Faculty of Bioscience, Fisheries and Economics

Department of Arctic and Marine Biology

Allochthonous Allelopathy

Effects of Empetrum nigrum chemical interference on plant development

—

Anna Katharina Pilsbacher
Master thesis in Biology BIO-3950- May 2016

Allochthonous Allelopathy

Effects of Empetrum nigrum chemical interference on plant development

Anna Katharina Pilsbacher

BIO-3950 Masters Thesis in Biology

Northern Population and Ecosystems

May 2015

Department of Arctic and Marine Biology

Faculty of Biosciences, Fisheries and Economics

University of Tromsø—The Arctic University of Norway

Supervisor:

Professor Kari Anne Bråthen, University of Tromsø—The Arctic University of Norway

Table of Contents

Abstract

The dwafshrub *Empetrum nigrum* uses phenolic compounds exuded by its leaves to depress the germination and growth of its surroundings. The allelopathic effect has, only been studied in areas immediately surrounding the plant (*autochthonous allelopathy*) but not in an open system apptoach that takes into account that a multitude of EMpetrum nigrum leaves get introduced into new environments through wind, rain or snowmelt (*allochthonous allelopathy*).

An observational study of 51 snowbed in Northern Norway found an accumulation of Empetrum nirgrum leaves on every one and in 96% of examined plots.

A growth and germination study grew 10 plant species native to either heath or snowbeds in soils mixed with varying concentrations of *Empetrum nigrum* leaves.Germination success was found to be mainly species specific and unaffected by leaf abundance for all but the highest concentration. Plant height and secondary leaf development continuously (p=>.0001 for height and leaf number) decreased with increasing abundance of *Empetrum* leaves in the soil, the decrease being most pronounced between the control group and plants at the lowest concentration suggesting a threshold effect.

The results suggest that the subsidy of allochemicals derived from *Empetrum nigrum* leaves into areas previously unaffected by the plant could lead to ecosystem degradation in those environments. This is especially pertinent to snowbed environments that already face environmental stress in terms of a warming climate.

Keywords: *Empetrum nigrum*, Community Ecology, Allelopathy, Dwarfshrub Tundra, Snowbed, Seedling Growth

Introduction

Presented with a world adjusting to a changing climate, it becomes more important than ever before to understand what causes species distribution patterns and what role plant-plant interaction (such as allelopathy) play.

A species' realized biological niche can first be explained by its evolutionary and dispersal history (*historical filter*), its ability to endure the abiotic characteristics of its environment (*physiological filter*) and persevere within the environment's the biotic interactions present (*biotic filter*) (Lambers, Chapin, and Pons 2008, Lortie 2004). While the influence of evolutionary history and the abiotic factors such as temperature, salinity, and water- and nutrient availability, on plant performance are easily measurable and discernable, biotic factors determining ecosystem composition, that is the interactions between the living organisms within a given environment are more difficult to parse and assess (Lambers, Chapin, and Pons 2008). Inter- as well as intraspecific plant-plant interaction can be classified according to whether coexistence yields positive (*facilitation*), neutral (*mutualism*) and negative (*competition, allelopathy*) results. Due to the difficulty achieving a clear separation between the effects of resource competition and allelopathy (Thijs et al. 1994) Muller established the use of the collective term interference in 1966 (Reigosa, Sánchez-Moreiras, and González 1999).

Plants using allelochemicalsare found in many different ecosystems, at all stages of succession and with different evolutionary histories (Reigosa, Sánchez-Moreiras, and González 1999). The phenomenon and the means by which it is achieved is widespread and has evolved independently in many different plant families (Reigosa, Sánchez-Moreiras, and González 1999). Production of allelochemicals and their

release into the environment is connected to a plant's low palatability to herbivores (Bråthen et al. 2010 Tybirk et al. 2000), litter production that retards key soil processes (Wardle and Lavelle, 1997, Wardle et al., 1998), long-term humus accumulation (Wardle et al., 1998, Tybirk et al. 2000), inhibition of seedling establishment (Lorenzo, Hussain and Gonzalez 2013), and on plant respiration and ATP synthesis (Koocheki, Lalegani, and Hosseini 2013). When released into soil, allelochemicals induce changes in the patterns of nitrogen cycling (Lorenzo, Hussain and Gonzalez 2013).

An area of well documented allelopathic interference is the alpine and subalpine tundra of Northern Norway. It is dominated by ericoid shrubs, specifically *Empetrum nigrum (*Bråthen et al. 2010). The dominance of this evergreen dwarf shrub common in the acidic and nutrient poor soils of temperate, arctic and alpine biomes (Nilsson et al. 1998; Tybirk et al. 2000; Lid and Lid 2005) has been attributed to the production of secondary metabolites, most notably the dihydrostilbene Batatasin-III (Odén et al. 1992; Wallstedt et al. 1997; Nilsson et al. 1998; Bråthen et al 2010), which inhibits seedling establishment and growth of associated species and retards microbial soil activity (Zackrisson and Nilsson 1992, Wallstedt et al. 1997). *Empetrum nigrum's* phenolic compounds are produced in superficial leaf glands (Nilsson et al 1998, Wardle et al. 1998; Tybirk et al. 2000), is temporarily stable (Wallsted et al. 1997; Nilsson et al. 1998) and persists in unpalatable leaf litter leading to enhanced Batatasin-III concentration in the immediate surrounding of *Empetrum nigrum* clones (Wardle et al. 1998; Tybirk et al. 2000; Bråthen et al 2010). Most commonly, however, the plants allelotoxins are released from both fresh senescing and dead leaves during snowmelt and rain events (Nilsson et al. 1998; Wardle et al. 1998; Tybirk et al. 2000;

Brännäs et al. 2004) as leachates (Reigosa, Sánchez-Moreiras, and González 1999; Brännäs et al. 2004). Thus allowing the plant to exercise chemical interference at the same time as it avoids autotoxicity (Reigosa, Sánchez-Moreiras, and González 1999).

Distributing its phenolic compounds by means of leakage from its aerial parts, *Empetrum nigrum* is a crucial in allowing the plant to expand its allelopathic spread into regions that are inaccessibly to it due to its immobility and slow growth and dispersal pattern. This pattern is in opposition to the conservative understanding of allelopathy as limited to the immediate area around the plant responsible for it, making the plant the centre of a closed allelopathic system. As already observed in *Empetrum nigrum* some plants however spread their allelopathic compounds far beyond the areas in which it is physically represented by means of water, wind and possible other factors. They act in an open system of allelopathy that is not limited to their immediate vicinity (please see Figure 1 for a conceptual illustration of an open versus a closed system approach to allelopathy). As nomenclature for this phenomenon is lacking, I propose autochthonous (derived from the same soil) allelopathy as a name for the traditional, closed system approach to allelopathy, while I propose to use the term allochthonous (derived from foreign soil) when talking about an allelopathic influx from another area.

Empetrum nigrum, as an example of a plant that is the source of allochthonous allelopathic means, possesses many small leaves (Lid and Lid 2005). Comparing the values of sampled *Empetrum nigrum* leaves (see the section about leaf collection in the Materials and Methods part) to the terminal velocity of wind dispersed seeds of similar proportions(Kleyer et al. 2008), the argument that *Empetrum* leaves show certain aerodynamic properties that benefit being spread with the wind, enable

persistence on top of the snow cover (Larsson and Molau 2001)as well as dispersal by wind and meltwater streams (Körner 2004) arrises.

As snowmelt and meltwater streams make up the majority of the annual water budget in alpine and arctic ecosystems (Körner 2003) they are of specific importance in determining the direction of the spread of *Empetrum nigrum* leaves. Those are locally conservative and directed by topographical patters (Kullman 2002) and often terminate in depressions where snowbeds are located. This leads to an annual influx of allelopathic *Empetrum nigrum* leaves into snowbed environments which might degrade the system.

While allochthonous allelopathic influx has likely been a persistent feature in Fennoscandic tundra environments due to the dominance of *Empetrum nigrum*, this phenomenon has become interesting due to the high vulnerability of this biome to the on going effects brought onto by global climate change.
It is projected that arctic and high latitude areas will be primarily affected by the changes in temperature (IPCC 2013), precipitation (SWIPA 2011), snow to rain-ratio (Wipf et al. 2009) and length of snow cover (Kullman 2002; SWIPA 2011; IPCC 2013).
In its report on Snow, Water, Ice and Permafrost in the Arctic (SWIPA) the Arctic Monitoring and Assessment Programme (AMAP) projected average duration of snow cover to decline by up to 20% by 2050 (SWIPA 2011). Allochthonous allelopathy might present as a confounding factor in further studies that attempt to assess the impact of these changes in snow cover and temperature.
Even though it has been established that allelopathy is mainly possible between plants that have not co-evolved or when the soil conditions stop microbial transformation of allelochemicals" (Rabotnov 1974 cited in Reigosa, Sánchez-

Moreiras, and González 1999), until now, the plants native to Northern Norwegian

snowbeds have evaded invasion and persisted against the allelopathic backdrop by

means of competitive exclusion (Björk and Molau 2007; Wipf et al. 2009; Hülber,

Bardy and Dullinger 2011). The projected loss of snowcover length will therefore

degrade the ecosystems resistance to invasion defined by Lonsdale 1999 as the

"intrinsic resistance of native ecosystem to invasion through community structure

(Lonsdale 1999)."

This study approaches the issue of chemical interference of *Empetrum nigrum* leaves

into snowfield ecosystems in two ways. First, by assessing the status quo of leaf influx

and soil chemistry in snowbeds via an observational study. My prediction for this

study is that: I) A high concentration of *Empetrum nigrum* leaves will be found in the

debris on snow beds, thus establishing the existence of allochthonous allelopathy into

snowbeds environments.

Additionally, I will perform growth experiments mirroring natural snowbed

conditions to monitor the germination and growth responses of snowbed and heath

plants to the presence of different concentrations of *Empetrum nigrum* leaves in the

soil. Hypothesizing that the phenolic compounds released by *Empetrum nigrum* will

affect plants and ecosystem stability both in the area where it is produced and

adjacent systems but that snowbed species might be less adapted to these phenolic

compounds I predict that: II. Germination and plant growth will decrease with

increasing concentration of *Empetrum nigrum* leaves added to the soil and that: III.

Snowbed plants will respond more strongly to the presence of phenolic compounds

in the soil.

Although it could be argued that plants of different growth forms and thus different strategies in resource allocation might have an influence on *Empetrum nigrum's* ability to affect growth and germination, no prediction will be made in regards to the germination or growth response of plants belonging to different growth forms, as a review of literature did not yield a clear idea of how a response would look like and which plants would be favoured. It was included as a variable nonetheless because of potentially interesting results.

Material and Methods

Study sites

There were two distinct study sites and in a phytotron. A snowbed study was conducted in Ifjordfjellet, Finmark county (Figure 2) in June 2013. The collection of material for the growth and germination study was conducted in July and October of 2012 in Troms country (Figure 2,3). Storage of seeds as well as the greenhouse experiments were conducted in autumn of 2012 in the phytotron at UiT- The Arctic University of Norway. Each study site will be described in more detail in its respective section.

Snowbed study

An observational snowbed study was conducted in order to assess the frequency of occurrence of dead *Empetrum* leaves on snow in snowbeds in an *Empetrum* dominated tundra environment in Northern Norway.

