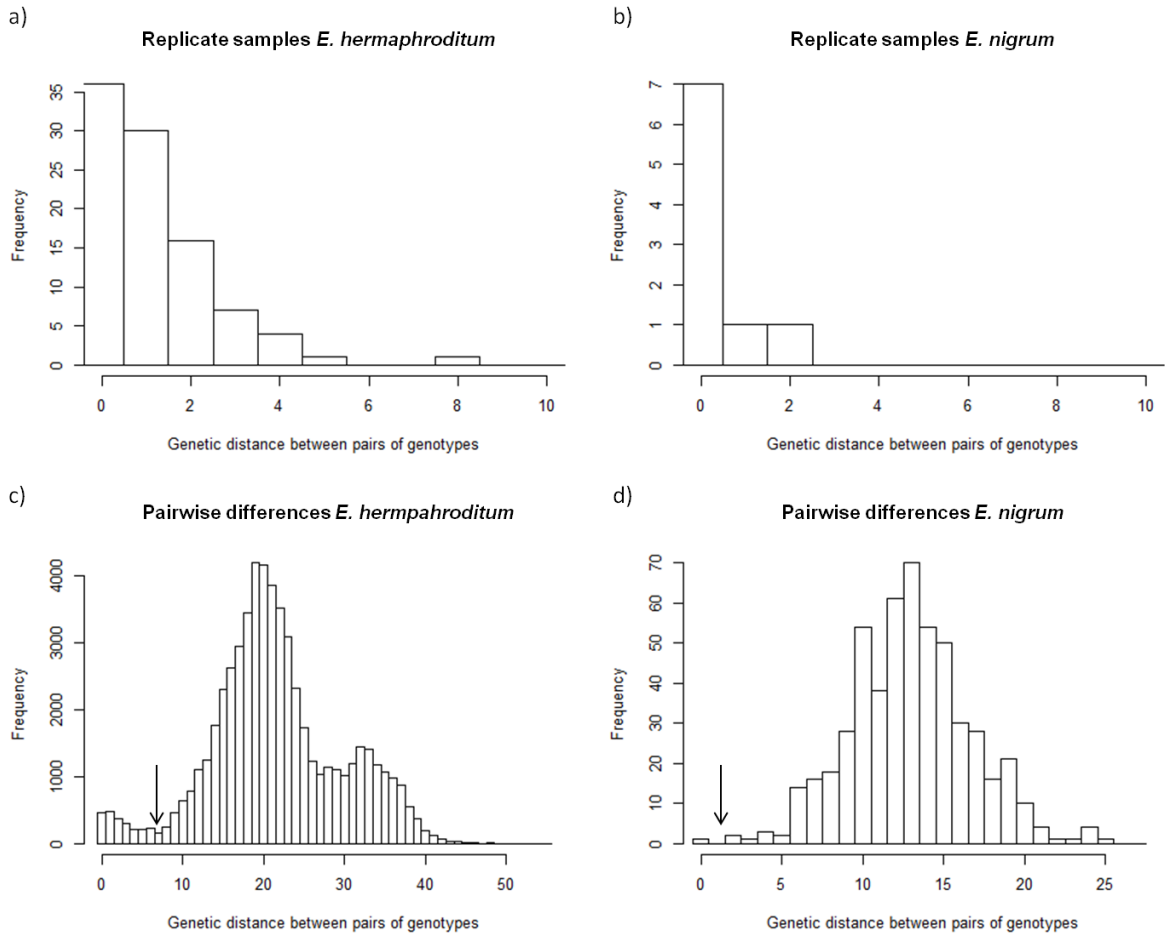


Figure 2: Frequency distributions of the number of pairwise differences among AFLP genotypes (Manhatten distance). Shown are results for comparison of pairs of replicate samples of the tetraploid *E. hermaphroditum* (a) and of the diploid *E. nigrum* (b), and comparisons of all samples of *E. hermaphroditum*

(c) and *E. nigrum* (d). *Bimodal thresholds* are indicated (arrow) at the frequency minimum, which was 7 for *E. hermaphroditum* and 1 for *E. nigrum*.

We used spatial autocorrelation methods to examine the small-scale spatial genetic structure (SGS). SGS analyzes the genetic relatedness between pairs of individuals regarding to their spatial distance (Vekemans and Hardy, 2004). We combined samples across regions within habitats but focused on plot scale relationships by restricting the distance range to the within plot scale. To cover all possible distances of the sampling design and to ensure sufficient distribution of pairs among distance classes, distance class limits were set at 1, 2, 4, 8, 12, 25, and >25m, the latter representing comparisons between habitats and regions. Within SPAGeDi 1.5a (Hardy and Vekemans, 2002) we used the pairwise kinship coefficient for dominant markers F_{ij} (Hardy, 2003) with an inbreeding coefficient of 0.5 and tested for significance of mean F_{ij} with 9999 permutations. To quantify the degree of SGS we calculated the *Sp* statistic as $Sp = -b_{log}/(1-F_{(1)})$, where b_{log} is the regression slope of mean F_{ij} on log geographic distance and $F_{(1)}$ is the mean F_{ij} of the first distance class (Vekemans and Hardy, 2004). Furthermore, we used GenAlEx 6.5 (Peakall and Smouse, 2012) to assess SGS and to test for differences of SGS between habitats by heterogeneity tests (Smouse et al., 2008). Number of permutations and bootstraps was 9999, respectively. Significance of heterogeneity tests was given if $p < 0.01$ (Banks and Peakall, 2012).

4.3 Results

Clonal diversity

Analysis of clonal structure showed generally higher clonal richness in ***s*** than in ***b*** and ***d*** (Fig. 3), with the exception of Vassijaure, where the ***s***-habitat had less clones than ***b*** and ***d***. Estimates of clonal diversity were strongly affected by the choice of threshold (Fig. 3, Tab. 1).

Clonal diversity was much higher, clonal evenness was lower and clone size was slightly larger for the *mean threshold* than for the *bimodal threshold* (Tab. 1). However, patterns of clonal diversity among habitats and regions were consistent between the two thresholds as evidenced by high correlation (*r* ranging betwe**e**n 0.44 and 0.73 for the

parameters in Table 1, excluding *E. nigrum*). In all regions and at both threshold levels we observed putative clones that spread across habitats. The single plot consisting solely of *E. nigrum*, i.e. the ***s***-habitat in Kongsvoll, had very high clonal diversity (R = 1.00 and 0.78 respectively for *mean threshold* and *bimodal threshold*, Tab. 1A and B) and small clone size, in contrast to *E. hermaphroditum* which had on average lower clonal diversity (R = 0.59 to 0.66 and 0.21 to 0.32, respectively). In *E. hermaphroditum*, on average, and irrespective of diversity estimator (*R, Sd, Hs, cf*), we observed highest clonal diversity in the alpine tundra habitat with shallow snow cover (***s***), intermediate values in the alpine tundra habitat with deep snow cover (***d***) and lowest values in the birch forest (***b***) (Tab. 1).

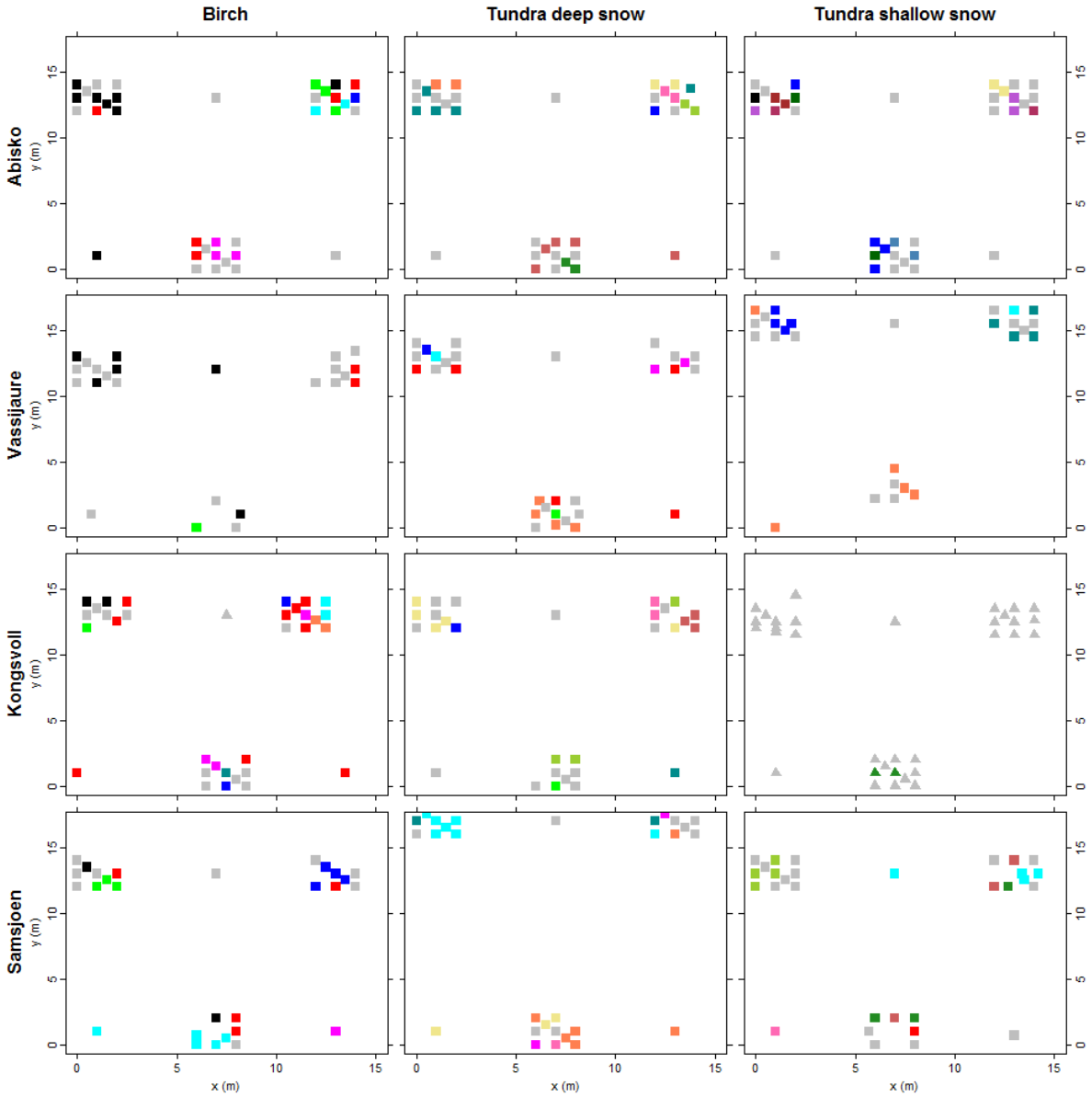
Table 1: Indices of clonal diversity of *Empetrum hermaphroditum* populations *(E. nigrum* in the ***s***-habitat of Kongsvoll) in different habitats in the four study regions. *N* sample size, *G* number of genotypes, *R* clonal diversity, *Sd* Simpsons complement, *Se* Simpons eveness, *Hs* Shannon diversity index, *CF* clonal fraction, *Amax* maximal clone size. Average values were calculated without *E. nigrum*. For clonal identification, we used either the *mean threshold* of AFLP mismatches (1 for *E. hermaphroditum* and 0 for *E. nigrum)* or the *bimodal threshold* (7 for *E. hermaphroditum* and 1 for *E. nigrum)*. ***b*** = birch forest, ***d*** = alpine tundra with deep snow cover, ***s*** = alpine tundra with shallow snow cover.