The study took place June 23-27th 2013 along the mountainpass Ifjordfjellet (70°25´N 27°20E; 250-450m asl.) in Finnmark county, Norway. The snowbeds were found above the treeline in the low or medium alpine zone (Moen 1998). Vegetation consists of dwarf shrub heaths dominated by *Empetrum nigrum*, *Vaccinium myrtillus* and *Betula nana*, with *Salix ssp* thickets and associated meadow patches (*Bistorta vivipara, Solidago virgaurea, Avenella flexuosa, Anthoxanthum nipponicum, Deschampsia cespitosa*) populating riparian depressions and wetlands. All late season snowbeds (defined as still being present June 23-26th 2013) in an area of 20 km² were visited as

long as they measured more than 20 m^2, exhibited a slope of less than 60° in snowbed centre and were accessible without danger.

Presence of *Empetrum* leaves was detected within a square of 40×40cm delimited by a metal frame. Depending on size, a minimum of four and a maximum of ten such squares (averaging 8 squares/snowbed) per snowbed were investigated for the presence of *Empetrum nigrum* leaves in the litter accumulating on top of the snowcover. As litter covers the snowbed not uniformly, but in patterns that are influenced by precipitation, wind, direction of snowmelt and animal activity (Figure 2), and because the objective of this study was not to quantify the *Empetrum nigrum* leaves as exactly as possible in one snowbed, but to provide an overview of the scale of *Empetrum nigrum* leaf influx across the studied area, a subjective way of choosing the position of the squares was chosen. While squares were placed across the snowbed to maximize cover, they always were placed in areas that were subjectively regarded as areas of high litter concentration. The presence or absence of *Empetrum nigrum* leaves in the accumulated litter was then recorded and if present recorded in one of the four following classes: 1-10 leaves, 10-20 leaves, 20-30 leaves, and more than 30 leaves.

Furthermore, the distance between the snowbed edge to the closest area of *Empetrum nigrum* dominance (ground cover of more than 50%) was recorded. The distance measures were exact up to a distance of 20 meters (i.e. the length of the measuring tape). When the destination was further away, distances were estimated to the closest 10m interval.

Experimental Greenhouse study

Collection of *Empetrum* leaves, seeds and soil

Leaves

Empetrum nigrum leaves, soil and seeds for the experiment were sampled at various
sites in Troms county, Norway in late August and early October of 2012 (Figure 1).
Because the study focused on the effect of the dispersal of dead *Empetrum nigrum*
leaves, all *Empetrum nigrum* leaves collected were clipped from branches that showed
clear signs of wilting or had been detached from the mother plant. A smaller amount
of fresh leaves was collected in order to allow for the chemical analyses of the relative
difference between samples of exclusively fresh leaves, exclusively old leaves or a
mixture of old and fresh leaves. NIRS analysis showed that the concentration of
Batatasin-III decreased in the dead leaves while bioassays showed no difference
between the phytotoxidity of old and fresh leaves proving that other phenolic
compounds can be found within the plant (González et al. 2014). Apendix I offers an in
depth description of the analysis and the results of the collected leaves.

A personal study of 50 *Empetrum nigrum* fresh leaves placed the average leaf weight
at 0.53 mg (+/- 0.11 mg), the average length at 4.5-5 mm and the width at the
broadest point at 1mm.

Seeds

Plant species to be used in the germination and growth experiments were chosen to
include different life strategies dependent on plant origin (snowbed species and that
are species common or rare in heath environments) and growth forms (forbs, woody
plants or graminoids) that represented the areas encountered. The collected species

included: *Empetrum nigrum, Avenella flexuosa, Pedicularis lapponica, Chamaepericlymenum suecicum, Salix herbacea, Vaccinium myrtillus, Sibbaldia procumbens, Bistorta vivipara, Omalotheca supina, Nardus stricta, Anthoxantum nipponicum, Dryas octopetala,* and *Solidago virgaurea*. Please see *Table 1* for a listing of how the aforementioned plants were separated into the categories regarding their origin and growth form. The choice of plants fell n plants that were present at a minimum of 3 of the collection sites. The categorization of into growth forms and plant origin through a study of the Pan Arctic Flora database (http://nhm2.uio.no/paf/) as well as a review of Ellenberg et al. (1992). The collection method depended on the physical properties of the fruits. If it was possible to distinguish between individual seeds, approximately 50 mature, non-diseased fruits were collected at each site. If the seed were part of complex inflorescences, about 25 of these inflorescences were picked. The collected seeds were stored in paper bags at room temperature for approx. and frozen at -10°C and stored until further use.

The wide range of collection sites (Figure 1) led to seeds from different communities, genetic populations and subjected to different environmental factors to be collected. This means that the results of the study are not limited to a small study area, but have wider applicability.

In order to obtain a baseline germination rate, 100 seeds of each species were placed in a petri dish with 4.5 cm diameter equipped with Whatmann filterpaper (Nr. 1), moistened with 1.5 ml distilled water and germinated at room temperature. These preliminary germination tests yielded the following species specific germination rates: *Empetrum nigrum* (2,47%)*, Avenella flexuosa (*60.34%)*, Pedicularis lapponica (*0%*),Chamaepericlymenum suecicum (*0%*), Salix herbacea (*89.79%*), Vaccinium*

myrtillus (76.80%), Sibbaldia procumbens(52.34%), Bistorta vivipara (94.67%), Omalotheca supine (57.12%), Nardus stricta(63%), Anthoxantum nipponicum(73.98%), Dryas octopetala(89.47%), and *Solidago virgaurea (78.11%)*. A visual representation of the results is given in Appendix IV.

In light of these germination rates, *Pedicularis lapponica*, *Chamaepericlymenum suecicum*, and *Empetrum nigrum*—which exhibited germination rates of under 2,50% —were excluded from the growth studies. The poor germination results could be explained by bad seed quality, faulty storage, specific growth requirements not met by the experimental set-up (Taylor 1999), and the generally low germination rates of a species (Baskin, Zackrisson and Baskin 2002).

Soil

Soil to be used in the greenhouse experiments was collected at 5 areas within Troms county, Norway (Figure 1). 10 collection sites in each area were chosen in the vicinity of seed collection sites with a minimum distance to areas of *Empetrum nigrum* dominance of at least 500m. The wide range of collection sites for soil samples gives robustness because the soil is taken from different environmental contexts.

Areas with a high percentage of vegetation cover were avoided in order to limit the collection of root material that would have to be separated at a later point in time. Taking out 10 cm soil cores determined whether the soil profile allowed for collection (i.e. the site had a proficiently deep organic layer). If the soil profile showed a layer of non-sandy topsoil of at least 6 cm, approximately 200-250g of topsoil was collected at each site with the topmost layer being discarded. The exact amount of soil collected and the depth at which it was collected was dependent on site specific factors such as

sandiness, prescience of vegetation or large stones or whether or not bedrock was present.

The collected soil was dried at room temperature and sieved to remove large non-organic matter and roots. It was then stored at -10°C until further use. (Please see Appendix II for further analysis of collected soil.)

Estimation of abundance of *Empetrum nigurum* leaves via soil cores

In order to assess in what abundance *Empetrum nigrum* leaves are found along snowbed edges, soil cores of the soil right next to snowbed edges were taken throughout the collection period in Troms county. Because of the time within the season (late August and early October) and the fact we stayed in the lower altitudinal ranges, only five snowbeds were encountered during the sampling period. Two soil cores (length 10 cm, cross-section: 5.5 cm)were taken for to represent a snowbed. The soil cores were weighed before they were dried at 95 degrees for 12 hours. Afterwards, the soil was sieved to discover *Empetrum nigrum* leaves contained in it. The leaves were counted and using the approximate estimate (1 leaf= 1mg), their abundance within 1 kg of soil was calculated. Leaf abundance in soil varied from 10g/kg soil to 43g/kg soil with 6 of 10 samples showing values between 25-35g/ kg soil. In order to represent both low and high abundance, a gradient with four levels (0, 15g, 30g, and 45g) was created. It is important to note that the areas along snowbed edges generally have very high abundance accumulated debris. The soil cores were never meant to give a completely accurate representation of leaves found in the centre of a snowbed, but more to give guidelines as to which treatment concentrations are feasible.

Germination- and growth experiments

Germination and growth experiments were carried out during the late fall and winter of 2012. Before the commencement of the experiments, the frozen soil was allowed to slowly thaw over a period of three days in a dark room of ca. 4°C. It was then homogenized by mixing as well as by the addition of sand in a 1:5 as suggested by Medina-Roldán, Paz-Ferreiro and Bardgett, (2012) in order to remove site-specific differences and then allowed to sit unexposed to light for 48h at 0.5°C. Then, it was mixed with dried *Empetrum nigrum* leaves in the following concentrations: 0g/kg, 15g/kg, 30g/kg and 45g/kg and transferred into standard cylindrical planting pots (9cm diameter, 7,5cm depth). Concentrations were chosen to represent an allelopathic gradient expected to occur on snowbed edges . For each of the 4 treatments 2 pots per species were established, equipped with 10 seeds each and covered with fine grade Perlite to avoid water loss.

 Bioassays were conducted to assess the possibility of Perlite buffering the chemical interference of Empetrum nigrum leaves by placing 150 pre-germinated *Lactuca sativa* seeds in petri dished with 3mg *Empetrum nigrum* leaves and 150 pre-germinated *Lactuca sativa* seeds in petri dishes with 3mg *Empetrum nigrum* leaves as well as 5g Perlite mixture. Apart from the concentrations of 3mg *Empetrum nigrum* leaves and Perlite mixture outlines above, the experimental set up was similar to, and performed alongside the bioassay study described in more detail in appendix I. After 3 days, no difference between seedling mortality in seeds exposed to both *Empetrum nigrum* leaves and Perlite and those only exposed to Empetrum nigrum leaves was discovered, indicating Perlite had no effect on the bioactivity of the *Empetrum* leaves.

The seeds were stratified by being placed in a darkened room at 0,5°C for 7 days after being thawed in order to break seed dormancy (Leidulf Lund, personal communication) after which they were left to germinate under 24h artificial light at 8°C, a temperature approaching average summer temperature in alpine areas of Northern Norway (weather data for the last 50 summers (May-September) taken from the weather station in Lebesby, Finnmark and accessed through: http://met.no/).

The seeds were revisited every three days for watering and assessment. In order to avoid seedling competition, seedling density in the pots was reduced to 5 germinated seedlings per pot once this density was reached whereas new germinating seeds were still counted but removed. In total, the experiment consisted of 2 replicates with 5 pseudo-replicates per species per treatment. Once a pot exhibited germination, plants were allowed to grow for 25 days after which their height and number of secondary leaves was recorded.

The pot- specific germination was so synchronized that the five seedlings allowed to grow geminated in between two visits at the experimental site (i.e. within 3 days), hence no further measures to control for varying seedling ages within pots was necessary. had taken and seedling age at harvest was set to be 26 days. ?

Statistical Analysis

All statistical analysis was performed with R v. 2.15.3 (R Development Core Team, 2013) using linear mixed models appropriate for the respective data type.

Germination, as response variable, was estimated setting treatment or species as predictor, and pot as random variable. The germination data were binomial and were modelled using the glmer function of the lme4 package. Germination was furthermore visualized in a stacked bar chart contrasting germination success and failure in the varying treatment types.