Region/habitat	N	G	R	Sd	Se	CF	Hs	Amax (m)
				Mean threshold				
Abisko								
b	36	20	0.543	0.929	0.748	0.444	1.747	17.7
d	35	22	0.618	0.958	0.818	0.371	1.815	13.9
s	35	25	0.706	0.976	0.854	0.286	1.873	14.6
Vassijaure								
b	24	18	0.739	0.942	0.294	0.25	1.841	14.5
d	30	22	0.724	0.961	0.622	0.267	1.853	17
s	27	17	0.615	0.937	0.701	0.37	1.785	16.5
Kongsvoll								
b	33	19	0.562	0.919	0.668	0.424	1.752	17.4
d	29	20	0.679	0.958	0.728	0.31	1.836	13.4
s (*E. nigrum*)	33	33	1	1		0	2	1
Samsjøen								
b	28	15	0.519	0.931	0.824	0.464	1.745	13.4
d	34	14	0.394	0.87	0.738	0.588	1.628	20.2
s	30	20	0.655	0.959	0.779	0.333	1.827	13.4
Average								
b		*18*	*0.591*	*0.93*	*0.634*	*0.396*	*1.771*	*15.7*
d		*19.5*	*0.604*	*0.937*	*0.727*	*0.384*	*1.783*	*16.1*
s		*20.7*	*0.659*	*0.957*	*0.778*	*0.33*	*1.828*	*14.8*

			Bimodal threshold					
Abisko								
b	36	6	0.143	0.594	0.557	0.833	1.304	18.4
d	35	10	0.265	0.869	0.878	0.714	1.574	13.9
s	35	9	0.235	0.871	0.912	0.743	1.561	14.6
Vassijaure								
b	24	8	0.304	0.732	0.554	0.667	1.5	14.5
d	30	12	0.379	0.811	0.599	0.6	1.575	17
s	27	8	0.269	0.815	0.791	0.704	1.524	19.8
Kongsvoll								
b	33	8	0.219	0.807	0.816	0.758	1.499	17.4
d	29	9	0.286	0.865	0.872	0.69	1.585	17.7
s (*E. nigrum*)	33	26	0.781	0.987	0.891	0.212	1.916	1
Samsjøen								
b	28	6	0.185	0.82	0.917	0.786	1.492	16.3
d	34	7	0.182	0.774	0.803	0.794	1.447	20.2
s	30	14	0.448	0.917	0.841	0.533	1.699	15.4
Average								
b		*7*	*0.213*	*0.738*	*0.711*	*0.761*	*1.449*	*16.6*
d		*9.5*	*0.278*	*0.83*	*0.788*	*0.7*	*1.545*	*17.2*
s		*10.3*	*0.317*	*0.868*	*0.848*	*0.66*	*1.595*	*16.6*

However, aside from this general trend some site specific patterns were observed, e.g. low clonal diversity in the ***s***-habitat in Vassijaure and in the ***d***-habitat in Samsjøen. Simpsons evenness increased from ***b*** to ***d*** to ***s*** in three of the regions (except Samsjøen) indicating that not only the number of clones increased but also clone abundance became more equal from ***b*** to ***d*** to ***s*** habitats. Clone size of *E. hermaphroditum*, restricting the analysis to the within plot level, was similar across all habitats, reaching at least 13 m.

Mean threshold



Bimodal threshold

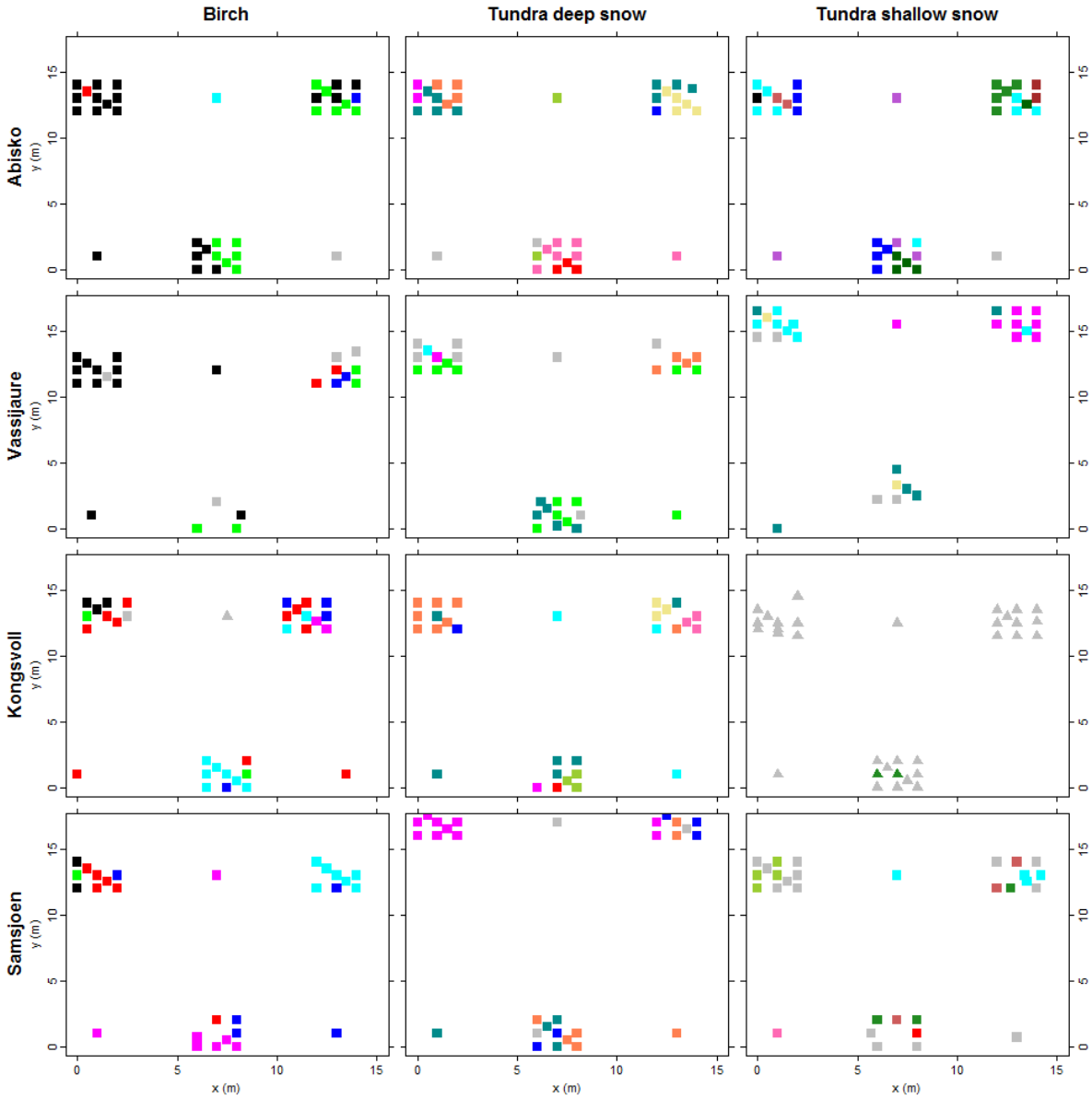


Figure 3: Spatial distribution of clones of *Empetrum* in different habitats in the four study regions Abisko, Vassijaure, Kongsvoll and Samsjøen at *mean threshold* (upper panel) and *bimodal threshold* (lower panel). Each grey dot indicates a different unique genotype, dots sharing a color belong to the same clone within one row (region). Note that in Kongsvoll, both *E. hermaphroditum* (squares) and *E. nigrum* (triangles) occurred, with *E. nigrum* mainly restricted to ***s***-habitat.

Small scale spatial genetic structure

All plots of *E. hermaphroditum* in the three habitats across the four regions showed significant SGS (supplement 3). In all populations of *E. hermaphroditum* autocorrelation was highest in the first distance class, and significant

autocorrelation was found up to 4 m in both ***b*** and ***d*** and up to 2 m in the ***s*** habitats. In *E. nigrum* only the first distance class (1 m) showed significant values. For *E. hermaphroditum* the heterogeneity tests revealed significant differences in SGS between all three habitats ($p < 0.007$). In particular ***d*** and ***s*** showed a stronger decline of autocorrelation in the first three distance classes than ***b*** (Figure 4). When combined per habitat across regions, the *Sp* values decreased from ***b*** (0.153) to ***d*** (0.128) to ***s*** (0.118) indicating strongest SGS in the birch forest. Also the diploid *E. nigrum* in the ***s***-habitat in Kongsvoll showed significant SGS, with *Sp* = 0.026, indicating much weaker SGS than in *E. hermaphroditum*.

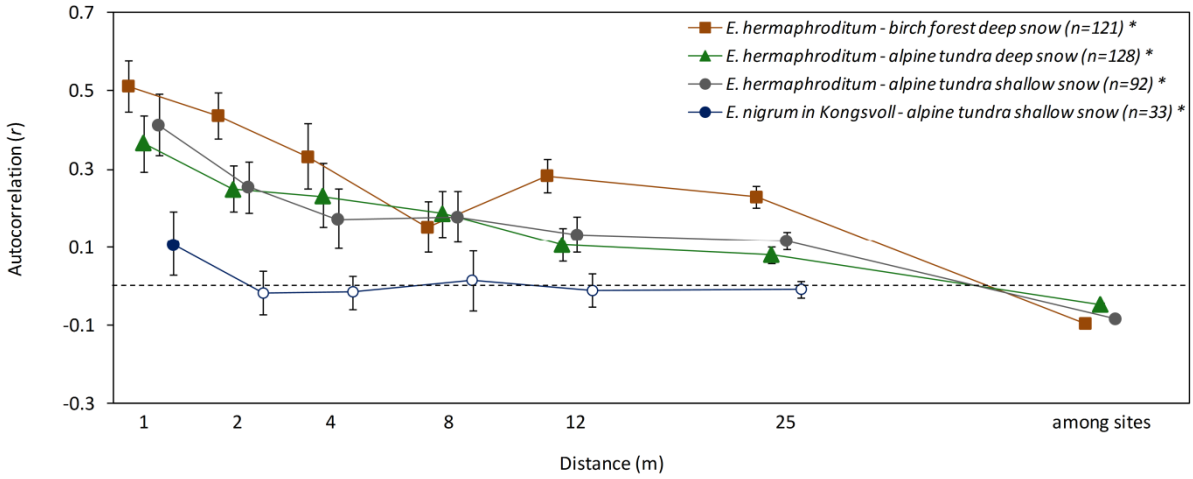


Figure 4: Spatial genetic autocorrelation in populations of *E. hermaphroditum* in the three habitats (birch forest, alpine tundra with deep snow cover, and alpine tundra with shallow snow cover) and of *E. nigrum* in the ***s***-habitat in Kongsvoll. Filled symbols indicate significant genetic autocorrelation for single distance classes. * within the legend indicate significant spatial genetic structure (SGS) for each habitat. Habitat specific analyses are shown in supplement 3.

4.4 Discussion

Irrespective of the method used to identify clones we found consistent differences of clonal diversity between habitats which were confirmed through analyses of spatial genetic structure. We found a strong influence of the method of clonal identification on absolute measures of clonal diversity. However, for both methods clone mates were identified in different habitats up to 2 km apart, which we consider biologically impossible to achieve by vegetative spread as discussed below.

Snow-related habitat effects on clonality and spatial genetic structure

Concerning our first study question, and excluding *E. nigrum*, we overall observed an increase of clonal diversity from birch forest over the sheltered depression habitat to the exposed ridge habitat, in conjunction with a decrease of snow cover and enhancement of snow melt (Bienau et al., 2014, 2015). Szmidt et al. (2002) investigated the genetic structure of *E. hermaphroditum* at three regions with different successional stages in Northern Sweden. The results of their study are comparable to ours for the indices of clonal diversity. Also, both studies revealed that sexual reproduction plays an important role in the slow growing *E. hermaphroditum*. In particular, the low proportion of clonal reproduction in the ***s***-habitat implies a higher reproduction by seedlings under such conditions (Eriksson, 1992). Within the ridge habitat, competition is lower and the proportion of open soil is higher, which likely facilitates recruitment from seeds (Callaghan et al., 1992; Szmidt et al., 2002). This result was also confirmed by a study of Boudreau et al. (2010), who found that most individuals in a subarctic sand dune ecosystem originated from seeds instead of clonal spread on early successional sites, where the conditions where comparable to the ***s***-habitat in our study regarding the amount of open soil.