Growth data (species height, number of secondary leaves) in response to the abundance of *Empetrum nigrum* leaves mixed into the soil, growth form (forbs, woody plants, graminoids) and species origin (snowbed, dominant heath, non-dominant heath) and their interaction as explanatory variable was modeled using linear mixed effects models (LMEs). Plant species nested within the pot set up was designated as random effect (random=~1|species/pot). The data were scaled to enable comparison between species independent of their innate differences such as the differences in growth and leaf production between graminoid and non-graminoid species using the "scale" function in R. Aikaike´s information criterion (AIC) and residuals assisted in model selection (Shahbaba 2012). Equation 2 was determined to best model the experimentally collected data.

Results

Snowbed study

51 easily accessibly snowbeds with a minimum size of 20 m² were discovered within the study area . They exhibited an average distance to the closest area of *Empetrum nigrum* dominance of 15.41(14.08; 16.73) meters after the data were treated for outliers. Only four of the snowbeds encountered were located ≥60 meters away from the *Empetrum* heath edge. Distance from the *Empetrum nigrum* heath did not seem to have an influence on the abundance of *Empetrum* leaves found within the sampled plots (Figure 5).

Empetrum nigrum leaves were found on top of the snowcover of all of the snowbeds observed and on 96% (395 of 408) 40x40cm sampling plots (Figure 5).

Germination study

The first seeds started to germinate 3 days after being placed in the climate chamber and new seedlings continued to appear until 18 days after the start of the experiment. After 21 days, the overall germination rate lay at 66,75 percent with treatment specific germination rates ranged from 57,36 percent in the control group to 48,05 percent with plants germinating in soil with the highest concentration of *Empetrum nigrum* leaves (45g/kg) (Figure 6).

Modeling treatment specific germination rates by means of a generalized linear mixed model showed that addition of *Empetrum nigrum* leaves to soil decreased germination in soil only with the highest leaf to soil ratio, i.e. 45g leaves to 1kg soil (Table 2).

Growth study

Height response

The modeling outputs showed a significant influence of treatment (i.e. abundance of *Empetrum nigrum* leaves) on seedling height (p=<.0001, Figure 9). Seedling height was, however, not influenced by seed origin (p=0.9964), growth form (p=0.9886), interactions between leaf concentration and origin (p=0.9054) or between leaf concentration and growth form (p=0.4229).

A simplified model with seedling height as the dependent and treatment as the independent variable, and species /pot as a random factor showed that the difference between the control group and all treatment types was significant.

A negative trend between seedling height and the increasing abundance of *Empetrum nigrum* leaves in the soil is observed, yet only the treatment with the lowest and the highest abundance of leaves (15g/kg soil and 45g/kg soil respectively) have significantly different seedling heights (Figure 7).

Leaf number

The modeling outputs showed an significant influence of treatment (i.e. concentration of *Empetrum nigrum* leaves in soil) on the development of secondary leaves (p=<.0001, Figure 10). Leaf number was, however, not influenced by seed origin,

growth form (p=0.9757), or interactions between leaf concentration and origin (p=0.4147) or leaf concentration and growth form (p=0.2367). A simplified model with leaf number as the dependent and treatment as the independent variable, and species/pot as a random factor showed that only the difference between the control group and all treatment types was significant, but that the various treatment types did not significantly vary from each other even though a negative correlation between number of secondary leaves and *Empetrum nigrum* concentration was observable (Figure 8).

Discussion

Discussion

In order to test whether or not *Empetrum nigrum* leaves could influence environments (such as snowbeds) in which it is not established, by means of allochthonous allelopathic on snowbed environments, we conducted both an observational snowbed study and an experimental germination and growth experiment.

This section will discuss whether or not the predictions that were connected to the experiments were supported or not and what these results mean for our understanding of the interactions causing plant species establishment and persistence in snowbed environment, specifically in a changing climate.

I) *Empetrum nigrum* leaves can be found within the debris collecting on top of snowbeds

When conducting the observational snowbed study we *expect[ed] to find a high concentration of leaves in the debris* on snowbeds, establishing the existence of *Empetrum* nigrum influx into snowbed environments. The data supported this claim. In Ifjordfjellet *Empetrum nigrum* leaves were found in the debris of every snowbed sampled and in 96.8 per cent of all sampled plots. The mean distance between the a snowbed and the closest area of *Empetrum nigrum* dominance was approximately 15 meters; yet debris was also found in most plots on snowbeds more than 50 meters away from an area dominated by *Empetrum nigrum*.

The presence of *Empetrum nigrum* leaf litter supports the idea that

snowbeds are already influenced by the phenolic compounds of the plant. This furthermore means that the areas around *Empetrum nigrum* heaths experience continuous stress through the presence of allochemicals in the soil.

On more general terms, this study shows that allelopathic plant-plant interactions are not limited to the distinct area surrounding the plant producing the compound but to be exuding its influence on a greater area through the transfer of allolopathic plant parts through dispersal via wind (as compared to the terminal seed velocity of wind-dispersing seeds of similar size and weight in Kleyer 2008), snowdrift (Körner 2003), or meltwater streams (Körner 2003). It seems to follow that plants with allelopathic parts that disperse easily are at an advantage when it comes to reaching critical mass or crossing an allelopathic threshold in another system.

The idea that allelopathy does not only operate in closed environment but operates in an area that exceeds its immediate surroundings is summarized in the idea of allochthonous allelopathy.

Prediction II) *Empertum nigrum* negatively affects growth but not necessarily germination

For the greenhouse experiment we predicted that the germination and the growth of the seedlings grown would decrease with increasing abundance of *Empetrum nigrum* leaves in the soil. The data only partially supported our predictions.

Germination

Germination success in the greenhouse experiment was mostly affected by species identity and occurred independent of the concentration of *Empetrum nigrum* leaves (Figure IV.3) except for the treatment with the highest concentration of *Empetrum nigrum* leaves, which negatively affected germination across the species boundaries (Figure 6). The experiment thus reaches results similar to older studies performed on germination inhibition in connection with *Empetrum nigrum,* which showed strong species-specific differences in germination but inconclusive results about direct effect of *Empetrum nigrum* on germination (González et al. 2014; Milbeau et al. 2009). The minor effect of the phytotoxicity on germination could be explained by the fact that seeds are equipped with a food source independent from the environment to subsidize seedling growth until the onset of photosynthesis (Lambers, Chapin and Pons, 2008) and thus possess the ability to be less affected by environmental influences in the germination phase.

It should however be noted, that greenhouse experiments offer seedlings open gaps in which they can establish themselves; conditions unlike the ones encountered in ecosystems with an already established layer of vegetation where the newly germinated seedling would face a higher degree of competition. Milbeau et al. furthermore points out that the most important stage in seedling establishment is not germination itself but seedling survival into adulthood (2009).

Growth

The seedling height and the number of secondary leaves in relation to the concentration of *Empertum nigrum* leaves in the soil showed that plant development was significantly delayed by the presence of leaves (Fig 7, 8). While the trend showed a gradual decrease of plant height and the number of secondary leaves with growing

leaf concentration, the only significant decrease could be found between the control group and the plants grown with the lowest abundance of *Empetrum nigrum* leaves (15 g/kg soil) for secondary leaf number (Figure 8). Plant height showed significant decreases between the control and the lowest abundance (15 g/kg soil) as well as between plants grown with a leaf concentration of 15 g/kg soil and 45g/kg soil (Figure 7). These results could point to the presence of an allelopathic threshold. Since the concentrations have been modeled on concentrations found within natural environments (see materials and methods), this would mean that the present saturation of snowbed environments by *Empetrum nigrum* leaves already has a significant influence on the functioning of the environment. It should be mentioned that the observational study was conducted in an area mostly made up of a dwarfshrub heath dominated by *Empetrum nigrum*. In areas where the plant is less abundant, there may be a threshold for *Empetrum nigrum* presence in order to have enough leaves accumulating in snowbeds to cause any allelopathic effect on the surrounding plants.

III) *Empetrum nigrum* added to soil does not affect snowbed plants more negatively than heath plants

Our prediction that common heath plants will react less negatively than snowbed species was based on the observation that the abundance of *Empetrum nigrum* leaves in dwarfshrub heaths leaves greatly exceeds the abundance of the leaves found in snowbed environments. In order to coexist, it heath plants could have evolved coping mechanisms.

No significant difference between the responses of snowbed species and heath species to the increasing concentration of *Empetrum nigrum* leaves was found, however

(Table 5). This means that even those heath plants that seem to be able to coexist alongside *Empetrum nigrum* (category: common heath) exhibit reduced development due to chemical interference; i.e. their seedling stage has not been adpated to the effects of the allochemicals excreted by *Empetrum nigrum*.

We did not have specific predictions as to whether plants with different growth forms (woody, graminoid, forb) showed different responses to *Empetrum nigrum* interference. I found no interaction between growth form and *Empetrum nigrum* concentration at the seedling stage within the first 21 days after germination.

Implications

Although the experiment showed that snowbed species are neither more nor less vulnerable to the allochemicals of *Empetrum nigrum* than?, impending climate change could introduce a further stressor as snowbed species are shown to have a lower heat tolerance than other species (Wipf, Rixen, and Mulder 2006; Schöb et al. 2008). According to the subsidy-stress gradient, when ecosystems experience increased stress the performance of plants decreases until a point of community replacement is reached (Odum, Finn and Franz 1979). The fluctuating resource theory (Davis, Grime, and Thompson 2000), furthermore predicts the heightened nitrogen availability expected as a result of the warming tundra (Sturm et al. 2005; Björk and Molau 2007) to increase replacement pressure on snowbed species. Both theories suggest ways that abiotic stresses unrelated to the chemical interference of *Empetrum nigrum* might facilitate its degenerating effects on snowbed communities. Accurate predictions about ecosystem changes in response to elevated temperatures are hard to make, however, because phenological and functional responses too might exhibit interspecific variation and established community interactions rather than individual

species might be affected (Aerts et al. 2006). Not only therefore, understanding the underlying factors (allochthonous allelopathy being one of them) shaping a specific ecosystem, such as snowbed environments is imperative to accurately predict species response to a warming climate.

Acknowledgements

In a short, but incomplete list, I would like to thank certain people and iinstitutions that were important in enabling my work on this thesis.

The university of Tromsø, who accepted me as a master student thus making it possible for me to pursue this degree and dive into this topic.

My thesis advisor for their help and expertise. For pushing me to see the greater picture and challenging me, but at the same time understanding and acknowledging when I needed to take time off to think and digest. Furthermore for her enthusiasm and trust in the project.

The phytotron at Holt, especially Leidulf Lund for allowing me to use their facility during the germination experiments along with being vey forthcoming with their expertise and their help.

The members of the Plants and Ecosystem group as well as the members of the Northern population group for hearing me out and giving me advice, and allowing me to bounce ideas off them.

All people who have allowed me to accompany them on fieldwork and learn from them especially my fellow botanists Xabi, Edurne, Freja, and Martin.

My family at homes, as well as my choir (Tromsø Akademiske Kvinnekor) which has become my second family, for their moral support.

References

Aerts, R., Cornelissen, J. H. C., & Dorrepaal, E. (2006). Plant performance in a warmer world: general responses of plants from cold, northern biomes and the importance of winter and spring events. *Plant Ecology*, *182*(1-2), 65-77.

Alsos, I. G., Ehrich, D., Thuiller, W., Eidesen, P. B., Tribsch, A., Schönswetter, P., Lagaye, C., Taberlet, P., & Brochmann, C. (2012). Genetic consequences of climate change for northern plants. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 2042-2051.

AMAP. (2011). Snow, water, ice and permafrost in the Arctic (SWIPA). *Oslo: Arctic Monitoring and Assessment Programme (AMAP).*

Baldwin, I. T. (2003). Finally, proof of weapons of mass destruction. *Science Signaling*, *2003*(203), pe42.

Baskin, C. C., Zackrisson, O., & 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.

Bazzaz, F. A. (1979). The physiological ecology of plant succession. *Annual review of Ecology and Systematics*, 351-371.

Brännäs, E., Nilsson, M. C., Nilsson, L., Gallet, C., Brännäs, K., Berglind, R., Eriksson L. O., Leffler, P. E., & Zackrisson, O. (2004). Potential toxic effect on aquatic fauna by the dwarf shrub Empetrum hermaphroditum. *Journal of chemical ecology*, *30*(1), 215-227.

Bråthen, K. A., Fodstad, C. H., & Gallet, C. (2010). Ecosystem disturbance reduces the allelopathic effects of Empetrum hermaphroditum humus on tundra plants. *Journal of vegetation science*, *21*(4), 786-795.

Bråthen, K. A., & Lortie, C. (2015). A portfolio effect of shrub canopy height on species richness in both stressful and competitive environments. *Functional Ecology*.

Bråthen, K. A., & Ravolainen, V. T. (2015). Niche construction by growth forms is as strong a predictor of species diversity as environmental gradients. *Journal of Ecology*, *103*(3), 701-713.

Büntgen, U., Hellmann, L., Tegel, W., Normand, S., Myers-Smith, I., Kirdyanov, A. V., Nievergelt D.& Schweingruber, F. H. (2015). Temperature-induced recruitment pulses of Arctic dwarf shrub communities. *Journal of Ecology*.

Chodak, M. (2008). Application of Near Infrared Spectroscopy for Analysis of Soils, Litter and Plant Materials. *Polish Journal of Environmental Studies*, *17*(5).

Choler, P., Michalet, R., & Callaway, R. M. (2001). Facilitation and competition on gradients in alpine plant communities. *Ecology*, *82*(12), 3295-3308.

Davis, M. A., Grime, J. P., & Thompson, K. (2000). Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, *88*(3), 528-534.

Ellenberg H., Weber H.E., Düll R., Wirth V., Werner W. & Paulissen D. (1992). *Zeigerwerte von Pflanzen in Mitteleuropa (Zweite Auflage). Scripta Geobotanica XVIII*. Göttingen: Goltze Verlag

Foley, W. J., McIlwee, A., Lawler, I., Aragones, L., Woolnough, A. P., & Berding, N. (1998). Ecological applications of near infrared reflectance spectroscopy–a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia*, *116*(3), 293-305.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American naturalist*, 1169-1194.

González, V. T., Junttila, O., Lindgård, B., Reiersen, R., Trost, K., & Bråthen, K. A. (2014). Batatasin-III and the allelopathic capacity of Empetrum nigrum. *Nordic Journal of Botany*.

Heegaard, E., & Vandvik, V. (2004). Climate change affects the outcome of competitive interactions—an application of principal response curves. *Oecologia*, *139*(3), 459-466.

Hülber, K., Bardy, K., & Dullinger, S. (2011). Effects of snowmelt timing and competition on the performance of alpine snowbed plants. *Perspectives in Plant Ecology, Evolution and Systematics*, *13*(1), 15-26.

Inouye, D. W. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, *3*(5), 457-463.

Klanderud, K., & Birks, H. J. B. (2003). Recent increases in species richness and shifts in altitudinal distributions of Norwegian mountain plants. *The Holocene*, *13*(1), 1-6.

Kleyer, M., Bekker, R. M., Knevel, I. C., Bakker, J. P., Thompson, K., Sonnenschein, M., Poschlod, P., Van Groenedael, J.M., Klimes, L., Klimesová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J.G., Jackel, A-K., Kühn, I., Kunzmann, D., Ozinga, W.A., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E., & Peco, B. (2008). The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology*, *96*(6), 1266-1274.

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.*

Kullman, L. (2002). Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. *Journal of ecology*, *90*(1), 68-77.

Lambers, H., Chapin III, F. S., & Pons, T. L. (2008). *Plant Physiological Ecology.* Springer New York.

Larsson, E. L., & Molau, U. (2001). Snowbeds trapping seed rain—a comparison of methods. *Nordic Journal of Botany*, *21*(4), 385-392.

Lid, J., & Lid, D. T. (2005). Norsk flora. 7 utgåve ved R. *Elven. Det Norske Samlaget, Oslo*.

Lonsdale, W. M. (1999). Global patterns of plant invasions and the concept of invasibility. *Ecology*, *80*(5), 1522-1536.

Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I., & Callaway, R. M. (2004). Rethinking plant community theory. *Oikos*, *107*(2), 433-438.

Mallik, A. U., & Pellissier, F. (2000). Effects of Vaccinium myrtillus on spruce regeneration: testing the notion of coevolutionary significance of allelopathy. *Journal of Chemical Ecology*, *26*(9), 2197-2209.

Medina-Roldán, E., Paz-Ferreiro, J., & Bardgett, R. D. (2012). Grazing-induced effects on soil properties modify plant competitive interactions in semi-natural mountain grasslands. *Oecologia*, *170*(1), 159-169.

Milbau, A., Graae, B. J., Shevtsova, A., & Nijs, I. (2009). Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany*, *104*(2), 287-296.

Moen, A. (1998). Nasjonalatlas for Norge: Vegetasjon (Norwegian national atlas: Vegetation). *Norwegian Mapping Authority, Hønefoss*.

Moosavi, A., Afshari, R. T., Asadi, A., & Gharineh, M. H. (2011). Allelopathic Effects of Aqueous Extract of Leaf Stem and Root of Sorghum bicolor on Seed Germination and Seedling Growth of Vigna radiata L. *Notulae Scientia Biologicae*, *3*(2), 114-118.

Muller, C. H. (1966). The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club*, 332-351.

Nilsson, M. C. (1994). Separation of allelopathy and resource competition by the boreal dwarf shrub Empetrum hermaphroditum Hagerup. *Oecologia*, *98*(1), 1-7.

Nilsson, M. C., & Zackrisson, O. (1992). Inhibition of Scots pine seedling establishment byEmpetrum hermaphroditum. *Journal of Chemical Ecology*, *18*(10), 1857-1870.

Odén, P. C., Brandtberg, P. O., Andersson, R., Gref, R., Zackrisson, O., & Nilsson, M. C. (1992). Isolation and characterization of a germination inhibitor from leaves of Empetrum hermaphroditum Hagerup. *Scandinavian Journal of Forest Research*, *7*(1-4), 497-502.

Odum, E. P., Finn, J. T., & Franz, E. H. (1979). Perturbation theory and the subsidy-stress gradient. *BioScience*, *29*(6), 349-352.

Perry, L. G., Thelen, G. C., Ridenour, W. M., Weir, T. L., Callaway, R. M., Paschke, M. W., & M Vivanco, J. (2005). Dual role for an allelochemical:(±)-catechin from Centaurea maculosa root exudates regulates conspecific seedling establishment. *Journal of Ecology*, *93*(6), 1126-1135.

Reigosa, M. J., Sánchez-Moreiras, A., & González, L. (1999). Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences*, *18*(5), 577-608.

Schöb, C., Kammer, P., Kikvidze, Z., Choler, P., & Veit, H. (2008). Changes in species composition in alpine snowbeds with climate change inferred from small-scale spatial patterns. *Web Ecology*.

Schöb, C., Kammer, P. M., Choler, P., & Veit, H. (2009). Small-scale plant species distribution in snowbeds and its sensitivity to climate change. *Plant Ecology*, *200*(1), 91-104.

Shahbaba, B. (2012). *Biostatistics with R*. Springer.

Sorensen, P. L., Clemmensen, K. E., Michelsen, A., Jonasson, S., & Ström, L. (2008). Plant and microbial uptake and allocation of organic and inorganic nitrogen related to plant growth forms and soil conditions at two subarctic tundra sites in Sweden. *Arctic, Antarctic, and Alpine Research*, *40*(1), 171-180.

Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V.,& Midgley, P. M. (2013). IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Taylor, K. (1999). Cornus suecica L.(Chamaepericlymenum suecicum (L.) Ascherson & Graebner). *Journal of Ecology*, *87*(6), 1068-1077.

Thijs, H., Shann, J. R., & Weidenhamer, J. D. (1994). The effect of phytotoxins on competitive outcome in a model system. *Ecology*, 1959-1964.

Tukey Jr, H. B. (1966). Leaching of metabolites from above-ground plant parts and its implications. *Bulletin of the torrey botanical club*, 385-401.

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

Virtanen, R., Eskelinen, A., & Gaare, E. (2003). *Long-term changes in alpine plant communities in Norway and Finland* (pp. 411-422). Springer Berlin Heidelberg.

Wallstedt, A., Nilsson, M. C., Odham, G., & Zackrisson, O. (1997). A method to quantify the allelopathic compound batatasin-III in extracts from Empetrum hermaphroditum using gas chromatography: applied on extracts from leaves of different ages. *Journal of chemical ecology*, *23*(10), 2345-2355.

Wardle, D. A., Nilsson, M. C., Gallet, C., & Zackrisson, O. (1998). An ecosystem-level perspective of allelopathy. *Biological Reviews*, *73*(3), 305-319.

Weidenhamer, J. D., Hartnett, D. C., & Romeo, J. T. (1989). Density-dependent phytotoxicity: distinguishing resource competition and allelopathic interference in plants. *Journal of Applied Ecology*, 613-624.

Weir, T. L., Park, S. W., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current opinion in plant biology*, *7*(4), 472-479.

Wipf, S., Rixen, C., & Mulder, C. P. (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., & 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.