Furthermore, the probability of seed dispersal to the ***s***-habitat by bird droppings may be high, because birds often roost on exposed, elevated rocks around which a lot of bird droppings can be found (pers. obs.). This interpretation is also corroborated by the results of our previous finding of higher fruit and seed production in the ***s***-habitat than in ***d*** and ***b*** (Bienau et al., 2014). In addition to long distance seed dispersal also seedling recruitment in the surrounding of the mother plant might be important for clonal diversity of *E. hermaphroditum*.

Clonal growth is expected to promote spatial autocorrelation (SGS), i.e. increasing genetic similarity with decreasing spatial distance (Reusch et al., 1999). Furthermore, the breeding system has been shown to correlate with SGS (Vekemans and Hardy, 2004). The mean *Sp* value was 0.14 in *E. hermaphroditum,* which thus ranges among the largest values listed in Vekemans and Hardy (2004), typically found in selfing species. The single plot of *E. nigrum* had Sp = 0.02, similar to other outcrossing dwarf shrubs like *Calluna vulgaris*. We observed higher SGS in the ***b***-habitat than in the ***d***- and ***s***-habitat. Thus, SGS increased, as expected, with

decreasing clonal diversity and increasing clone size. We observed that *E. hermaphroditum* in the ***b***-habitat grows more like guerilla species and in the ***s***-habitat more like phalanx species, whereas in the ***d***-habitat it growth intermediate. In a former study we investigated shoot growth of *E. hermaphroditum* (see Bienau et al. 2014) an found significantly lower ramet height, shorter annual shoot segments, lower number of lateral shoots and lower total biomass is the ***s***- than in ***d***- and ***b***-habitat. In contrast, leaf density and relative leaf mass were highest in ***s***-habitats, intermediate in ***d***-and lowest in ***b***-habitats. This pattern is conforming to the clonal growth habit in the present study. The more procumbent growth form in ***s*** with lower ramet height and shorter shoot segments but higher leaf density protects the species during periods of unstable snow cover from cold winter temperatures and prevailing strong winds and reduced freezing and desiccation (Körner 2003). In the ***b***- and ***d***-habitat protection by snow during winter (Körner 2003) and surrounding vegetation (Sturm et al. 2001; Fletcher et al. 2010) during growing season is higher, promoting prolonged growth. Further aspects promoting growth of *E. hermaphroditum* in sheltered habitats were higher water (Sturm et al. 2001; Fletcher et al. 2010) and nutrient availability during melt of a higher snowpack, which stores atmospherically deposited inorganic nitrogen (Bowman 1992; Weih 1998).

Nevertheless, the more phalanx growth form in the ***s***-habitat might facilitate a high density of flowers in one clone, whereby the possibility of selfing increases (cf. Handel, 1985) and thus SGS decreased. However, effective sexual propagation does not only depend on pollen flow, also seed dispersal, seed germination and seedling establishment are of high importance (Alberto et al., 2005). Thus, the ***s***-habitat might be the more favorable for seedling establishment due to seed dispersal into this habitat by bird droppings and a higher amount of open soil and less competition than in ***b*** and ***d***.

Empetrum nigrum

In Scandinavia, *E. hermaphroditum* and *E. nigrum* are partly allopatric with the latter being confined to more southern and coastal areas, where the former is missing (Hultén & Fries 1986). Both species occurred in our study region Kongsvoll. Analyses of additional samples in the three habitats in Kongsvoll proved that *E.*

hermaphroditum made up 99% in the ***b***-habitat, whereas *E. nigrum* made up 45% in ***d*** and 73% in ***s*** (Bienau et al., unpublished data). Nearly all sampled ramets of *E. nigrum* were different genotypes which was also reflected in SGS which was spatially restricted to the first distance class. Therefore, we expect that most of the ramets of *E. nigrum* in the ***s***-habitat are results of sexual reproduction. Moreover, the dioecious *E. nigrum* is obligatorily outcrossing (Bell and Tallis, 1973) and thus seed derived offspring will be genetically heterogeneous. In contrast, *E. hermaphroditum* has strong vegetative reproduction and is selfing, resulting in genetically more homogenous offspring (Barrett, 2003). Both *E. nigrum* and *E. hermaphroditum* build up a perennial seed bank (Klimešová and de Bello, 2009). Even though germination of both species was rarely observed in the field (*E. hermaphroditum*: Graae et al., 2011, *E. nigrum*: Mallik and Gimingham, 1985), sexual reproduction clearly is the major mode of reproduction in *E. nigrum* in this population.

Threshold definition, clone identification and the role of selfing

To address the methodological difficulties inherent in the identification of clones, we used two different thresholds of genotypic difference above which ramets were considered to belong to the same genet. The *mean threshold* determined from doubly analyzed samples (e.g. Vonlanthen et al., 2010) neglects the fact that the error rate of multiple replicates follows a Poisson distribution and thus likely overestimates clonality. The *bimodal threshold*, in contrast, takes into account the whole distribution of genotypic differences (de Witte et al., 2012) but depends on a clear separation of the two modal distributions. In our case the minimum of the bimodal distributions coincided with the upper range of distances observed between replicates. Therefore, individual clones are smaller and clonal diversity consequently is higher using the *mean threshold* than the *bimodal threshold*.

However, irrespective of the method used, in all regions we detected clones that apparently spread across different habitats up to 2 km apart which we consider biologically impossible to achieve by vegetative spread. In a former study we measured mean shoot elongation of 2.2 cm a^{-1} (range: 0.5 – 5.9 cm a^{-1} across all habitats in the four study regions) (Bienau et al., 2014). Burges (1951) observed similar growth rates of 2 cm a^{-1}. Using 6 and 1 cm a^{-1} as upper and lower bounds, the

age of a clone with a size of 15 m – the size achieved in most of our plots – would be between 125 and 750 years assuming continuous bidirectional horizontal growth. A clone of 2 km extent as observed here would thus be between ~17,000 and 100,000 years old. Such ages are at odds with both age estimates and history. Since the retreat of the ice-sheet at the end of the last glaciation (Weichselian glaciation) around 8000 years have passed (Backéus, 1999), which sets the absolute maximum size achievable for a clone to between 160 m and 960 m assuming continuous horizontal growth. In summary, we consider distances of hundreds of meters or up to 2 km as biologically impossible. But how to explain the large extent of *Empetrum* clones? With clonal spread the probability of geitanogamous selfing might increase (Handel, 1985), which is in line with the reported high level of selfing in *E. hermaphroditum* (Tikhmenev, 1984). We therefore hypothesize that at least a part of the selfed seeds of a particular genotype cannot be distinguished unequivocally from vegetatively propagated clones. If maternal plants are highly homozygous as a result of previous cycles of selfing, then offspring may be genetically virtually identical with the mother plant making it impossible to distinguish sexual from vegetative offspring. In this case only demographic observations can unequivocally determine the origin of a particular ramet. With respect to the threshold of genetic distance allowed within a clone, this underlines the importance to study halfsib families and whether they can be discriminated from vegetative clones (Schleuning et al., 2001; Douhovnikoff and Dodd, 2003).

In consequence, we therefor consider clone sizes of > 25m, i.e. outside the size of our study plots as highly unlikely and consider such "clones" to be result of sexually derived seeds after selfing, potentially of a highly homozygous mother plant. Thus, long distance seed dispersal becomes an important process determining the genotypic structure of *E. hermaphroditum*. Important vectors for *Empetrum* seeds are birds and the Arctic fox (*Alopex lagopus*; Graae et al., 2004) and red fox (*Vulpes vulpes*; Boudreau et al., 2010). In spring, large swarms of snow bunting (*Plectrophenax nivalis*) are e.g. observed in the Abisko area, which feed on shoots and berries on early melting sites and move from the valley to the alpine tundra (personal communication Nils Åke Andersson). Although less mobile, also rock ptarmigan (*Lagopus muta*) might be an important vector for *Empetrum* seeds (personal communication Nils Åke Andersson). Therefore, long distance seed

dispersal by birds and fox might help to establish individuals far away from the mother clone that are genetically indistinguishable from vegetative clones.

4.5 Conclusions

Our study demonstrates that clonal structure of *Empetrum* is affected by the prevailing local habitat conditions. We overall observed an increase of clonal diversity from birch forest over the sheltered depression habitat to the exposed ridges habitat. High clonal diversity and smaller clone size was found on exposed ridges with lower snow depth during winter, higher proportion of open soil and less competition than in the depression and the birch forest habitats (Callaghan et al., 1992; Szmidt et al., 2002). The low proportion of clonal reproduction implies a relatively higher reproduction by seedlings under such conditions (Eriksson, 1992; Boudreau et al., 2010).

An important aspect in this context might be the effect of selfing and the increasing probability of selfing in large clones (Handel, 1985). We expect that at least a part of the selfed seeds of a particular genotype cannot be distinguished unequivocally from vegetatively propagated clones. Also long distance dispersal of seeds by birds and Arctic fox becomes an important process determining the genotypic structure of *E. hermaphroditum*.

Our study revealed that sexual reproduction in addition to vegetative spread is important in clonal species.

Acknowledgments

Financial support was obtained from the Deutsche Forschungsgemeinschaft (DFG, grant EC209/9-1).

Additionally, the research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement n_262693 [INTERACT]. Field assistance was provided by Josef Scholz-vom Hofe and lab assistance by Ina Geier, Martina Herrmann and Michael Jager. We further thank Nils Åke Andersson for fruitful discussions, the director and staff of the Abisko Scientific Research Station for climate data, logistic support and accommodation. All help is gratefully acknowledged.

References

Alberto, F., Gouveia, L., Arnaud-Haond, S., Pérez-Lloréns, J.L., Duarte, C.M., and Serrão, E.A. (2005). Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. *Molecular Ecology*, *14*(9), 2669–2681. http://doi.org/10.1111/j.1365-294X.2005.02640.x

Alsos, I.G., Ehrich, D., Thuiller,W., Bronken Eidesen, P., Tribsch, A., Schönswetter, P., Lagaya, C., Taberlet, P., and Brochmann, C. (2012). Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society of London B: Biological Sciences*, *279*, 2042–2051. http://doi.org/10.1098/ rspb.2011.2363

Arnaud-Haond, S., Duarte, C.M., Alberto, F., and Serrão, E.A. (2007). Standardizing methods to address clonality in population studies. *Molecular Ecology*, *16*(24), 5115–5139. http://doi.org/10.1111/j.1365-294X.2007.03535.x

Backéus, I. (1999). The late Quaternary vegetation history of Sweden. *In: Rydin, Håkan, Pauli Snoeijs, and Martin Diekmann. „Swedish plant geography: dedicated to Eddy van der Maarel on his 65th birthday." (1999).*

Banks, S.C., and Peakall, R. (2012). Genetic spatial autocorrelation can readily detect sex-biased dispersal. *Molecular Ecology*, *21*(9), 2092–2105. http://doi.org/ 10.1111/ j.1365-294X.2012.05485.x

Barrett, S.C.H. (2003). Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*(1434), 991–1004. http://doi.org/10.1098/ rstb.2003.1301

Bell, J. N. B., and Tallis, J. H. (1973). *Empetrum nigrum* L. *Journal of Ecology*, *61*(1), 289–305. http://doi.org/10.2307/2258934

Bienau, M.J., Hattermann, D., Kröncke, M., Kretz, L., Otte, A., Eiserhardt, W.L., Milbau, A., Graae, B.J., Durka, W., and Eckstein, R.L. (2014). Snow cover consistently affects growth and reproduction of *Empetrum hermaphroditum* across latitudinal and local climatic gradients. *Alpine Botany*, *124*(2), 115–129. http://doi.org/ 10.1007/s00035-014-0137-8

Bienau, M.J., Kröncke, M., Eiserhardt, W.L., Otte, A., Graae, B.J., Hagen, D., Milbau, A., Durka, W., and Eckstein, R.L. (2015). Synchronous flowering despite

differences in snowmelt timing among habitats of *Empetrum hermaphroditum*. *Acta Oecologica*, (69), 129–136. http://doi.org/10.1016/j.actao.2015.10.005

Bliss, L. C. (1971). Arctic and Alpine Plant Life Cycles. *Annual Review of Ecology and Systematics*, *2*, 405–438.

Bonin, A., Bellemain, E., Bronken Eidesen, P., Pompanon, F., Brochmann, C., and Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, *13*(11), 3261–3273. http://doi.org/10.1111/j.1365-294X.2004.02346.x

Boudreau, S., Ropars, P., and Harper, K. A. (2010). Population dynamics of *Empetrum hermaphroditum* (Ericaceae) on a subarctic sand dune: evidence of rapid colonization through efficient sexual reproduction. *American Journal of Botany*, *97*(5), 770–781. http://doi.org/10.3732/ajb.0900304

Bowman, W. D. (1992). Inputs and Storage of Nitrogen in Winter Snowpack in an Alpine Ecosystem. *Arctic and Alpine Research*, *24*(3), 211. http://doi.org/10.2307/ 1551659

Burges, A. (1951). The Ecology of the Cairngorms: III. The *Empetrum-Vaccinium* Zone. *Journal of Ecology*, *39*(2), 271–284. http://doi.org/10.2307/2257912

Callaghan, T.V., Carlsson, B.Å., Jónsdóttir, I.S., Svensson, B.M., and Jonasson, S. (1992). Clonal Plants and Environmental Change: Introduction to the Proceedings and Summary. *Oikos*, *63*(3), 341–347. http://doi.org/10.2307/3544959

Callaghan, T.V., & Emanuelsson, U. (1985). Population structure and processes of tundra plants and vegetation. In *The population structure of vegetation* (S. 399–439).

Cook, R.E. (1983). Clonal Plant Populations: A knowledge of clonal structure can affect the interpretation of data in a broad range of ecological and evolutionary studies. *American Scientist*, *71*(3), 244–253.

De Frenne, P., Rodríguez-Sánchez, F., Coomes, D.A., Baethen, L., Verstraeten, G., Vellend, M., Bernhardt-Römermann, M., Brown, C.D., Brunet, J., Cornelis, J., Decocq, G.M., Dierschke, H., Eriksson, O., Gilliam, F.S., Hédl, R., Heinken, T., Hermy, M., Hommel, P., Jenkins, M.A., Kelly, D.L., Kirby, K.J., Mitchell, F.J., Naaf, T., Newman, M., Peterken, G., Petrik, P., Schultz, J., Sonnier, G., van Calster, H., Walther, G.R., White, P.S., Woods, K.D., Wulf, M., Graae, B.J., and Verheyen, K.

(2013). Microclimate moderates plant responses to macroclimate warming. *Proceedings of the National Academy of Sciences*, *110*(46), 18561–18565. http://doi.org/10.1073/pnas.1311190110

de Witte, L.C., Armbruster, G.F.J., Gielly, L., Taberlet, P., and Stöcklin, J. (2012). AFLP markers reveal high clonal diversity and extreme longevity in four key arctic-alpine species. *Molecular Ecology*, *21*(5), 1081–1097. http://doi.org/10.1111/j.1365-294X.2011.05326.x

Dorken, M.E., and Eckert, C.G. (2001). Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, *89*(3), 339–350. http://doi.org/10.1046/j.1365-2745.2001.00558.x

Douhovnikoff, V., and Dodd, R.S. (2003). Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. *Theoretical and Applied Genetics*, *106*(7), 1307–1315. http://doi.org /10.1007/s00122-003-1200-9

Ellstrand, N.C., and Roose, M.L. (1987). Patterns of Genotypic Diversity in Clonal Plant Species. *American Journal of Botany*, *74*(1), 123–131.

Eriksson, O. (1989). Seedling Dynamics and Life Histories in Clonal Plants. *Oikos*, *55*(2), 231–238. http://doi.org/10.2307/3565427

Eriksson, O. (1992). Evolution of seed dispersal and recruitment in clonal plants. *Oikos*, *63*(3), 439–448. http://doi.org/10.2307/3544970

Escaravage, N., Questiau, S., Pornon, A., Doche, B., and Taberlet, P. (1998). Clonal diversity in a *Rhododendron ferrugineum* L. (Ericaceae) population inferred from AFLP markers. *Molecular Ecology*, *7*(8), 975–982. http://doi.org/10.1046/j.1365-294x.1998.00415.x

Fletcher, B. J., Press, M. C., Baxter, R., and Phoenix, G. K. (2010). Transition zones between vegetation patches in a heterogeneous Arctic landscape: how plant growth and photosynthesis change with abundance at small scales. *Oecologia*, *163*(1), 47–56. http://doi.org/10.1007/s00442-009-1532-5

Graae, B.J., Ejrnaes, R., Lang, S.I., Meineri, E., Ibarra, P.T., and Bruun, H.H. (2011). Strong microsite control of seedling recruitment in tundra. *Oecologia*, *166*(2), 565–576. http://doi.org/10.1007/s00442-010-1878-8

Graae, B.J., Pagh, S., and Bruun, H.H. (2004). An Experimental Evaluation of the Arctic Fox (*Alopex lagopus*) as a Seed Disperser. *Arctic, Antarctic, and Alpine Research*, *36*(4), 468–473. http://doi.org/http://dx.doi.org/10.1657/1523-0430(2004) 036[0468:AEEOTA]2.0.CO;2

Handel, S.N. (1985). The Intrusion of Clonal Growth Patterns on Plant Breeding Systems. *The American Naturalist*, *125*(3), 367–384.

Hardy, O.J. (2003). Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology*, *12*(6), 1577–1588. http://doi.org/10.1046/j.1365-294X.2003.01835.x

Hardy, O.J., and Vekemans, X. (2002). spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, *2*(4), 618–620. http://doi.org/10.1046/j.1471-8286.2002.00305.x

Jonas, T., Rixen, C., Sturm, M., and Stöckli, V. (2008). How alpine plant growth is linked to snow cover and climate variability. *Journal of Geophysical Research: Biogeosciences*, *113*(G3). http://doi.org/10.1029/2007JG000680

Jonasson, S. (1981). Plant communities and species distribution of low alpine *Betula nana* heaths in Northernmost Sweden. *Vegetatio*, *44*(1), 51–64. http://doi.org/10.1007/BF00119804

Klimeš, L., Klimešová, J, Hendriks, R., and van Groenendael, J. (1997). Clonal plant architecture: a comparative analysis of form and function (S. 1–29). Backhuys Publishers Leiden.

Klimešová, J, and de Bello, F. (2009). CLO-PLA: the database of clonal and bud bank traits of Central European flora. *Journal of Vegetation Science*, *20*(3), 511–516. http://doi.org/10.1111/j.1654-1103.2009.01050.x

Körner, C. (2003). *Alpine plant life: functional plant ecology of high mountain ecosystems* (2nd Aufl.). Berlin, Heidelberg: Springer Verlag.

Hultén, E., and Fries, M. (1986). *Atlas of North European vascular plants, vol. 1-3.* Königstein: Koeltz.

Lenoir, J., Graae, B.J., Aarrestad, P.A., Alsos, I.G., Armbruster, W.S., Austrheim, G., Bergendorff, C., Birks, H.J.B., Bråthen, K.A., Brunet, J., Bruun, H.H., Dahlberg, C.J., Decocq, G., Diekmann, M., Dynesius, M., Ejrnaes, R., Grytnes, J.-A., Hylander, K.,

Klanderud, K., Luoto, M., Milbau, A., Moora, M., Nygaard, B., Odland, A., Ravolainen, V.T., Reinhardt, S., Sandvik, S.M., Schei, F.H., Speed, J.D.M., Tveraabak, L.U., Vandvik, V., Velle, L.G., Virtanen, R., Zobel, M., and Svenning, J.C. (2013). Local temperatures inferred from plant communities suggest strong spatial buffering of climate warming across Northern Europe. *Global Change Biology*, *19*(5), 1470–1481. http://doi.org/10.1111/gcb.12129

Lovett Doust, L. (1981). Population Dynamics and Local Specialization in a Clonal Perennial (*Ranunculus Repens*): I. The Dynamics of Ramets in Contrasting Habitats. *Journal of Ecology*, *69*(3), 743–755. http://doi.org/10.2307/2259633

Mallik, A.U., and Gimingham, C.H. (1985). Ecological Effects of Heather Burning: II. Effects on Seed Germination and Vegetative Regeneration. *Journal of Ecology*, *73*(2), 633–644. http://doi.org/10.2307/2260500

McGraw, J.B., and Shaver, G.R. (1982). Seedling density and seedling survival in Alaskan cotton grass tussock tundra. *Ecography*, *5*(2), 212–217. http://doi.org/10.1111 /j.1600-0587.1982.tb01039.x

Molau, Ulf, and Larsson, E.L. (2000). Seed rain and seed bank along an alpine altitudinal gradient in Swedish Lapland. *Canadian Journal of Botany*, *78*(6), 728–747. http://doi.org/10.1139/b00-049

Nilsson, M.-C., and Wardle, D.A. (2005). Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Frontiers in Ecology and the Environment*, *3*(8), 421–428. http://doi.org/10.1890/1540-9295(2005)003 [0421:UVAAFE]2.0.CO;2

Odland, A., and Munkejord, H.K. (2008). Plants as indicators of snow layer duration in southern Norwegian mountains. *Ecological Indicators*, *8*(1), 57–68. http://doi.org/10.1016/j.ecolind.2006.12.005

Peakall, R., and Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, *28*, 2537–2539. http://doi.org/10.1111/j.1471-8286.2005. 01155.x

Pielou, E.C. (1969). *An introduction to mathematical ecology.* New York: Wiley.

Pluess, A.R., and Stöcklin, J. (2004). Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany*, *91*(12), 2013–2021. http://doi.org/10.3732/ajb.91.12.2013

R Core Team. (2015). R: The R Project for Statistical Computing. Abgerufen von http://www.r-project.org/

Reusch, T.B.H., Hukriede, W., Stam, W.T., and Olsen, J.L. (1999). Differentiating between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity*, *83*(2), 120–126. http://doi.org/10.1046/j.1365-2540.1999.00546.x

Saarinen, T., and Lundell, R. (2010). Overwintering of *Vaccinium vitis-idaea* in two sub-Arctic microhabitats: a reciprocal transplantation experiment. *Polar Research*, *29*(1), 38–45. http://doi.org/10.1111/j.1751-8369.2010.00152.x

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanawald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, L.N., Sykes, M.T., Walker, B.H., Walker, D. and Wall, D.H. (2000). Global Biodiversity Scenarios for the Year 2100. *Science*, *287*(5459), 1770–1774. http://doi.org/10.1126/science.287.5459.1770

Schleuning, M., Becker, T., Vadillo, G.P., Hahn, T., Matthies, D., and Durka, W. (2011). River dynamics shape clonal diversity and genetic structure of an Amazonian understorey herb. *Journal of Ecology*, *99*, 373–382. http://doi.org/10.1111/j.1365-2745.2010.01775.x

Smouse, P.E., Peakall, R., and Gonzales, E. (2008). A heterogeneity test for fine-scale genetic structure. *Molecular Ecology*, *17*(14), 3389–3400. http://doi.org/10.1111 / j.1365-294X.2008.03839.x

Sturm, M., Holmgren, J., McFadden, J.P., Liston, G.E., Chapin, F.S., and Racine, C.H. (2001). Snow–Shrub Interactions in Arctic Tundra: A Hypothesis with Climatic Implications. *Journal of Climate*, *14*(3), 336–344. http://doi.org/10.1175/1520-0442(2001)014<0336:SSIIAT>2.0.CO;2

Suda, J. (2002). New DNA polidy level in *Empetrum* (Empetraceae) revealed by flow cytometry. *Annales Botanici Fennici*, *39*(2), 133–141.

Szmidt, A.E., Nilsson, M.-C., Briceño, E., Zackrisson, O., and Wang, X.-R. (2002). Establishment and genetic structure of *Empetrum hermaphroditum* populations in northern Sweden. *Journal of Vegetation Science*, *13*(5), 627–634. http://doi.org/ 10.1111/j.1654-1103.2002.tb02090.x

Tikhmenev, E.A. (1984). Pollination and self-pollinating potential of entomophilic plants in arctic and mountain tundras of the northeastern USSR. *The Soviet journal of ecology*, *15*(4), 166–172.