Zackrisson, O., & Nilsson, M. C. (1992). Allelopathic effects by Empetrum hermaphroditum on seed germination of two boreal tree species. *Canadian Journal of Forest Research*, *22*(9), 1310-1319.
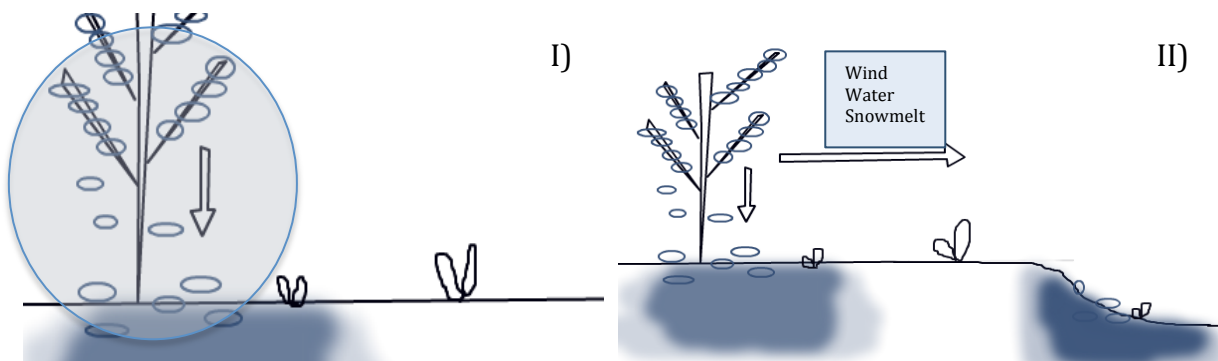
Figures

Figure 1. Conceptual illustration of **autochthonous allelopathy** (closed system approach) (I) as opposed to **allochthonous allelopathy** (open system aproach) (II).
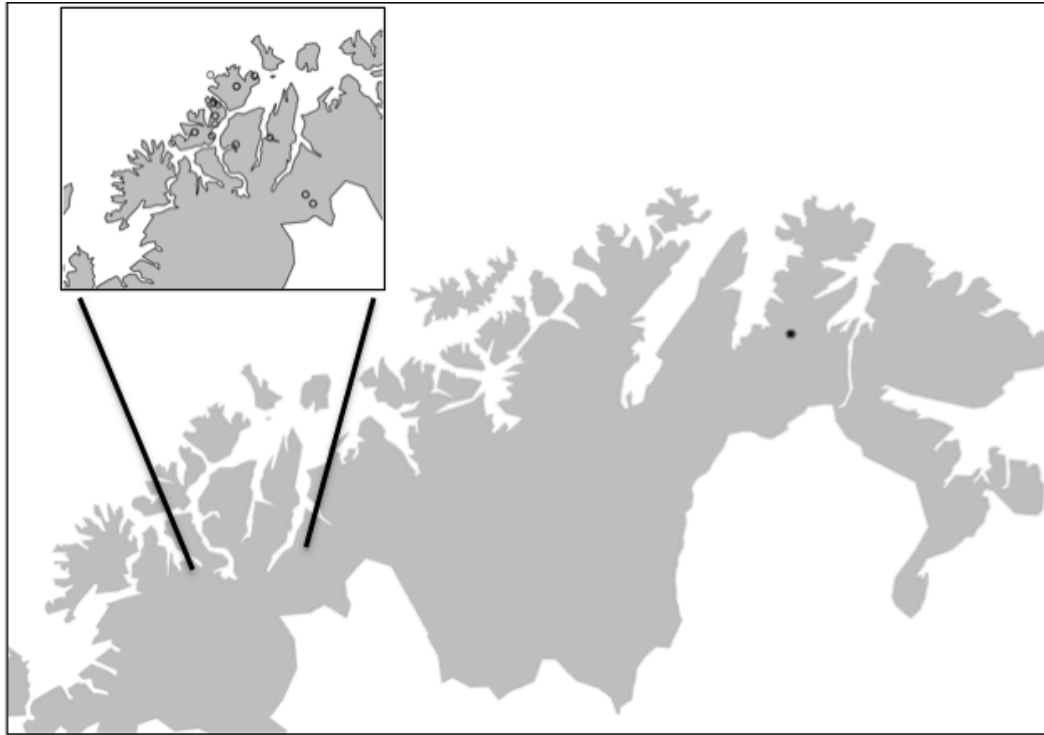
Figure 2. Map of Northern Norway displaying location of observational snowbed study in Ifjordfjellet performed in June 2013 (asterisk) and locations of sampling biological material for experiments (small picture, unfilled circles).

Figure 3. Map showing the 5 sites of used for the collection of the soil
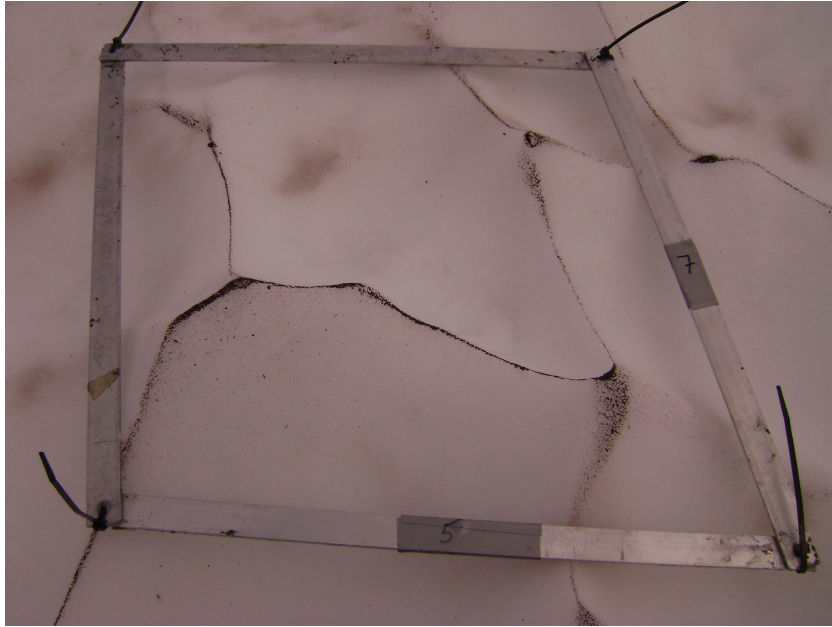used in the greenhouse experiment (small picture, unfilled circles).

Figure 4. Illustration of snowbed sampling procedure with a 40×40cm metal frame as well as litter-patterns.

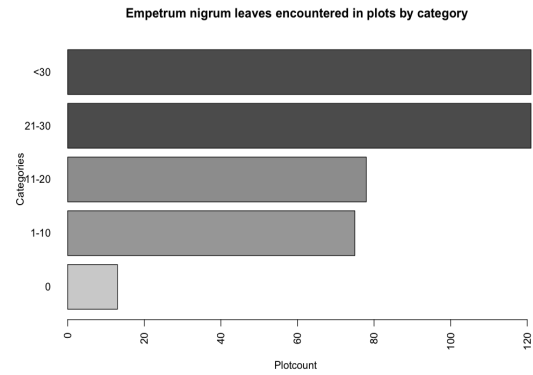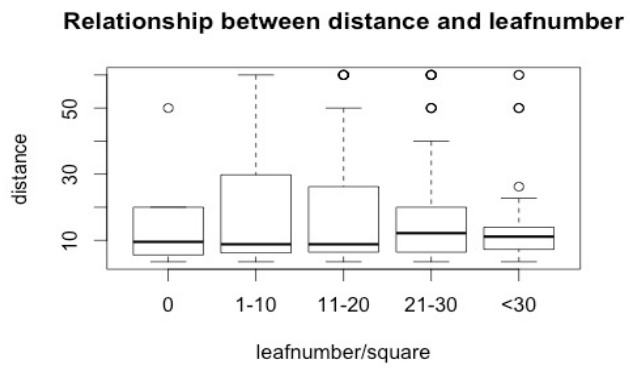Figure 5. Descriptive representation of observational snowbed study illustrating the relationship between a snowbed's distance from the next area of *Empetrum nigrum* dominance in meters and the number of leaves per square (divided into the various categories) (left) and the numerical breakdown of the 408 plots in regards to the number of *Empetrum nigrum* leaves counted within each square (right).
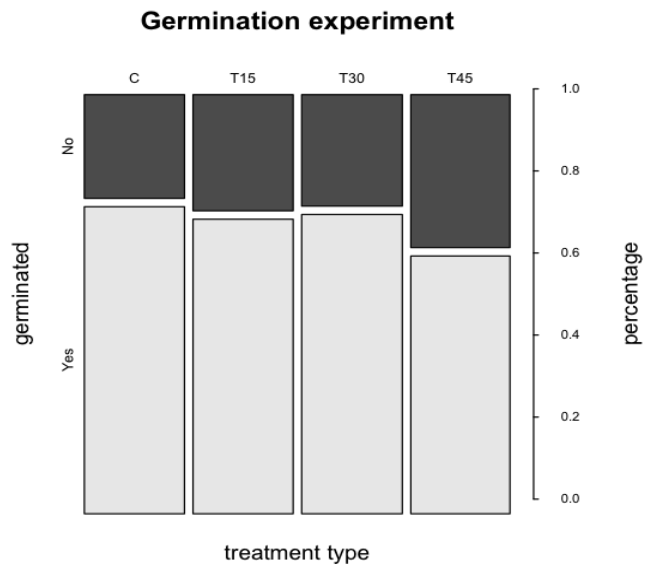
Figure 6. Germination rate of 10 alpine species as affected by different levels of *Empetrum nigrum* leaves added to soil.
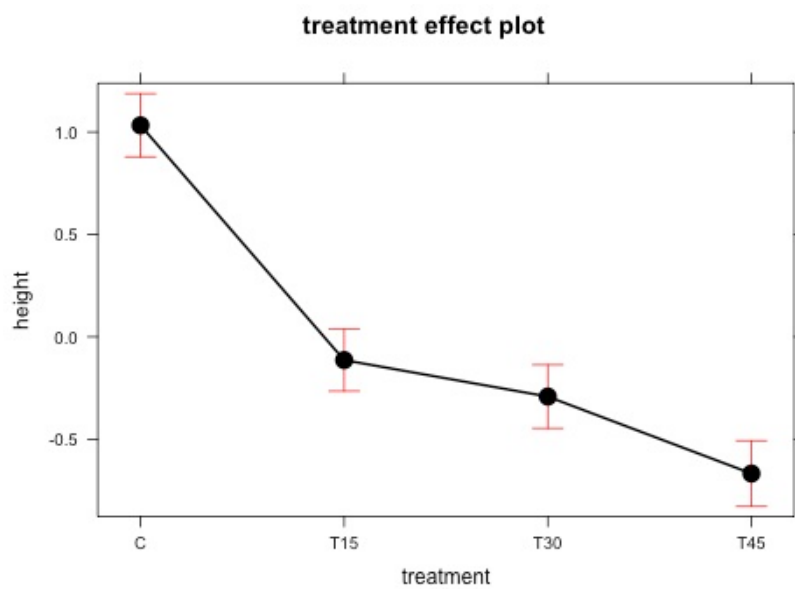
Figure 7. Model output of concentration of *Empetrum nigrum* leaves on plant height in plant seedlings with error bars representing the 95% confidence intervals. Height was modeled as response variable to treatment with species/pot as a nested random factor.
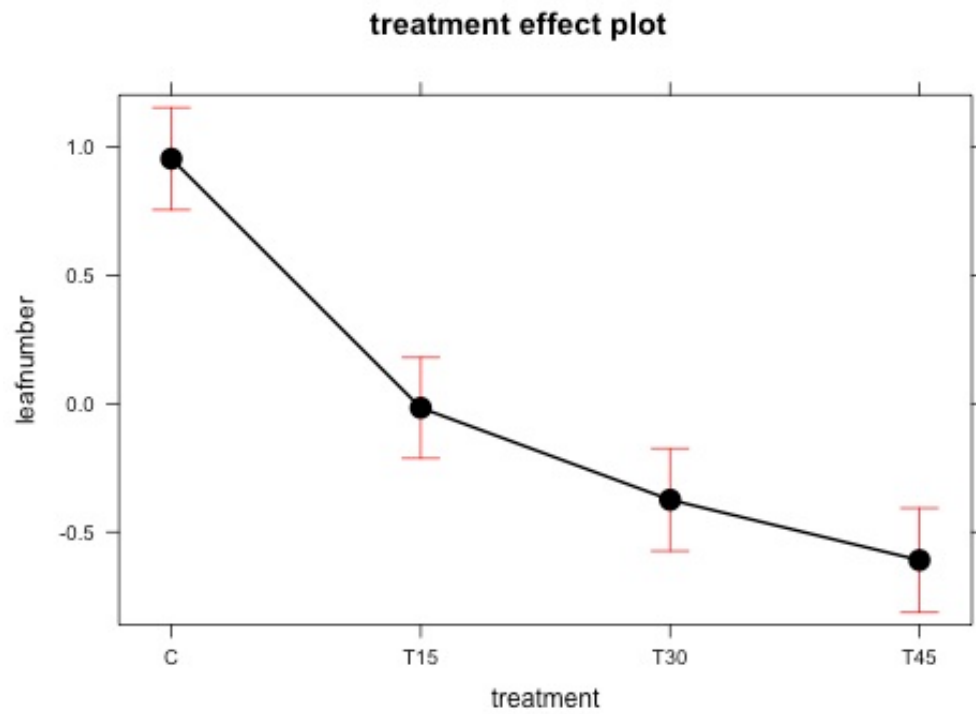
Figure 8. Model output of the concentration of *Empetrum nigrum* leaves on seedling secondary leaf production with th error bars representing the 95% confidence intervals. Leaf number was modeled as response variable to treatment with species/pot as a nested random factor.
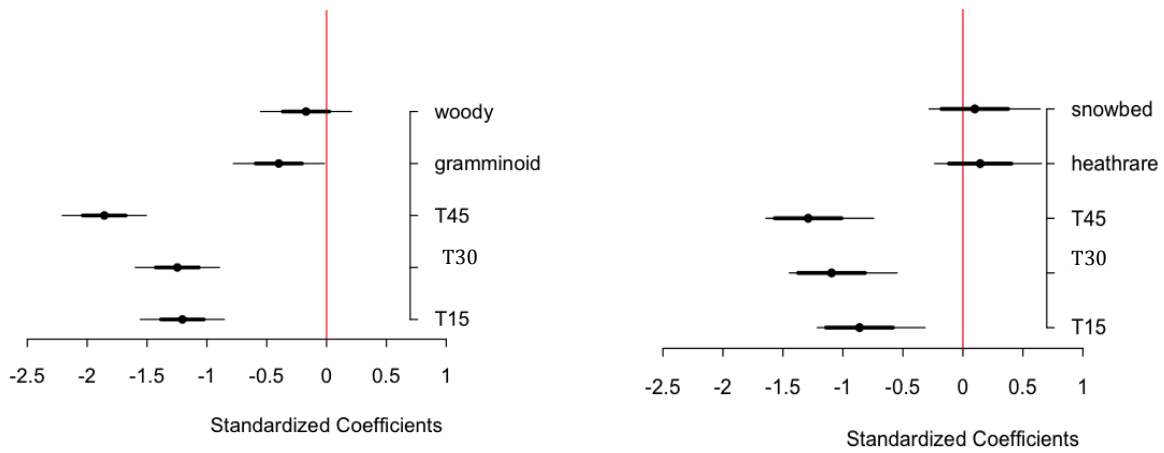
Figure 9. Height response of seedlings exposed to varying levels of *Empetrum nigrum* leaf concentration in soil arranged by growth from (left) and origin (right). The red line represents the average scaled height of forbs grown in soils with no added leaves (left), or of common heath plants grown in soils with no added leaves (right).
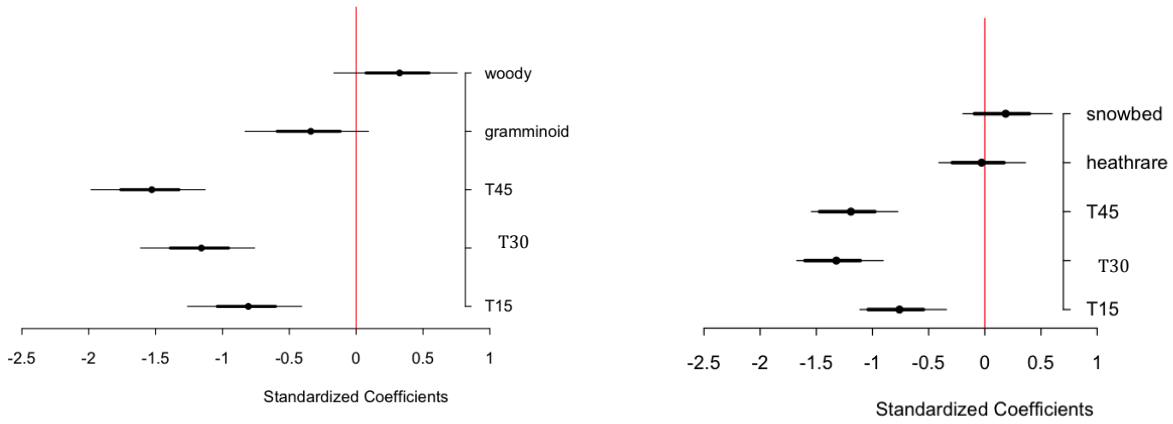
Figure 10. Secondary leave development of seedlings exposed to varying levels of *Empetrum nigrum* leaf concentration in soil arranged by growth from (left) and origin (right). The red line represents the average scaled number of secondary leaves produced by forbs grown in soils with no added leaves (left), or by common heath plants grown in soils with no added leaves (right).