Tybirk, K., Nilsson, M.-C., Michelsen, A., Kristensen, H. L., Shevtsova, A., Strandberg, M.T., Johansson, M., Nielsen, K.E., Riis-Nilsen, T., Strandberg, B. and Johnsen, I. (2000). Nordic *Empetrum* dominated ecosystems: function and susceptibility to environmental changes. *AMBIO: A Journal of the Human Environment*, *29*(2), 90–97. http://doi.org/10.1579/0044-7447-29.2.90

Vekemans, X., and Hardy, O.J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*(4), 921–935. http://doi.org/10.1046/j.1365-294X.2004.02076.x

Vonlanthen, B., Zhang, X., and Bruehlheide, H. (2010). Clonal structure and genetic diversity of three desert phreatophytes1. *American Journal of Botany*, *97*(2), 234–242.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*(21), 4407–4414. http://doi.org/ 10.1093/ nar/23.21.4407

Weih, M. (1998). Seasonality of Nutrient Availability in Soils of Subarctic Mountain Birch Woodlands, Swedish Lapland. *Arctic and Alpine Research*, *30*(1), 19. http://doi.org/ 10.2307/1551741

Supplementary Material

Electronic Supplement 1

AFLP protocol

For restriction and ligation (RL; Table S1) 6.0 µl genomic DNA were combined with 5.0 µl RL reaction mix (Table S1). The reaction was incubated for 2 h at 37 °C and afterwards diluted 1:1.

Table S1: Content of the RL reaction mix with used primers.

Product	***Volume (µl)***
BSA (1mg/ml; New England Biolabs, NEB)	0.55
T4 DNA ligase buffer (NEB)	1.1
0.5 M NaCl (NEB)	1.1
*Eco*RI (10u/µl; NEB)	0.1
*Mse*I (10000 u/ml; NEB)	0.1
T4 DNA ligase (NEB)	0.05
*Eco*RI adapter (5 pmol/µl)	1.0
*Mse*I adapter (50 pmol/µl)	1.0

Adaptors

Primer	**Sequence**
*Eco*RI adapter top	5’ – CTCGTAGACTGCGTACC – 3’
*Eco*RI adapter bottom	5’ – AATTGGTACGCAGTCTAC – 3’
*Mse*I adapter top	5’ – GACGATGAGTCCTGAG – 3’
*Mse*I adapter bottom	5’ – TACTCAGGACTCAT – 3’

For the preselective amplification (PCR1; Table S2), 4 µl diluted RL product were combined with 16 µl PCR1 reaction mix (Table S2). The thermocycler protocol was 72.0°C (2 min) followed by 20 cycles of 94.0°C (20 s), 56.0°C (30 s) and 72.0°C (2 min) and at the end 60.0°C (30 min), performed on an Eppendorf Mastercycler gradient. The PCR1 product was diluted 1:9.

Table S2: Content of the PCR1 reaction mix with used primers.

Product	***Volume (µl)***
H_2O	9.84
*Eco*RI preselective primer (1.5 ng/µl)	1.0
*Mse*I preselective primer (1.5 ng/µl)	1.0
dNTPs (2 mM; Roth)	2.0
10 x Dream Tag buffer (QIAGEN)	2.0
Dream Tag polymerase (5u/ µl ; QIAGEN)	0.16

Preselective primers	
Primer	**Sequence**
*Eco*RI +A	5' – GACTGCGTACCAATTCA – 3'
*Mse*I +C	5' – GATGAGTCCTGAGTAAC – 3'

For the selective amplification (PCR2; Table S3), 1 µl diluted PCR1 product was combined with 3.4 µl PCR2 reaction mix. The thermocycler protocol was 94.0°C (2 min) followed by 10 cycles of 94.0°C (20 s), 66.0°C (30 s, decreasing 1°C per cycle) and 72.0°C (2 min) and 20 cycles of 94.0°C (20 s), 56.0°C (30 s) and 72.0°C (2 min), and at the end 60.0°C (30 min), performed on an Eppendorf Mastercycler pro 384.

Table S3: Content of the PCR2 reaction mix with used primers.

Product	***Volume (µl)***
Multiplex PCR kit (QIAGEN)	2.2
fluorescent *Eco*RI primer (1 pmol/µl)	0.6
fluorescent *Mse*I (5 pmol/µl)	0.6
Selective primer AFLP	
Primer	**Sequence**
*Eco*RI + ACT-FAM	5' – GACTGCGTACCAATTCACT – 3'
*Eco*RI + ACA-VIC	5' – GACTGCGTACCAATTCACA – 3'
*Eco*RI + AAG-NED	5' – GACTGCGTACCAATTCAAG – 3'
*Eco*RI + AGC-PET	5' – GACTGCGTACCAATTCAGC – 3'
*Mse*I + CAG-FAM	5' – GATGAGTCCTGAGTAACAG – 3'
*Mse*I + CTC-VIC	5' – GATGAGTCCTGAGTAACTC – 3'
*Mse*I + CAG-NED	5' – GATGAGTCCTGAGTAACAG – 3'
*Mse*I + CAC-PET	5' – GATGAGTCCTGAGTAACAC – 3'

Electronic Supplement 2

Demo script using functions from Clonal_diversity_descriptors.R

(Walter Durka 21.04.2016)

```
setwd('D:/Empetrum/Auswertung_Klonale_Studie/Datensatz_neu')

source("Clonal_diversity_descriptors.R")
library("vegan")

# set the threshold for the genetic distance allowed within clones
ourthreshold <- 7

# plot a histogram of genetic distance among samples
clonal_hist(inputfile ="Abisko.txt",xy=T, Main="Abisko",
            distmethod="manhattan",clustermethod="complete")

# plot a hierachical cluster using dendrogram of genotypes
clonal_clust(inputfile ="Abisko_xy.txt",xy=T, Main="Abisko",

distmethod="manhattan",clustermethod="complete",threshold=ourthreshold)

# use two plot windows to plot both a histogram and a cluster, as above
par(mfrow=c(1,2))
clonal_hist_clust(inputfile ="Abisko.txt",xy=T,Main="Abisko",

distmethod="manhattan",clustermethod="complete",threshold=ourthreshold)

# plot spatial distribution of clones
# step 1: extract list of clones
cl       <-       clone_list("Abisko_xy.txt",xy=T,distmethod="manhattan",
clustermethod="complete",ourthreshold)
# step 2: do the plot
clonal_identity_plot (cl, Main="Abisko birch", pop_to_plot = "AB",
                      symbolsize=1.25,symbols.=15,"xx",xlim.=c(0,15),
                      ylim.=c(0,17),T,T,F,F)


# Extract clonal diversity descriptors; includes a hist + clust plot
par(mfrow=c(1,2))
clonal_diversity_descriptors(inputfile ="Abisko_xy.txt",xy=T,
                                outputname="Abisko",Main="Abisko",

distmethod="manhattan",clustermethod="complete",

threshold=ourthreshold,appendFiles=F,Fis=0.5)


# Extract spatial size of clones
clonal_spatial_descriptors(inputfile ="Abisko_xy.txt",
                           outputname="Abisko", Main="Abisko",
                           distmethod="manhattan",
clustermethod="complete",
                           threshold=ourthreshold,appendFiles=F)
```