Tables

Table 1. List of native plant species used in growth experiment and classification classes of origin and growth form as defined by the experimental design.

| Species name	Growth form	Origin
Anthoxantum nipponicum Honda	graminoid	heath common
Avenella flexuosa (L.) Trin.	graminoid	heath common
Bistorta vivipara (L.) Delarbre	forb	heath non-common
Chamaepericlyneum sueccica (L.) Graebn.	woody	heath common
Dryas octopetala L.	woody	heath non-common
Empetrum nigrum L.	woody	heath-common
Nardus stricta L.	graminoid	heath non-common
Omalotheca supina (L.) DC.	forb	snowbed
Pedicularis lapponica L.	forb	heath non-common
Salix herbacea L.	woody	snowbed
Sibbaldia procumbens L.	forb	snowbed
Solidago virgaurea L.	forb	heath non-common
Vaccinium myrtillus L.	woody	heath common

Table 2. Treatment effect on germination rates of ten Northern Norwegian plant species as modeled with generalized linear modeling.

Formula: germ ~ treatment + (1 | pot)
Family: binomial (logit)

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.08519	0.16356	6.635	**3.25e-11**
treatmentT15	-0.15471	0.22726	-0.681	0.4960
treatmentT30	-0.09744	0.22839	-0.427	0.6696
treatmentT45	-0.56346	0.22040	-2.557	**0.0106**

Appendices

I) Pre-experiments concerning Batatasin-III content and bioactivity of _Empetrum nigrum_ leaves

Batatasin-III content in the dead, dried Empetrum leaves was measured by means of Near Infrared Spectroscopy (NIRS) [12] analysis using a FieldSpec3 (ASD Inc, Boulder, Colorado). NIRS technology exploits the fact that the chemical make up of a certain material can be deduced by its reflective properties. Batatasin III content was inferred by comparing the amplitude of the spectra of dried _Empetrum nigrum_ leaves to standardized reflection curves with a known concentration of the chemical in R. As secondary metabolites in _Empetrum nigrum_ are not limited to Batatasin III[3], bioassays using lettuce (_Lactuca sativa_) seeds were emloyed to obtain a more accurate understanding of the allelopathic abilities inherent in _Empetrum nigrum_ leaves. Before the growth-experiment, 20 pre-germinated _Lactuca sativa_ seeds were placed in petri dishes of 4.5 cm diameter equipped with Whatmann filterpaper (Nr. 1) moistened with 1.5 ml distilled water. Petri dishes were surrounded with Parafilm "M" to avoid water-loss and incubated at room temperature under 24h artificial light. 15 replicates per leaf-concentration (0, 1,5mg, 3mg, 4,5mg) were created and seedling survival as well as root length was recorded after 3 days.

---

[1] Chodak, M. (2008). Application of Near Infrared Spectroscopy for Analysis of Soils, Litter and Plant Materials. _Polish Journal of_

[2] Foley, W. J., McIlwee, A., Lawler, I., Aragones, L., Woolnough, A. P., & Berding, N. (1998). Ecological applications of near infrared reflectance spectroscopy–a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. _Oecologia_, _116_(3), 293-305.

[3] González, V. T., Junttila, O., Lindgård, B., Reiersen, R., Trost, K., & Bråthen, K. A. (2014). Batatasin-III and the allelopathic capacity of Empetrum nigrum. _Nordic Journal of Botany_

Root bioassays showed a gradual, and significant, decrease of seedling root length when exposed to increasing concentration of *Empetrum nigrum* leaves (Table I.1).

Figure I.1 Rootlenght (cm) of *Lactuca sativa* seedlings in response to exposure to various *Empetrum nigrum* leaf concentrations (mg).
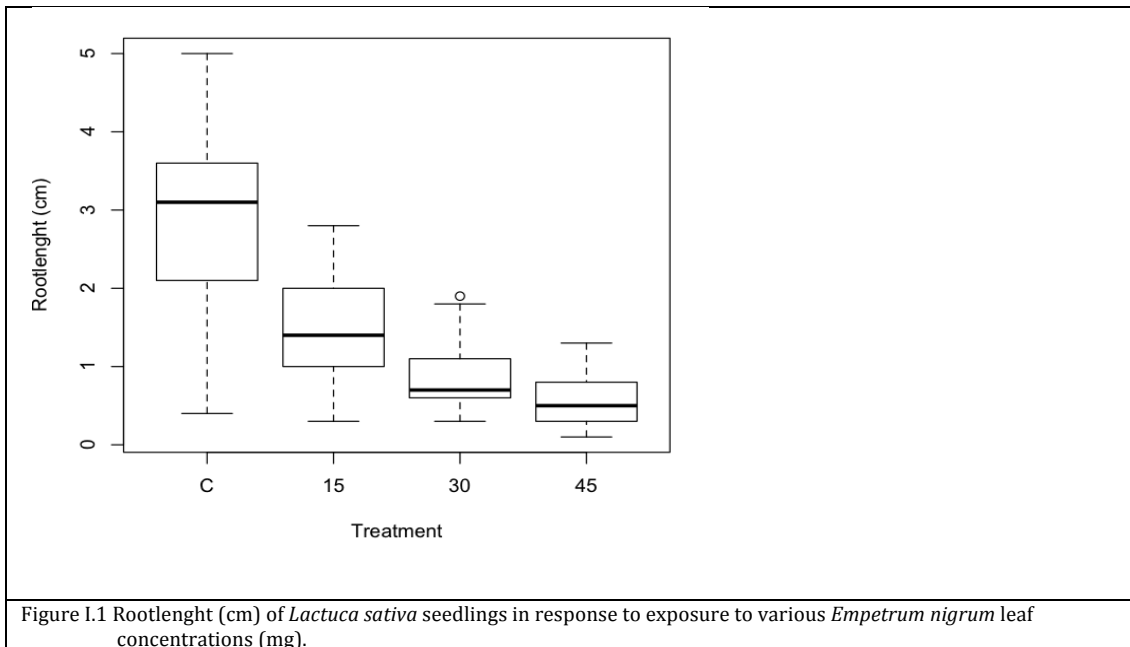
Table I.1 Estimations for *Lactuca sativa* root length

Estimates= root length ~ treatment

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.9333	0.1030	28.48	**<2e-16**
bio$treatment15	-1.5089	0.1457	-10.36	**<2e-16**
bio$treatment30	-2.0711	0.1457	-14.22	**<2e-16**
bio$treatment45	-2.3733	0.1457	-16.29	**<2e-16**

NIRS analysis showed that the Batatasin-III concentration of mixed leaf samples, such as the ones used in the growth experiments, resembled that of strictly fresh leaves

more than that of strictly old leaves and that averages for mixed samples ranged from

17,63 to 20,8 mg/g compared to 27,43 mg/g in the fresh, green specimen (Table I.2).

Table I.2 Batatasin III. content of old, new and mixed (Ex_Ki, Ex_Kval(1), Ex_Kval(2)) *Empterum nigrum* leaves
collected from various locations in Kvaløya, Troms in August 2012 as predicted by means of the NIRS methods

Sample	Constituent	Pred_Value (mg/g)	M_Distance	
Ex_Ki1.spc	SatBat	19,53212664	1,688527575	
Ex_Ki2.spc	SatBat	20,95506512	2,259373658	
Ex_Ki3.spc	SatBat	16,31607416	2,446204464	**18,93442197**
Ex_Kval(1)1.spc	SatBat	22,59284505	1,596193328	
Ex_Kval(1)2.spc	SatBat	21,59216819	1,458155121	
Ex_Kval(1)3.spc	SatBat	18,21728324	1,876594222	**20,80076549**
Ex_Kval(2)1.spc	SatBat	16,48005683	2,487993416	
Ex_Kval(2)2.spc	SatBat	20,82446993	2,01117834	
Ex_Kval(2)3.spc	SatBat	15,58598735	1,943700247	**17,63017137**
Fre1.spc	SatBat	30,15159968	1,918135347	
Fre2.spc	SatBat	24,94193516	1,869889023	
Fre3.spc	SatBat	28,43745105	1,835435535	**27,84366196**
Old1.spc	SatBat	-6,993128073	12,79069843	
Old2.spc	SatBat	-6,357370344	10,6380675	
Old3.spc	SatBat	-7,342029125	12,39306728	**-6,897509181**

II) Soil analysis
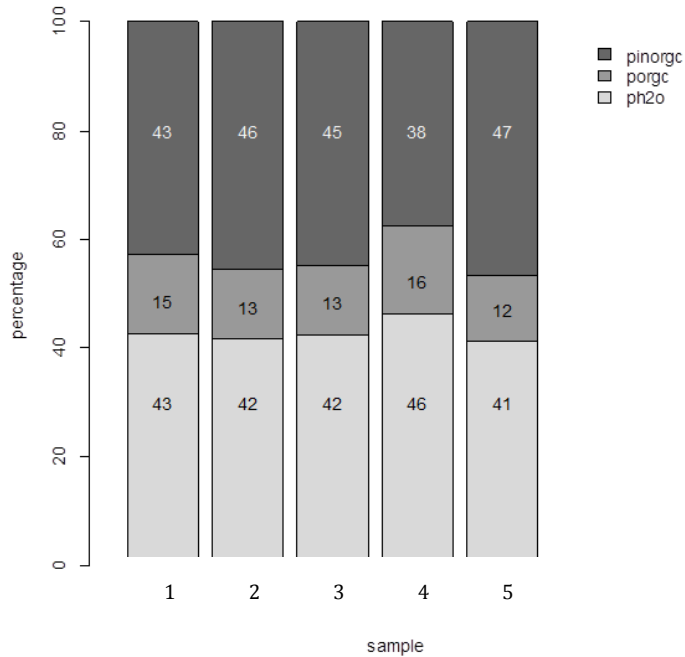
Figure II.1 Percentage of organic carbon (porg), inorganic carbon (pinorg) and water (ph2o) contained within the soils sampled from the 5 sampling sites and used for greenhouse experiment.

In order to ensure a similar make-up of soils from the five sampling locations, the inorganic and organic carbon content of the samples was determined experimentally. For each of the 5 sampling site, 10 5g samples of soil were placed in pre-weighed crucibles resulting in 50 crucibles. The samples were then dried at 95 degrees Celsius for 12 hours and allowed to cool and weighed. Thereafter, the samples were incinerated at 450 degrees Celsius for 4 hours, allowed to cool and weighed. The soils organic and Organic Carbon content as well as the water content was determined through calculation after the initial weight of the crucible was disregarded.

- Water Content: (weight of fresh sample-weight of dried sample)
- Organic Carbon Content: (weight of burnt sample-weight of dried sample)

- Inorganic Carbon Content: weight of burnt sample

Figure II.1 illustrated the results of the experiment.

Additionally, 15 (3 per site) 25g samples were placed in air tight sampling bags and sent to the Institute for Sustainable Plant Production (NPP) of the Austrian Agency for Health and Food Safety (AGES)[4] for further chemical analysis. Table II.1. summarizes the results of analyses undertaken to determine soil pH, acetate concentration, phosphorous, nitrogen and potassium levels as well as the percentage of humus in the sampled soil.

Table II.1. Soil analysis for topsoil samples collected at different sites in Troms county, Norway executed by the Austrian Agency for Health and Food Safety (AGES).

	sample origin					
	1	2	3	4	5	
pH Cacl/ pH Ca	4.80	4.50	4.30	4.60	4.40	**4.52**
Acetat	6.03	6.01	6.04	6.01	6.09	**6.04**
P (mg/kg)	14.00	13.00	13.00	15.00	11.00	**13.00**
K(mg/kg)	96.00	101.00	101.00	126.00	86.00	**102.00**
Humus(%)	16.50	18.30	19.80	25.20	15.90	**19.14**
N (%)	0.971	0.86	1.01	1.08	0.87	**0.958**

[4] Institut für nachhaltige Pflanzenproduktion/Abteilung für Bodengesundheit und Pflanzenernährung Spargelfeldstraße 191, 1220 Vienna, Austria

III) Additional pictures illustrating *Empterum nigrum* leaf-influx into snowbed environments

To illustrate the concept of allochthonous influx of *Empetrum nigrum* leaves into snowbed environments, additional pictures were taken in connection with an excursion into Varangerhalvøya National Park in July 2015.

Figure III.1 Collection of leaf debris on the snowcover and surrounding area of a late season snowbed. Photocredit: Kari Anne Bråthen.

Figure III.2 Picture of late season snowbed surrounded by leaf debris. Photocredit: Kari Anne Bråthen.

Figure III.3 Close up of leaf debris deposited on the immediate edge of a late season snowbed.
Photocredit: Kari Anne Bråthen.

Figure III.3 Close up of vegetation developing in and around leaf debris deposited at site through snowmelt.

Figure III.4 Portrait next to the study area. Photocredit: Kari Anne Bråthen.

IV. Additional graphs and tables

Table IV. 1. Model output of plant height as a function of the interaction of growth form and treatment type and plant origin and treatment type (see formula below). Light grey shaded areas represent variables that yielded significant results. The intercept represents seeds categorized as common heath forbs in the control group.

height ~ treatment * growthform + treatment * origin					
	Value	**Std.Error**	**DF**	**t-value**	**p-value**
Intercept	1.465526	0.3976669	55	3.685312	0.0003
T15	-1.8892293	0.5593148	55	-3.377757	0.0013
T30	-1.9215138	0.5619345	55	-3.419463	0.0012
T45	-2.0151903	0.5644304	55	-3.570308	0.0007
gramminoid	-0.5742499	0.3888514	5	-1.476785	0.1998
woody	-0.3775262	0.3166874	5	-1.192110	0.2867
uncommon heath	-0.1988556	0.3481429	5	-0.571190	0.5926
snowbed	-0.2284991	0.4097560	5	-0.557647	0.6011
T15:gramminoid	0.8092113	0.5458056	55	1.482600	0.1439
T30:gramminoid	0.8634462	0.5498281	55	1.570393	0.1221
T45:gramminoid	0.5531388	0.5516160	55	1.002761	0.3204
T15:woody	0.7431889	0.4482195	55	1.658092	0.1030
T30:woody	0.1883194	0.4490522	55	0.419371	0.6766
T45:woody	0.6022872	0.4511620	55	1.334969	0.1874
T15:uncommom heath	0.3421317	0.4881708	55	0.700844	0.4864
T30:uncommon heath	0.4001624	0.4917830	55	0.813697	0.4193
T45:uncommon heath	0.0174715	0.4930464	55	0.035436	0.9719
T15:snowbed	0.4692783	0.5772765	55	0.812918	0.4198
T30:snowbed	0.4920014	0.5793985	55	0.849159	0.3995
T45:snowbed	-0.1698864	0.5829066	55	-0.291447	0.7718

Table IV. 2. 95% confidence intervals for the model output of plant height as a function of the interaction of growth form and treatment type and plant origin and treatment type (see formula below) . Light grey shaded areas represent variables that yielded significant results. The intercept represents seeds categorized as common heath forbs in the control group.

height ~ treatment * growthform+ treatment * origin

	lower	estimate	upper
Intercept	0.6828809	1.46552659	2.2481723
T15	-3.0101213	-1.88922935	-0.7683374
T30	-3.0476556	-1.92151379	-0.7953720
T45	-3.1463340	-2.01519025	-0.8840465
gramminoid	-1.5738242	-0.57424992	0.4253244
woody	-1.1915971	-0.37752618	0.4365447
uncommon heath	-1.0937854	-0.19885561	0.6960742
snowbed	-1.2818105	-0.22849912	0.8248123
T15:gramminoid	-0.2846076	0.80921130	1.9030302
T30:gramminoid	-0.2384339	0.86344623	1.9653264
T45:gramminoid	-0.5523243	0.55313884	1.6586019
T15:woody	-0.1550630	0.74318893	1.6414408
T30:woody	-0.7116012	0.18831940	1.0882400
T45:woody	-0.3018616	0.60228723	1.5064360
T15:uncommom heath	-0.6361844	0.34213172	1.3204479
T30:uncommon heath	-0.5853928	0.40016237	1.3857175
T45:uncommon heath	-0.9706157	0.01747147	1.0055586
T15:snowbed	-0.6876096	0.46927834	1.6261663
T30:snowbed	-0.6691391	0.49200137	1.6531418
T45:snowbed	-1.3380574	-0.16988645	0.9982845

Table IV. 3. Model output of number of secondary leaves as a function of the interaction of growth form and treatment type and plant origin and treatment type (see formula below). Light grey shaded areas represent variables that yielded significant results. The intercept represents seeds categorized as common heath forbs in the control group.