Clonal_diversity_descriptors

(Walter Durka 19.02.2016, updated 21.04.2016)

```
# Function defines clones from AFLP-like data sets using hierarchical
# clustering and applying a user-defined cutoff threshold
# For clones defined, a number of descriptive statistics are computed
# Parameters needed:
#     inputfile   file name of input data;
#                 fileformat: 1st line = header
#                 1st column = individual ID
#                 2nd column = population ID
#                 >2nd column = AFLP phenotypes (0/1)
#                 optionally, the last two columns are carthesian x and
#                 y coordinates
#
#     xy          boolean indicating whether the inputfile has xy
#                 coordinates or not
#     outputname  base name for output files, will be supplemented for
#                 - distance-matrix: "_dist.txt"
#                 - sample list with clonal identity: "_clID.txt"
#                 - clone list with clonal frequency per population
#                   "_clFreq.txt"
#                 - AFLP data-file of clones (single genotype per clone
#                   using the majority rule for assigning genotypes of
#                   AFLP markers that are polymorphic within clones):
#                   "_clgenotypes.txt"
#                 - clonal diversity descriptors:
#                   "_clonal_diversity_ descriptors.txt"
#     Main        title to be plotted on figures
#     distmethod  function used in the R function dist: e.g. "manhattan"
#                 (which is an Euclidian distance)
#     clustermethod method used by hclust, e.g. "single", "average",
#                   "complete"
#     threshold   distance-threshold accepted within clonals
#     appendFiles should result files be append to files with identical
#                 names?:True/False
#     inbreeding coefficient   is used in the calculation of gene
#                 diversity (He) to estimate allele frequencies from
#                 AFLP band frequency
#
# Descriptive parameters calculated per population:
#     "n"               number of samples
#     "n_gt"            number of clones (genotypes)
#     "R_cl_div"        clonal diversity R = (G-1)/(N-1) (Dorken and
#                       Eckert 2001)
#     "Simpsons_comp"   Simpson's index of diveristy (complement),
#                       modified for finite population size
#                       (Pielou 1969)
#                        d = 1-sum[(ngt_i*(ngt_i-1))/(n*(n-1))]
#     "Simpson_eve"     Simpsons evenenss = (d-min(d))/(max(d)-min(d))
#     "eff_n_gt"        Effective numer of genotypes   = Simpsons
#                       reciprocal
#                       eff_n_gt = 1/sum[(pi)^2] ; pi = ngt_i/n
#     "clonal_fraction" Clonal fraction (Szmidt 2002)
#                       clonal_fraction = (n-ngt)/n
#     "Hs"              Normalized Shannon diversity index
#                       Hs = -sum[pi * ln(pi) / ln (n)]
#     "gene diversity"
#########################################################################
```

```
clonal_diversity_descriptors <-function(inputfile,
                                        xy=T,
                                        outputname,
                                        Main,
                                        distmethod,
                                        clustermethod,
                                        threshold,
                                        appendFiles,
                                        Fis=0.5
                                        ){
# inputfile<- "Abisko.txt"
# Main<-inputfile
# clustermethod <-"complete" #"average" # average = UPGMA;
# distmethod<- "manhattan"
# threshold <- 12
# outputname<-"Abisko"
# Fis <- 0.5
      d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
      if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
                  d       <- d[,-c(ncol(d)-1,ncol(d))]}
      d[,2]<- as.factor(d[,2])
      dist<-dist(d[,-c(1:2)],method=distmethod);#dist # pairwise
            # differences
      y<-data.frame(as.matrix(dist));   names(y)<-   d[,1];row.names(y)<-
      d[,1]
      write.table(y,file=paste(outputname,"_dist.txt",sep=""),sep="\t",
      quote=F)
      hist(dist, breaks=seq(.5,max(dist)*1.1+0.5,by=1),
      xlim=c(0,max(dist)*1.1),
      main=Main,xlab=paste(distmethod,"distance"))

      cl<-hclust(dist,clustermethod); # UPGMA-cluster
      cl$labels<-d[,1]
      plot(cl,ylab=distmethod,main=Main,cex=1)
      if    (threshold    >0)    rect.hclust(cl,h=threshold,border="red")
      #clones by  threshold

      Clonelist<- data.frame(cutree(cl,h=threshold));
      Clonelist<- cbind(d[,1],d[,2],Clonelist)
      names(Clonelist)<-c(names(d)[1],names(d)[2],"clID");
      #head(Clonelist)
      write.table(Clonelist,file=paste(outputname,"_clID.txt",sep=""),se
      p="\t",quote=F,row.names=FALSE)
      Clonelist <- Clonelist[,-c(1)]

      write.table(table(Clonelist[,2],Clonelist[,1]),
      file=paste(outputname,"_clFreq.txt",sep=""),sep="\t",quote=F)
      pops <- levels(Clonelist[,1]); # pops
      npop <- length(pops)

#  cat(names(genotypes),file=paste(outputname,"_clgenotypes.txt",sep=""),
#  "\n", sep="\t")
  # populationwise calcualtion of diversity indices
  div <- matrix(NA, npop, 10) # matrix for diversity indices
  for (i in 1:npop){
  #i<-1
      popdata        <-   Clonelist[which(Clonelist[,1]   ==   pops[i]),-
      c(1)];#popdata
      popsize   <- length(popdata)              # population size
      freqs     <- table(popdata)/popsize;#freqs # relative frequency
            # table of clones of one population
```

```
      freqsabs  <- table(popdata);#freqsabs #absolute frequency
      # table of clones
      div[i,1] <- popsize    # number of samples
      div[i,2] <- threshold
            div[i,3] <- length(freqs) # number of genotypes
      div[i,4] <- round((length(freqs)-1)/(popsize-1),3)  # R, clonal
            # diversity (Dorken + Eckert 2001)
      Simpson <- 0      # Unbiased estimator of Simpsons diversity index
            # = Neis Genotype diversity unbiased estimator;
      Hs <- 0          # Shannon diversity
      for (gt in 1:dim(freqs)) {
            Simpson <- Simpson + freqsabs[gt]*(freqsabs[gt]-
            1)/(popsize*(popsize-1))
            Hs <- Hs + freqs[gt] * log(freqs[gt])/log(popsize)}
      Simpson_complement <- 1-Simpson
      div[i,5] <- round(Simpson_complement,3)
      # Eveness of Simpsons complement
      Ngt <- length(freqs); Nsp <- popsize
      Simpson_min <- ((2*Nsp-Ngt)*(Ngt-1)/Nsp^2)*(Nsp/(Nsp-1))
      Simpson_max <- (Ngt-1)/Ngt * (Nsp/(Nsp-1))
      Simpson_eveness <- (Simpson_complement-Simpson_min)/(Simpson_max-
      Simpson_min)
      div[i,6] <- round(Simpson_eveness,3)
# Effective numer of genotypes   = Simpsons reciprocal
      div[i,7] <- round(1/sum(freqs^2),3)
      div[i,8] <- round((Nsp-Ngt)/Nsp,3) # Clonal fraction (Szmidt et
            # al. 2002)
      div[i,9] <- round(1- Hs,3) # Shannon diversity
      popdata        <-   Clonelist[which(Clonelist[,1]   ==   pops[i]),-
      c(1)];#popdata

# Neis gene diversity
    attach(Clonelist)
            genotypes <- cbind(clID,d);detach(Clonelist) # attach clID
    genotypes <- genotypes[which(genotypes[,3]==pops[i]),] # select pop
    genotypes <- genotypes[,-c(2,3)]
    genotypes <- aggregate(genotypes,
    by=list(genotypes$clID),FUN=mean);
    genotypes <- genotypes[,-c(1)]
    for (j in 2:ncol(genotypes)) genotypes[,j] <- round(genotypes[,j],0)
      # clonal genotype = majority rule genotype
            if (length(freqs) > 1) {  # if more than one genotype ...
            freqs1 <- apply(genotypes[,-c(1)], 2, mean);#freqs1 # vector
            # band-frequency of loci
            freqs1 <- sqrt( (1-freqs1)*(1-Fis)+ (1-freqs1)^2*Fis)
            #;freqs1   #(Lynch & Milligan 1994)
            div[i,10] <- round(mean((1-(freqs1^2+(1-
            freqs1)^2)))*(length(freqs)/(length(freqs)-1)),3)
            }
            else div[i,10] <- 0

# write genotype file
      sn<-rep(pops[i],length(freqs) )
      gt<-cbind(sn,genotypes)
      if(i == 1)  write.table(genotypes,file=paste
      (outputname,"_clgenotypes.txt",sep=""),sep="\t",quote=F)
      else
      write.table(genotypes,file=paste(outputname,"_clgenotypes.txt",sep
      =""),sep="\t",col.names=F,quote=F,append=T)
            }
```

```
# write table of descriptive statistics
      div <- cbind(pops, data.frame(div));
      names(div) <- c(names(d)[2], "n", "threshold","n_gt",
            "R_cl_div","Simpsons_comp","Simpson_eve","eff_n_gt",
            "clonal_fraction","Hs","gene_diversity");
      write.table(div, file=paste(outputname,"_clonal_diversity_
            descriptors.txt",sep=""), sep="\t", col.names=T,
            row.names=FALSE, quote=FALSE, append=appendFiles)
            return(div)
}#########################################################################

#########################################################################
#  clonal_identity_plot                          Walter Durka 17.03.2016
#########################################################################

# Function defines clones from AFLP-like data sets using hierarchical
# clustering and applying a user-defined cutoff threshold
# For clones defined, a number of descriptive statistics are computed
# Parameters needed:
#    inputfile    file name of input data;
#                 fileformat: 1st line = header
#                             1st column = individual ID
#                             2nd column = population ID
#                             >2nd column = AFLP phenotypes (0/1)
#                 optionally, the last two columns are carthesian x and
#                 y coordinates
#     xy          boolean indicating whether the inputfile has xy
#                 coordinates or not
#     distmethod  function used in the R function dist: e.g.
#                 "manhattan" (which is an Euclidian distance)
#     clustermethod    method used by hclust, e.g. "single", "average",
                      "complete"
#     threshold   distance-threshold accepted within clonals
#########################################################################
clone_list <-function(inputfile, xy,
                      distmethod,
                      clustermethod,
                      threshold  ){
#inputfile<- "Abisko.txt"
#xy<-T
#Main<-inputfile
#clustermethod <-"complete" #"average" # average = UPGMA;
#distmethod<- "manhattan"
#threshold <- 12
  d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
  if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
           d       <- d[,-c(ncol(d)-1,ncol(d))]}
  d[,2]<- as.factor(d[,2])
  dist<-dist(d[,-c(1:2)],method=distmethod);#dist #pairwise differences
  cl<-hclust(dist,clustermethod); # UPGMA-cluster;
  cl$labels<-d[,1]

  Clonelist<- data.frame(cutree(cl,h=threshold));
  if (xy) { Clonelist<- cbind(xy_coor,Clonelist);
  names(Clonelist)<-c("ID","pop","X","Y","clID");}
  else names(Clonelist)<-c("clID")
  return(Clonelist)
}#########################################################################
```

```
#########################################################################
#  clonal_identity_plot                          Walter Durka 17.03.2016
#########################################################################
clonal_identity_plot <-function(Clonelist, Main,
                                pop_to_plot,
                                symbolsize, symbols.,
                                colorcode,
                                xlim.,ylim.,#xlab.,ylab.,   #   x,y-range,
                                    # x,y-label
                                l1,l2,l3,l4   # boolean to specify labels
                                    # below, left, above, right

                                        ){
# inputfile<- "Abisko.txt"
# xy<-T
# Main<-inputfile
# clustermethod <-"complete" #"average" # average = UPGMA;
# distmethod<- "manhattan"
# threshold <- 6
# Main<-inputfile
# l1<-F;l2<-F;l3<-F;l4<-F
# pop_to_plot<-"AS"
# symbolsize  <- 1.25
# colorcode <-  "xx"
# xlim.<-c(0,16);ylim.<-c(0,16)
#  d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
#  if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
#           d       <- d[,-c(ncol(d)-1,ncol(d))]}
#  d[,2]<- as.factor(d[,2])
#  dist<-dist(d[,-c(1:2)],method=distmethod);#dist #pairwise differences
#  cl<-hclust(dist,clustermethod); # UPGMA-cluster;
#  cl$labels<-d[,1]
#
#  Clonelist<- data.frame(cutree(cl,h=threshold));
#  Clonelist<- cbind(xy_coor,Clonelist);
#  names(Clonelist)<-c("ID","pop","X","Y","clID");Clonelist
#
  Clonefre<-data.frame((table(Clonelist$clID)));Clonefre ;#str(clonefre)
  pops <- levels(Clonelist[,2]);  pops
  npop <- length(pops)  ;npop
  nclo <- nrow(Clonefre);nclo

  # define colors for clones with more than 1 incidence, otherwise =
  # gray
  allcols<-c("black","red","green","blue","cyan","magenta","coral",
             "darkcyan","khaki","hotpink","yellowgreen","indianred",
             "forestgreen","brown","darkgreen","mediumorchid","maroon",
             "khaki","steelblue","yellowgreen","gold")
  if (colorcode == "rainbow")
  allcols<-rainbow(nclo, s = 1, v = 1, start = 0, end = max(1, nclo -
  1)/nclo, alpha = 1);
  if (colorcode == "heat")
  allcols<-heat.colors(nclo, alpha = 1)
  if (colorcode == "terrain")
  allcols<-terrain.colors(nclo, alpha = 1)
  if (colorcode == "topo")
  allcols<-topo.colors(nclo, alpha = 1)
  if (colorcode == "cm")
  allcols<-cm.colors(nclo, alpha = 1)
  if (colorcode == "brewer")
  allcols<-brewer.pal(nclo, "Paired")
```

```
  # colors for clones with more than 1 incidence
  Clonefre$ccol<-"gray"
  colcount<-1
  for (i in 1:nrow(Clonefre))
    {if (Clonefre [i,2]>1)
        {Clonefre$ccol[i]<-allcols[colcount];
        colcount<-colcount+1} }

  Clonelist$clcol<-"yellow";
  for (i in 1: nrow(Clonelist)) Clonelist$clcol[i] <-
  Clonefre$ccol[Clonelist$clID[i]]


  if(pop_to_plot != "") cl<-Clonelist[which(Clonelist$pop==pop_to_plot),
      ]
      else  cl<-Clonelist ;


#  names(Clonelist)<-c("ID","pop","X","Y","clID");Clonelist
  cl$X <- cl$X-min(cl$X);cl$Y <- cl$Y-min(cl$Y);
  plot(cl$Y                    ~                    cl$X                    ,
pch=symbols.,col=cl$clcol,cex=symbolsize,main=Main,xlab="",ylab="",
                  xlim=xlim.,ylim=ylim.,axes=F,frame=T)
  axis(1,labels=l1,tck=-0.02);axis(2,labels=l2,tck=-
0.02);axis(3,labels=l3,tck=-0.02);axis(4,labels=l4,tck=-0.02);
#  axis(1,labels=l1,tck=-0.02);axis(2,labels=T,tck=-0.02);
#  axis(3,labels=l3,tck=-0.02);axis(4,labels=l4,tck=-0.02);

#  plot(c$Y ~ c$X , pch=15,col=c$clcol,cex=symbolsize,
#  main=Main,xlab="",ylab="")

}#####################################################################
  clonal_hist_clust            <-function(inputfile,
                                          xy=T,
                                          outputname,
                                          Main,
                                          distmethod,
                                          clustermethod,
                                          threshold
                                          ){
#inputfile<- "Abisko.txt"
#Main<-inputfile
#xy<-F
#clustermethod <-"complete" #"average" # average = UPGMA;
#distmethod<- "manhattan"
#threshold <- 12
#outputname<-"Abisko"
#inputfile<-"AFLP_Genotyping_clones_replicate_samples.txt"
  d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
  if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
           d       <- d[,-c(ncol(d)-1,ncol(d))]}
  d[,2]<- as.factor(d[,2])
  dist<-dist(d[,-c(1:2)],method=distmethod);#dist # pairwise differences
  hist(dist, breaks=seq(-.5,max(dist)*1.1+0.5,by=1),
  xlim=c(0,max(dist)*1.1),main=Main,xlab=paste(distmethod,"distance"))

  cl<-hclust(dist,clustermethod); # UPGMA-cluster;
  cl$labels<-d[,1]
  plot(cl,main=Main,cex=1,ylab=paste(distmethod,"distance"))
  if (threshold >0) rect.hclust(cl,h=threshold,border="red")  #clones
      # by threshold
```

```
}#########################################################################
clonal_clust            <-function(inputfile,
                                   xy=T,
                                   outputname,
                                   Main,
                                   distmethod,
                                   clustermethod,
                                   threshold
                                   ){
  d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
  if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
           d       <- d[,-c(ncol(d)-1,ncol(d))]}
  d[,2]<- as.factor(d[,2])
  dist<-dist(d[,-c(1:2)],method=distmethod);#dist # pairwise differences
  hist(dist, breaks=seq(-.5,max(dist)*1.1+0.5,by=1),
  xlim=c(0,max(dist)*1.1),main=Main,xlab=paste(distmethod,"distance"))

  cl<-hclust(dist,clustermethod); # UPGMA-cluster;
  cl$labels<-d[,1]
  plot(cl,main=Main,cex=1,ylab=paste(distmethod,"distance"))
  if (threshold >0) rect.hclust(cl,h=threshold,border="red")  #clones
      # by threshold

}#########################################################################
clonal_hist             <-function(inputfile,
                                   xy=T,
                                   outputname,
                                   Main,
                                   distmethod,
                                   clustermethod
                                 ){
  d<-read.table(inputfile,head=T,sep="\t");#str(d)  ;head(d)
  if (xy){ xy_coor <- d[,-c(3:(ncol(d)-2))]
           d       <- d[,-c(ncol(d)-1,ncol(d))]}
  d[,2]<- as.factor(d[,2])
  dist<-dist(d[,-c(1:2)],method=distmethod);#dist # pairwise differences
  hist(dist,breaks=seq(-.5,max(dist)*1.1+0.5,by=1),
  xlim=c(0,max(dist)*1.1),main=Main,xlab=paste(distmethod,"distance"))
}#########################################################################


#########################################################################
#  Clonal_spatial_descriptors                    Walter Durka 22.02.2016
#########################################################################
# Function defines clones from AFLP-like data sets using hierarchical
# clustering and applying a user-defined cutoff threshold
# For clones defined, a number of spatial descriptive statistics are
# computed
# Parameters needed:
#     inputfile   file name of input data;
#                 fileformat: 1st line = header
#                             1st column = individual ID
#                             2nd column = population ID
#                             >2nd column = AFLP phenotypes (0/1)
#                 the last two columns are carthesian x and y
#                 coordinates
#     outputname  base name for output files, will be supplemented for
#                 - sample list with clonal identity: "_clIDsp.txt"
#                 - clone list with clonal frequency per population
#                   "_clFreqsp.txt"
#                 - clonal diversity descriptors:
```

```
#                   "_clonal_sp_descriptors.txt"
#     Main        title to be plotted on figures
#     distmethod  function used in the R function dist: e.g.
#                 "manhattan" (which is an Euclidian distance)
#     clustermethod   method used by hclust, e.g. "single", "average",
#                     "complete"
#     threshold   distance-threshold accepted within clonals
#     appendFiles should result files be append to files with identical #
      names? :True/False
#
# Descriptive parameters calculated per population:
#     "n"               number of samples
#########################################################################
clonal_spatial_descriptors <-  function(inputfile,
                                        outputname,
                                        Main,
                                        distmethod,
                                        clustermethod,
                                        threshold,
                                        appendFiles
                                        ){
# inputfile<- "North-SO_xy.txt"#"Abisko.txt"
# Main<-inputfile
# clustermethod <-"complete" #"average" # average = UPGMA;
# distmethod<- "manhattan"
# threshold <- 12
# outputname<-"Abisko"
# appendFiles<-F

  d<-read.table(inputfile,head=T,sep="\t");#d;str(d)  ;head(d)
  xy_coor <- d[,-c(3:(ncol(d)-2))]
  d       <- d[,-c(ncol(d)-1,ncol(d))]
  # calculate phenotypic distance
  dist<-dist(d[,-c(1:2)],method=distmethod);#dist # pairwise differences
  #calculate minxy-distance between any two samples in the plot
  minxydist <- min(dist(xy_coor[,-c(1,2)],method="euclidian"))

  cl<-hclust(dist,clustermethod); # UPGMA-cluster;
  cl$labels<-d[,1]
  plot(cl,ylab=distmethod,main=Main,cex=1)
  if (threshold >0) rect.hclust(cl,h=threshold,border="red")   #clones by
      # threshold

  Clonelist<- data.frame(cutree(cl,h=threshold));
  Clonelist<- cbind(d[,1],d[,2],Clonelist)
  names(Clonelist)<-c(names(d)[1],names(d)[2],"clID");#head(Clonelist)
  # calculate maximum diameter of each clone; if genotype is single
  # assign min xy dist as calculated above
  xy_coor<-cbind(xy_coor,Clonelist$clID)
  for (i in 1:nrow(Clonelist)){
#i <-27#14
     # overall size across all plots
     xy_c <- xy_coor[which(xy_coor[,5] == Clonelist$clID[i]),-c(1,2,5)]
     if         (nrow(xy_c)>1)         Clonelist$tot_clDiam[i]         <-
      round(max(dist(xy_c,method="euclidian")),3) else
      Clonelist$tot_clDiam[i] <- round(minxydist,3)
     # size in plots
     xy_c <- xy_coor[which(xy_coor[,5] == Clonelist$clID[i] &
     xy_coor[,2] == Clonelist[i,2]),-c(1,2,5)]
     if( nrow(xy_c)>1) Clonelist$plot_clDiam[i] <-
     round(max(dist(xy_c,method="euclidian")),3) else
```

```
      Clonelist$plot_clDiam[i] <- round(minxydist,3)
  }

write.table(Clonelist,file=paste(outputname,"_clIDsp.txt",sep=""),sep="\t
",quote=F,row.names=FALSE, append=appendFiles)

Clonelist <- Clonelist[,-c(1)]
tot_clDiam<-aggregate(Clonelist[,-
c(1,4)],by=list(Clonelist$clID),FUN=mean)[,-c(1,2)] # clone size across
      # plots
p_cldiam<-aggregate(Clonelist[,-
c(1,3)],by=list(Clonelist$clID,Clonelist[,1]),FUN=mean)[,-c(1)]  #  clone
      # size within plots
# assign plotsize to table clID x plot
p<-as.data.frame.matrix(table(p_cldiam[,2],p_cldiam[,1]))
for (i in 1: nrow(p))
   for (j in 1: ncol(p)){
     p_<-p_cldiam[which(p_cldiam[,1]==names(p)[j] & p_cldiam[,2]==i),]
      if (nrow(p_) >0) p[i,j] <- p_$plot_clDiam else   p[i,j] <- NA
    }
names(p)<- paste(names(p),"_popclDiam",sep="")
# output of clonelist with frequency across populations, clone diameter #
within plots and diameter across all plots
write.table(cbind(as.data.frame.matrix(table(Clonelist[,2],Clonelist[,1])
),p,tot_clDiam),
      file=paste(outputname,"_clFreqsp.txt",sep=""),    sep="\t",quote=F,,
      append=appendFiles)
    return(cbind(p,tot_clDiam))
}#########################################################################
```