leafnumber ~ treatment * growthform + treatment * origin					
	Value	**Std.Error**	**DF**	**t-value**	**p-value**
Intercept	1.0809453	0.3332116	55	3.244021	0.0013
T15	-1.0794621	0.4663538	55	-2.314685	0.0244
T30	-1.8816461	0.4704489	55	-3.999682	0.0002
T45	-1.3138387	0.4743969	55	-2.769493	0.0076
gramminoid	-0.3881614	0.3268963	5	-1.187415	0.2884
woody	0.2089416	0.2632168	5	0.793800	0.4633
uncommon heath	-0.1820459	0.2928837	5	-0.621564	0.5615
snowbed	-0.0123635	0.3430925	5	-0.036035	0.9726
T15:gramminoid	0.4061553	0.4557605	55	0.891159	0.3767
T30:gramminoid	0.8551754	0.4621284	55	1.850515	0.0696
T45:gramminoid	0.2089190	0.4649397	55	0.449346	0.6549
T15:woody	-0.2885368	0.3727764	55	-0.774021	0.4422
T30:woody	-0.3223115	0.3739788	55	-0.861844	0.3925
T45:woody	-0.2404410	0.3773638	55	-0.637160	0.5267
T15:uncommom heath	0.2308126	0.4076070	55	0.566263	0.5735
T30:uncommon heath	0.6811135	0.4131855	55	1.648445	0.1050
T45:uncommon heath	-0.2247183	0.4152784	55	-0.541127	0.5906
T15:snowbed	-0.0527685	0.4816956	55	-0.109547	0.9132
T30:snowbed	0.4366111	0.4850304	55	0.900173	0.3720
T45:snowbed	-0.5028060	0.4906178	55	-1.024843	0.3099

Table IV. 3. 95% confidence intervals for the model output of leaf number as a function of the interaction of growth form and treatment type and plant origin and treatment type (see formula below) . Light grey shaded areas represent variables that yielded significant results. The intercept represents seeds categorized as common heath forbs in the control group.

leafnumber ~ treatment * growthform+ treatment * origin

	lower	estimate	upper
Intercept	0.42515378	1.08094525	1.7367367
T15	-2.01405590	-1.07946210	-0.1448683
T30	-2.82444689	-1.88164615	-0.9388454
T45	-2.26455127	-1.31383868	-0.3631261
gramminoid	-1.22847513	-0.38816144	0.4521523
woody	-0.46767871	0.20894158	0.8855619
uncommon heath	-0.93492757	-0.18204592	0.5708357
snowbed	-0.89431083	-0.01236349	0.8695838
T15:gramminoid	-0.50720920	0.40615525	1.3195197
T30:gramminoid	-0.07095068	0.85517542	1.7813015
T45:gramminoid	-0.72284090	0.20891901	1.1406789
T15:woody	-1.03559738	-0.28853684	0.4585237
T30:woody	-1.07178174	-0.32231153	0.4271587
T45:woody	-0.99669497	-0.24044099	0.5158130
T15:uncommom heath	-0.58605009	0.23081260	1.0476753
T30:uncommon heath	-0.14692877	0.68111348	1.5091557
T45:uncommon heath	-1.05695475	-0.22471828	0.6075182
T15:snowbed	-1.01810797	-0.05276850	0.9125710
T30:snowbed	-0.53541148	0.43661107	1.4086336
T45:snowbed	-1.48602611	-0.50280604	0.4804140

Table IV. Summarized model outputs for plant height and leaf number per variable analyzed.

height ~ treatment * growthform+ treatment * origin

	numDF	denDF	F-value	p-value
Intercept	1	293	0.00357	0.9524
treatment	3	55	35.36354	<.0001
growthform	2	5	0.00362	0.9964
origin	2	5	0.01153	0.9886
treatment:growthform	6	55	1.01883	0.4229
treatment:origin	6	55	0.35253	0.9054

leafnumber ~ treatment * growthform+ treatment * origin

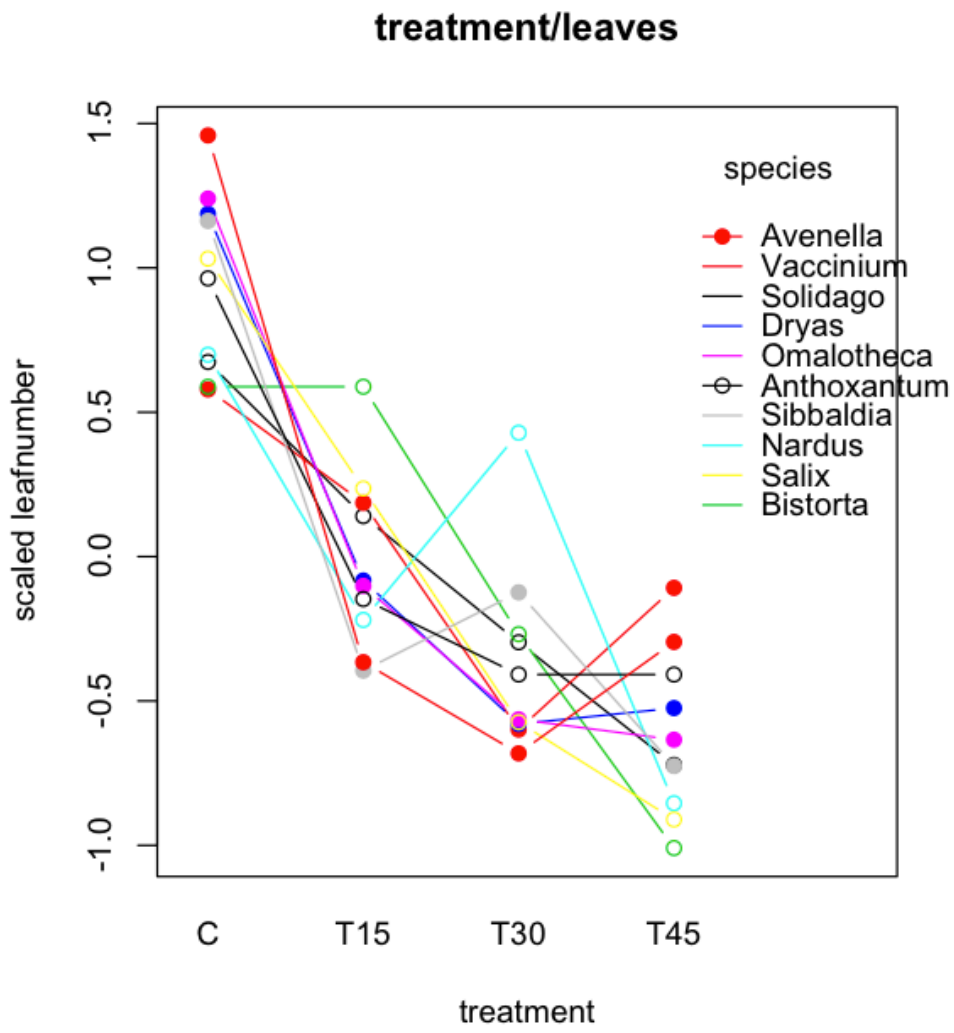|	numDF	denDF	F-value	p-value
Intercept	1	293	0.00947	0.9226
treatment	3	55	44.54518	<.0001
growthform	2	5	0.00029	0.9997
origin	2	5	0.02477	0.9757
treatment:growthform	6	55	1.38591	0.2367
treatment:origin	6	55	1.03193	0.4147

Figure IV. 1 Secondary leaf development response to varying *Empetrum nigrum* concentrations in soil by species.
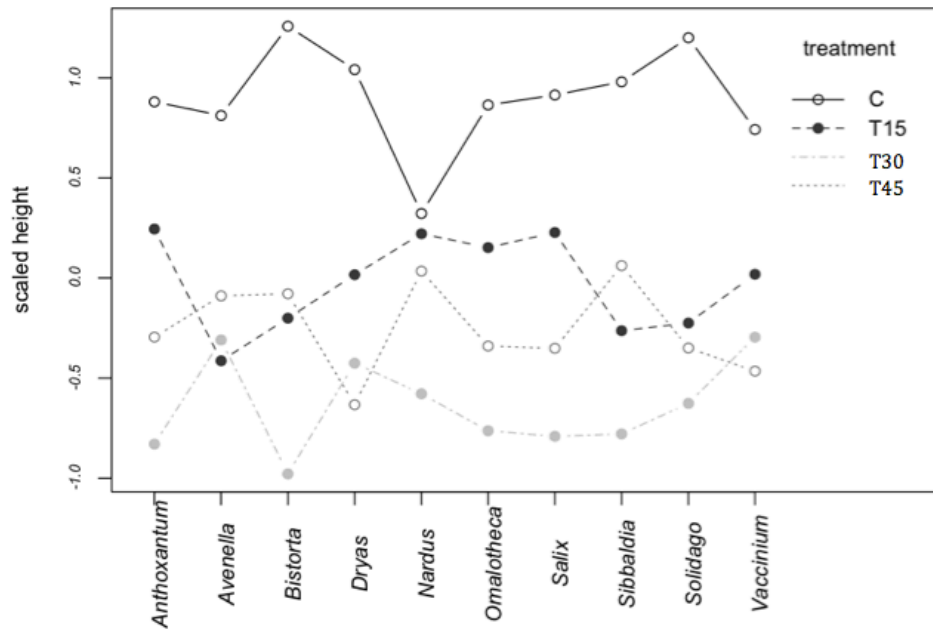
Figure IV.2 Seedling height response to varying *Empetrum nigrum* concentrations in soil by species.
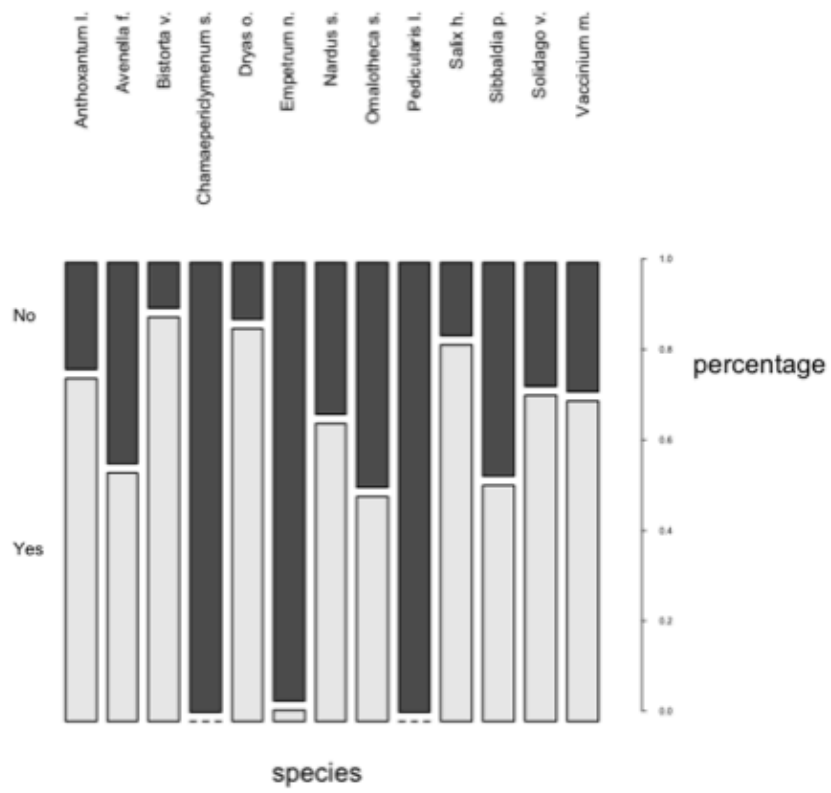
Figure IV.3. Species specific germination rate of 13 alpine species.