Electronic Supplement 3

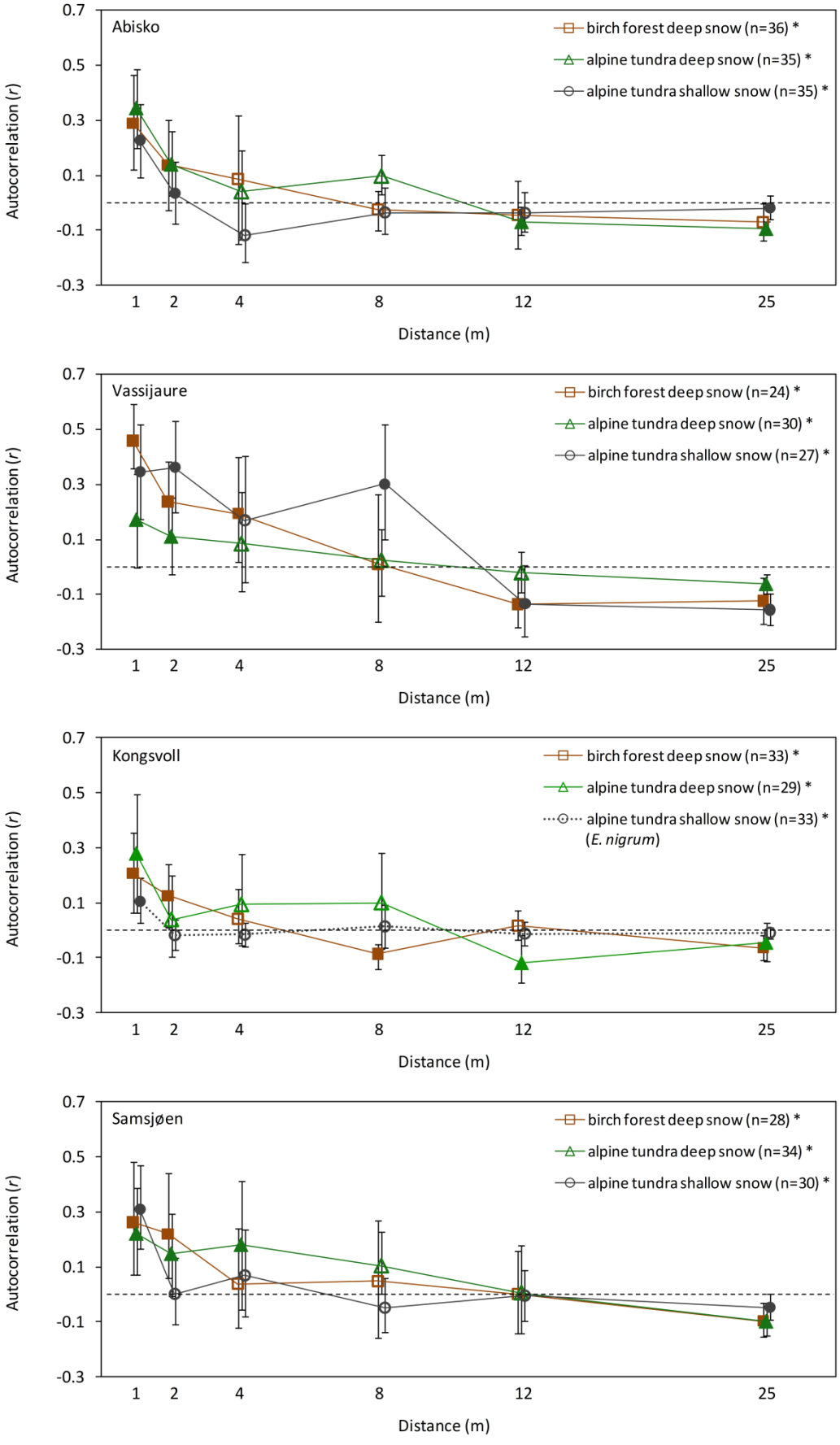


Figure S1: Spatial genetic autocorrelation in populations of *E. hermaphroditum* in the three habitats (birch forest, alpine tundra with deep snow cover, and alpine tundra with shallow snow cover) in the four study regions and of *E. nigrum* in the ***s***-habitat in Kongsvoll. Filled symbols indicate significant genetic autocorrelation for single distance classes. * within the legend indicate significant spatial genetic structure (SGS) for each habitat.

Summary

Arctic and alpine ecosystems are characterized by a cold and relatively short growing season. Within these landscapes topography and prevailing wind directions shape heterogeneous snow distribution patterns. The heterogeneous snow distribution leads to habitats differing in snow depth during winter and snow melt timing in spring. The alpine tundra represents a mosaic of early-melting habitats on wind-exposed ridges with shallow snow cover, and late-melting habitats in wind-sheltered depressions with deep snow cover. Also in sub-arctic birch forest, birch stems act as snow traps, leading to accumulation of snow. Consequently, the various habitats are characterized by plant species and communities, which prefer and avoid snow cover, respectively. However, some species occupy a wide range of habitats and intraspecific differences in responses to variation in snow depth and duration can affect growth habit, phenology and reproduction. One such species with a broad habitat range is *Empetrum hermaphroditum,* a prominent evergreen dwarf shrub in several subarctic heath and mountain birch forest communities.

The present study investigated growth, flowering phenology, reproduction and clonal structure of *Empetrum hermaphroditum* along a natural snow cover gradient in four study areas. The study areas are located along a latitudinal gradient (northern Sweden vs. central Norway), and at each latitude along a local climatic gradient (sub-continental vs. sub-oceanic climate).

Along the natural gradient, significant differences in snow depth during winter, snow melt timing in late spring and summer irradiation were observed. Snow depth increased from the exposed ridge habitat in the alpine tundra to the sheltered depression habitat in the alpine tundra to birch forest. Consequently, snow melt occurred first on ridges and later in depressions and birch forest. During growing season, the birch forest habitat experienced the lowest light availability and the ridge habitat the highest, light availability in the depression habitat is intermediate.

The results show that *Empetrum hermaphroditum* shoots from shallow snow cover and high summer irradiation habitats had significant higher numbers of flowers and fruits, lower plant heights, shorter shoot segments, lower numbers of lateral shoots and total biomass but higher leaf density and higher relative leaf allocation than shoots from habitats with higher snow depth and lower summer irradiation. Hence,

Empetrum hermaphroditum showed high phenotypic trait variation, with a consistent match between local habitat conditions and its growth and reproduction. Although study areas varied strongly with respect to latitude and local climatic conditions, response patterns of growth and reproduction to habitats with different environmental conditions were consistent.

Furthermore, the study demonstrates that *Empetrum hermaphroditum* flowers about the same time in early and late melting habitats, independent of snowmelt timing. Therefore, the plants in the exposed and sunny, early melting habitat have a longer time-lag which might increase the risk of frost damage, whereas plants in the late melting habitats flowers directly after snowmelt when temperatures are higher. Flowering of *Empetrum hermaphroditum* does not seem to be related to snowmelt time across all habitats, but to temperature conditions during the lag-phase between snowmelt and flowering. In this way small scale variation seems to matter less to flowering of *Empetrum hermaphroditum* than interannual differences in snowmelt timing.

The results show that clonal structure of *Empetrum hermaphroditum* is affected by the prevailing local habitat conditions. A decrease of clonal diversity from the exposed ridges over the sheltered depression habitat to birch forest was observed across all study areas. A high proportion of clonal reproduction implies a relatively low reproduction by seedlings. An important aspect in this context might be the effect of selfing and the increasing probability of selfing in large clones. We expect that at least a part of the selfed seeds of a particular genotype cannot be distinguished unequivocally from vegetatively propagated clones. The study revealed that sexual reproduction in addition to vegetative spread is important in clonal species.

The response of plant species along natural gradients might be similar to temporal changes of environmental conditions. Thus, studies along environmental gradients, encompassing the range of climate change predictions, is more likely to give a realistic picture concerning extent of intraspecific phenotypic trait variation, which may determine the long-term adaptive potential of plant species to climate change. Arctic ecosystems face strong changes in snow conditions due to global warming by an increase in temperature, most pronounced in winter and spring, causing an earlier onset of snowmelt and an earlier start of the growing season.

This thesis revealed that *Empetrum hermaphroditum* has a broad ecological niche, which enables the potential to cope with changing snow conditions in the course of climate change. Furthermore, changes in snow cover and temperature are not likely to cause changes in flowering synchrony but general changes in flowering time. The high phenological overlap might indicate that reproductive isolation and genetic differentiation among the habitats is rather unlikely. However, while phenotypic plasticity will allow individuals to immediately adapt to changing conditions, locally adapted populations may locally go extinct. The latter will offer the possibility for seedling recruitment of adapted genotypes. The analyses of the clonal structure of *Empetrum hermaphroditum* revealed that sexual reproduction in addition to vegetative spread is important in this species. Long distance dispersal of seeds by birds and Arctic fox becomes an important process determining the genotypic structure of *Empetrum hermaphroditum* and might promote the survival of the species in spite of climate change.

Zusammenfassung

Arktische und alpine Ökosysteme sind gekennzeichnet durch eine kalte und kurze Vegetationsperiode. In diesen Landschaften formen die Topographie und die vorherrschende Windrichtung unterschiedliche Muster hinsichtlich der Schneeverteilung und der daraus resultierenden Schneeschmelze im Frühjahr. Die alpine Tundra ist ein Mosaik aus wind-exponierten Kuppen mit geringer Schneedecke, mit früherer Schneeschmelze und geschützten Senken in denen sich eine hohe Schneedecke akkumulieren kann, die deutlich später im Frühjahr schmilzt. Ähnliche Bedingungen finden sich auch im Birkenwald, in dem Birkenstämme zur Akkumulation einer hohen Schneedecke beitragen. Diese unterschiedlichen Habitate sind geprägt durch Pflanzen-Arten und -Gesellschaften, die eine hohe Schneedecke entweder bevorzugen oder meiden. Allerdings gibt es auch Arten, die verschiedene Habitate besiedeln und im Zusammenhang mit der Schneebedeckung eine intraspezifisch hohe Variabilität hinsichtlich der Wuchsform, Phänologie und klonalen Struktur aufweisen.

Eine Art, die in den verschiedenen Habitaten der subarktischen Heiden und in Birkenwäldern vorkommt, ist der immergrüne Zwergstrauch *Empetrum hermaphroditum*.

Die vorliegende Arbeit untersucht das Wachstum, die Blüh-Phänologie, Reproduktion und klonale Struktur von *Empetrum hermaphroditum* entlang eines natürlichen Schneedeckungsgradienten in vier verschiedenen Untersuchungsgebieten. Diese sind entlang eines Nord-Süd Gradienten angeordnet (Nord-Schweden bzw. Zentral-Norwegen) und weisen zudem einen klimatischen Gradienten auf (sub-kontinentales Klima bzw. sub-ozeanisches Klima).

Entlang des natürlichen Gradienten wurden signifikante Unterschiede in der Schneehöhe im Winter, dem Zeitpunkt der Schneeschmelze, sowie der Lichtverfügbarkeit während der Vegetationsperiode beobachtet. Die Schneehöhe ist am geringsten auf den wind-exponierten Kuppen und steigt über die wind-geschützten Senken der alpinen Tundra bis hin zum Birkenwald an. Demnach erfolgt die Schneeschmelze zuerst auf den Kuppen und deutlich später in den Senken und im Birkenwald. Während der Vegetationsperiode ist die Sonneneinstrahlung im

Birkenwald am geringsten und auf den Kuppen am höchsten, während sie in den Senken dazwischen liegt.

Die Ergebnisse zeigen, dass *Empetrum hermaphroditum* auf den Kuppen mit geringer Schneedecke und hoher Sonneneinstrahlung eine deutlich höhere Anzahl Blüten und Früchten bilden, sowie niedrigwüchsiger sind, mit kürzeren Sprossen, einer geringeren Anzahl an Seitentrieben und weniger Biomasse bildet in den beiden anderen Habitaten. Dahingegen ist die Blattdichte von Empetrum hermaphroditum auf den Kuppen höher, folglich bildet *Empetrum hermaphroditum* in diesem Habitat mehr Blätter pro Millimeter Stamm aus. Diese Wachstums-Unterschiede zwischen den Habitaten wurden in allen vier Gebieten nachgewiesen. Demnach weist *Empetrum hermaphroditum* eine hohe Plastizität in der Merkmalsausprägung in Abhängigkeit von den Habitat-Bedingungen auf.

Ein weiteres Ergebnis der Studie zeigt, dass der Blühzeitpunkt von *Empetrum hermaphroditum* trotz unterschiedlicher Schneeschmelze in den Habitaten synchron verläuft. Demnach hat *Empetrum hermaphroditum* auf den Kuppen eine längere Phase zwischen der Schneeschmelze und dem Blühen als in den Senken und im Birkenwald. Dadurch steigt das Risiko von Frostschäden an den Blütenknospen, da die Temperaturen nach der Schneeschmelze auf den Kuppen noch sehr niedrig sein können. Während der Schneeschmelze in den Senken und im Birkenwald sind die Temperaturen meistens schon höher und das Blühen erfolgt kurz nach der Schneeschmelze. Generell scheint das Blühen von *Empetrum hermaphroditum* nicht in allen Habitaten von der Schneeschmelze abhängig zu sein, stattdessen spielen die Temperaturen zwischen der Schneeschmelze und dem Blühzeitpunkt eine wichtige Rolle. Demzufolge spielen kleinräumige Unterschiede im Zeitpunkt der Schneeschmelze eine untergeordnete Rolle. Allerdings konnten Unterschiede im Blühzeitpunkt zwischen Jahren mit früher und später Schneeschmelze festgestellt werden, dennoch blühte *Empetrum hermaphroditum* in den verschiedenen Habitaten synchron.

Die klonale Struktur von *Empetrum hermaphroditum* unterscheidet zwischen den verschiedenen Habitaten. Auf den Kuppen ist die klonale Diversität am höchsten, gefolgt von den Senken und dem Birkenwald. Ein hoher Anteil an klonaler Reproduktion weist auf eine geringe Vermehrung durch Keimlinge hin. Im Zusammenhang mit der Klongröße spielt die Befruchtung der Blüten eine wichtige

Rolle. Findet Selbstbefruchtung statt, sind die entstandenen Genotypen untereinander ähnlicher und können denen gleichen, die durch klonales Wachstum entstanden sind. Es zeigte sich, dass sexuelle Reproduktion bei *Empetrum hermaphroditum* eine ebenso wichtige Rolle spielt wie klonales Wachstum.

Die Reaktion von Pflanzen entlang eines Umweltgradienten ist ähnlich der Reaktion von Pflanzen auf sich zeitlich verändernde Bedingungen, wie beispielsweise durch den Klimawandel. Demnach können Studien entlang natürlicher Umweltgradienten eine realistische Einschätzung der Reaktion und Adaptation einzelner Pflanzenarten auf den Klimawandel ermöglichen. Für arktische Ökosysteme wird eine gravierende Veränderung der Schneebedeckung im Winter und Frühling als Folge der globalen Erwärmung erwartet.

Die vorliegende Studie zeigt, dass *Empetrum hermaphroditum* eine breite ökologische Nische aufweist, welche es der Art ermöglichen kann mit veränderten Schnee-Bedingungen zurechtzukommen. Auch die Blüh-Synchronität von *Empetrum hermaphroditum* scheint nicht durch die Veränderungen in Folge des Klimawandels betroffen zu sein. Demnach scheint eine genetische Isolation durch asynchrones Blühen, was den Pollenaustausch verhindert, unwahrscheinlich zu sein.

Die phänotypische Plastizität ermöglicht den Individuen zwar eine schnelle Anpassung an veränderte Bedingungen, dennoch können lokal angepasste Populationen stellenweise aussterben. Dadurch besteht aber die Möglichkeit der Ansiedlung angepasster Genotypen. Die Analyse der klonalen Struktur von *Empetrum hermaphroditum* zeigte deutlich, dass sexuelle Reproduktion als Ergänzung zur vegetativen Ausbreitung eine wichtige Rolle spielt. Die Verbreitung von Samen über große Distanzen durch Vögel und Füchse bestimmt somit die genetische Struktur von *Empetrum hermaphroditum* und kann somit zum Erhalt der Art trotz sich verändernder Umweltbedingungen beitragen.

Acknowledgements

The completion of thesis would not have been possible without the support and helpful contributions of numerous people. I would like to express my deepest gratitude to all of them. In particular, I thank …

- Prof. Dr. Lutz Eckstein who initiated this project and gave me the opportunity to work on this interesting research topic. With helpful advises, fruitful discussions and patience he alway supported me in all aspects of research and writing.
- Prof. Dr. Dr. Dr. Annette Otte for enabling a very pleasant and constructive working environment in the division of Landscape Ecology and giving me a wide scope to develop and build on my scientific skills. And for her willingness to be the second referee.
- Dr. Walter Durka for mentoring me in the fields of molecular ecology.
- Josef Scholz vom Hofe for his invaluable help in almost all aspects of field and greenhouse work.
- Adrian Bienau, Dirk Hattermann, Michael Kröncke, Lena Kretz, PD Dr. Tobias Donath, Michael Jager, Martina Herrmann, Ina Geier, and all student assistants of the project for their assistance during field and laboratory work.
- I am grateful to all my colleagues from the Division of Landscape Ecology for their support, exchange of ideas, the pleasant working atmosphere and inspiring discussions in the coffe and lunch breaks.
- The Deutsche Forschungsgemeinschaft (DFG), the European Union Seventh Framework Programme (INTERACT) and the Justus-Liebig-University Giessen for providing me financial support with the scholarship for doctoral studies through the Equal Opportunity Office
- All my friends for always being a great support, for their patience and encouragement.
- Finally, I would like to thank my parents and my brother for everything!